ISSN: 2320-8694

Journal of Experimental Biology And Agricultural Sciences

Volume 11 || Issue III || JUNE, 2023

Production and Hosting by Horizon Publisher India[HPI]
(http://www.horizonpublisherindia.in)

ISSN No. 2320 - 8694

Peer Reviewed - open access journal

Volume No – 11
Issue No – III
June, 2023

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Journal of Experimental Biology and Agricultural Sciences

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ISSN No. 2320 – 8694

Cinnamon as a Potential Feed Additive: Beneficial Effects on Poultry Health and Production Performances – An Update

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Received – May 28, 2023; Revision – June 25, 2023; Accepted – June 29, 2023
Available Online – June 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(3).444.461

KEYWORDS

Cinnamon

Natural feed additive

Antibiotic alternative

Essential oil

Poultry health

ABSTRACT

According to the Food and Agricultural Organization, global poultry output increased from approximately 115 million tons in 2016 to around 136 million tons in 2023. Poultry production has increased significantly with the dramatic uptick in meat and egg demand. Feed accounts for between 65 and 70 percent of total production costs, making it the largest chicken industry expense. This is why it's important to maximize the transformation of poultry feed into feed with a high biological value while taking as many steps as possible to protect feed quality and reduce feed costs. The use of feed additives in poultry feed has recently gained popularity and has been essential to increase feed efficiency and growth rate, which typically leads to reduced costs. The meat's texture, consistency, and nutritional content are all improved, and its shelf life is lengthened as a bonus. Feed additives are a fantastic tool for boosting a

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Gut microbiota

poultry farm's bottom line. For example, cinnamon (*Cinnamomum verum*) is often used as a traditional feed supplement. Rather than antibiotics, the poultry industry could benefit from using cinnamon as a natural antibiotic replacement, which would benefit animal welfare, consumer health, and the bottom line. The performance index, feed intake, FCE performance, and weight growth of poultry can all be improved by including cinnamon in the feed at varied concentrations. The digestive health and intestinal microbial population of hens are enhanced by a diet containing bioactive components of cinnamon. Cinnamon essential oils' popularity stems from their many valuable features, such as their ability to increase gastric enzyme synthesis and other biofunctional benefits. This review focuses on the possible advantages of cinnamon as a natural feed supplement for chickens, particularly about their intestinal microbiota, blood chemistry, nutrient absorption, gene expression, and immunology.

1 Introduction

Improvements in chicken farming have come a long way in recent decades. The Food and Agriculture Organization reported that the global poultry output increased from 115 million tons to around 136 million tons in 2022. This trend is being driven in high-income nations by the increasing popularity of white meats as a healthier and more convenient alternative to red meat (Buttar et al. 2022; Mitra et al. 2022). Lower pricing for poultry meat relative to other meats contributes to the increased trend in low and middle-income countries. For instance, the average annual rate of increase in India's poultry meat consumption (in 1000 metric tons) from 2013 to 2022 is depicted in Figure 1. Chicken will be the most common source of protein among meats, accounting for 47% of total consumption. The Economic Survey 2021-22 reported that India's domestic egg production increased from 91 eggs per year per capita in 2020 to 122.11 billion eggs in 2022, making India the third-largest producer of eggs and the eighth-largest producer of meat globally. The feed cost is the primary barrier to the profitable production of broilers (Hashemi et al. 2010). In India, 58% of the feed market is devoted to poultry. The current demand for chicken feed in India is around 22 million tons (Habib et al. 2021; Chandran et al. 2022). Maintaining feed quality while cutting feed costs to achieve optimal conversion of animal feed into feed of high biological value is a top priority (Thirumalaisamy et al. 2008; Chandran 2021; Rajan et al. 2023).

Feed efficiency and growth rate can be improved, and therefore savings on feed can be expected when nutritional supplements are incorporated into chicken feed (Abd El-Hack and Alagawany

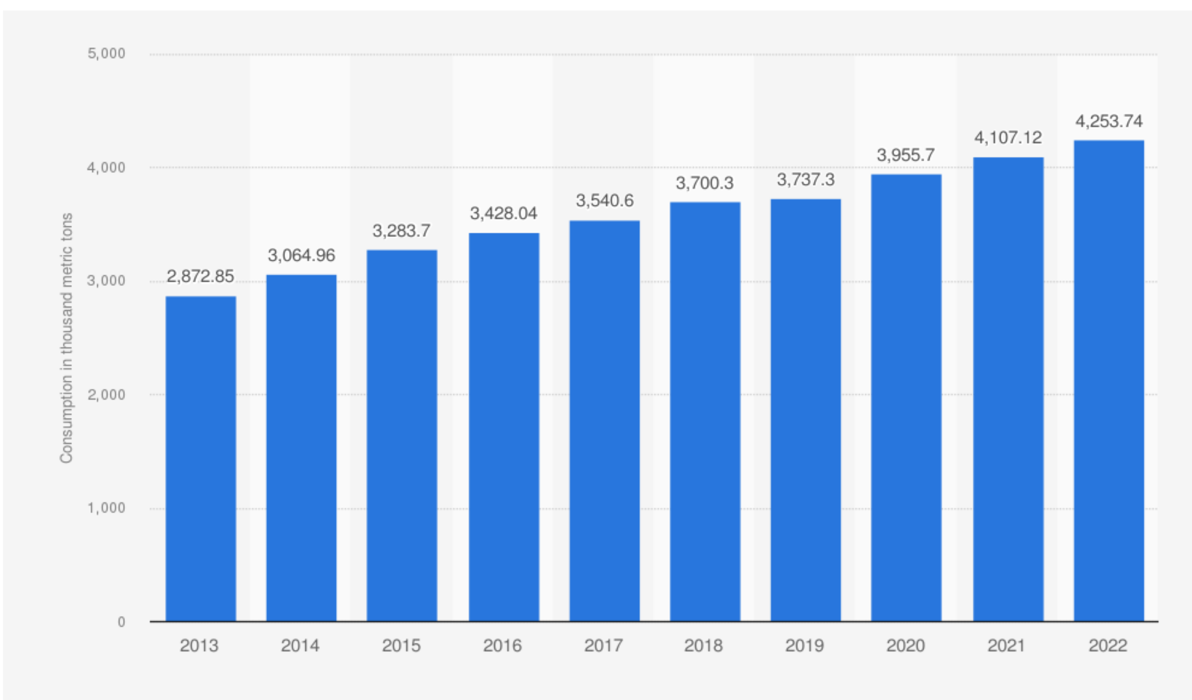

Figure 1 Average annual rate of increase in India's poultry meat consumption (in 1000 metric tons) from 2013 to 2022 (adopted, modified and updated from: Statista Research Department)

2022). Moreover, it enhances the meat's flavour, tenderness, shelf life, and nutritional value. Profit margins for the chicken industry have been greatly helped by the widespread availability of nutritional supplements (Rajan et al. 2022; Rajan et al. 2023). Research on the efficacy of traditional and potential herbal remedies in maximizing livestock and poultry production is being conducted in many countries. Phytochemicals and bioactive ingredients in plants and medicinal herbs have revealed multiple beneficial applications in promoting animal and poultry health and enhancing production performances, as well as found promising for safeguarding human health (Dhama et al. 2015a; Abdelli et al., 2021; Hartady et al. 2021; Seidavi et al. 2022; El-Sabrout et al. 2023; Zebeaman et al. 2023). These provide vital nutrients, act as potent immunomodulatory regimens, and possess antioxidants, antimicrobial and other activities, also suggested as alternatives to antibiotics and play supportive / ādjunctive roles in the current scenario of emerging antimicrobial and drug resistance (Dhama et al. 2014; Dhama et al. 2015b; Yadav et al. 2016; Dhama et al. 2018; Tiwari et al. 2018; Kuralkar and Kuralkar 2021; Uddin et al. 2021; Parham et al. 2020).

Antibiotics are commonly used to speed up the growth of chickens and other fowl (Ali et al. 2021; Habiba et al. 2021). However, antibiotic use is not generally promoted because of the detrimental implications of these residues in products; thus, the creation of a reliable and efficient antibiotic alternative is necessary, as reported by Hashemi et al. (2010) and Farag et al. (2021). Thus, natural ingredients have been investigated as potential replacements for synthetic substances in producing safe and healthy human meals. Spices and other herbs have been used to incorporate alternative medicines into animal feed (Habiba et al. 2021). Traditional fish meals, bone meals, and other plant-derived items like peanut or soybean meal, used in poultry diets, have become prohibitively expensive in developing countries. Rising raw material costs and increased human competition mean that even though these food ingredients are readily available, they are not nearly enough. Finding alternative feed sources to reduce feed prices has become critical (Swain et al. 2014; Prakash et al. 2022; Rajan et al. 2022).

Antibiotic growth promoters (AGPs) are used as a common practice for treating gut infections and mitigating the negative effects of stress on chicken digestion. However, as public awareness grows about antibiotics' negative impact on human health, as does the prevalence of bacterial resistance, and as concerns grow about food safety, measures have been taken to limit antibiotic incorporation in poultry production (Alagawany et al. 2020; Abd El-Hack and Alagawany 2022). To combat this problem, scientists and businesses are looking at feasible

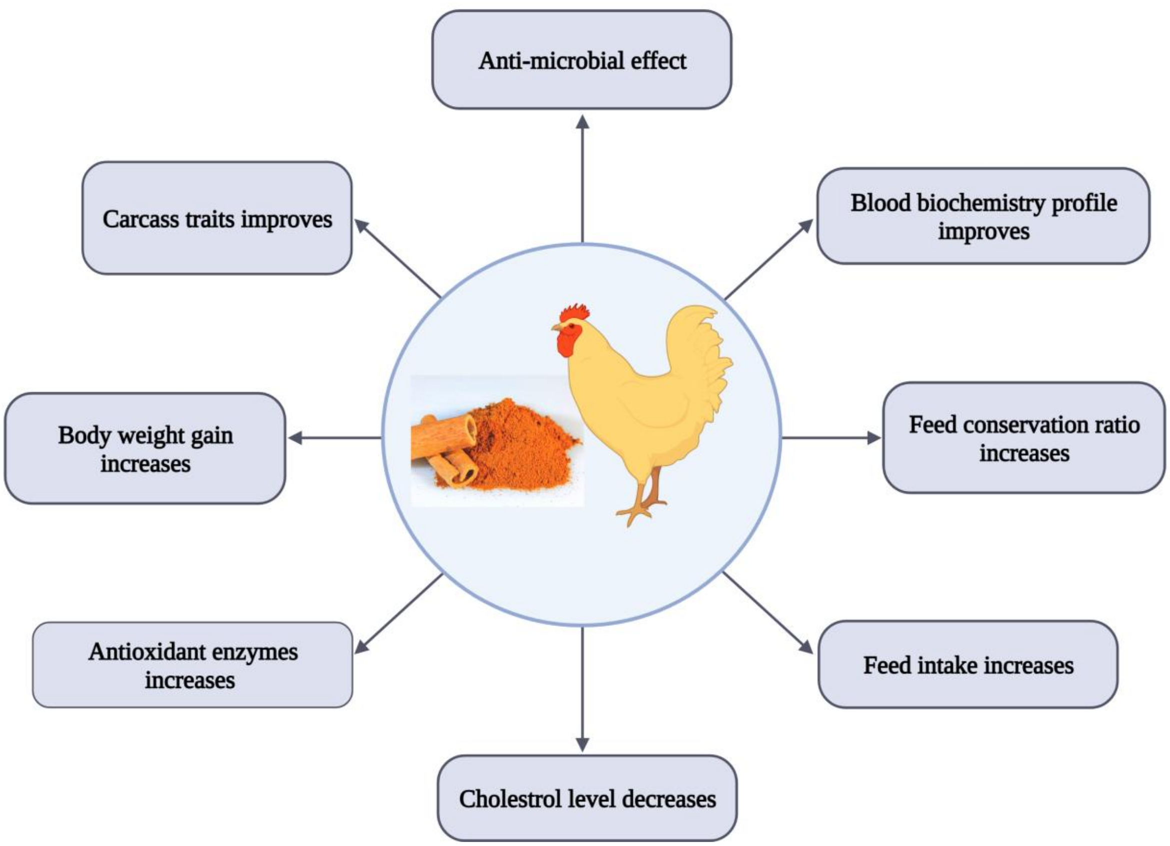

Figure 2 Positive health and nutrition impact of cinnamon as a feed additive in poultry

alternatives to AGPs, such as dietary therapies that boost the microbiome and general health of chickens without resorting to antibiotics. Potentially replacing AGPs, phytogenic feed additives (PFAs) have entered the market and show significant promise in the poultry sector. The immune system, health performance, and stress response are all boosted in chickens exposed to heat stress, for instance (Ali et al. 2021; Prakash et al. 2022; Rajan et al. 2022; Rajan et al. 2023).

Additionally, there has been a rise in the demand for leaner poultry, which has prompted scientists to focus on developing safe growth promotions and carcass modifiers. To that end, PFAs have been investigated as potential replacements for AGPs and growth modifiers in producing healthy and safe food. Several PFAs contain natural antioxidants that increase poultry products' longevity, quality, and marketability. With their therapeutic characteristics, phytochemicals found in herbs and spices have gained attention as potential replacements for AGPs. The poultry industry was pleased with their availability, natural safety, and lack of chemical residue. Natural plant compounds have been shown to have therapeutic benefits in animals, including an aperitif, an increase in digestive enzyme secretion, immunity stimulation, killing bacteria and viruses, and acting as an antioxidant. Potentially beneficial to poultry, cinnamon is one of the most potent PFAs (Prakash et al. 2021a; Prakash et al. 2021b; Kumar et al. 2022; Kumari et al. 2022).

Ceylon cinnamon, or cinnamon (*Cinnamomum verum*), is a spice derived from the bark of a tree in the Lauraceae family (Jakhetia et al. 2010; Arain et al. 2018). It's a staple seasoning in kitchens throughout the globe. In the agricultural sector, cinnamon has the potential to replace antibiotics, boosting health, food security, and the bottom line for commercial chicken operations. Adding cinnamon to broiler feed, even in small amounts, positively affects the birds' growth, supplement consumption, and feed conversion efficiency (FCE) (Chowlu et al. 2019). Therapeutic potential in cinnamon includes its ability to aid digestion and stimulate hunger (Taback et al. 1999), kill bacteria (Chang et al. 2001), neutralize free radicals (Singh et al. 2007), and reduce inflammation and acid reflux (Singh et al. 2007; Jakhetia et al. 2010). The potential benefits of cinnamon's addition to chicken feed in terms of health and nutrition are shown in Figure 2.

This review focuses on the possible advantages of cinnamon as a natural feed supplement for chickens regarding intestinal microbiota, blood chemistry, nutrient absorption, gene expression, and immunology.

2 Chemical composition and bioactive compounds in cinnamon

Many resinous substances, such as cinnamaldehyde, cinnamate, cinnamic acid, and other essential oils, make up cinnamon (Senanayake et al. 1978; Alagawany et al. 2016). The chemical composition of different parts of cinnamon is presented in Table 1; key bioactive compounds responsible for the functional characteristics of cinnamon are shown in Figure 3. Cinnamaldehyde, created by oxygen absorption, gives the food its pungent smell and taste. Cinnamon's colour darkens over time, and the amount of the resinous ingredient likewise rises (Singh et al. 2007). Cinnamon contains a variety of essential oils, including trans-cinnamaldehyde, cinnamyl acetate, eugenol, L-borneol, L-acetate-bornyl, beta-caryophylene, E-nerolidol, alpha-cubebene, alpha-terpineol, caryophyllene oxide, terpinolene, and alpha-thujene, (Tung et al. 2008; Farag et al. 2016). Procyanidins and catechins are present in cinnamon bark (Nonaka et al. 1983). The primary chemical components responsible for the aroma and other biological functions include cinnamaldehyde, trans-cinnamaldehyde (Cin), and eugenol (Chang et al. 2001). According to Marongiu et al. (2007), cinnamic aldehyde from *C. zeylanicum* has anti-tyrosinase activity. According to recent studies, cinnamaldehyde and cinnamon powder, alone or

Table 1 Important bioactive compounds present in different parts of cinnamon

Part of the plant	Bioactive compound	References
Leaves	Cinnamaldehyde: 1.00 to 5.00% Eugenol: 70.00 to 95.00%	Saeed et al. (2010); Abd El-Hack et al. (2020); Ali et al. (2021)
Bark	Cinnamaldehyde: 65.00 to 80.00% Eugenol: 5.00 to 10.00%	Saeed et al. (2010); Abd El-Hack et al. (2020)
Root bark	Camphor: 60.00%	Saeed et al. (2010); Abd El-Hack et al. (2020)
Fruit	*trans*-Cinnamyl acetate: 42.00 to 54.00% and caryophyllene: 9.00 to 14.00%	Saeed et al. (2010); Ali et al. (2021)
Buds	Terpene hydrocarbons: 78.00% *alpha*-Bergamotene: 27.38% *alpha*-Copaene: 23.05% Oxygenated terpenoids: 9.00%	Saeed et al. (2010); Habib et al. 2021; Ali et al. (2021)
Flowers	(E)-Cinnamyl acetate: 41.98% *Trans-alpha*-Bergamotene: 7.97% Caryophyllene oxide: 7.20%	Rao et al. (2014); Abd El-Hack et al. (2020)

Source: Jayaprakasha et al. (2002); Vangalapati et al. (2012); Saeed et al. (2018)

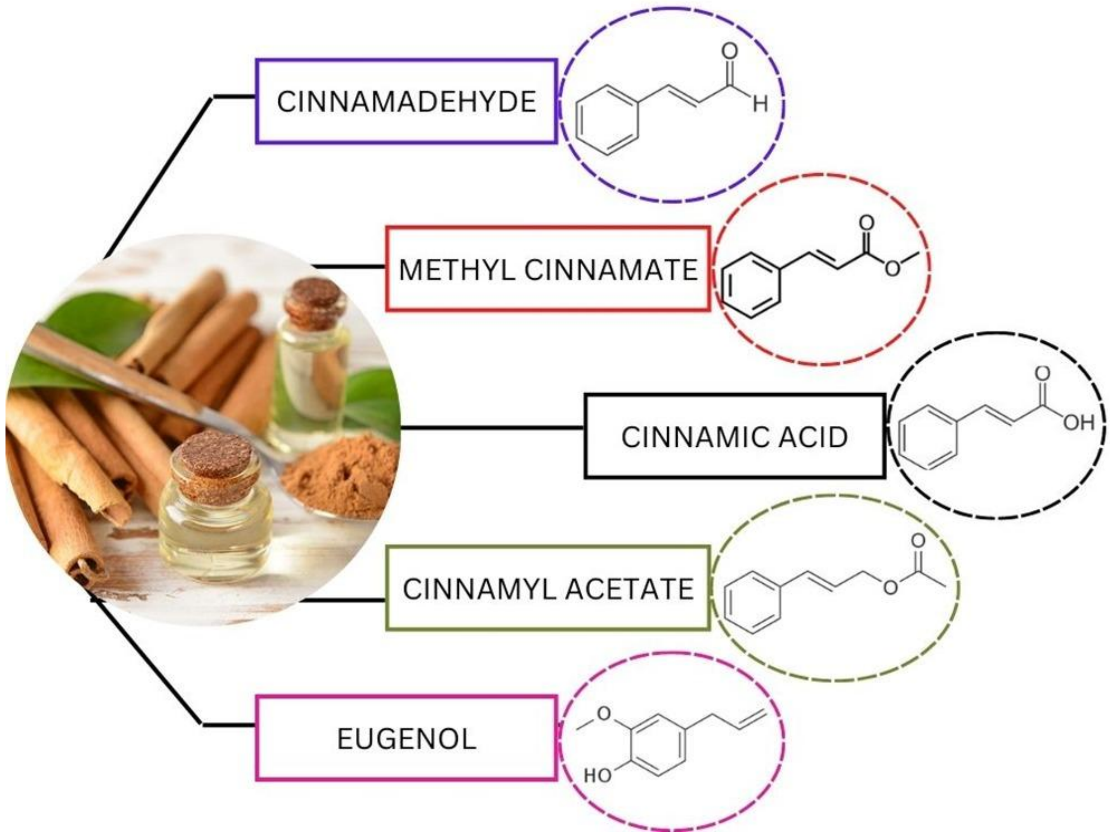

Figure 3 Key bioactive ingredients in cinnamon that contribute to its beneficial effects

in combination with other essential oils, have a variety of advantageous benefits on chickens. Among these results is a rise in feed consumption (Al-Kassie 2009), more excellent growth rates and meat of higher quality (Sang-Oh et al. 2013), increased output and effectiveness of feed (Kamel 2001; Al-Kassie 2009; Isabel and Santos 2009), elevated intestinal and pancreatic lipase activity (Kim et al. 2010), increased production of breast flesh (Isabel and Santos 2009), increased state of health (Kamel 2001; Al-Kassie 2009), protection against *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, epidermal Staphylococcus, Salmonella sp., *Helicobacter pylori* and *Vibrio parahemolyticus* (Taback et al. 1999; Chang et al. 2001; Chang et al. 2008) are reported.

3 Impact of cinnamon on digestion and absorption of nutrients

Cinnamon is now legal to use as a PFA in poultry feed. To improve the health, growth, carcass quality and carcass features of chickens, including bioactive chemicals such as essential oils, cinnamaldehyde, and phenolic compounds in chicken food is necessary (Ali et al. 2021). Studies have shown that the bioactive compounds present in extract of cinnamon are efficient enough to slow the process of digestion in the small intestine to augment the efficiency of utilization of nutrients (Baskara et al. 2021). Cinnamon's bioactive components have potent antimicrobial, antioxidant, anti-inflammatory, and free radical scavenging effects and inhibit NF-κB activity to minimize NO production. Broiler chicken research shows that cinnamon and its essential oils are antimicrobial and antioxidant (Zhu et al. 2020). In addition, cinnamon oil is effective as an anti-inflammatory, anti-fungal, anti-pain, and antioxidant. Concurrently, the bioactive chemicals in cinnamon can inhibit the growth of pathogenic germs in the intestines of chickens while encouraging the expansion of commensal bacteria (Lin et al. 2003).

When fed properly, broiler chickens benefit from a higher feed efficiency (FCR), greater body weight growth (BWG), and better health. Feed utilization can be enhanced, and growth-restricting metabolic and digestive disorders can be mainly avoided by stabilizing the gut microbial environment and stimulating the release of digestive enzymes (Bento et al. 2013). Cinnamon oil may influence intestinal mucosa and pancreas digesting enzyme release, according to chicken research (Bento et al. 2013; Zhu et al. 2020). Furthermore, cinnamon's bioactive components influence lipid metabolism via mediating fatty acid transport in broilers' intestines. Cinnamon oil aids digestion by stimulating the production of digestive enzymes and facilitating nutrient absorption (Garcia et al. 2007).

Additionally, the gut morphology and integrity protection provided by cinnamon oil may increase nutrient absorption. One study found that the cinnamon-fed group absorbed more nutrients than the control group (Devi et al. 2008). Cinnamon oil supplementation in the broiler diet increased duodenal and jejunal villus height (VH), villus surface area, and nutrient absorption and digesting efficiency. In addition, increased mucosal digesting enzyme activity is associated with a higher VH, leading to better nutrient absorption. Cinnamon oil's antioxidant properties are responsible for improving VH (Mehdi et al. 2018; Zhai et al. 2018). Digestion generates reactive oxygen species (ROS), which act on the intestinal mucosa and shorten intestinal villi but are bound by antioxidant enzymes (Windisch et al. 2008). One manner in which cinnamon oil safeguards the intestinal villi from oxidative damage is by elevating the activity of these antioxidant enzymes. The antimicrobial properties of cinnamon oil enhance intestinal morphology (Chowdhury et al. 2018).

Poultry benefit from cinnamaldehyde because it stimulates their digestive function. Cinnamaldehyde helped broiler chickens break down their food more efficiently by stimulating saliva production. This, in turn, stimulated the production of digestive enzymes in the pancreas and intestines. Cinnamon tannins have been found to improve poultry health and nutrition by precipitating proteins in the intestine (Zeng et al. 2015; Kumar et al. 2022). Groups given cinnamaldehyde supplements saw dramatic increases in their nutritional, amino acid, and crude fat digestibility. Cinnamon oil supplementation in broiler diets enhanced protein digestion by stimulating gastric production of hydrochloric acid (HCl) and pepsin (Platel and Srinivasan 2004; Redondo et al. 2014).

On the other hand, broiler chicks fed 200 ppm of an essential oil extract containing oregano, pepper, and cinnamon did not exhibit any changes in nutritional digestibility (Hernandez et al. 2004). Improvements in nutrient absorption and a return to a more normal state of gut ecology have been observed in hens treated with cinnamon oil (Jamroz et al. 2003; Mountzouris et al. 2011), which may be due to the terpenoids in cinnamon. Phytobiotic growth promoters that remained active throughout the digestive tract enhanced nutrient uptake changed intestinal histomorphology, reduced microbial load, and raised host immunity. Since it encourages the production of digestive enzymes and bile acid, it makes food and fat seem easier to break down. It also affects the digestive process by mediating interactions between feed and digestive enzymes (Zhai et al. 2018). The impact of cinnamon on nutrient digestion and absorption is summarized in Figure 4.

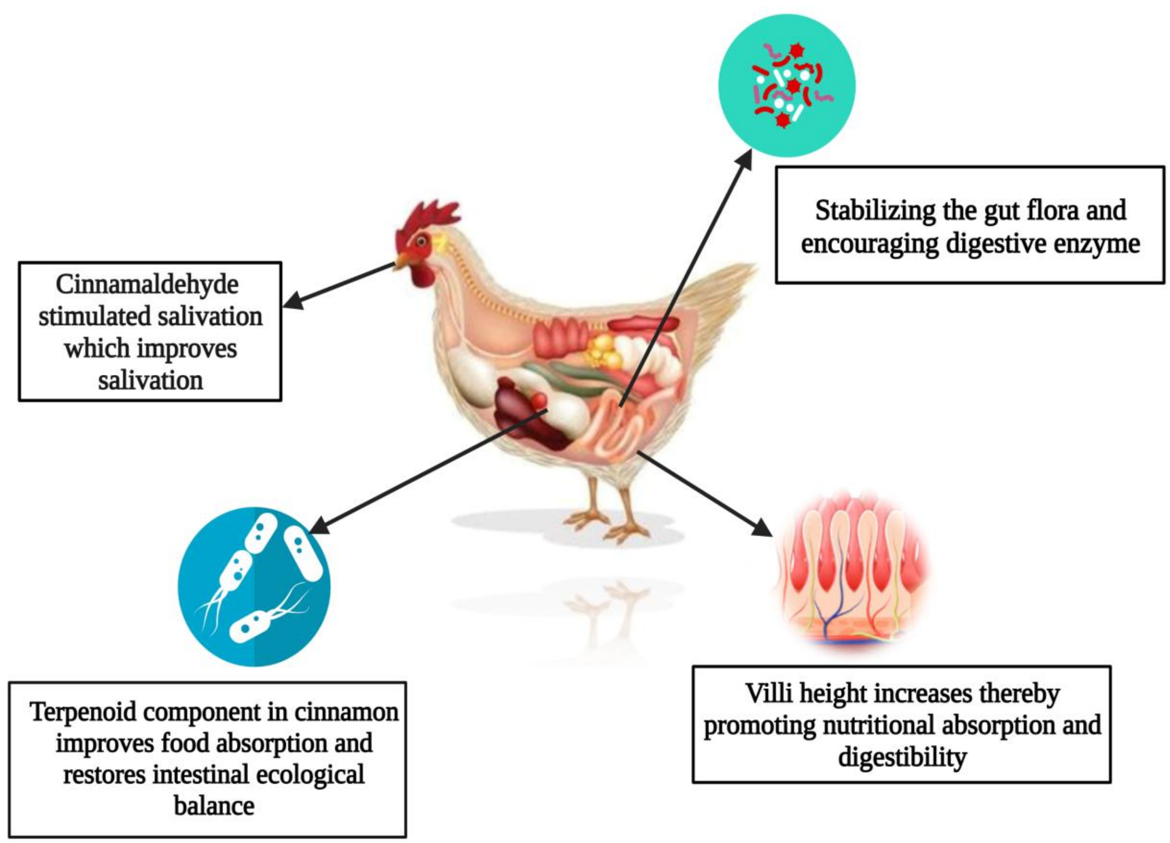

Figure 4 Potential impact of cinnamon on nutrient digestibility and absorption

4 Impact of cinnamon on body weight gain

It has been the subject of numerous scientific investigations into the effect of cinnamon and its derivatives on poultry diets. Researchers found that adding cinnamon essential oil to broiler feed led to a greater weight increase than giving the feed to broilers without any added cinnamon (Al-Kassie 2009). Consumption of cinnamon products at the rate of 2g/kg similarly enhanced the weight of chicks (Toghyani et al. 2011; Shirzadegan 2014). It has been shown by another group of researchers (Lee et al. 2003) that female broilers who consume cinnamon oil and its byproducts don't gain weight, but they do drink less water. Adding cinnamon products at a rate of 0.5-2 g/kg, as shown in the study of Koochaksaraie et al. (2011), did not affect body weight or broiler growth. The body weight of broilers does not change during the sale period when they consume 0.5 to 1 mL/kg (Symeon et al. 2014). Alternate strategies have concentrated on improving health and immune system function by decreasing the number of pathogenic bacteria and modifying the gut microbiota composition to increase output. Essential oils improve feed efficiency and growth performance by influencing immune function, regulating intestinal microflora, stimulating the production of gastric enzymes, and promoting antimicrobial and antioxidant properties. Though the inclusion of cinnamon in poultry diets has been shown to have no direct effect on body weight, few researchers reported that cinnamon has a positive impact on the birds' overall health and wellbeing, particularly their intestinal health and immunity, both of which contribute to increased growth and production (Saeed et al. 2018; Abo-Ghanima et al. 2020). In broiler chickens under heat stress conditions, it has been found that there is a significant improvement in the gain of body weight by feeding cinnamon powder (Khan et al. 2022).

5 Impact of Cinnamon on feed conversion efficiency and feed intake

Cinnamon oil has improved poultry immune systems, reduced feed consumption, and improved feed conversion efficiency; however, studies have reached conflicting conclusions. Three studies have confirmed this effect (Mehdipour et al. 2013; Sim sek et al. 2015; Torki et al. 2015). Poultry fed a meal containing 200 ppm of an essential oil blend of thyme and cinnamon performed better in terms of feed efficiency and feed intake, as reported by Al-Kassie (2009). Broilers fed a diet containing 500 ppm of essential cinnamon oil outperform antibiotic-treated groups in terms of feed conversion efficiency, according to Ciftci et al. (2009). However, it was reported that using cinnamon oil in the diet decreased the feed conversion rate (Sim sek et al. 2015). Torki et al. (2015) found that layers fed a diet containing zinc and cinnamon oil had a worse feed conversion rate when exposed to chilly stress. Antibiotics can be reinstated with organic acid and essential oil, as shown by the fact that the feed control rate increased after two weeks when poultry were given either enramycin at 125 ppm or a mixture of cinnamaldehyde (500mg/kg) and calcium formate. Broilers fed 200 ppm of an essential oil extract containing oregano, pepper, and cinnamon did not exhibit any changes in feed conversion efficiency during weeks 2 and 3, as reported by Hernandez et al. (2004). In broilers under heat stress conditions, it has been found that there is a significant improvement in the intake of feed and feed conversion ratio by feeding cinnamon powder (Khan et al. 2022).

6 Impact of Cinnamon on gene expression profiling

Gene expression profiling is one of the most cutting-edge methods for understanding the genetics behind complicated features like residual feed intake (RFI) in chickens and other animals (Izadnia et al. 2019). The genes and processes that contribute to complex traits in chickens have been categorized using RNA sequencing and next-generation sequencing technologies, which have been widely used to study molecular genetic pathways (Erfan and Marouf 2019). For example, by manipulating gene expression in chickens for up- and down-regulations, 121 and 279 previously identified genes were revealed to be involved with RFI and growth rate, respectively (Izadnia et al. 2019). Quantitative trait loci (QTL) analysis revealed the down-regulation of genes involved in feed efficiency features (Ali et al. 2021). In a study conducted by Tabatabaei et al. (2015), a chicken who received *E. coli* injections in addition to dietary cinnamon extracts (100-200 mg) showed significantly lower expression levels of nuclear factor beta (NF-β) and tumour necrosis factor-alpha (TNF-α). Another study found an upregulation of gene expressions of cationic and neutral amino acid transporters in birds fed with encapsulated cinnamaldehyde (Yang et al. 2021).

7 Impact of Cinnamon on Immunomodulation

The delicate balance of diet, bacteria, and mucus necessary to maintain the gut's immune system properly functioning is not easy. The phenolic components in cinnamon have free radical-scavenging properties; therefore, eating cinnamon can help keep your skin looking young. Multiple studies (Ciftci et al. 2010; Simsek et al. 2013; Alves-Santos et al. 2020) support this notion. Consuming cinnamon regularly increases the beneficial bacteria in the gut, which protects against disease and offers essential nutrients. As a result of their bacteriostatic properties, short-chain fatty acids inhibit the growth of pathogen-causing bacteria, increase the colon's absorption surface by stimulating cell division, and attempt to halt the production of new bile byproducts. Cinnamonaldehyde has been found to reduce the production of inflammatory cytokines in cells stimulated with lipopolysaccharide (Habiba et al. 2021). The expression of critically important mRNA in epithelial cell inflammation is also suppressed in response to these cytokines. Chickens' resistance to disease is improved by cinnamonaldehyde, which contains antiinflammatory effects (Dawson et al. 2003; Pannee et al. 2014). The antigen and immune response pathways are involved in cinnamon's immune regulatory actions (Lillehoj et al. 2011). Improvements in intestinal immunocompetence and an increase in IgA content in chickens fed cinnamon essential oil were reported by Kettunen et al. (2006). Increased serum immunoglobulin was seen in broilers fed a cinnamon diet (Sang-Oh et al. 2013). The serum immunoglobulin M (IgM) concentration increased in hens after being fed cinnamon essential oil for 42 days (Yang et al. 2019), and the MDA concentration dropped after just 21 days.

8 Impact of Cinnamon on blood biochemistry profile

Blood biochemistry is a useful diagnostic tool in both human and veterinary medicine for determining the effects of the environment, diet, and treatment. The positive effect of cinnamon oil in reducing the level of sugar in the body is evident. Both the albumin-to-globulin (A/G) ratio and total cholesterol levels were affected by the cinnamon-derived PFAs. PFAs derived from cinnamon could lower cholesterol levels in consumers' favourite meat, increasing its appeal (Chandran 2021; Krauze et al. 2021; Kumari et al. 2022). The effects of cinnamon on chicken blood biochemistry, however, are inconsistent. There is a notable impact on blood biochemistry from using cinnamon essential oil, cinnamon powder, cinnamaldehyde, or any combination of these, as chicken feed additives. Total protein content, hemoglobin, RBCs and WBCs increased in chickens given 200 ppm of cinnamon essential oil as supplemental feed (Al-Kassie 2009). The heterophils/lymphocytes ratio and cholesterol concentration both decreased. Cholesterol and malondialdehyde (MDA), total saturated fatty acid, and alanine aminotransferase activity were all found to be decreased in chickens fed 1000 ppm cinnamon essential oil, while unsaturated fatty acids, omega-6 fatty acids, catalase activities and blood phagocytic activity were all found to be increased (Ciftci et al. 2010; Prakash et al. 2021a, b). Superoxide dismutase (SOD) activity, antioxidant capacity, catalase, and corticosteroid levels were all enhanced after 42 days of supplementation with cinnamon powder in chickens (Mehdipour and Afsharmanesh 2018). Blood levels of uric acid, malondialdehyde, and lactate dehydrogenase were all lower in heat-stressed Ross 300 broiler chicks fed cinnamon powder (Kanani et al. 2016). Poultry fed a diet containing 7.5g of cinnamon powder per kilogram of body weight experienced a reduction in the heterophil/lymphocyte ratio, suggesting that the spice may have a calming effect (Naderi et al. 2014). These results were accomplished because cinnamaldehyde is an antioxidant that prevents ROS from damaging the liver. Figure 5 shows how cinnamon changes the biochemistry of poultry blood.

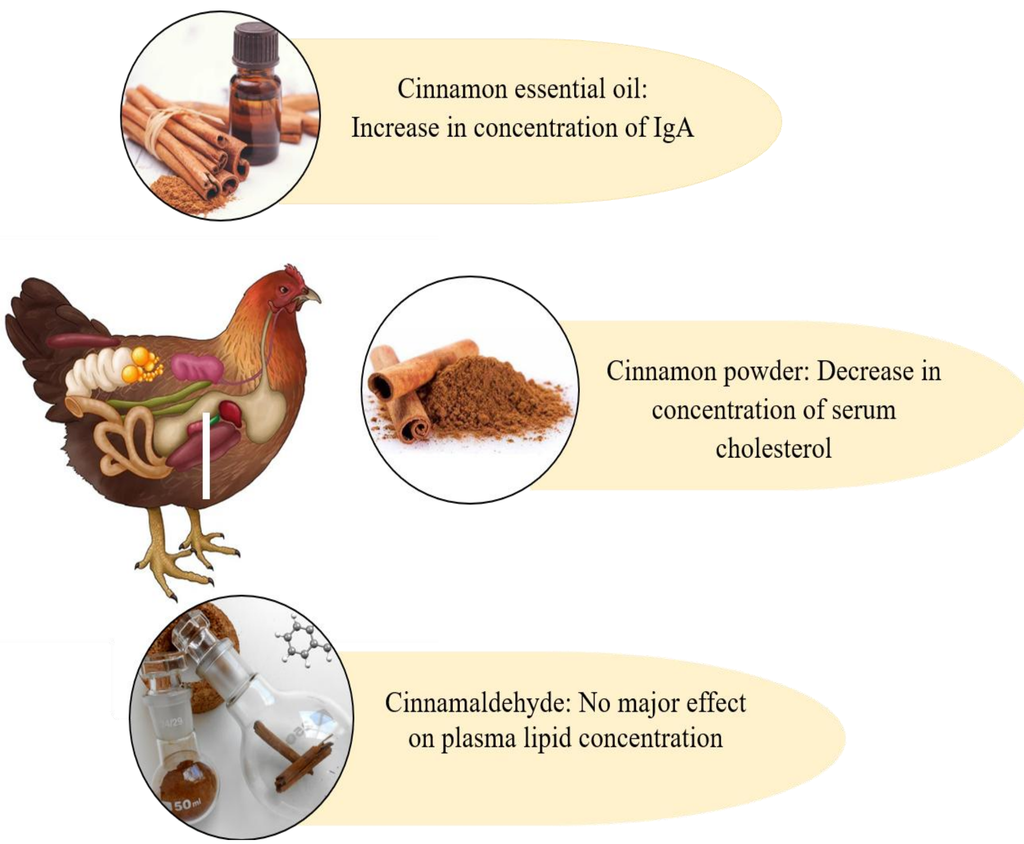

Figure 5 Potential impact of cinnamon on blood biochemistry profile of poultry

Adding cinnamon essential oil to broiler feed increased HDL cholesterol and decreased triglycerides and LDL cholesterol (Faghani et al. 2014). When added to chicken feed at a dosage of 0.8%, cinnamon has been shown to decrease total protein, low-density lipoprotein (LDL) cholesterol, plasma cholesterol, and blood glucose concentrations in birds (Najafi and Taherpour 2014; Kumari et al. 2022). Blood sugar levels were lowered and the proportion of antibody-positive controls increased when cinnamon powder at a concentration of 1.0% was added to the chicken feed (Hossain et al. 2014). Torki et al. (2015) found that adding cinnamon and zinc to the diets of broilers exposed to cold stress reduced the animals' triglyceride and blood glucose levels. A lower blood cholesterol concentration was observed when ground cinnamon was added to the food of broiler chickens at 250 and 500 ppm (Gopi et al. 2012). The plasma lipid concentrations of broilers were not noticeably affected by the addition of cinnamaldehyde to their diet (Lee et al. 2017). The use of the medium and highest doses of cinnamon (0.1 and 0. 25 ml/l) resulted in a beneficial increase in the concentration of low-density lipoprotein receptor-related protein 1 and acetylcholinesterase as well as a decrease in the level of cholesterol and hyperphosphorylated Tau protein (Krauze et al. 2023).

9 Impact of cinnamon on gut microbiota and overall gut health

Eliminating infectious pathogens and promoting great intestinal health in chickens depend on the proper development of meal digestion and absorption, the immune system, and the gut's balance of water and electrolytes. The content of the diet, the presence or absence of feed additives (such as prebiotics, probiotics, phytobiotics, etc.), heat stress, genetic profile and chicken farm feeding practices all affect the gut microbial ecology. Chickens' health and the composition of their gut microbiota are profoundly impacted by these factors (Nawab et al. 2018). It is well known that a healthy poultry intestine with enough microbiota to perform its necessary functions is essential for peak performance. This is because all of these factors—energy balance, sustained inflammatory balance, tissue metabolism, and correct gut physiological function—are intertwined with structural consistency and antibody production. Chicken health is affected by the composition and activity of the gut microbiome (Diaz Carrasco et al. 2019).

A wide variety of bacteria make up the microbiota of a chicken's digestive tract. There are hundreds of different types of bacteria in the intestinal microbiota of chickens, including actinomycetes, bacteroides, firmicutes, fusobacteria, and proteobacteria (Iqbal et al. 2020). The feed efficiency of broilers is increased, increasing the growth rate and productivity of poultry (Bedford 2000). The parasite 5 infects the eggs of laying hens because it resides in the intestines of these birds and then spreads to other organs, including the fallopian tubes. Including trans-cinnamaldehyde in laying hens' diets has improved their health by reducing the spread of infections from *Salmonella enteritidis* eggs (Upadhyaya et al. 2015). Iqbal et al. (2020) estimate that anywhere from 500 to 1100 distinct bacterial species are present in the GIT, each accounting for 100 trillion individual cells. Protozoa, bacteria, and fungi all have roles in the avian digestive system. Poultry had varying microbial concentrations throughout the intestine, with the highest levels towards the tail end. To ward off microbial invasion and play a role in cell signalling, intestinal epithelial cells form tight interactions with one another. Oxidative stress, which is the result of interactions between pathogenic bacteria and their toxins and the mucosa, is known to cause damage to the intestinal mucosa, the intestinal epithelial barrier, tight junctions, and lipid peroxidation, as shown in many studies (Gopi et al. 2012; Upadhyaya et al. 2015; Habiba et al. 2021).

Broilers are designed to gain the most weight quickly to satisfy the ever-growing demand for chicken meat (Lee et al. 2017). Antioxidant compounds added to meals reduce free radicals and protect the intestinal lining (Mandal et al. 2015). Thus, the poultry industry needs to figure out how to implement this finding. The high expense of testing and the need for a final sampling of a large number of birds to capture stomach contents have limited the application of this technique, even in commercial settings. The health, longevity, and antioxidative capacity of chickens can be improved by adding bioactive components to their diet. Essential oils and phenolics are two of cinnamon's active components that have been shown to have antiinflammatory and antimicrobial benefits in animal studies. Modifying the intestinal microbiota and its activity with dietary adjustments can counteract cinnamon's potential effect on the microbiome (Mandal et al. 2015; Lee et al. 2017).

To this end, PFAs are being tested in poultry feed as a non-toxic and safe way to improve the birds' health by fostering a more diverse and stable gut microbiota. In order to improve nutritional absorption through the intestinal walls of chickens, phytobiotics are utilized to alter their gastrointestinal systems (Mueller et al. 2012). This is accomplished by promoting antibacterial, antiinflammatory, and antioxidant responses. The caecal organism's aid in the host's nitrogen metabolism is associated with food's positive health effects (Oakley et al. 2014). Although the small intestine is where most digestion and absorption of nutrients occurs, the bacteria found in the cecum ferment any food that was only partially digested in the ileum (Lee et al. 2017).

The ileum of the intestine is dominated by aerobic bacteria (Enterococcus spp. and Lactobacillus spp.), whereas the cecum is dominated by pathogenic obligate anaerobic bacteria (Campylobacter spp. and Enterococcus spp.) (Yin et al. 2010; Boguslawska-Tryk et al. 2015). It has been hypothesized that a chicken's digestive tract's health directly affects its productivity.

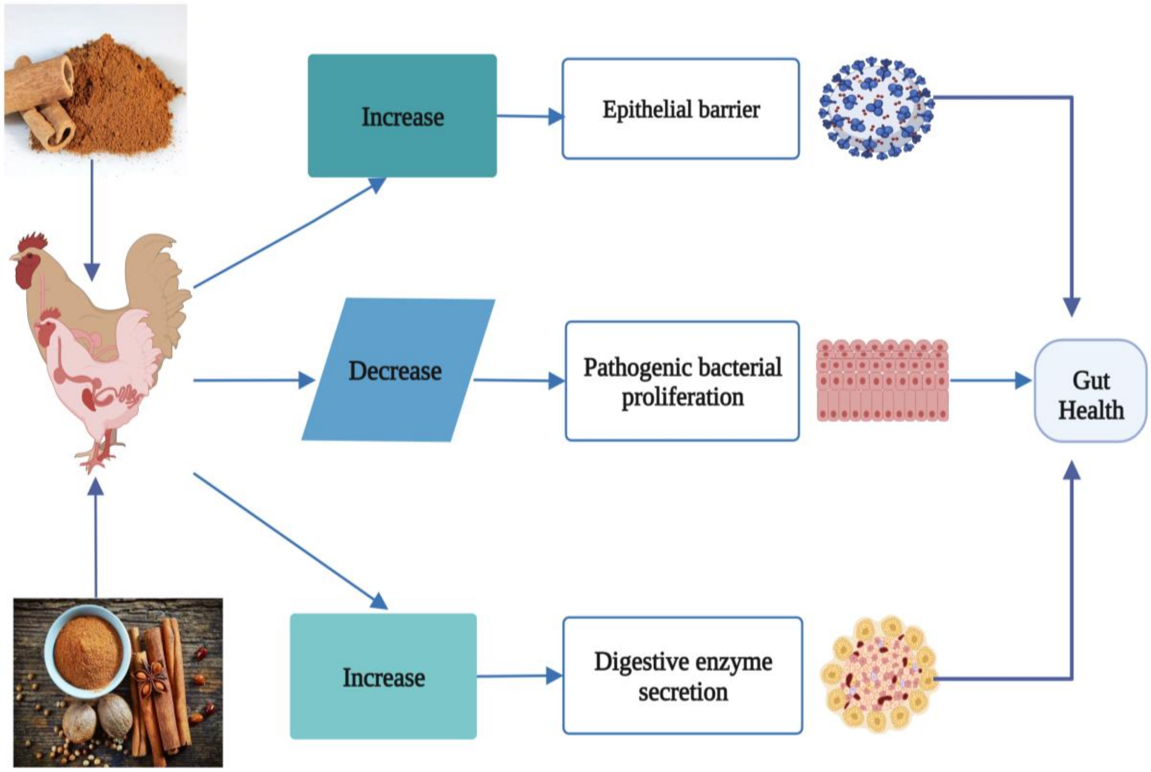

Figure 6 Potential impact of cinnamon on poultry gut health

Including cinnamon in a chicken's food has been shown to improve intestinal microbiota and digestive health (Mehdi et al. 2018). The gut ecology functioned better after consuming a cinnamon-based diet, as beneficial bacteria proliferated and detrimental bacteria decreased (Rashid et al. 2020). The cinnamon diet promoted the growth of Lactobacillus spp. in chickens ileum and caecum while dramatically inhibiting Campylobacter spp., *E. coli*, and other microorganisms. There are bioactive components in cinnamon that kill bacteria, such as Enterococcus spp. and *Pseudomonas aeruginosa* (Chang et al. 2001).

Volatile components such as cinnamaldehyde, eugenol, and carvacrol make up the bulk of cinnamon essential oils. Compared to cinnamon extract, cinnamon oil was more effective against bacteria. Further research showed that cinnamon essential oil has a bactericide minimum bactericidal concentration (MBC) of 125–250 g/mL and a minimum inhibitory concentration (MIC) of 25–100 g/mL (Pathak et al. 2017). It is important to note that cinnamaldehyde in encapsulated form may not prove toxic to the birds (Zaefarian et al. 2019). Combining thymol and cinnamaldehyde has been recommended in several studies for its potential to reduce disease transmission and give animals better stomach wellbeing (Ouwehand et al. 2010; Tiihonen et al. 2010). Cinnamaldehyde, capsaicin, carvacrol, and other compounds in cinnamon have been shown to reduce the number of *E. coli* and increase the number of Lactobacillus in the intestines of broilers (Jamroz et al. 2005). The ability of cinnamon oil to disrupt bacterial cell membranes may explain why it helps reduce *E. coli* populations. Again, cinnamaldehyde (encapsulated) alone or in combination with citral has been found to cause a reduction in the cases of necrotic enteritis (Yang et al. 2020). Poultry with a higher antioxidant status, immunity, and antimicrobial activity can benefit by including cinnamon essential oil as a feed element in poultry feed (Tiihonen et al. 2010). As Solanki et al. (2022) reported, Cinnamon oil can be used as a substitute for chemical growth enhancers in the chicken business. Caecal *E. coli*, *Salmonella*, total yeast, mold, and total microbial count were reduced in chickens fed cinnamon oil, but the total microbial count was identical to that of antibiotic-treated chickens. Broiler chickens can benefit from a diet supplemented with cinnamon oil instead of antibiotic growth enhancers at doses of 10 mg/kg avilamycin, 500 mg/kg, 1000 mg/kg, and 1500 mg/kg, respectively (Saied et al. 2022). The potential impact of curcumin on poultry gut health is illustrated in Figure 6.

Conclusion and future prospects

Using cinnamon as a feed additive improved a variety of characteristics in poultry, including their ability to digest and use nutrients, their rate of growth, their body weight gain, their appetite, their feed conversion efficiency, the traits of their carcasses, the quality of their meat, their blood chemistry, the expression of their genes, the composition of their gut microbiota, and their immune function. Increased demand for broilers resulted in the stress-reducing effects of cinnamon on their feed, which reduces the heterophil to lymphocyte ratio. As a result, cinnamon has the potential to replace antibiotics, as it protects against food poisoning, lessens exposure to harmful chemicals in feed, and promotes environmental health. Incorporating cinnamon into poultry feed has been demonstrated to have positive effects. In addition to boosting the immune system and lowering illness prevalence, cinnamon has been shown in certain tests to increase broiler chickens' growth rate and feed efficiency. Cinnamon may also have positive effects on human health and the environment, and it is also a safe and natural feed ingredient that can replace synthetic additives. A rising number of people are concerned about the environmental effects of the poultry business and are working to increase the efficiency of poultry farming. Improved poultry health and growth with less reliance on antibiotics and other synthetic chemicals might be possible with cinnamon's inclusion in poultry feed. Demand for natural and organic poultry products is also rising, thanks to increasing consumer awareness of the negative effects of conventional poultry agriculture on the environment and human health. Cinnamon's timely inclusion as a feed additive could help farmers gain a competitive edge and higher prices for their wares. Overall, cinnamon's potential as a feed additive for poultry is encouraging, and more study in this area is expected to provide additional insights and benefits in the future. While cinnamon has shown promise as a feed additive, its efficacy may vary based on individual chicken farms' environmental factors and management techniques; experiments should be conducted and performance closely monitored.

Using PFAs like cinnamon can make poultry production more cost-effective, risk-free, and healthy without negatively impacting the productivity of the birds. Even yet, further investigation is needed to elucidate the fundamental link of these PFAs with enhanced gut microbiota. Heat stress, in particular, has a major effect on the health and productivity of chickens and may be mitigated by applying cinnamon oil. There has been a rise in interest in using cinnamon oil for its immunomodulatory characteristics in poultry production, although this area still lacks sufficient study. To monitor the metabolites and fate of cinnamon's bioactive components in the chicken intestine, it is necessary to establish methodologies for the analytical analysis of these compounds. The effectiveness of the poultry industry's use of cinnamon or cinnamon oil depends on our having a more solid understanding of the biological activities of cinnamon and related components. More research is needed into the relationship between cinnamon's chemical composition and its Influence on poultry health and productivity.

Acknowledgement

All the authors acknowledge and thank their respective Institutes and Universities.

Author's contribution

All the authors contributed significantly.

Funding

This is a compilation written by its authors and required no substantial funding to be stated.

Disclosure statement

All authors declare that there exist no commercial or financial relationships that could, in any way, lead to a potential conflict of interest.

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Journal of Experimental Biology and Agricultural Sciences, June - 2023; Volume – 11(3) page 462 – 478

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Potential benefits of *Glycyrrhiza glabra* (Liquorice) herb, its chemical make-up and significance in safeguarding poultry health: Current scientific knowledge

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Received – May 25, 2023; Revision – June 28, 2023; Accepted – June 30, 2023
Available Online – June 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(3).462.478

KEYWORDS

Glycyrrhiza glabra

Liquorice

Therapeutic benefits

Pharmaceutical

ABSTRACT

Positive results have been seen when bioactive components from herbal plants are added to poultry diets. Efficacy in feeding, digestion of nutrients, antioxidant health, immunological indices, and other factors can all be improved with the help of these additives, which in turn increases growth rates and improves poultry welfare. Several researchers have used sophisticated herbal formulae that included *Glycyrrhiza glabra* (Liquorice) as an ingredient. Epidemic illnesses, mainly in the respiratory, digestive, and immunological systems, pose the greatest threat to the poultry business. Flavonoids and glycyrrhizin are two of the bioactive compounds in Liquorice. The roots of this plant contain glycyrrhizin at concentrations of 1-9%, which has numerous pharmacological benefits, including anti-infectious, antioxidant, antiviral,

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI]
(http://www.horizonpublisherindia.in/).

Poultry

Health

and anti-inflammatory properties. Liquorice extracts are helpful in the treatment of multiple common illnesses. These include problems with the liver, the lungs, and the immunological system. Adding Liquorice to chicken diets improves their productivity in several ways, including fostering organ growth and stimulating digestion and appetite. Liquorice has many beneficial effects on birds, including helping them grow larger bodies, cleansing their systems, and protecting them from free radicals, bacteria, and inflammation. In this article, we'll look at the chemical make-up of liquorice herb, its role in protecting poultry health, and its recent applications and benefits.

1 Introduction

In developing countries like India, the majority of people make their living in agriculture and related industries. Although the agricultural sector's share of India's economy is shrinking, it disproportionately impacts the country's workforce disproportionately. As a result of rising costs and uneconomical holdings, farming in the country has become unprofitable, notwithstanding the country's impressive achievements in food production (Chandran 2021; Buttar et al. 2022; Chandran et al. 2022; Mitra et al. 2022; Rajan et al. 2023). Farmers must therefore rely on unrelated industries such as animal husbandry, dairy farming, poultry industry, and milk manufacturing. Due to its low investment cost and short gestation period, poultry farming has emerged as a key industry in India, with the potential to spur rapid economic growth and assist people experiencing poverty. Backyard poultry also helps the poorest of the poor generate more revenue and feed their families (Kaur et al. 2009; Sheikh et al. 2018; Chandran 2021; Egorov et al. 2021; Prakash et al. 2022; Rajan et al. 2023).

Plants and medicinal herbs possess several useful potentials in protecting the health of humans, animals and poultry and increasing production in animals and poultry (Hartady et al. 2021; El-Sabrout et al. 2023; Zebeaman et al. 2023). These are a rich source of essential nutrients and play critical roles as immunomodulators, antioxidants, antimicrobials, and also as alternative and supportive (adjunctive) to antibiotics under the present evolving menace of emerging antimicrobial drug resistance (Dhama et al. 2014; Dhama et al. 2015b; Yadav et al., 2016; Dhama et al. 2018; Tiwari et al. 2018; Kuralkar and Kuralkar 2021; Uddin et al. 2021; Parham et al. 2020). Medicinal plants have been traditionally used for many years to treat and prevent illness in animals and poultry and have shown promising beneficial, scientifically evidenced impacts in recent times (Verma and Singh 2008; Abdelli et al. 2021; Dhama et al. 2015a; Kuralkar and Kuralkar 2021; Chandran et al. 2022; Seidavi et al. 2022; El-Sabrout et al. 2023). Phytobiotics have been shown to improve growth, gastrointestinal health, antioxidant action, nutrient absorption, and immunity and decrease diarrheal syndrome in poultry (Prakash et al. 2021a; Rafiq et al. 2022). Herbal remedies are frequently employed in the industry. In particular, small- and medium-scale farmers often rely on traditional treatments derived from plants rather than synthetic pharmaceuticals. Alkaloids, saponins, flavonoids, phenols, and tannins are only a few examples of the phytochemicals found in medicinal plants that give them therapeutic properties and make them inexpensive. Despite the advances in modern medicine, many Indian farmers still rely on traditional herbal treatments for poultry health (Egrov et al. 2021; Prakash et al. 2021a; Prakash et al. 2021b; Kumar et al. 2022; Kumari et al. 2022; Rajan et al. 2023).

Glycyrrhiza glabra Linn (Liquorice or licorice) is one of the most used herbs in Ayurvedic medicine (Jatav et al. 2011). It is also most often traditionally used medicine in Europe and China for long before (Zhou et al. 2019). *Glycyrrhiza glabra*, commonly called Liquorice or sweetwood or Mulaithi, is a medicine and a flavouring agent. The host's metabolism and immune system are improved using Liquorice, mainly used to treat oral, gastrointestinal, and liver problems. The blood testosterone level of women is lowered by its use, and aplastic anemia is helped by it. It is also a herbal medicine for gastritis and respiratory tract infections. Immunodeficiency illnesses, such as acute immunodeficiency syndrome, are also treatable with liquorice extract, which has been used for years to treat autoimmune conditions (Kaur et al. 2013; Sharma and Agrawal 2013). There are both estrogenic and anti-estrogenic properties in licorice root. According to research conducted by Kaur et al. (2013), this plant is also effective in treating hormone-related issues in females.

Glycyrrhiza, the genus from which Liquorice comes, is a family of roughly fourteen species. The roots of this plant have been used medicinally for more than 4000 years. As Ajaib et al. (2013) reported, *G. glabra* is a plant whose roots treat genito-urinary conditions due to their tonic, demulcent, laxative, and emollient properties. Roots of the glycyrrhiza plant are used to alleviate a cough due to their demulcent and expectorant qualities. Liquorice roots, when chewed fresh, have multiple uses as a breath mint which helps in teething, and make whiten teeth. Liquorice root is a popular supplement for treating gastrointestinal issues, menopausal symptoms, and bacterial infections in modern times. Liquorice tea is also used to cure a sore throat, and topical gels help with skin issues such as acne and eczema. Liquorice leaves when used fresh, work as a natural antiseptic for cuts and scrapes. It is also used to deal with conditions like diabetes, stomach ulcers, Addison's disease, renal issues, and infections caused by bacteria and viruses. Increasing oxygen and nutrition delivery to the scalp helps hair grow from its base. Diarrhea, fever, fever with delirium, and anuria are all conditions that benefit from their inclusion in therapeutic oils. Liquorice powder can also be found in desserts, ice creams, baked products, and soft drinks (Bala et al. 2021).

Numerous pharmacological benefits, including immunomodulatory, antioxidant, antiviral, and anti-inflammatory, have been ascribed to various bioactive components of Liquorice, such as glycyrrhizin and flavonoids (Farag et al. 2012). Liquorice, when added to poultry diets, promotes healthy organ development, boosting growth and productivity. Quails fed a diet containing 200 ppm of liquorice root extract and 1% probiotic gained more weight and muscle and consumed more feed than control quails (Gowthaman et al. 2021). Broiler hens with 2 g/kg of Liquorice in their feed or 0.3 g/l of licorice in their water had lower levels of abdominal fat. Alagawany et al. (2019a, b) found that laying hen performance improved when Liquorice was added to chicken diets at concentrations up to 0.5% before sexual maturity. Poultry birds fed Liquorice in drinking water at 1, 2, or 4 mg/ kg body weight saw an improvement in the flavor and aroma of their carcasses. The present review paper tried to outline the morphology of the plant, the chemical make-up of Liquorice, and the therapeutic and pharmacological effects of Liquorice on poultry.

2 Characterization of plant

Glycyrrhiza glabra L. (Figure 1) belongs to the Fabaceae family (Sheidai et al. 2008). Nearly 30 different species of *Glycyrrhiza* have been identified, and even within the *G. glabra* species, there are many different cultivars. Some examples include *G. glabravar typica*, which grows in horizontal stolons and has brown bark, similar characteristics have been reported for *G. glabra glandulifera* and *G. glabravar violaceae* (Nomura et al. 2002; Martins et al. 2016). *G. glabra* is a perennial herb that can reach a height of 2.5 meters. Pinnately complex leaves accompany the purple or white flowers. The fruit is oblong, and it's filled with seeds. About 1.5cm in length, the taproot branches out into smaller, 1.25cm long roots, from which the woody stolons emerge. Together with the root, they make up commercial Liquorice and can grow to 8 meters when dried and cut. Root fragments break apart fibrously, exposing a yellowish core with a distinct odor and flavor. The flower spikes on this plant resemble peas and are around 10 to 15 centimetres long. You can find purple, white, and yellow spikes. Narrow, papilionaceous flowers in shades of lavender and violet bloom on spikes in the plant's axils. The calyx is covered in glandular hairs in short and campanulate, with lanceolate apexes (Kaur et al. 2013).

Pinnately complex leaves with 9–17 oblong to lanceolate leaflets arranged in pairs on the stem. The flowers are tiny and range in hue from purple to bluish-white, while the pods are flat and oblong to linear. The seeds are tiny and dark in color, with a diameter of around 2.5 mm and a weight of 6.2 g per thousand seeds. Up to 1.5 cm in length, the pod-like fruit is erect, glabrous, and pitted reticulately with 3–5 brown reniform seeds (Kaur et al. 2013). Using nitrogen-fixing bacteria in association with *G. glabra*, nitrogen may be extracted from the air. There is

Figure 1 *Glycirrhiza glabra* (Liquorice)

evidence that certain Mesorhizobium strains cause nodules on *G. glabra* and other *Glycyrrhiza* species. *Agrobacterium*, *Sinorhizobium*, *Rhizobium*, and *Paenibacillus* bacteria have also been found on rare occasions, although these bacteria are either not very effective at fixing nitrogen or are quite rare (Montonen et al. 2012).

3 Chemical composition and bioactive compounds of Liquorice

Liquorice has a wide variety of compounds like phytosterols, starch, sugars, amino acids, tannins, coumarins, choline, and vitamins (such as ascorbic acid/ vitamin C) (Alagawany et al. 2019a; Alagawany et al. 2019b). Liquorice root contains mainly glycyrrhizin as an active ingredient. The glycyrrhizinic acid content of a plant is highest in the primary root, then in the lateral roots, and finally in the above-ground sections of the plant, which are typically thrown away as garbage (Farag et al. 1984). An extract of liquorice root may contain as much as 25 percent glycyrrhizin. Magnesium, potassium, and calcium salts are also reported to be abundant in this substance, consisting of one molecule of glycyrrhetinic acid and two molecules of glucuronic acid. Since glycyrrhizin in liquorice extract (LE) is around 50 times sweeter than sucrose, it has been used instead of sucrose (Hayashi et al. 2009). Raw Liquorice comprises carbohydrates, fiber, protein, silica, and very little fat. In addition, liquorice root ash and moisture levels were measured at 7.70% and 6.80%, respectively. In addition, LE had a calcium level of 1720 mg/100 g and phosphorus content of 78 mg/100 g; its main amino acid components were proline, aspartate, alanine, and glutamic acid (Omer et al. 2014).

Table 1 Liquorice's bioactive constituents are responsible for many of its useful characteristics

S. No	Functional property	Bioacitve constituents
1	Antioxidant activity	Licochalone, Glabridin, Isoliquiritigenin, Licocoumarin
2	Anti-HIV	Glycyrrhizin
3	Anticancer activity	Glycyrrhetic acid, Glycyrrhizin
4	Antitussive activity	Glycyrrhizin
5	Antiulcer activity	Glabridin, glabrene, glycyrrhizinic acid
6	Analgesic and uterine relaxant	Isoliquiritigenin
7	Antihyper glycemic activity	18-β-glycyrrhetinic acid, glycyrrhizin
8	Memory enhancer	Glabridin
9	Antimycobacterial activity	Glabridin
10	Antiviral activity	Glycyrrhizin, licochalcones, glycyrrhetinic acid
11	Corticosteroid activity	18-β-glycyrrhetinic acid
12	Hepatocellular carcinoma	Glycyrrhizin
13	Antithrombin activity	Glycyrrhizin, isoliquiritin
14	Estrogenic activity	Glabrene, liquiritigenin
15	Antimycobacterial activity	Glabrene, liquiritigenin
16	Chronic hepatitis C	Glycyrrhizin
17	Spasmolytic	Liquirtin
18	Muscle relaxant	Rhamnoglucoside
19	Hepatoprotective activity	Glycyrrhizin
20	Anti-allergic activity	8-β-glycyrrhetinic acid, liquiritigenin, glycyrrhizin
21	Immunostimulatory activity	Glycyrrhetinic acid
22	Anti-inflammatory activity	Liquiritoside, Lichochalcone A, glycyrrhetic acid
23	Antioxidant activity	Licochalcone, glabridin, licocoumarin

Source: Kaur et al. (2013); Alagawany et al. (2019a,b); Prakash et al. (2021a): Prakash et al. (2021b): Kumari et al. (2022); Reda et al. (2021)

Liquorice has been analyzed and contains bioactive components like saponins, flavonoids, and triterpenes (Farag et al. 2012). Liquorice's distinctively pleasant flavour originates in its saponin content. Because of variations in processing, geographical origin, and harvesting techniques, the concentration of these chemicals can range widely. Liquorice root also contains 49 phenolic compounds and 15 distinct saponins. Liquorice gets its distinctive yellow hue from a variety of flavonoids. Some of the essential flavonoids are the glycoside liquiritigenin (4',7-dihydroxyflavanone) and isoliquiritigenin (2',4,4'-tri-hydroxychalcone) found in liquiriti fruit. Root dry weight typically contains between 0.08 to 0.35% glabridin, the primary isoflavone (Wang et al. 2015; Rizzato et al. 2017). Stilbene derivatives from liquorice leaves and derivatives of caffeic acid esters are two examples of chemicals that have been isolated in trace levels. In addition to fatty acids and phenols (guaiacol), saturated linear gamma-lactones have been isolated (Martins et al. 2016).

Many people utilize Liquorice for its ethnopharmacological uses. Moreover, the antioxidant and anti-tumour activities of Liquorice and the compounds derived from it have made their use widespread. 2.5% glycyrrhizin is estimated to be included in at least 71% of traditional Japanese Kampo remedies (Hayashi et al. 2009; Leite et al. 2022). Injectable and tablet (Glycyron) formulations of Liquorice are used in Japan as prescription drugs for treating various medical conditions. Many medications contain liquorice extracts, and one derivative of glycyrrhetinic acid, 3-β-O-hemisuccinate, is currently on the market for medical usage (Petropoulos et al. 2016). Hepatitis A and C, Human Immunodeficiency Virus, Herpes Zoster, Herpes simplex, and Cytomegalovirus are only some of the RNA and DNA viruses that have been proven to be inhibited by glycyrrhizin and glycyrrhizic acid. Pseudoaldosterone syndrome is brought on by glycyrrhizin and its metabolites, which prevent aldosterone from metabolizing in the liver and reduce 5(beta) reductase activity. This research was conducted by Ajaib et al. (2013). Liquorice's bioactive components help the plant achieve many valuable effects, as in Table 1.

Methods for extracting bioactive compounds from Liquorice include supercritical carbon dioxide-assisted, microwave-assisted, ultrasound-assisted, and multistage counter-current extraction. Several of the proposed procedures employ potentially dangerous solvents in the liquid phase of the extraction process. Extraction yields are increased, and organic solvent usage is decreased when solid-liquid extraction technologies are used as opposed to traditional methods (Karkanis et al. 2018). Increased yields can be

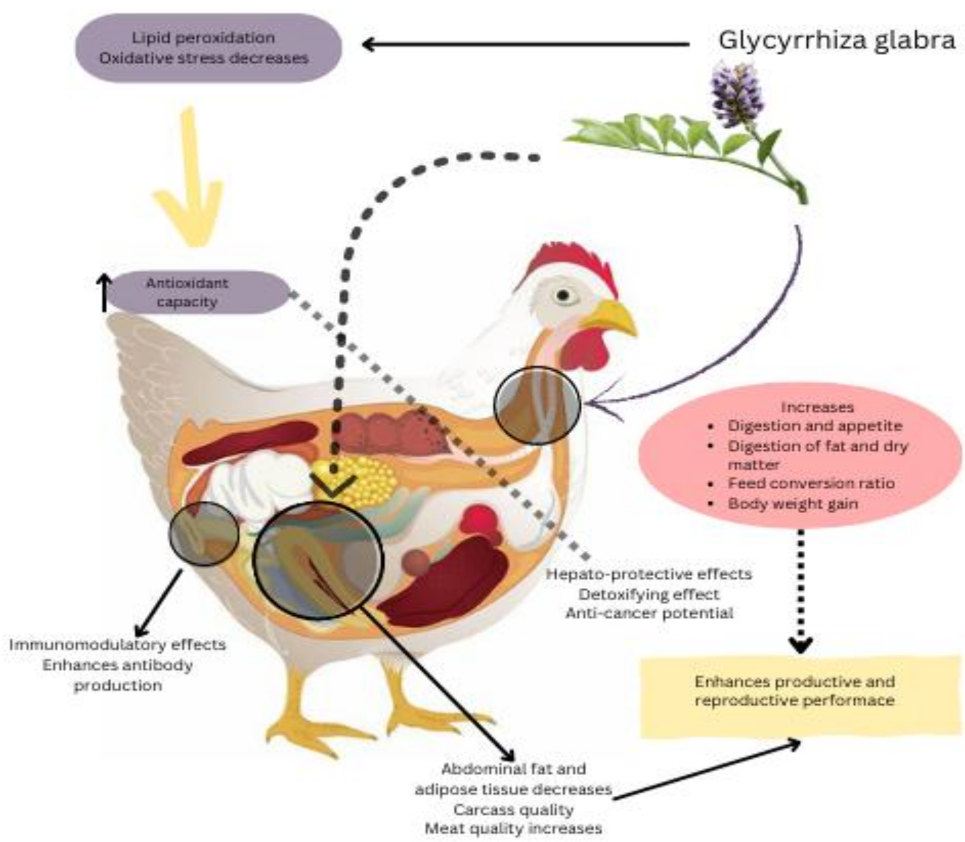

Figure 2 Therapeutic and health benefits of *Glycyrrhiza glabra* supplementation in poultry

achieved in about 4-5 minutes of microwave-aided extraction of roots in an ethanol-water mixture, compared to the typical extraction time of 20-24 hours (Pan et al. 2016). The hazardous effects of chemical solvents can be reduced using supercritical carbon dioxide. More study is needed before the herbal business can use the supercritical extraction process, although it is more efficient and has fewer negative impacts on the environment than conventional methods (Hedayati and Ghoreishi 2016)

4 Beneficial effects of *Glycyrrhiza glabra* on poultry

Glycyrrhiza glabra, commonly known as Liquorice, has been valued for centuries due to its medicinal and therapeutic characteristics (Karkanis et al. 2018). Glycyrrhizin, found in licorice root, is a triterpene saponin a thousand times sweeter than sucrose (Pastorino et al. 2018). The roots contain glycyrrhizin, a pharmacologically active compound with actions as diverse as antioxidant, antiviral, anti-infectious, and anti-inflammatory. Liquorice has been used to treat anything from mouth ulcers to arthritis, and it may even have anti-cancer, immune-modulating, antibacterial, detoxifying, and growth-promoting effects. Liquorice supplements, when fed to chickens, have been shown to improve productivity by promoting organ growth and stoking the animals' appetites and digestive fires (Alagawany et al. 2019a; Alagawany et al. 2019b). However, it also serves an essential role as a natural anticoccidial agent, with preliminary evidence suggesting that a

Table 2 Poultry specific studies incorporating the inclusion of Liquorice in feed

Category of poultry	Plant part and its dosage	Functional property	Results	References
Broilers	Liquorice extract (0.4 g/L in drinking water)	Immunomodulatory effect	Gains in body mass, feed consumption, and feed conversion ratio. Hypolipidemic, hypoglycemic, hepatoprotective, immunostimulant, and antioxidant properties are only few of the many biological activity demonstrated by licorice. The morphometric examination of various intestinal characteristics showed that the group supplemented with Liquorice at a dose of 0.4 gm/L had significantly longer and wider intestinal villi and a greater ratio of villi length to crypt depth than the other groups. Liquorice 0.4 gm/L enhanced the proportion of CD3+ T cells in the duodenum and ileum, relative to the control group. Superoxide dismutase and catalase mRNA expression, in addition to growth-related gene expression and lipid-metabolism-related gene expression, were all significantly upregulated in the liver in response to liquorice supplementation. By influencing the expression of genes involved in growth, lipid metabolism, and antioxidant activity, we found that liquorice supplementation improved the productivity of broiler chickens	Abo-Samaha et al. (2022)
Broilers	Liquorice extract (1 g/kg of diet)	Improves gut health and effective against *Campylobacter jejuni*	Lowered *Campylobacter jejuni* shedding from sick birds, boosted growth performance, and affected intestinal integrity maintenance.	Ibrahim et al. (2020)
Chicken		Effective against Mycoplasmosis	Reduced inflammation in the trachea and lungs after Mycoplasma infection. Inhibits Mycoplasma-induced inflammation and apoptosis by downregulating virulence genes (MMP2/MMP9) via the JNK and p38 signaling pathways. The findings suggest it has potential as an alternate antibiotic for the prevention of Mycoplasmosis infection.	Wang et al. (2022)
Japanese quails	Liquorice powder (750-1000 mg/kg diet)	Improves the gastrointestinal health	Resulted in significant decreases in total bacteria, coliforms, *E. coli*, and Salmonella. Benefits include optimizing performance, immunity, and antioxidant capacity while also preserving a balanced gut microbiome.	Reda et al. (2021)
Chickens	Liquorice extract (0.4% in drinking water)	Antioxidant property	Inhibit abdominal fat accumulation	Alagawany et al. (2021)

combination of *G. glabra* and *Echinacea purpurea* could be used to effectively combat coccidiosis in the poultry sector (Ghafouri et al. 2023). The beneficial effects of liquorice supplementation in poultry are depicted in Figure 2. Evidence from chicken-specific studies (Table 2) shows that *G. glabra* could play a significant effect if added to poultry feed.

5 Antimicrobial effect of Liquorice

The most widely studied pharmacological effects of licorice are its antiviral and antibacterial capabilities. Viral and other microbial infections have a substantial impact on a variety of diseases, especially in developing countries. Liquorice deserves more attention for its extraordinary qualities because of the importance of developing safe and effective antiviral or antibacterial medications. More than 20 triterpenoids and about 300 flavonoids can be found in liqorice. These active components are glabridin, glycyrrhizin, liquiritigenin, licochalcone E, licochalcone A, and 18-β-glycyrrhetinic acid (Wang et al. 2015).

6 Antibacterial effect

Various *in-vitro* studies have shown that methanolic extracts of Glycyrrhiza roots are significantly more effective than water extracts at inhibiting the growth of various bacteria, including *Bacillus subtilis*, *Pseudomonas syringae* pv. tomato, *Agrobacterium tumefaciens*, and *B. cereus*. In a laboratory setting, it was discovered that Liquorice's ethanolic extract inhibited the growth of three different types of bacteria, including *B. subtilis*, *Staphylococcus aureus* and *Candida albicans* (Gowthaman et al. 2021). Chloroform, ether, and acetone solvents are also used to extract active ingredients of Liquorice, and all these extracts showed antibacterial activity against both gram-positive (*Staphylococcus aureus* and *B. subtilis*) and gram-negative (*P. aeruginosa* and *E. coli*) bacteria (Nitalikar et al. 2010). The glycyrrhizic acid present in Liquorice is effective against *P. aeruginosa* bacteria (Chakotiya et al. 2016). Yeast and mould growth, lactic acid bacteria, and enterococci prevalence were not affected by five weeks of supplementation with different concentrations of Liquorice in Japanese quail. On the other hand, adding Liquorice to the diet drastically decreased the number of bacteria, particularly coliforms. The highest concentrations of the studied microorganisms were found in the control group that was not fed Liquorice (Alagawany et al. 2021).

7 Antiviral effect

There are 73 bioactive components and 91 potential targets from Liquorice's separated components. GL and 18-beta-glycyrrhetinic acid (GA) are two triterpenoids that have been the subject of extensive research and have been found to have antiviral properties. Glycyrrhizin refers to a compound found primarily in liquorice roots. Numerous studies conducted in recent years have demonstrated GL's antiviral effectiveness. Introducing infectious anti-hepatitis C virus (HCV) particles into a cell was the precise point of attack for GL. Glycyrrhizin is also a potent antiviral agent, inhibiting the expression and replication of viruses by decreasing the adhesion force and stress experienced by viruses and decreasing the binding of HMGB1 to DNA. Fewer studies have been conducted on GA's antiviral activity than GL.

18-beta-glycyrrhetinic acid treatment, administered after virus entry, inhibited rotavirus replication. After viral adsorption, adding GA to infected cultures resulted in a 99% reduction in rotavirus yields. Both GA and GL have antiviral properties; however, they are different. Human respiratory syncytial virus (HRSV) and rotavirus do not match GA's antiviral properties. The mechanisms by which these chemicals fight viruses, however, are similar. GA prevents viruses from attaching to cells, slows their growth, and stimulates the activity of the host cells.

In vitro research on the herpes simplex virus 1 (HSV-1) has demonstrated that aqueous extracts of liquorice roots have an antiherpetic effect. Possible mechanisms for this antiherpetic effect include *G. glabra*'s role in blocking HSV attachment to host cells when in contact with the extract. *G. glabra* aqueous extract has an antiadhesive activity that prevents HSV-1 from sticking to Vero cells *in vitro*, and the virus can be inactivated. Evidence suggests that pigeons given LE are less likely to spread Paramyxovirus type 1 (Dziewulska et al. 2018). Glycyrrhizin is a powerful immune stimulant, and it has been shown to have significant antiviral effects against duck hepatitis virus (DHV) (Soufy et al. 2012). Newcastle disease virus (NDV) has also been demonstrated to be susceptible to inhibition by *G. glabra* extracts (Omer et al. 2014). Higher antibody titers against NDV and an increased cellular immunological response in broilers treated with glycyrrhizic acid at 60 g/mL drinking water were observed (Ocampo et al. 2016). In an *in vivo* antiviral study, *G. glabra* extract at a concentration of 300 g/mL was found to have significant antiviral efficacy against NDV. Compared to untreated embryonated eggs, those injected with NDV and afterwards treated with the herbal extract had a higher survival rate and no detectable virus in their allantoic fluids (Ashraf et al. 2017). The anti-flu properties of glycyrrhizin have also been demonstrated in laboratory studies. Contact between the virus and the cell membrane is essential for virus entry and results in decreased endocytotic activity and virus uptake (Wolkerstorfer et al. 2009). In addition to its effectiveness against the Hepatitis C virus, glycyrrhizin also has hepatoprotective properties by shielding the liver from oxidative damage.

8 Antioxidant effect of Liquorice

Several mechanisms contribute to licorice's antioxidant and anti-inflammatory effects: the mitochondria are protected from lipid peroxidation, free radicals are scavenged, antioxidant enzymes are activated, and the activity of phospholipase A2, a key player in many inflammatory processes, is suppressed by licorice extracts. Cellular oxidation is slowed by licochalcone A, but liquorice flavonoids trigger inflammation. The flavonoid NF-kB signaling pathway may be targeted by liquiritigenin, glycyrrhizic acid and liquiritin to inhibit the secretion of inflammatory cytokines; these compounds reduce the expression levels of pro-inflammatory cytokines in the liver. Glycyrrhizic acid reduces prostaglandin E2 synthesis and cyclooxygenase activity (Alagawany et al. 2019a, b), and it also has indirect effects on platelet aggregation and inflammatory markers. In addition, it was discovered that glycyrrhetinic acid's delayed release of cortisol might cause high oxidation levels and increased cardiac weight in chickens (Awadein et al. 2010).

The antioxidant effects of Liquorice have been studied extensively in the laboratory and on living organisms. Glabridin was determined to be the most powerful antioxidant out of seven substances found to have antioxidant properties against the oxidation of low-density-lipoproteins by Vaya et al. (1997). In a separate investigation, various liquorice species were tested for their ability to scavenge ABTS+ radicals and prevent lipid peroxidation. In particular, lipid peroxidation was dose-dependently correlated with antioxidant activity (Fu et al., 2013; Gowthaman et al. 2021). Antioxidant feed levels for Japanese quails were also monitored in a separate experiment. Malondialdehyde, superoxide dismutase and total antioxidant capacity were shown to be significantly altered upon administration of Liquorice (Alagawany et al. 2021).

9 Immunomodulatory effect of Liquorice

Extracts of Liquorice have a beneficial effect on the immune systems of chickens. They can boost their immune system's response and increase their output with the help of this herb. Antibody titers against nonspecific and specific antigens were induced in the broilers by supplementing their diet with 0.1% liquorice extract. Liquorice root extract was augmented to the commercial broiler chicks to examine if it altered their immune profile. Total serum protein, albumin, globulin, as well as the albumin/globulin (A/G) ratio were estimated from three sets of chicks to characterize their biochemical status. This criterion divided the chicks into three groups: those given 1% of powdered *G. glabra* crude extract powder, those shown 0.1%, and those given no *G. glabra* extract. Measures of humoral immunity included total and differential white blood cell (WBC) counts, hemagglutination (HA) inhibition titre against LaSota strain of NDV, and HA titre against sheep red blood cells antigens. The results showed that chicks fed a powdered extract of 0.1% liquorice had markedly enhanced immune responses. Feed supplements made from natural ingredients are also used to boost immunity because they increase white blood cell counts and, in consequence, interferon levels. The laying hens' cellular immunity improved after LE (50 g/mL) was added to their diet. Strong immunological activity has been attributed to the glycyrrhiza polysaccharide, which is involved in numerous facets of immune regulation. The ability to phagocytose was also improved in chicken mononuclear cells and granulocytes after exposure to LE (Alagawany et al. 2019a,b).

Adding Liquorice to broilers' diet improved the animals' immunity and general health by increasing the weight of immune organs (Alexyuk et al. 2019). Immunomodulation and the production of interleukins by glycyrrhetinic acid promote the development of T cells, antibodies and IFN-γ (gamma interferon), all hinting at the compound's antiviral potential. Feeding broilers, LE did not affect the immune system (Alagawany et al. 2019a; Alagawany et al. 2019b). Liquorice's immunostimulatory, anti-inflammatory, and antimicrobial properties are because of the active components found in the herb. Glycyrrhiza polysaccharides (GPS) enhance both Th1 as well as Th2 responses by promoting IL-1β; IL-2; IL-4; and IFN-γ expression while augmenting the proportion of both T helper cells and cytotoxic T cells in the gastrointestinal tract (Wu et al. 2022a). Not only these, but such polysaccharides also show an immune boosting effect in a dose-dependent manner which can augment immunity of the New Castle disease virus vaccine in chickens (Wu et al. 2022b). Ocampo et al. (2016) found that influencing humoral and cell-mediated immune responses can improve poultry immunity, reduce the likelihood of viral infections, and serve as an adjuvant treatment for existing viral disorders. The levels of immunological markers (IgG, IgM, and lysozyme) in Japanese quail that had been fed were measured in an experiment. While plasma lysozyme activity was comparable between groups, both IgM and IgG were significantly modified. These parameters were shown to be elevated after being given Liquorice (Alagawany et al., 2021).

10 Effect of Liquorice on growth performance

Feed intake increased at 21 and 42 days after adding 0.4% LE to the broilers' drinking water, but there was no change in body weight across the ages. It was discovered that feeding broilers an additional 1% LE in their base diet led to more significant gains in body weight and feed efficiency at 42 days compared to the control group. In addition, the output of heat-stressed broiler chickens was enhanced by LE. *G. glabra*-supplemented diet improved their growth performance by fostering organ growth. Additionally, 2.5 g/kg *G. glabra* added to broiler feed improved digestion and appetite. In addition, feeding up to

0.5% *G. glabra* to developing pullets enhanced their performance, leading to healthier, more productive hens later. Compared to untreated controls, broilers fed glycyrrhizic acid (60 g/mL in water) had higher final body weight, better FCR, and lower mortality. The feed consumption of laying hens was not significantly altered by supplementing the standard diet with 0.5, 1.0, 1.5, or 2.0% liquorice powder. Adding 5g of Liquorice per kilogram of broiler feed had no discernible effect on any of the abovementioned metrics, including feed intake, feed conversion rate, body weight, livability index, or productivity index. The addition of 0.1, 0.2, or 0.3 mg LE/L to the drinking water of broiler chicks did not affect the animals' body weight, feed intake, or feed efficiency as compared to the control group (Alagawany et al. 2019a; Alagawany et al. 2019b). It has been found that when the diet of broilers is supplemented with 600mg/kg of Glycyrrhiza polysaccharide, it shows an optimal effect on growth. In the poultry production industry, the polysaccharide can also be used as a feed additive (Zhao et al. 2023).

Japanese quail and broiler chickens have been the primary subjects of studies on the effects of adding liquorice root supplements to their diets, with the greatest benefits seen in the latter when the supplement was fed at a rate of 2.5g/kg. When LE was added to the drinking water of quails at concentrations of 100 mg and 450 mg/L, the birds gained more weight overall and at harvest. However, only male birds displayed these outcomes. Similar weight increases and lower FCR were observed in quails fed a combination of 200ppm LE and 1% probiotic. Feeding broiler chickens a variety of Liquorice and garlic has been proven to improve the birds' performance in terms of growth (Gowthaman et al. 2021). A considerable weight gain was observed in broiler chickens when aqueous LE was added to their drinking water (Beski et al. 2019). This was accompanied by no changes in the chickens' appetite, thirst, or feed consumption. The addition of Liquorice to broiler chickens, on the other hand, resulted in lower rates of feed consumption and lower rates of weight gain compared to the control group. Liquorice's flavonoids may help you lose weight by decreasing your body's appetite and cravings for fatty foods. As the herbal plants in a diet increase, the risk of nutritional imbalances and adverse effects on body metabolism rises. This has been linked to a decline in growth performance.

A study on 40-week-old hundred egg-laying hens found that diets supplemented with LE increased functional egg production and modulated laying hen productivity by decreasing egg cholesterol and low-density lipoprotein (LDL) while increasing high-density lipoprotein (HDL) and total antioxidant capacity in plasma. Liquorice in feed or water has been demonstrated to improve poultry performance, carcass traits, and meat quality. Broiler chicks exposed to a high stocking density gained weight more quickly when given a probiotic and LE (500 ppm). Adding LE to broiler hens' drinking water is a viable alternative to giving them antibiotics to speed up their growth. The body weight increase of birds raised at high stocking density was stimulated by LE (500 ppm) but not by the feed conversion rate. Probiotics (200 ppm) were added, and the results improved even more.

Most research indicates that administering licorice root extract to broiler chickens has no discernible effect on the birds' body weight, feed consumption, or feed efficiency. Liquorice supplementation had a significant impact on body weight, daily body weight growth, daily feed consumption, and feed conversion rate in 3-week-old Japanese quail, and this effect persisted even after the feeding trial was over (5 weeks). At three weeks of age, the groups supplemented with 750 and 1000 mg/kg of Liquorice had the highest body weight, daily body weight growth and the lowest feed efficiency values. The most outstanding feed intake values were found in the groups that were given meals containing either 0 or 250 mg/kg of Liquorice. After five weeks, the group fed a diet containing 750 mg/kg of Liquorice had the highest body weight and fat-free mass values, while the groups fed diets containing 0 and 250 mg/kg of Liquorice had the lowest feed conversion rate values (Alagawany et al. 2021). Similarly, supplementing broiler chickens' diets with 2 and 3 g/kg of licorice extracts improved their physiological health and growth performance (Toson et al. 2023).

11 Effect of Liquorice on carcass quality and Yield

There is scanty published research on how feeding the licorice plant affects carcasses and meat quality. The hydrophobic flavonoids in the licorice, as reported by Moradi et al. (2017) and confirmed by Gowthaman et al. (2021), were responsible for the significant reduction in abdominal fat in broiler chicken that was supplemented with 0.3g of liquorice/L of water. While adding 0.45g liquorice extract/L water significantly reduced belly fat in broiler chickens, it did not affect the carcass output of birds raised in a heat-stressed environment. Broiler chickens given LE supplementation did not show any discernible changes in the relative weights of their internal organs (Sedghi et al. 2010; Moradi et al. 2014). Similarly, supplementing Japanese quail with 200 ppm, LE had no appreciable effect on the weights of their spleens or gizzards while leading to increased relative liver weights. In one study, supplementing Japanese quail with liquorice root extract considerably boosted the carcass yield percentage (Myandoab and Hosseini 2012); however, in another study, supplementing with LE had no significant potential effect on the carcass yield (Hosny et al. 2020). Beski et al. (2019) found no evidence that increasing broiler chickens' drinking water with aqueous LE altered their visceral organ weights, carcass cuts, or intestine histomorphology. Liquorice supplementation led to a rise in organ weights (Rezaei et al. 2014). Liquorice supplements dramatically enhanced the bursa and spleen weight of broiler chickens, and immunological organs, according to Kalanter et al. (2017). Liquorice's antioxidant activity, which influences lipid and protein metabolism, has been associated with improved chicken carcass features and meat quality, as reported by Michaelis et al. (2011). Compound herbal additive B (CHAB) containing Liquorice apart from *Atractylodes, Codonopsis pilosula* and *Poria cocos* can cause an improvement in the quality of meat in Hungarian white geese (Fu et al. 2023).

12 Effect of Liquorice on blood chemistry

Human and animal studies found that Liquorice is effective in dyslipidemia and hyperglycemia in cases of metabolic syndrome (Jafari et al. 2021). However, broiler chicken and Japanese quails had a dose-dependent increase in plasma glucose when given liquorice root extract or powder in their diets (Al-Daraji 2012; Dogan et al. 2018a; Dogan et al. 2018b). Since the saponin glycoside glycyrrhizin is 60 times sweeter in taste than sucrose (Roshan et al. 2012), it may be responsible for this increase in blood sugar. However, only a few trials in broiler chickens supplemented with LE (Moradi et al. 2017) or *G. glabra* (Rezaei et al. 2014) have indicated a reduction in serum glucose levels. Supplementation with LE did not significantly affect plasma glucose levels in broiler chicken (Sedghi et al. 2010) or Japanese quail (Myandoab and Hosseini 2012; Al-Sofee 2018, Abdul-Majeed 2019). Toson et al. (2023) found that when LE was added to the chickens' diets, the animals had lower levels of plasma triglycerides, cholesterol, urea, uric acid, and total cholesterol while developing more significant levels of antioxidant markers, RBCs, haemoglobin, WBCs, plasma complete protein, and albumin.

13 Effect on total proteins and their fractions

Japanese quail serum TP significantly increased when crushed liquorice root was introduced to their meal (Al-Sofee 2018). *G. glabra* supplementation in broiler chicken diets led to similar results (Rezaei et al. 2014). On the other hand, another study indicated that the serum total protein levels of Japanese quail treated with liquorice root powder were unaffected (Abdul-Majeed 2019). However, a study of broiler chickens at six weeks of age reported no significant changes in serum albumin, globulin, and albumin: globulin ratio (Jagadeeswaran et al. 2012). Blood total protein and globulin levels were highest in the birds given the 750-mg licorice diet, with the A/G ratio being lowest in those given the 250-mg licorice diet (Reda et al. 2021).

14 Effect on blood glucose

The plasma glucose levels of Japanese quails and broiler chickens were found to rise dose-dependent when liquorice root extract or powder was added to their diets (Al-Daraji 2012; Dogan et al. 2018b). The saponin glycoside glycyrrhizin, which is sixty times sweeter than sucrose, may be to blame for this increase in blood sugar (Roshan et al. 2012; Dogan et al. 2018a). However, some studies show that giving LE or *G. glabra* to broiler chicks reduces their blood sugar. The plasma glucose levels of Japanese quail and broiler chickens were not significantly different after receiving LE supplementation (Gowthaman et al. 2021).

15 Effect on heterophils, lymphocytes and heterophil-to-lymphocyte ratio

Heterophils and the H/L ratio were shown to change in response to heat stress in chicken (Yalcin et al. 2003) and Japanese quail (Mahmoud et al. 2013). An accurate measure of heat stress in hens is the H/L ratio (Gowthaman et al. 2021). Adding LE to the water supply for broiler chickens raised in hot conditions has significantly reduced the H/L ratio (Al-Daraji 2012). According to two previous studies, LE did not affect the percentage of lymphocytes, heterophils, monocytes, or the H/L ratio in broiler chickens (Sedghi et al. 2010; Moradi et al. 2014).

16 Effect on triglyceride, cholesterol, very low-density lipoprotein, low-density lipoprotein, and high-density lipoprotein concentrations

Moradi et al. (2017) reported that giving broiler chickens LE in their water dramatically decreased their serum low-density lipoprotein (LDL) cholesterol and total cholesterol, while Al-Sofee (2018) reported the same for Japanese quail. Feeding Japanese quail, a powder made from liquorice roots, decreased their triglyceride and cholesterol levels (Myandoab and Hosseini 2012). Rezaei et al. (2014) found that supplementing broiler chicken feed with *G. glabra*, *Thymus vulgaris*, or both herbs, remarkably reduced the birds' serum triglyceride levels. Cholesterol and LDL cholesterol levels fell when Liquorice and a prebiotic mixture were added to the feed of broiler chickens (Sedghi et al. 2010), but there was little to no effect on blood triglycerides, VLDL, or HDL cholesterol levels. Liquorice has been linked to a reduction in LDL cholesterol because it prevents the oxidation of LDL cholesterol by blocking cyclooxygenase (COX) and lipoxygenase (LPX) enzymes (Abdul Majeed 2019). In another study, adding liquorice root powder to the diets of broiler chickens resulted in a considerable rise in plasma HDL cholesterol while not affecting plasma LDL cholesterol, total cholesterol, or triglycerides (Gowthaman et al. 2021). The quail fed a 750-mg licorice diet had lower contents of all lipid profile parameters (LDH, TC, TG, and LDL) (Reda et al. 2021).

17 Effect of Liquorice on other blood parameters

Supplementation with Liquorice Broiler hens showed elevated levels of uric acid, alkaline phosphatase (ALP), aspartate aminotransferase (AST), calcium, and phosphorus, as well as RBCs, WBCs, platelets, erythrocyte sedimentation rate (ESR) and plasma uric acid (Al-Daraji 2012). Amen and Muhammad (2016) discovered a similar effect when they fed LE to broiler chickens; they saw a rise in platelets, haemoglobin, WBCs and RBCs. However, Al-Sofee (2018) found that supplementing Japanese quail with licorice root had no appreciable effect on their packed cell volume (PCV). Also, giving broiler chickens Liquorice increased their white blood cell count (Sedghi et al. 2010), and giving Japanese quail LE in their water increased their triiodothyronine (T3) and thyroxine/ tetraiodothyronine (T4) levels (Hosny et al. 2020). However, a different study on broiler chickens at six weeks of age reported no significant changes in serum WBC count or cell-mediated immunity (Jagadeeswaran et al. 2012). Liquorice extract can reduce malondialdehyde's concentration in broiler chicken breast meat experimentally exposed to aflatoxin-B_1 (Rashidi et al. 2020).

Conclusion and future prospects

Liquorice contains bioactive compounds with powerful pharmacological and therapeutic effects, such as flavonoids and glycyrrhizin. It has been hypothesized that LE's immunogenic and antioxidant properties could make it an effective alternative to antibiotics for treating and preventing respiratory, gastrointestinal, and immunological diseases in poultry. To increase productivity and bird health, however, better distribution of this essential herb is required in poultry. This review has also postulated that *G. glabra* supplementation to poultry diets may enhance development, productivity, feed efficiency, carcass characteristics, and meat quality. Furthermore, the liquorice herb's excellent role in increasing the activities of the digestive system may treat several illnesses and issues in chicken farms. Standardizing the use of Liquorice (in water or feed) is important to guarantee consistent outcomes and appropriate dosing. Therefore, this standardization should be the focus of future studies if Liquorice is to be used effectively in the poultry sector.

Glycyrrhiza glabra extract may be a key component in creating numerous pharmaceutical compounds useful in the poultry industry. Liquorice has medicinal properties because of the diverse bioactive components it contains. The immunogenic and antioxidant effects of licorice extract improve the growth performance, carcass characteristics, feed conversion efficiency and hemato-biochemical indicators of poultry and have potential biomedical applications for a wide range of digestive, respiratory and immunological ailments. Poultry that consumed water containing up to 0.4 g/L of LE had better immunological responses, higher antioxidant indices, and a healthier lipid profile. When added to the diets of laying hens, 50 g/mL of LE has been shown to affect cellular immunity positively. Extraction of *G. glabra* at 300 g/mL was highly effective against NDV. Additional study is required to evaluate the benefits of licorice herb as a chicken feed supplement and explore different characteristics of this medicinal plant that can enhance poultry productivity and health. *In ovo* delivery and nano delivery, strategies should be researched to optimize the delivery of this important herb in poultry, which could increase productivity and safeguard the health of the poultry birds.

Acknowledgement

All the authors acknowledge and thank their respective Institutes and Universities.

Author's contribution

All the authors contributed significantly.

Funding

This compilation was written by its authors and required no substantial funding.

Disclosure statement

All authors declare that there exist no commercial or financial relationships that could, in any way, lead to a potential conflict of interest.

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Journal of Experimental Biology and Agricultural Sciences, June - 2023; Volume – 11(3) page 479 – 499

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Beneficial impacts of biochar as a potential feed additive in animal husbandry

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Received – May 25, 2023; Revision – June 28, 2023; Accepted – June 30, 2023
Available Online – June 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(3).479.499

KEYWORDS

Biochar

Activated charcoal

Animal husbandry

Livestock production

Feed additive

ABSTRACT

In the last decade, biochar production and use have grown in popularity. Biochar is comparable to charcoal and activated charcoal because it is a pyrogenic carbonaceous matter made by pyrolyzing organic carbon-rich materials. There is a lack of research into the effects of adding biochar to animal feed. Based on the reviewed literature, including its impact on the adsorption of toxins, blood biochemistry, feed conversion rate, digestion, meat quality, and greenhouse gas emissions, adding biochar to the diet of farm animals is a good idea. This study compiles the most important research on biochar's potential as a supplement to the diets of ruminants (including cows and goats), swine, poultry, and aquatic organisms like fish. Biochar supplementation improves animal growth, haematological profiles, meat, milk and egg yield, resistance to illnesses (especially gut pathogenic bacteria), and reduced ruminant methane emission. Biochar's strong sorption capacity also helps efficiently remove contaminants

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI]
(http://www.horizonpublisherindia.in/).

Animal nutrition

and poisons from the animals' bodies and the farm surroundings where they are raised. Animal farmers are predicted to make greater use of biochar in the future. Biochar could potentially be of value in the healthcare and human health fields; hence research into this area is encouraged. The present review highlights the potential benefits of biochar as an additive to animal feed and demonstrates how, when combined with other environmentally friendly practices, biochar feeding can extend the longevity of animal husbandry.

1 Introduction

Carbonaceous substances from heating wood include charcoal, activated charcoal, and biochar (BC). All three items are made under identical conditions and have similar characteristics and applications. Bio-based carbon materials are solids high in carbon created via pyrolysis from biomass high in carbon. Wood-based charcoal has been a heating and cooking fuel for thousands of years (Dayang et al. 2022). The term "activated charcoal" refers to charcoal that has been subjected to either a physical activation process or a chemical activation process. Activating charcoal improves its physicochemical qualities (Hagemann et al. 2019; Shi et al. 2021; Haider et al. 2022). Biochar has multifunctional values due to its use for various purposes as a nutrient and microbial carrier, soil amendment to enhance soil quality, heavy metal and organic pollution immobilizing agent for water and soil remediation, porous material for reducing the emissions of greenhouse gas and odorous compounds, catalyst for industrial uses, and feed additives to enhance animal production, health and feed efficiency (Bolan et al. 2022). Further, water treatment, biodiesel production, syngas upgrading, composting of organic waste, and soil conditioning are just a few of the many applications of BC. Soil remediation also uses it due to its enhanced water-holding capacity, adsorption ability, and microbial variety. Biochar is an effective soil addition for cleaning up polluted areas. In the remediation of contaminated soils like mine tailings, it has been used to eliminate heavy metals/metalloids and pesticides. BC improves soil moisture retention and nutrient movement by limiting runoff and altering soil biota (Huang et al. 2021; Kumar et al. 2021; O'Reilly et al. 2021; Patel et al. 2022).

Biochar is created by pyrolyzing organic matter. Hydrogen, water vapour, methane, carbon dioxide, ethane, carbon monoxide, and "char" are generated when biomass is heated in an oxygen-free environment. The ability of BC to bind to a wide variety of chemicals and adsorb bacteria and toxins makes it a promising ingredient for use in animal feeding systems to improve animal performance while having a more negligible environmental impact. Much of that stuff is left over from municipal or agricultural waste management and must be thrown away (Man et al. 2021; Yang et al. 2021). Energy and carbon may be recovered by using this waste to make BC with superior qualities for environmental protection, agriculture and animal husbandry. The properties of BC are determined by the feedstock material and the pyrolysis conditions, especially the temperature and process duration (O'Reilly et al. 2021; Dayang et al. 2022). Biochar reduced bulk density, more porosity and surface area, less oxygen and hydrogen, and higher carbon content as the pyrolysis temperature is raised. The BC, made from various biomass sources, has many properties. The bulk of BCs is alkaline, suggesting they can act as a pH buffer in the rumen and increase weight gain in livestock-fed high-energy diets. BC's cascading utility was proved by its addition to various feeds, beddings, and liquid manures in small increments (Huang et al. 2021). Interestingly, they have gotten special attention due to the ease of manufacturing BCs. Further, BC's eco-friendly and economically profitable nature and its use as a sustainable bio-adsorbent must also be considered (Haider et al. 2022; Patel et al. 2022). Adding organic nutrients to BC helps with animal husbandry, boosts the province's economy, and has many positive (reverberating) effects on the natural world.

Over the past decade, various studies have been conducted to study the impact of a BC-supplemented diet on ruminants, pigs, poultry, and fish (Winders et al. 2018; Wang et al. 2019; Jinija et al. 2022). Biochar can increase both the quantity and quality of eggs laid by hens. Improved mineral intake from BC can help minimise cracked eggs' occurrence when wood charcoal is added to the hens' diet. It boosts growth and survival, as well as high-density lipoprotein, and lowers low-density lipoprotein, all linked to enhanced immunity. Due to its adsorption capabilities, it was also able to diminish the prevalence of chicken diseases such as *Campylobacter hepaticus* and *Gallibacterium anatis*. Because of this, it can be used in animal husbandry as ab antibiotic (Shehata et al. 2013; Wilson et al. 2019). The high porosity of Biochar could help the host gut bacteria like methanogenic archaea lower the methane emissions from ruminants, contributing to global warming. Emissions of greenhouse gases are an essential contributor to the planet's warming. Biochar has been found to reduce methane production from ruminants, which significantly contribute to agricultural GHG emissions, and increase microbial fermentation. In addition, the ability of BC to absorb nutrients from the digestive tracts of cattle and then excrete them as soil fertilizer might lead to increased farm output even if it also helps in decreasing the input of chemical fertilizer (Man et al. 2021; Dayang et al. 2022; Haider et al. 2022).

Biochar has shown promise as a feed supplement, with studies finding that it improves various animal health and production

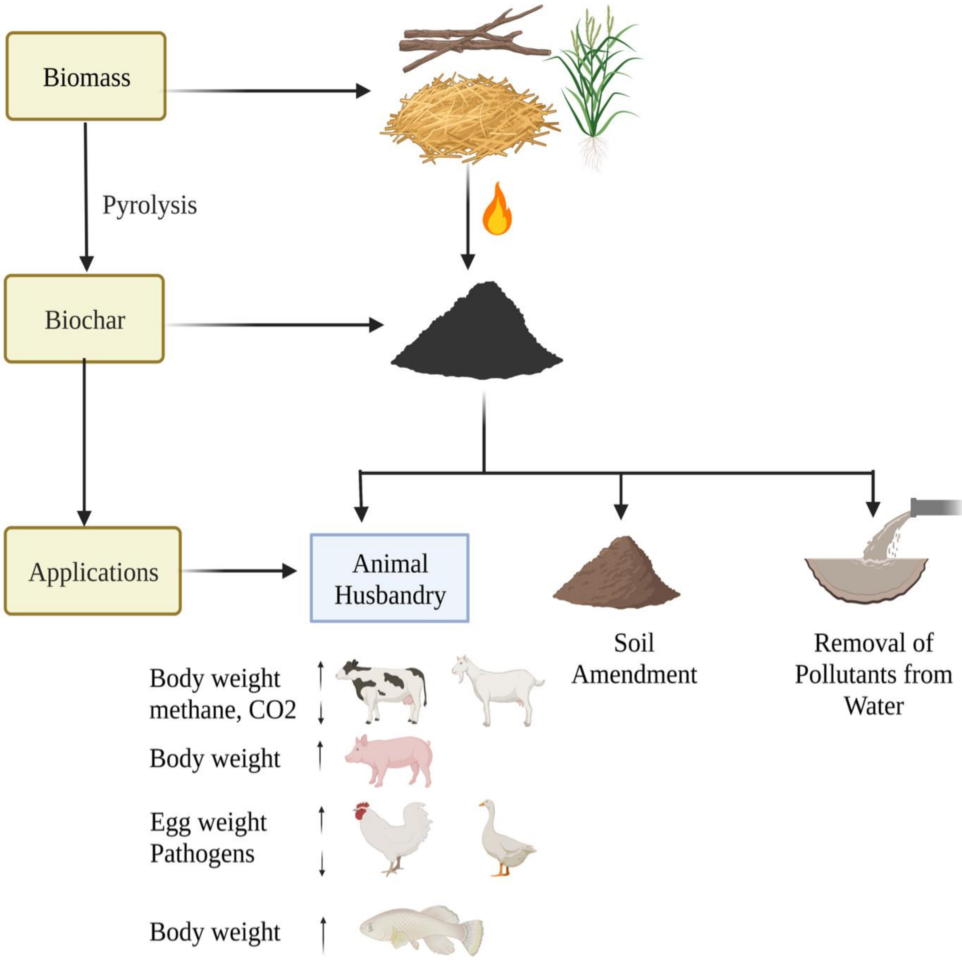

Figure 1 Biochar for livestock farming, from production to final uses

indices. Biochar for animal husbandry, from production to final applications, is depicted in Figure 1. Biochar production and its potential use in environmental cleaning have generated a lot of data in recent years. However, the use of BC produced from various feedstocks severely lacks data. This review article summarizes the present knowledge about BC feedstocks and their effects as feed additions for livestock and poultry production and identifies knowledge gaps and future research priorities in this area.

2 Raw materials used for the production of biochar

Biochar's primary constituents come from food production, preparation, and consumption. Aquaculture, livestock, poultry breeding, and agricultural output contribute to total output (Schmidt et al. 2019; Schmidt et al. 2021; Dayang et al. 2022). Depending on the raw materials used, BC can be roughly categorized as straw BC, shell BC, wood BC, sludge BC, animal fecal BC, bamboo BC, and others (Dai et al. 2019), and most of these are part of some of the food chain. Agricultural wastes, as well as animal and poultry manure, are always produced while creating food. Using rice straw, Yang et al. (2021) produced BC at temperatures between 500 and 900 °C, with the surface area of the BC reaching a maximum of 520.71 m^2/g at the highest temperature. Shi et al. (2021) pyrolyzed cow dung at temperatures between 350 and 750 °C for two hours to create BC. The pyrolysis process produced BC with a specific surface area of up to 308.1 m^2/g at 750 °C. Produced and consumed goods can generate a wide variety of trash, such as banana peels, eggshells, apple chip pomace, sugarcane bagasse, walnut shells, peanut hulls, grape pomace, etc. Those that eat eggs tend to make eggshells as a by-product of their diet (Xu et al. 2020; Li et al. 2016, Jiang et al. 2018). The final stage of the food chain, the kitchen, generates the most garbage. The trash from kitchens can consist of a wide variety of items; BC can be made, in part, from kelp, tea leaves, and crab shells. For instance, Huang et al. (2021) used kelp as raw material and high-temperature pyrolysis to create BC. The BC used by Altaf et al. (2021) was created by pyrolyzing tea leaves at 500 °C. Across a temperature spectrum of 300–900 °C, Dai et al. (2017) synthesized calcium-rich BC from crab shells. Furthermore, BC can be manufactured from everyday household waste by pyrolyzing food at temperatures between 300 and 700 °C for two hours (Kumar et al. 2021). By pyrolyzing kitchen waste at 300-600 °C for two hours, Xu et al. (2021) created BC with a surface area of 1.19-10.27 cm^3/g. Up the food chain, wastes can be used as BC source materials. The food chain's solid waste can be managed by converting these wastes into BC.

Feedstock for the production of BC includes different types of organic resources. Raw resources for BC production include cow dung, wood chips, wheat straw, rice husk, grass, and cassava rhizome (Ronsee et al. 2013; Kiran et al. 2017). Various input materials and pyrolysis settings have been shown to affect the synthesis of BC with high nutritional value (Chan et al. 2007). BC is produced from waste biomass by modern pyrolysis technology. This waste biomass includes agricultural, manure, wood, and green waste. Producing and utilizing agricultural, industrial and urban/municipal wastes has also contributed to waste management (Novotny et al. 2015; Kameyama et al. 2016). Several authors have discussed the prevalence of different feedstock materials in BC manufacturing (Reddy 2015; Sohi et al. 2009; Tumuluru et al. 2011). Peterson et al. (2012) found that 40% BC was extracted from maize stover. Sullivan and Ball (2012) noted that most of the biomass used to produce BC consists of the polymers such as cellulose, hemicellulose, and lignin. It has been found that cellulose is the primary component of plant-based biomasses, while lignin plays a substantial role in woody biomass.

3 Production of biochar as a feed additive or feed supplement

Biochar can be produced on a small scale with inexpensive adapted stoves or kilns and on a large scale with costly larger pyrolysis facilities and more feedstocks. As noted above, BC may be made from various biomass feedstocks via pyrolysis (Zhu et al. 2018). The acquired dry trash is chopped into bits no bigger than three centimetres before being put to use. It is heated to 350 and 700 °C (662 and 1292 °F) to prepare either oxygen-free or with deficient oxygen concentrations feedstock. Over 500°C (heating rates of 1000 °C/min), fast pyrolysis can occur in seconds. Classifying pyrolysis processes according to the required heating temperature and time are standard practices. In these conditions, bio-oil production is at its peak. On the other hand, slow pyrolysis takes longer to complete (30 minutes to several hours at heating speeds of 100 °C/min), but more charcoal is produced (Brown et al. 2011).

Depending on the used biomass, heating rate and heating temperature, several types of BC can be produced. Incontrovertibly, higher temperatures increase char yields. The carbon structure of BC produced at moderate temperatures (550°C) is less aromatic than that of BC produced at high temperatures (Joseph et al. 2015). Char yield is reduced in all pyrolysis methods as the temperature increases (Antal et al. 2003). According to Peng et al. (2011), BC yield was affected by charring time, with yield decreasing with increasing charring time at a constant temperature. Biochar quality and its capacity for carbon sequestration or agronomic performance are greatly affected by pyrolysis. Slow pyrolysis of biomass yields 24–77% BC (Dutta et al. 2010). All biomass used to produce BC should be pyrolyzed (Mohan et al. 2006), regardless of whether activation is used. To create BC, raw materials will be pyrolyzed at temperatures between 200 to 1000 °C in an oxygen-depleted atmosphere. Because of this, the final goods will have different characteristics and capabilities (Brendova et al. 2012; Koltowski et al. 2017). Biochar products' performance as feed supplements depends on heating rate, temperature and residency duration (Waheed et al. 2013).

4 Use of biochar as a feed additive

The organic matter used to create BC feed additives is heated at rates between 7 °C/min and 40 °C/min for durations ranging from 3 minutes to 12 hours, with temperatures ranging from 350 °C to 1100° C (McFarlane et al. 2017). A pyrolysis temperature variation of no more than 20 °C is required to comply with European Union requirements for BC as animal feed. Activation is not necessary for BC products intended for use as feed additives. As early as the turn of the last century, veterinarians in Germany were looking into the health benefits of both activated and non-activated BC feed for animals. Since 1915, studies by Skutetzky and Starkenstein (1914) on activated BC have shown that it can decrease and absorb dangerous clostridial toxins from *Clostridium botulinum* and *C. tetani*. Mangold (1936) researched the effects of BC when fed to animals and reported that charcoal in young animals' diets appears to have a prophylactic approach. Coccidiosis and other coccidial diseases in pets can be efficiently treated by adding BC to their diet. Totusek and Beeson (1953) later remarked that charcoal by-products had been used in American hog breeding since 1880 and in chicken feed since 1940. Steinegger and Menzi (1955) reported around the same time that BC was given to Swiss chick feed and laying hen meal to avoid digestive disorders and achieve a regulating action on digestion. BC has been thought to purge water and soil of chemicals, heavy metals and other pollutants (Tan et al. 2016; Shakoor et al. 2020). Most pollutants and toxins are found in the animal's regular feed, which several sources, including ambient pollution, insects, and microbial activities, can taint. Reduced mortality and improved development were two positive outcomes of feeding broilers a diet supplemented with 0.5% BC to mitigate the harmful effects of aflatoxins. Carbon enterosorbents (biochar) made from rice husks

Table 1 Research studies investigating the beneficial effects of different feedstock materials as biochar

Feedstock material	Biochar dose	Beneficial effects	References
Woody green waste	1% to 4% by mass of laying hens' feed	Gains in egg weight of upto 5% Egg production could rise by up to 13% Decrease in feed consumption by 7% Upto 14% better feed conversion ratio 19% boost in the shell's tensile strength Excreta nitrogen content decreased by upto 26%	Prasai et al. (2016)
Activated charcoal	1% of goats' typical diet in dry matter	Aflatoxin elimination was cut by 76% The composition of the milk remained unaffected	Nageswara Rao and Chopra (2001)
Charcoal	Daily doses of up to 1g of charcoal for each cow	Reduced levels of *Clostridium botulinum* antibodies by up to 30%	Gerlach et al. (2014)
Poultry litter	7% of BC's total tonnage of feed for chicken broilers	Intake of feed increased by 8% Reduction in body weight gain by 2% Feed conversion efficiency drops by 11%	Evans et al. (2015)
Poultry litter	2–4% of the total weight of feed given to chicken broilers	Improved feed conversion efficiency by 7% Weight growth slowed by 9%	Evans et al. (2017)
Bamboo	BC at a rate between 0.5 and 1.0 g/kg of goat body weight	Weight gain of 17% each day Decreased urine nitrogen content by 61%	Van et al. (2006)
Oakwood	Feed for chicken broilers and laying hens, between 1 and 10% by mass	Increased feed conversion rates by as much as 7% Gain in body weight of up to 23% Egg cracking can be reduced by up to 65%.	Kutlu et al. (2001)
Jarrah wood	Cows are given a mixture of BC and molasses, 3:1, on a regular basis.	Sorption of toxins Facilitate the recycling of cow manure and digestive byproducts	Joseph et al. (2015)
Woody green waste	Laying hens' feed at the rate of 4% by mass	Productivity of eggs rose by 1.2% Weight gain of 3% in eggs Feed efficiency improved by 8% Feed consumption decreased by 2%	Prasai et al. (2016)
Woody green waste	1-4% of the total amount of feed given to layer chickens and broiler chickens	Lower levels of water in laying hen poop Excreta nitrogen content can be reduced by up to 27% Carbon concentration in excreta increase by upto 45%. Increased ammonia emission of upto 47%	Prasai et al. (2018b)
Whole pine trees	In terms of feed mass, the range is between 0.8 to 3%	Improved digestion Reduction in methane emissions of upto 18.4% Possible reduction in carbon dioxide output of upto 9.7%	Winders et al. (2019)

Source: Kalus et al. (2019)

reduced uremic toxins to a clinically significant level, just like commercial enterosorbents. The carbon surface of these enterosorbents was treated with ozone oxidation and then ammonia to modify their characteristics. Figure 2 depicts the uses of biochar as a supplemental feed ingredient for livestock and poultry, and Table 1 displays the findings of some of the most significant studies investigating different feedstock materials as biochar.

5 Positive effects of biochar on livestock farming

Charcoal's purported detoxifying abilities have been the subject of numerous studies. Mycotoxins of several types can be bound to charcoal through a process proposed (Galvano et al. 1996a; 1997; 1998). A wide surface area, low surface acidity, and mesopores are ranged between 2 and 50 nm in size. Microporous charcoals (2 nm)

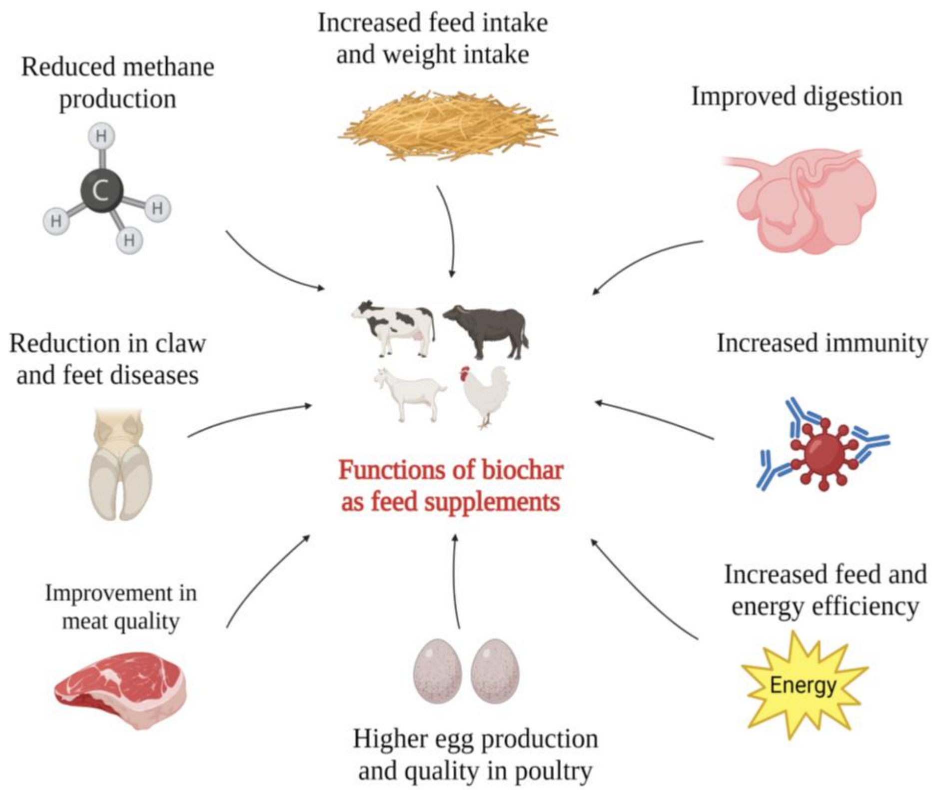

Figure 2 Functions of biochar as livestock and poultry feed supplements

exhibit reduced sorption capabilities due to slower mycotoxin diffusion and the repulsion of electrons by the positively charged surface of aflatoxin molecules. Mycotoxin contamination of animal feed can affect up to 25% of global feed production (Mézes et al. 2010). Fungal toxins are typically created in humid environments, making it challenging to prevent mold fungus from growing on freshly prepared and stored animal feed. Feed tainted with mycotoxin poses a significant health danger to farm animals. Adsorbents are often added to the diet to prevent animals from becoming ill from ingesting mycotoxins. There has been an increase in the use of activated carbon and specific polymers in addition to the more typical aluminosilicates (Huwig et al. 2001).

The adsorption behaviour of BC has been studied using aflatoxin, one of the most common mycotoxins, as a model drug (Galvano et al. 1996a). Researchers have deduced that BC blocks the absorption of aflatoxins in the intestines and, by extension, in the blood and milk of animals. When added to animal feed, 2% activated BC lowered the concentration of extractable aflatoxin by up to 74% and reduced aflatoxin concentration in milk by up to 45% (Galvano et al.1996b). However, the adsorption effectiveness of various BCs varied greatly, as demonstrated by a random sampling of activated BCs. In an *in vitro* batch study of sorption, four different activated carbons absorbed 99.9%. At a concentration of 0.5% aflatoxin B in a solution, 1.11 g of aflatoxin B per 100 ml of activated BCs was toxic (Diaz et al. 2004). In contrast to the 1% concentration in *in-vitro* experiments, the *in vivo* test fed a poorly defined BC at a low concentration of 0.25% of the meal's fresh weight without a feed matrix. Interestingly, Rashidi et al. (2020) found that when biochar made from poultry litter is fed to broiler chickens suffering from aflatoxicosis at the rate of 5g/ kg, body weight is restored. Side by side, the performance of the birds also increased. Galvano et al. (1996a) also evaluated the adsorption capabilities of 19 activated carbons for two mycotoxins, ochratoxin A and deoxynivalenol, and found that activated BC adsorbed 0.80–99.86% of ochratoxin A and up to 98.93% of deoxynivalenol, depending on the type of activated BC. The wide range of findings unequivocally demonstrates the value of thorough analysis and classification of BC characteristics. Activated BC was shown to have the highest toxin reduction capacity of the adsorbent feed additions studied by Di Natale et al. (2009) to lower aflatoxin levels in milk produced by dairy cows. Researchers found some positive effects by examining the milk's organic acid, lactose, chloride, protein, and pH levels. The authors explained the high specific surface area of the BC, its optimal micropore size distribution, and the great affinity of aflatoxin for the polyaromatic surface.

Zearalenone is an extremely dangerous estrogenic metabolite produced by the *Fusarium* fungus. Bueno et al. (2005) examined its binding with different concentrations of activated BC (0.1%, 0.25%, 0.5%, and 1%) at various concentrations. No cure for this drug had been found before that time. The zearalenone could be bound *in vitro* at the four BC concentrations. Many chemical molecules, including mycotoxins, compete with the free adsorption surfaces of BC, making selective adsorption difficult or impossible to achieve *in vivo*. In the *in vitro* studies utilizing swine digestive juices, activated charcoal showed high adsorption of Fusarium toxins (Avantaggiato et al. 2004; Döll et al. 2004). In contrast, no noticeable effect was observed when activated BC at a concentration of 0.3% was given to pigs' diets. Supplementation with uncharacterized industrial BC at this low dose did not affect ochratoxin levels in the body's fluids (Jarczyk et al. 2008). However, no unfavourable outcomes were uncovered either.

Liver damage caused by mycotoxins is common in chickens. Biochar at 0.02% of body weight daily improved critical liver enzyme activity (Ademoyero and Dalvi 1983; Dalvi and Ademoyero 1984 Dalvi and McGowan 1984). Broiler chickens' feed intake and weight increase were unaffected by aflatoxin (10 ppm) when fed 0.1% BC (w/w). Activated BC performed better than hydrated sodium calcium aluminosilicate. The alumina product did not significantly increase aflatoxin B levels in the liver or blood when paired with 0.25 and 0.5% BC therapy (Kubena et al. 1990; Denli and Okan 2006). In another investigation by Edrington et al. (1996), fattening chicks' feces had less aflatoxin B when activated BC was given with its diet. Kim et al. (2017) revealed that three BCs administered at 0.5% to the same basal meal reduced aflatoxin absorption by up to 100%.

Another study showed the importance of dose by reducing the amount of aflatoxin B1 in the birds' livers by 16-72% by adding 0.25 or 0.5% activated BC to a meal contaminated with the toxin (Bhatti et al., 2018). There is conflicting evidence, and the study by Toth and Dou (2016) adds to the confusion. Most *in vitro* studies of sorption in water showed discrepancies with corresponding *in vivo* examinations (Huwig et al. 2001). Matrix variables significantly affect mycotoxin sorption; therefore, *in vitro* trials must be thoroughly assessed. Activated carbon was found by Jaynes et al. (2007), for example, to adsorb up to 200 g/kg of aflatoxin. Matrix effects led to a one-hundredfold reduction in sorption capacity when maize meal was suspended in water. Matrix effects are anticipated to be far more nuanced in the digestive tract, which features a wide range of pH and redox conditions. Different studies have shown that while activated BC did not affect aflatoxin, it mitigated the hazardous effects of other fungal toxins like zearalenone to a large extent (Avantaggiato et al. 2004) and deoxynivalenol (Devreese et al. 2012; Devreese et al. 2014; Usman et al. 2016). The most important benefits of using biochar in animal husbandry are shown in Figure 3.

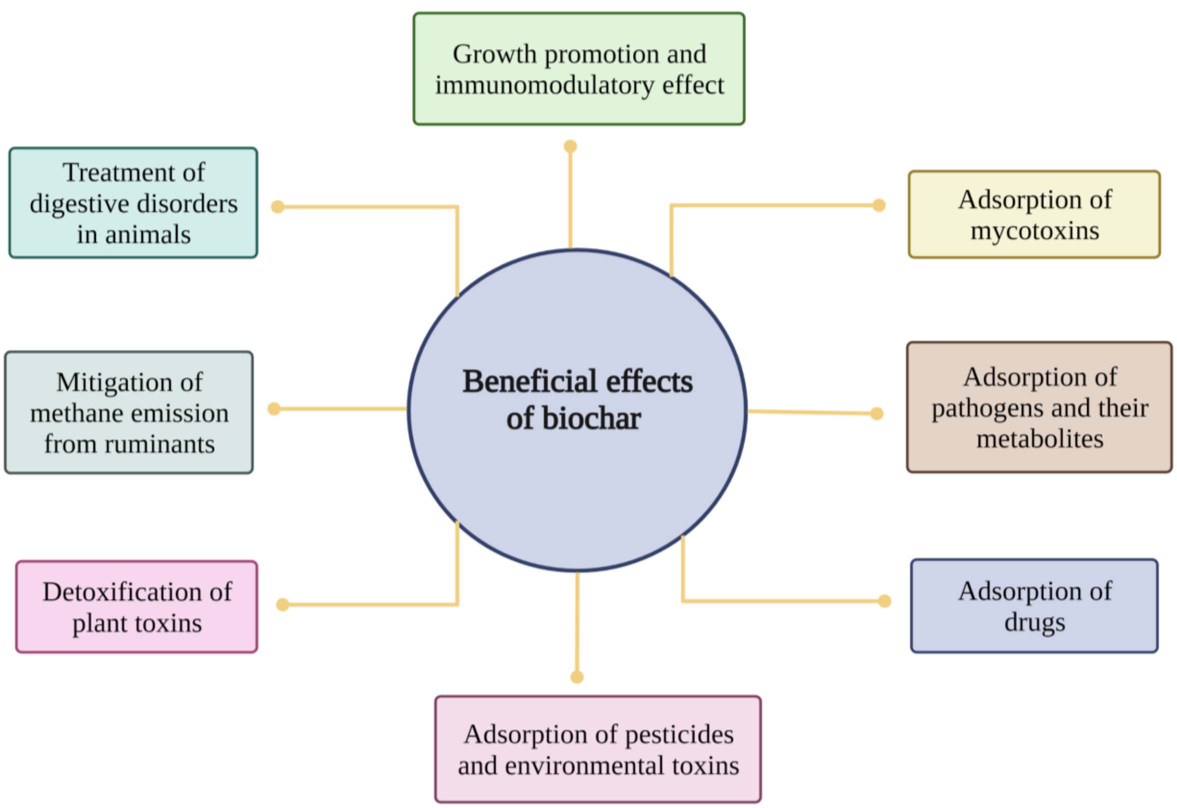

Figure 3 Major positive implications of biochar inclusion in livestock management

6 Adsorption of drugs

Numerous 1980s human medical research shed light on using activated BC as feed, especially for feed toxicity treatment (Erb et al. 1989). Most medications and toxins can be prevented from entering the gastrointestinal tract by activated carbon's adsorption. Cardiac glycosides, aspirin, dextropropoxyphene, carbamazepine, dapsone, and others were removed faster after an overdose when BC was ingested repeatedly, according to Neuvonen and Olkkola (1988). More quickly removed industrial and environmental contaminants were also identified. Adults are given 50–100 g of activated BC for acute poisoning, while children get 1 g/kg of body weight. The inadvertent consumption has no devastating repercussions. Finnish medical professionals recommend multiple doses of activated carbon given orally in acute poisoning to reduce the likelihood that poisons will be absorbed from the BC-toxin combination during digestion (Olkkola and Neuvonen 1989). The ability of BC to remove toxins from the body is enhanced when it is taken orally on multiple occasions (Crome et al. 1977; Dawling et al. 1978). Antibacterial drugs (namely tylosin and doxycycline), as well as the coccidiostats (namely salinomycin and diclazuril), were not significantly affected by the daily inclusion of 0.2% activated charcoal in the chicken feed. It was found that the use of activated carbon-enhanced feed and pharmaceutical products worked synergistically (De Mil et al. 2017).

7 Adsorption of pesticides and environmental toxins

Biochar is increasingly employed in animal feed due to its significant adsorption of insecticides, herbicides and pesticides (Shehata et al. 2013; Safaei Khorram et al. 2016; Cederlund et al. 2017). This is especially important when considering the absorption of glyphosate, a herbicide that has contaminated nearly every feed. While it has been illegal in Germany to use crop desiccation herbicides for pre-harvest treatment since May 2014, this is not the case in many other countries. Glyphosate has a powerful antimicrobial impact and can also immobilise magnesium and zinc (which may explain why it is linked to or promotes botulism) (Shehata et al. 2013). Effective sorption of glyphosate by BC particles is pH (high sorption at low pH) and temperature dependent (high sorption on high-temperature BC) (Herath et al. 2016). Hall et al. (2018) showed that a 0.1M monopotassium phosphate solution could remobilize the glyphosate charcoal had sorbed from pure water. The results of this study suggest that glyphosate from biochar-sorbed feed could be remobilized in the intestines due to ion competition. Due to the possibility that low pH, such as that found in the gastrointestinal tract, could enhance glyphosate sorption, additional *in vivo* and/or *in vitro* study in relevant matrices is necessary. Research with 380 dairy cows found that the intake of 200 g of BC and 500 g of sauerkraut juice per day (for four weeks) significantly decreased the amount of glyphosate in the cows' urine when they were fed glyphosate-contaminated silage (Gerlach et al. 2014). In the 1970s, BC was used in very few studies for pesticide adsorption (Smalley et al. 1971; Humphreys and Ironside 1980). Activated BC was employed to adsorb pesticides in the gastrointestinal tracts of ruminants, which were subsequently ejected (Wilson and Cook, 1970). However, similar trials in hens did not demonstrate any meaningful impacts on the residual quantities in tissues and eggs (Foster et al., 1972). A significant amount of contaminated BC was fed an organochlorine pesticide called Dieldrin, which was commonly used until the 1970s and is still traceable in the environment despite being outlawed.

Many common environmental toxins, namely dibenzo-p-dioxin (PCDD), dibenzofuran (PCDF), and polychlorinated biphenyls (PCBs), are fat-soluble organochlorine compounds. Adipose (fat) tissue accumulates these chemicals in humans and other animals. Several studies have used activated BC to remove these contaminants from water in Japan (Yoshimura et al. 1986; Takekoshi et al. 2005). Organochlorine compounds were found to have a strong affinity for activated charcoal in every experiment (Iwakiri et al. 2007). Twenty-four egg-laying hens participated in a controlled experiment in which they were administered feed containing the aforementioned organochlorine compounds for 30 weeks, with or without 0.5% BC. The organochlorine compounds' structure and aromaticity can lower PCDDs and PCDFs, non-ortho PCBs, and mono-ortho PCBs in eggs laying hens and tissues by more than 90%, 80%, and 50%, respectively (Fujita et al. 2012). Biochar binds many organochlorine chemicals, according to previous studies of polluted fish oil (Kawashima et al. 2009). Polycyclic aromatic hydrocarbons, in general, and higher aromatic molecules, in particular, show a substantial attraction to BC, as reported by Bucheli et al. (2015). Olkkola and Neuvonen (1989) found that supplementing human and animal diets with BC can considerably increase the elimination of PCB and dioxins. In terms of efficacy, BC may remove heavy metals and harmful elements and increase the use of biochar technology in water treatment (Inyang et al. 2016).

8 Detoxification of plant toxins

Regular BC consumption has various positive impacts, including reducing the negative outcomes of consuming naturally existing but potentially hazardous components like tannins, which are present in many diets (Struhsaker et al. 1997). Tannins are a chemical class spanning a broad spectrum, from being useful to poisonous, especially ruminants. Animals avoid eating beans and other high-protein foods because their tannins have a strong flavour that makes it difficult for them to digest and put on weight (Naumann et al. 2013). Some research has examined how BC feeding modifies the effect of tannin-rich diets. Goats fed a diet rich in tannins from acacia leaves gained 17% more weight per day when given 50-100 g of bamboo BC per kg in addition to their regular diet, compared to goats fed a control diet without BC (Van et al. 2006). Both nitrogen conversion and crude protein digestion were found to have improved significantly. Weight increases of goats fed 50 or 100 g of bamboo BC feed additives were similar, indicating an optimum BC dose.

Banner et al. (2000) found that the absorption of compounds rich in tannins and terpenes was considerably enhanced by adding 10-25 g of activated charcoal to rye daily. Sage and other terpenic and tannin-rich bushes showed comparable results for Rogosic et al. (2006; 2009); however, Villalba et al. (2002) could not prove that lambs ingested significantly more sage because of BC-enhanced feed. Since there aren't enough new pasture plants for sheep to graze on during the winter, they eat bitterweed containing toxic amounts of sesquiterpene lactones. That's why Poage et al. (2006) experimented with feeding bitter weed to lambs at a rate of 0.5-1.5 g of BC per lamb per day. The lambs refused to take the feed containing bitter weed when BC was not there, but when BC was present, they ingested up to 26.4% of the total amount of feed given to them without exhibiting signs of toxicosis. The toxic effects of the invasive flowering plant *Lantana camara* are mitigated in sheep and goats by supplementation with BC at 5g/kg body weight (Pass and Stewart 1984; McLennan and Amos 1989). In a study on *L. camara* toxicity, five of six calves survived after receiving activated charcoal therapy, while all six of the untreated calves died (McKenzie 1991). Bentonite therapy had a similar success percentage in curing patients, albeit it took twice as long for patients to recover fully. Treatments for oleander poisoning in sheep and yellow tulip (*Moraea pallida*) poisoning in cattle yielded comparable, highly significant results (Snyman et al. 2009; Tiwary et al. 2009; Ozmaie 2011). In this regard, biochar is recognized as an essential candidate for treating common contaminants like inorganic contaminants, heavy metals, microbial contaminants, pharmaceuticals, endocrine-disrupting chemicals, volatile organic compounds, and personal care products occurring in drinking water (Palansooriya et al. 2020).

9 Mitigation of methane emission from ruminants

In support of production in the agricultural sector in a sustained manner, mitigation of the emission of methane from ruminants is a crucial concept (O'Reilly et al. 2021). An important source of atmospheric methane (CH_4) comes from the digestive tracts of ruminants (animals having four-compartment stomachs that ferment food as a major element of the digestive process). In omnivores like chickens, pigs, fish, and others, the breakdown of solid and liquid wastes is the primary source of greenhouse gas emissions, but in ruminants, waste results in direct gaseous emissions from gas and belching (burping) (Johnson and Johnson 1995). Hristov et al. (2013) found that ruminants account for about 81% of all greenhouse gases emissions from livestock production. Greenhouse gases are major contributors to air pollution. Some 1.7 billion cattle, buffalo, and 2.2 billion sheep and goats make up the world's ruminant population (Searchinger et al. 2021). Tapio et al. (2017) found that 90% of cattle-related greenhouse gas emissions come from methane emissions, predominantly from rumen microbial methanogenesis. Livestock enteric fermentation produced 171 million metric tons of carbon dioxide equivalents in 2016. Manure management accounts for 10% of methane emissions, whereas enteric fermentation in ruminants accounts for 26%. The symbiotic relationships between these ruminants and the bacteria, fungus, and protozoa in their rumen allow them to get their energy from a fibrous diet (Castillo-Gonzalez et al. 2014). Microorganisms that can break down cellulose are abundant in the rumen of ruminants. When anaerobic microbes in the rumen break down organic matter, "enteric methane" is produced. Domestic animal enteric methane emissions are expected to grow by 50% by 2050, from a projected 100 million tonnes of carbon dioxide-equivalent in 2018 to more than a fifth of all agricultural emissions (Searchinger et al. 2020). Approximately 85% of worldwide emissions come from cattle and buffalo, while just 12% comes from sheep and goats (FAO 2019; Searchinger et al. 2021). Most methane is expelled through belching, while some make their way into the bloodstream and is expelled through the lungs (Danielsson et al. 2017).

Intestinal methane is produced by bacteria known as methanogenic archaea and methanotrophic archaea, respectively. Containing BC in soil amendments encourages the growth of methanotrophs, which in turn reduces methane emissions from the intestines of livestock by providing a habitat for methane oxidation in the stomach (Leng et al. 2012a). Methanotrophic proteobacteria and methanogenic archaea mostly carry out intestinal methane production. It was shown that methanotrophs produced more methane than they consumed (Feng et al. 2012). BC's ability to adsorb and absorb gases is crucial in lowering intestinal methanogenesis (Pereira et al. 2014; Danielsson et al. 2017). Thus, providing animals with BC can efficiently reduce their methane production in the digestive tract. Methane emissions were reduced by 15% when BC was incubated with rumen fluid (Leng et al. 2012b,c; Leng 2018). Furthermore, 9% BC (w/w) reduced intestinal methane emissions by 11%-17% (Hansen et al. 2012). Adding 1% BC (w/w) to cow diets has been shown to reduce methane output by 11-13% (Leng et al. 2012a). Using BC alone or in combination with nitrates lowered methane emissions by 22% and 41%, respectively (Leng et al. 2012b,c). When cows were co-fed with BC at a concentration of 3.8% (w/w), methane emissions were reduced by 12.6L per cow per day (Khoa et al. 2018). Adding 0.8% BC to a cattle's diet during the growth and fattening stages resulted in a 9.5% and 18.4% reduction in gut methane emissions, respectively, and improved digestion (Kalus et al. 2019; Winders et al. 2019). Adding 0.5% BC to *in vitro* rumen testing reduced methane generation by 25% (Saleem et al. 2018). Dairy ewes can minimize methane emissions and ammonia concentrations using BC made from chicken manure and walnut shells. Due to this reason, the incorporation of BC can be done effectively in the diet of dairy ewes as a cost-effective feed additive (Mirheidari et al. 2019). In conclusion, BC has been found to dramatically lower intestinal lumen methane emissions in both *in vivo* and *in vitro* experiments.

10 Treatment of digestive disorders in animals

O'Toole et al. (2016) noted that charcoal had been used for decades to cure diarrhoea in people and animals. Feeding animals with charcoal was common in the late 19th and early 20th centuries. Horses can suffer from colic (Edmunds et al. 2016), dogs can experience flatulence (Giffard et al. 2001), and horses can absorb toxins (Kaye et al. 2012). Claw and foot disease causes substantial economic loss due to decreased body weight, milk output, dry matter intake, herd lifespan, and reproductive efficiency in chickens. The severity of the disease is significantly reduced by BC, which positively impacts animal health and productivity. Material "cow fortifiers" were commonly promoted in the late 19th and early 20th-century agricultural literature. The makers of these tonics claim to improve milk production, appetite, and stomach issues. It is a universal antidote for venoms and has been used to treat various venomous animal diseases, including botulism in chickens, tetanus, and *Campylobacter jejuni* bacterial toxin (Toth and Dou 2016).

11 Growth promotion and immunomodulatory effect

Like the mechanisms by which antibiotics boost growth, the mechanisms by which charcoal does so are likely to be convoluted (Van et al. 2006; Allen et al. 2013). Charcoal has been shown to improve nutrient absorption, fat digestion, complex plant secondary metabolites (Mekbungwan et al. 2008), promote the development of intestinal villi, and decrease stress hormones (Kana et al. 2010; Chu et al. 2013a). Weight increases (live) and feed conversion rates have been observed in fattening pigs supplemented with biochar in the diet (Lao and Mbega 2020). Growing pigs fed a diet containing 2% BC had no detrimental effects on the measured performance metrics (Schubert et al. 2021). In particular, Chu et al. (2013b) and Islam et al. (2014) investigated whether charcoal products might substitute antibiotics in enhancing the development of food animals and found that they could. Most of Europe's BC output—90%—goes toward animal husbandry, including cattle and poultry production, where it is utilized as a feed additive (Gerlach and Schmidt 2012; Kammann et al. 2016). The use of BC in agriculture is expected to increase at a CAGR of about 12.5% over the next eight years, starting in 2018. O'Toole et al. (2016) found that 0.1% to 4.0% of the daily feed intake was blended with feed grade BC. According to a plethora of studies, including those by Leng et al. (2013), Winders et al. (2018), and Khoa et al. (2018), adding BC to feed has many benefits, including increased body weight gain and feed consumption, decreased usage of antibiotics, neutralization of chemical residues and other toxins in feed, and reduced gaseous methane emissions from the digestive tract.

12 Removal of pollutants and toxins in animals

According to Tan et al. (2016), BC can adsorb and remove heavy metals and contaminants (both organic and inorganic) from water and soil. Environmental pollutants and toxins can enter the body of animals through the food they consume. This contamination can be from human activity, pests, or even microorganisms. Including 0.5% BC in broiler diets improved growth and reduced the detrimental effects of aflatoxins (Teleb et al. 2004). The harmful bacterium *Campylobacter jejuni* was reduced in the gut microbiome of pullets after BC administration, as Prasai et al. (2016) reported. After oxidation by ozone and ammonia, carbon enterosorbents from rice husks could remove clinically relevant levels of uremic toxins (urea and creatinine) from the *in vitro* uremic toxin adsorption model tests (Jandosov et al. 2017). Biochars can catalyze abiotic reactions, particularly in the rhizosphere, that improve nutrient supply and absorb by plants, stimulate plant development, reduce phytotoxins, and increase resistance to environmental stressors and disease. On average, by Meta-analyses, biochars improve phosphorus availability by 4.6; decrease the concentration of heavy metals in plant tissues by 17%–39%, and reduce the emissions of non-CO_2 greenhouse gas from the soil by 12%–50% (Joseph et al. 2021). Also, biochar addition increased the average crop yield by 10%–42% (Joseph et al. 2021).

13 Biochar feed management and quality assurance

Raw resources for making BC mostly come from biomass and organic materials. Similar ones are employed in the production of activated charcoal. The European Biochar Feed Certificate states that a complete BC analysis and control of all applicable feed regulation criteria must be performed before using BC in animal feed. If BC has been treated with an alkali or acid before its use as a feed supplement, the activator must be flushed out with water analysis. Furthermore, the EBC suggests preventing dust generation by constantly preparing and delivering BC when damp. We usually blend BC with all standard feed mixes because it may be applied to any feed. Animals fed BC may also be exposed to BC in their drinking water. When treating acute poisoning, an aqueous suspension of activated BC is recommended (Neuvonen and Olkkola 1988). BC can also be supplied to animals at pasture or stable drinking holes without being mixed in with regular feed, though this varies by species. Molasses and sweeteners such as saccharin, sugar, and related compounds are common BC additions

(Cooney and Roach 1979). O'Toole et al. (2016) found that some farmers in Germany and Switzerland employ mechanized injection systems to add 1% (vol.) BC to silage towers and silage bales. In many cited tests, the combined effect was greater than that of the individual components fed separately (Galvano et al. 1996a).

Biochar may be combined with many other feed supplements to create compositions for specific purposes and animal types. Biochar's chemical absorption capability depends on its pore size distribution, surface area and charge. Even if the total surface area of activated BC grows (from about 300 m to >900 m) (Galvano et al. 1996b), the specific surface area grows even more. Micropores are typically too tiny to transport large molecules or pathogens vital to animal digestion. When tested against non-activated BC, highly-activated BC did not appreciably reduce the harmful effects of aflatoxin in chickens (Edrington et al. 1997). This was similarly true for other toxic compounds being tested. Thus, activating BC may not increase the target compound or organism adsorption capability. BC with an incredibly high concentration of accessible meso and macro holes can be made by adjusting the pyrolysis settings, eliminating the need for downstream activation (Brewer et al. 2014). Depending on the invocation technique, BC's ability to mediate electrons (and protons) is drastically altered during activation and acidification (Chen and McCreery 1996). However, no comprehensive studies of the effects of feeding animals modified BCs have been conducted as of yet.

It appears that pyrolysis temperature is the sole major factor impacting redox behaviour, with temperatures between 600 and 800°C being the most effective (Sun et al. 2017). Keep PAH levels below approved limits and lessen condensate deposition on BC surfaces. For the pyrolysis procedure to end successfully, the cooling BC must be actively degassed for a significant amount of time. Using an inert gas or adequate counterflow ventilation during discharge constitutes two methods proposed for achieving this goal (Bucheli et al. 2015). Bamboo (Chu et al. 2013a), corncob (Kana et al. 2012), straw (Cabeza et al., 2018), coconut shells, rice husk, rice, and rice hull were also used to produce BC in addition to wood. Recent articles assert that solid evidence supports favouring one type of biomass over another when making high-quality feed BC. If certain conditions are followed, biochar from woody or non-woody precursors can be fed to animals. Polycyclic aromatic hydrocarbons, other organic contaminants, and the degree of carbonization are all measured and held to these requirements.

14 Possible side effects of biochar

According to the evidence reviewed here, neither activated nor unactivated BCs used as feed additives or veterinary treatments were hazardous or damaging to animals or the environment. No serious adverse effects were reported in the acute and chronic studies. There have been no reported adverse effects from the long-term, daily use of BC supplements by a rising number of farmers who give them to their cattle (Kammann et al. 2017). There have been few clinical studies of BC feeding over the long term (Struhsaker et al. 1997; Joseph et al. 2010). According to human studies, oral administration of activated carbon appears to pose few risks. However, long-term therapeutic feeding experiments using BC are lacking. Patients with uremia who were given 20-50 g of activated BC once or twice daily for 4-20 months saw no adverse effects (Yatzidis 1972). Human patients provided 10-20 g three times a day for several months by Olkkola and Neuvonen (1989) saw no adverse effects. There are two significant dangers associated with BC eating for an extended time: (1) alterations to the microbiome (the community of microorganisms that live in the digestive tract) and (2) the possible absorption of necessary feed ingredients and/or medications. Regarding the microbiome, research into the adsorptive potential of activated BC for gram-positive bacteria in dairy cow digestive tracts was conducted (Naka et al. 2001).

Adsorption of the harmful gram-negative *E. coli* O157: H7 strain was much higher on activated BC than adsorption of typical, benign bacterial flora strains. The beneficial to pathogenic microorganisms ratio seemed to improve once BC was introduced. However, before any broad conclusions can be formed, a more comprehensive range of digestive and pathogenic microbes must be rigorously researched and their mechanisms understood. The effect of BC on microorganisms depends on the cell envelope, as the gram stain indicates, with gram-positive bacteria being either not sorbed to BC or poorly sorbed to BC. Cell envelope structure and gram positivity or negativity are not, however, reliable indicators of whether or not a bacterium is pathogenic.

Unlike vitamin E, which was lowered by 40% when chickens were given 0.5% BC daily, vitamins A and D3 did not demonstrate a statistically significant trend toward decreasing egg yolk levels. The eggs' fatty acids, mineral content and oxidative stability, did not alter after BC feeding, but this was the first indication that BC feeding could severely drop vitamin levels. Changes in egg yolk color indicate a loss of carotenoids, lending credence to the idea that, before recommending industrial-scale, long-term BC co-feeding, more research is needed, specifically focusing on animal fat-soluble supplements. Some may argue that the hazards associated with routinely supplementing with quality-controlled BC are negligible in light of the prevalence of herbicide and fungal toxin contamination in the feed given to livestock animals (Prasai et al. 2018a).

Conclusion and future prospects

Biochar's use in animal farming as a feed supplement is founded on activated charcoal's application in treating animal gastrointestinal diseases. Biochar can be used as a supplemental feed ingredient after being pyrolyzed, chemically, or physically activated. Biochar's physiochemical qualities are strongly linked to the feedstocks used and the pyrolysis temperature at which they were produced. More BC is formed from feedstock with greater lignin content; hence this usually results in a higher BC production. Some feedstuffs improve animal development and flavour due to the presence of specific components. The low surface area in BC may come from micropores blocked by the ash in the non-wood feedstock. BC as a feed additive has been proposed as beneficial for agriculture, the environment, and the animal husbandry industry. Positive benefits on animal growth have been observed when feeding biochar to animals, including cattle, goats, pigs, poultry, and fish. Cattle, pigs, and chickens could all benefit from a diet that includes biochar made from rice husks. Biochar's ability to create hospitable environments for methanogenic-methanotrophic microbial interactions in the gut and increase anaerobic methane oxidation in ruminants' digestive systems contributes to its ability to mitigate the animals' enteric methane emissions.

Because of its porous structure and huge surface area, BC, like activated charcoal, has a high sorption potential for removing hazardous substances from animals and the environment. The physiochemical features of biochar and the method used to make it determine its sorption capacity for toxicants. The sorption capacity of biochar generated at higher temperatures was typically greater. Forage digestibility and rumen fermentation kinetics were improved due to the higher pyrolysis temperature, producing smaller biochar particles. Adsorption of pathogens from meals containing biochar is one way to enhance poultry blood profiles and lessen their need for antibiotics. Biochar supplementation of animal meals looks to be a highly effective method for animal husbandry. Food and Agricultural Organization, World Health Organization, and European Biochar Foundation have established classification and certification requirements for standard biochar for use as a feed supplement and soil amendment, and their use is on the rise. These findings may benefit future efforts to develop BC for human consumption and control its use in animal diets.

Acknowledgement

All the authors acknowledge and thank their respective Institutes and Universities.

Author's contribution

All the authors contributed significantly.

Funding

This is a compilation written by its authors and required no substantial funding to be stated.

Disclosure statement

All authors declare that there exist no commercial or financial relationships that could, in any way, lead to a potential conflict of interest.

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Journal of Experimental Biology and Agricultural Sciences, June - 2023; Volume – 11(3) page 500 – 505

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Role of IGF-1 in goat semen freezing: A Review

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Received – January 27, 2023; Revision – May 22, 2023; Accepted – June 09, 2023
Available Online – June 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(3).500.505

KEYWORDS

IGF-1

Semen

Goats

Spermatozoa

Seminal

ABSTRACT

This review is based on the importance of Insulin-like Growth Factor-1 (IGF-1) in goat semen cryopreservation. Recent studies indicate that certain growth factors determine the seminal quality due to the interaction between seminal plasma and spermatozoa. Cryopreservation is the technique used to preserve semen at extremely low temperatures for extended periods, which is essential for artificial insemination (AI) and selective breeding programs. IGF-I promotes the proliferation and maturation of spermatozoa. IGF-I is involved in sperm motility, DNA fragmentation, membrane integrity and fertilizing capacity. There was a significant positive correlation between the weight of animals and IGF-1 genotype diversity. This review aims to investigate the effect of IGF-1 fortification in semen cryopreservation. Further, the review article also assesses the role of IGF-1 in improving the post-thaw quality and viability of goat semen, with the ultimate goal of enhancing the success rates of AI. The research gap this review aims to fill is the limited understanding of the role of IGF-1 fortification on goat semen cryopreservation.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI]
(http://www.horizonpublisherindia.in/).

1 Introduction

Insulin-like growth factor I (IGF-I) regulates the cell cycle, promotes cell proliferation, or prevents apoptosis (Ziegler et al. 2015; Werner et al. 2016). In the context of goat semen cryopreservation, adding egg yolk to the semen extender before freezing is a common practice to protect the sperm membrane from the sudden drop in temperature. However, the presence of lecithin in egg yolks can interact with phospholipase enzymes from the bulbourethral goat gland, leading to the production of lysolecithin, which may have toxic effects on goat sperm, causing membrane damage and loss of motility (Sauerwein et al. 2000; Macpherson et al. 2002). IGF-I is found in seminal plasma and helps in testicular spermatogenesis and steroidogenesis, with dysregulation potentially contributing to male infertility (Sauerwein et al. 2000; Macpherson et al. 2002).

Milk and dairy products contain significant amounts of IGFs and IGF-binding proteins (IGFBPs). While research has primarily focused on IGFs, there have been reports of shortened IGFBPs and IGFBP glycosylation in milk, although IGFBPs have received less attention than IGFs (Meyer et al. 2017). High concentrations of IGF-1 in milk promote the proliferation of human colon cancer cell culture (Purup et al. 2007). This raises concerns about the potential health risks associated with consuming milk and other dairy products, highlighting the importance of considering IGF-1 levels when evaluating their safety.

IGF-I is structurally and functionally similar to insulin and act via endocrine, autocrine and paracrine processes to regulate the growth, development and differentiation of cells and tissues (Partridge et al. 2011; Kim 2014; Thornton et al. 2016). The level of this hormone can be influenced by various factors, including the duration of breastfeeding and individual traits (Rasouli et al. 2017). Studies have shown that IGF-1 levels in milk and dairy products can vary based on breed, lactation stage, and management practices (Meyer et al. 2017). Considering the potential risks associated with high IGF-1 levels, it is crucial to investigate further and understand the relationship between IGF-1 in dairy products and its potential impact on human health.

The coding gene of growth hormone (calpastatin, IGF-1, leptin, pituitary transcription factor-1) are the key genetic determinant influencing goat weight (Schenkel et al. 2005; Casas et al. 2006; Misrianti 2009; Arman et al. 2012).

2 Mechanism of action of IGF-1

The mechanism of action of IGF-1 involves promoting cell proliferation and differentiation and preventing apoptosis (Ziegler et al. 2015; Werner et al. 2016). It acts as a growth factor that regulates the cell cycle and plays a significant role in various physiological processes, including spermatogenesis and steroidogenesis (Sauerwein et al. 2000; Macpherson et al. 2002).

The regulation of IGF-1 and insulin levels in the blood is influenced by dietary factors, particularly protein and calorie intake. Studies have shown that food consumption, especially the amount of protein consumed, can affect the circulating levels of IGF-1 and insulin (Magistrelli et al. 2005). This highlights the importance of proper nutrition in modulating the levels of these growth factors.

In the context of milk and dairy products, it is worth mentioning that they contain significant amounts of IGF and IGFBP. While the focus has primarily been on IGFs, the role of IGFBPs in milk has received less attention (Meyer et al. 2017). High concentrations of IGF-1 in milk serum have been associated with the growth and development of human colon cancer cells (Purup et al. 2007). Therefore, the presence of IGFs in dairy products raises concerns regarding their potential impact on human health, particularly concerning cancer risk.

The IGF-1 gene is a key gene influencing goat weight; its genotype diversity has also been associated with this (Lestari et al. 2020). This phenomenon suggests that variations in the IGF-1 genotype contribute to goats' growth and weight differences. Identifying and characterizing genes associated with favourable phenotypes, including the IGF-1 gene, can provide valuable information for improving goat breeding programs and ensuring their sustainability.

Furthermore, climatic variables such as photoperiod, temperature, and the temperature-humidity index have been found to impact the level of IGF-1 in young goats. Increases in photoperiod and ambient temperature have been associated with higher IGF-1 output in goats (Pehlivan 2019). This highlights the influence of environmental factors on regulating IGF-1 levels and suggests the need to consider these factors when studying the role of IGF-1 in goat physiology.

In conclusion, the mechanism of action of IGF-1 involves its regulation of cell proliferation, differentiation, and survival. Dietary factors influence its levels, and its presence in milk and dairy products raises concerns regarding its potential effects on human health. The IGF-1 gene plays a crucial role in goat weight, and environmental factors can impact the levels of IGF-1 in goats. Understanding the complex interactions and functions of IGF-1 in various biological processes can provide valuable insights for both animal breeding programs and human health considerations.

3 Types of IGF-1

IGF-I, IGF-II and IGFBP-2 have been found in goat milk (Prosser 1996). Studies in goats have demonstrated that these peptides can cross from circulation to breast secretions (Prosser and Schwander

Table 1 Different type of IGF and its role

Type of IGF	Location	Role	Reference
IGF-I	Seminal plasma (horse)	Presence confirmed after immunoprecipitation and Western ligand blotting	Macpherson et al. 2002
IGF-II	Human seminal plasma	Identified in human seminal plasma along with other IGFBPs and protease activity of IGFBP-3	Baxter et al. 1984; Ramasharma et al. 1986; Ovesen et al. 1995
IGFBP-1	Human seminal plasma	Found in human seminal plasma along with other IGFBPs and protease activity of IGFBP-3	Baxter et al. 1984; Ramasharma et al. 1986; Ovesen et al. 1995
IGFBP-2	Seminal plasma (horse, equine)	Quantified in equine seminal plasma; levels unaffected by sexual activity	Macpherson et al. 2002
IGFBP-4	Human seminal plasma	Identified in human seminal plasma along with other IGFBPs and protease activity of IGFBP-3	Baxter et al. 1984; Ramasharma et al. 1986; Ovesen et al. 1995
IGFBP-5	Equine seminal plasma	Present in equine seminal plasma	Macpherson et al. 2002
Fragments of IGFBP-3	Human seminal plasma	Identified in human seminal plasma along with other IGFBPs and protease activity of IGFBP-3	Baxter et al. 1984; Ramasharma et al. 1986; Ovesen et al. 1995

1996). Similarly, the most prevalent IGFBPs in pig milk include IGFBP-2, IGFBP-3, IGFBP-4 and an unidentified IGFBP with a molecular weight of 28 kDa (Monaco et al. 2005). IGFBP-5 was recently discovered using mass spectrometry in the milk of tammar wallabies (Modepalli et al. 2016). During parturition, the concentration of IGF-I, IGF-II and IGFBPs are typically higher compared to later stages of breastfeeding (Meyer et al. 2017). IGF-II has been investigated as a modulator of prolactin dependant morphogenesis, as its production restored alveologenesis in prolactin receptor-defective mammary epithelial cells (MEC).

In-vitro studies have shown that sodium butyrate, fortified as dose-dependent in milk, increases messenger RNA expression and IGFBP-3 secretion. This, in turn, leads to the enhancement of growth hormone and IGF-I concentrations in the blood of calves (Wang et al. 2017). Dairy processing techniques affect the amount of IGF 1 and its binding protein. The intact and fragmented IGF-I, IGFBP-2 and IGFBP-5 forms were found in milk samples (Meyer et al. 2017).

Furthermore, in cattle, GH and IGF-I play a vital role in regulating osteogenesis and muscle development (Curi et al. 2005). However, it is worth noting that the content provided does not directly address the role of IGF-I in semen freezing or its impact on sperm parameters. The information primarily focuses on the presence and effects of IGF-I, IGF-II, and IGFBPs in milk and their significance in various contexts.

Levels of IGF-I and IGFBP-2 were determined in seminal plasma using radioimmunoassay. IGFBPs were found in seminal horse plasma after immunoprecipitation, and Western ligand blotting techniques were used to confirm their presence. Equine seminal plasma included IGF-I, IGFBP-2, and IGFBP-5. IGF-I, IGF-I/protein, total IGF-I, IGFBP-2, IGFBP-2/protein, and total IGFBP-2 levels in seminal plasma were not markedly different ($P < 0.13$) (Macpherson et al. 2002). It has also been investigated how IGF proteins work as post-testicular regulators of reproductive function. Human seminal plasma has been shown to include IGF-I, IGF-II, IGFBP-1, IGFBP-2, IGFBP-4, fragments of IGFBP3 and IGFBP-3 protease ability (Baxter et al. 1984; Ramasharma et al. 1986; Ovesen et al. 1995). Equine seminal plasma samples were used to quantify IGF-I and IGFBP-2. Sexual activity did not alter IGF-I and IGFBP-2 levels.

The freezing of goat semen is a crucial technique in preserving genetic material for long-term storage and breeding programs. However, it is important to consider the potential implications of IGF-1 in both semen freezing and milk production. This comprehensive review aims to examine the role of IGF-1 in goat semen freezing while addressing its presence and effects in milk.

4 IGF-1 in Semen Freezing

Previous studies have highlighted a correlation between seminal plasma IGF-1 levels and sperm motility, appearance, and fertility rates. To further understand the impact of IGF-1, investigations have been conducted to assess DNA fragmentation, membrane integrity, and fertilizing capacity. These studies have shed light on the important aspect of IGF-1 in sperm function and its importance in maintaining the quality of frozen goat semen.

5 Source of IGF-1

IGF-1 and its binding proteins in colostrum are more compared to the serum of Holstein neonates (Gale et al. 2004). Leydig and Sertoli cells release IGF-I, present in seminal plasma from the testes. IGF-I is crucial in spermatozoa movement, germ cell differentiation, and development (Roser and Hess 2001; Henricks et al. 1998). Photoperiod significantly affects the levels IGF-1 in goats (Flores et al. 2018).

6 Role of IGF-1 in semen diluents

The IGF system impacts the physiology and operation of sperm in several animals (Selvaraju et al. 2012). The differences in IGF-I concentration in seminal plasma affected the signal for germ cell growth, maturation, and spermatozoa viability (Selvaraju et al. 2012). The IGF-I complex from seminal plasma affected goat spermatozoa membrane intactness, capacitation, and DNA integrity. Skim milk-egg yolk extender formulations included diluted buck semen. IGF-I supplementation enhances the quality of semen in various species, including goats and bulls (Kumar et al. 2019). The sustainability of semen samples and their ability to maintain optimal quality may be attributed to the presence of antioxidant activities. However, these antioxidant activities can be depleted or lost during the handling of seminal plasma, especially when dilution is required, leading to a decrease in their levels and potentially placing strain on the cells. When discussing the importance of antioxidants in maintaining sperm quality and protecting against oxidative stress, the concept of glutathione (GSH) and its role in seminal dilution can be introduced. GSH, as an essential antioxidant, serves as a vital function in protein synthesis that may be lost during oxidative stress. Additionally, it forms a protective shield over sperm membranes to prevent lipid peroxidation and the generation of harmful free radicals (Almeida et al. 2021).

In seminal dilution, the loss of GSH can occur due to various factors, such as dilution during sample processing or exposure to reactive oxygen species. This reduction in GSH levels may have implications for sperm health and viability, compromising the ability to repair oxidative damage and maintain optimal cellular function.

7 Status of IGF-1 in semen diluents

This topic concerns numerous national and international research projects, works, and conferences. The effects of IGF-I on sperm capacitation, membrane intactness, and DNA integrity in goat spermatozoa have been reported by Susilowati et al. (2015). Gauthier et al. (2006) explored the physiological importance of IGFs and IGFBPs in milk. IGFBP ranking was proposed in bovine mammary secretions (Blum and Baumrucker 2002; 2008). Work has been done on stallions at the international level; 51 light-breeds, 3 to 20-year-old stallions were sampled for ejaculates from January through August 2000 in Florida, Maryland, and Texas.

8 Future Prospects

IGF-1 fortification could be a valuable tool for improving goats' artificial insemination and selective breeding programs. However, the effects of IGF-1 supplementation on the cryopreservation of goat semen is still needed more study. Specifically, future research should focus on the (i) optimal dose of IGF-1 for supplementation, (ii) timing of IGF-1 supplementation, (iii) duration of IGF-1 supplementation and (iv) the effects of IGF-1 supplementation on other aspects of semen quality, such as DNA fragmentation and capacitation. With further research, IGF-1 supplementation could become a standard practice for improving the cryopreservation of goat semen. This would significantly impact the goat industry, enhancing the artificial insemination per cent and selective breeding programs.

Conclusion

In conclusion, this review has shed light on the role of IGF-1 in goat semen cryopreservation and its implications for seminal quality. Various growth factors influence the interaction between seminal fluid and sperm cells, with IGF-I being recognized as one of the most significant factors due to its promotion of germ cell proliferation and sperm maturation. The freezing aspect of goat semen cryopreservation is a crucial factor to consider when examining the role of IGF-1 in seminal quality. Cryopreservation, which involves storing semen samples at extremely low temperatures, is essential for long-term storage and transportation. The review has aimed to highlight the potential involvement of IGF-I in sperm function by exploring the correlation between IGF-I and important attributes such as spermatozoa motility, appearance, and fertility rates.

Additionally, identifying genes associated with favourable phenotypes has been a focus, as it holds promise for enhancing goat breeding programs and ensuring their sustainability. Considering the safety of dairy products, the review suggests that IGF-I should be considered when assessing their safety profile. High levels of this hormone have been associated with potential cancer risk in individuals consuming milk and other dairy products. Thus, more study is required on the implications and potential health risks associated with IGF-I in dairy products.

Furthermore, the review has revealed a significant correlation between goat weight and the genetic diversity of IGF-1. This finding underscores the importance of genetic factors in influencing IGF-1 levels and highlights the potential for using IGF-1 as a marker for desirable traits in goat breeding programs.

Overall, this review serves as a roadmap for future studies in the field, providing valuable insights into the role of IGF-1 in goat semen cryopreservation and its broader implications for reproductive processes and dairy product safety. Continued research in this area will contribute to further advancements in goat breeding and the development safe and sustainable dairy products.

Conflict of Interest

I hereby declare that there is no conflict of interest for publication of this manuscript on behalf of all authors.

Acknowledgements

Authors thank the Department of Bio-Technology, Ministry of Science and Technology, GOI, New Delhi, India (Project No. 102008) for financial support and the Director, ICAR-CIRG, Mathura, for facilitating all necessary facilities for the smooth and timely conduct of the different experiments.

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Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Sources, blood concentrations, and approaches for reducing exposure to lead: A critical appraisal on lead poisoning

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Received – May 20, 2023; Revision – June 21, 2023; Accepted – June 27, 2023
Available Online – June 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(3).506.519

KEYWORDS

Lead

Sources of exposure

Ayurvedic preparations

Workplace exposure

Lead poisoning

EDTA

Amelioration

ABSTRACT

Lead, a non-essential metal, enters the body in various ways, making it a major public health issue. Painters and smelters report lead poisoning in children and staff. Mining and battery workers risk lead exposure. Traditional and cultural remedies may include dangerous quantities of lead, producing lead poisoning. These drugs must be properly understood and regulated to avoid toxicity. Lead poisoning symptoms vary by duration and severity. Lead first impairs cognition, development, and behaviour by damaging the neural system. Time degrades reproductive and haematological systems. Lead's quiet entry into the body makes it deadly. Acute lead nephropathy damages kidneys at 100mg/dL. Lead levels exceeding 150mg/dL may induce encephalopathy. Blood lead levels indicate lead poisoning severity. Lead levels over 10g/dL in children and 40g/dL in adults are hazardous. Lead toxicity affects various organs. Lead may induce hypertension and cardiovascular disease. It may also cause chronic kidney disease and renal failure. Lead exposure may impede fertility, cause miscarriages, and alter foetal development; hence the reproductive system is vulnerable. Symptoms and lead levels may be treated with different approaches. Lead chelation treatment is frequent. Other vitamins and medications may enhance

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

organ function and treat lead poisoning. Lead poisoning prevention requires widespread awareness. Strict standards and education regarding lead-contaminated products and conventional remedies should reduce occupational lead exposure. Regular blood lead level monitoring, especially in youngsters and lead workers, may help detect and treat lead poisoning early. Lead poisoning has serious health consequences. Understanding lead exposure pathways, identifying symptoms, and preventing lead poisoning is essential to public health and organ system protection.

1 Introduction

Lead, an abundant environmental metalthe most common industrial hazard, with a lengthy history reaching 360 B Lead reference levels in different media serve as critical benchmarks: blood lead levels should be less than 2 micrograms per decilitre, ambient air levels should be less than 1.5 to 3 micrograms per cubic metre, and paint levels should be less than 90 parts per million. The occurrence of increased lead levels in various mediums necessitates rapid medical treatment. Traditional treatments have been linked to many recorded instances of lead poisoning in India (Kute et al. 2013).

A source of lead found potentially everywhere is tap water, typically secondary to the presence of this metal in plumbing. In several nations where gasoline (leaded) is available, air contaminated with lead from emissions is a vital exposure source (Whitehead and Buchanan 2019; Halmo and Nappe 2023). Adults may be exposed to lead through a wide range of occupations and hobbies. The working parents may bring home lead where the children are exposed second-hand. The occupations and hobbies involving the highest risk include welding metal, manufacturing of battery, use of lead in firing range or instruction and bullet salvaging, smelting as well as refining lead, use of lead in construction work or painting, plumbing and fitting pipe, building ship and shipbreaking etc. Several other exposures that are less ubiquitous have been implicated in adults as well as children with the heightened concentrations of lead that, include ceramic dining ware (contaminated), spices as well as cosmetics imported, and ingestion of foreign bodies made up of lead and retained bullets that are led (Quail 2018; Egan et al. 2019; Halmo and Nappe 2023).

Lead poisoning has a wide-ranging effect on the body, impacting several systems. The central nervous system is the primary target, followed by the haematological, hepatic, and renal systems. This harms both systolic and diastolic blood pressure, as well as abnormalities in cellular processes and enzyme systems, culminating in severe diseases (Figure 1).

While acute lead poisoning is rare, chronic lead toxicity is prevalent, with blood lead levels commonly ranging between 40 and 60 micrograms per decilitre (Srinivasan et al. 2016; Estévez-García et al. 2022). Chronic vomiting, narcosis, dementia, seizures, and coma are all symptoms of lead poisoning. Apart from all these, due to lead toxicity, the nerve conduction may be slow, mood may swing, and there may be drowsiness and fatigue along with impairment of concentration. There also may be fertility disorders, reduced libido and constipation, and if the condition is severe, there may be encephalopathy. The illness may quickly develop and become deadly if not treated promptly (Nedelescu et al. 2022). Notably, the neurological system takes the brunt of lead's damage, with excessive lead deposition harming both the peripheral and central nervous systems.

Lead also directly affects the haematological system, interfering with haemoglobin formation by inhibiting important enzymes involved in the heme synthesis pathway, a vital stage in the process (Dsouza et al. 2022). Furthermore, lead weakens cell membranes, reducing the lifetime of circulating erythrocytes. Renal failure, including acute or chronic nephropathy, occurs when lead levels are above 60 micrograms per decilitre, adding to the previously described vascular and cardiac damage (Mabrouk 2021). Hypertension may develop even at low levels of lead exposure, providing a considerable health concern (Gajewska et al. 2021; Ramírez Ortega et al. 2021). This review emphasizes lead exposure sources, blood concentrations, and prevention techniques. Lead exposure may occur via lead-based paint, fuel, soil, and dust. Examining lead exposure and BLLs helps investigate primary, secondary, and tertiary lead prevention techniques. Lead is a potent neurotoxin that, particularly in children, may cause cognitive and behavioural difficulties, delayed growth and development, and an increased risk of cardiovascular disease and cancer. Notably, priority should be given to protecting children from pollution caused by heavy metals like lead (Hou et al. 2019). Because lead is a severe public health risk, the review study strongly urges reducing lead exposure, particularly in children. Lead exposure may have substantial and long-term effects on children's health and development, making it vital to prioritize prevention measures. This review also highlighted that reducing lead exposure should be a public health priority and offers preventative options.

Anxieties about lead poisoning have been rising as of late because of the difficulty in diagnosing asymptomatic lead toxicity caused by prolonged exposure to low amounts of the metal. Appropriate investment in suitable environmental, nutritional, and medical resources is required to prevent and effectively cure lead

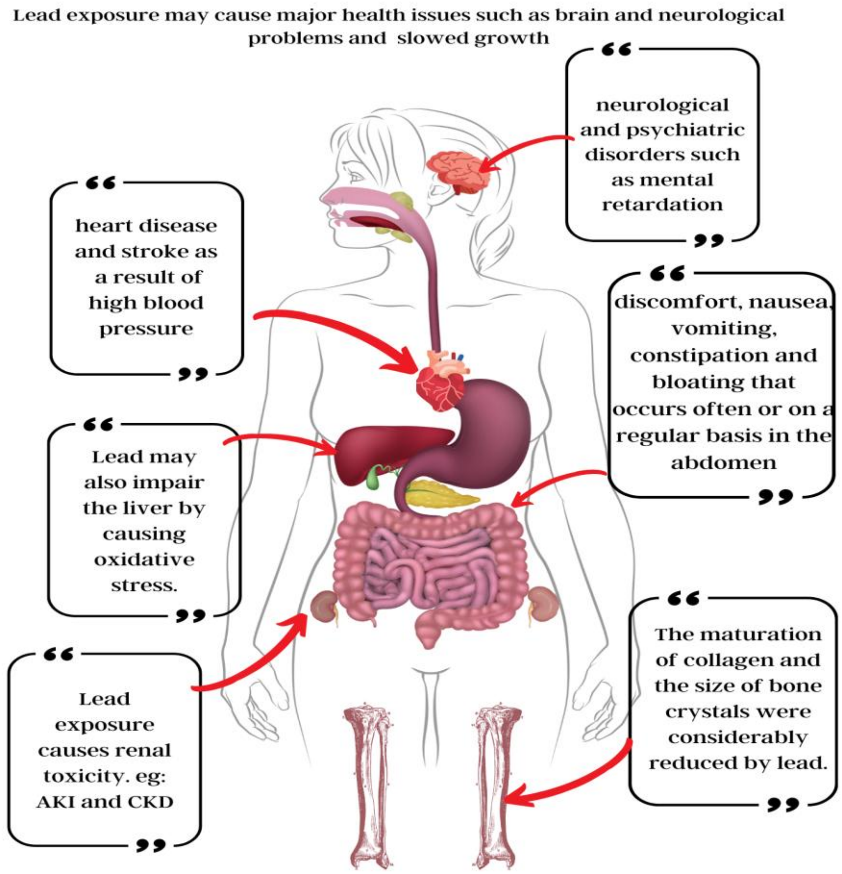

Figure 1 Illustrative scheme of lead exposure effect in various parts of the body

poisoning. Therefore, it is important to know where the lead came from to recognize the signs of lead poisoning in patients of varied ages and blood lead concentrations (Cantor et al. 2019; Lakka et al. 2023; Niu et al. 2023). The data provided by case reports is invaluable. Unfortunately, as far as we can tell, there aren't many reviews of lead poisoning case reports in the literature. An exhaustive literature review can shed light on the causes of lead poisoning that are currently unknown. It can help bring to light typical symptoms and realistic treatment options. In light of this, the current research reviewed published accounts of lead poisonings over the past decade, compiling data on exposure routes, symptoms, treatments, and preventative measures.

2 Lead exposure through Ayurvedic preparations

Ayurveda, an ancient Indian medical system, has received international acclaim for its holistic approach to treatment. Herbal medications, powders, and nutritional supplements are popular Ayurvedic formulations that enhance health and well-being (Gerritsen and Cleetus 2023). Concerns have been raised, however, about the presence of lead in several Ayurvedic medicines. Even though over 95 per cent of the exposure to lead is occupational, a potent source of lead poisoning is Ayurvedic medicine, which is responsible for 2 per cent of the non-occupational exposures (Yanamandra et al. 2020). This article aims to shed light on the subject by investigating the causes of lead pollution, possible health hazards, regulatory measures, and techniques to reduce exposure.

Several recent case reports of lead poisoning have been linked to using Ayurvedic supplements. A 58-year-old woman presented to the emergency room complaining of abdominal pain, anaemia, abnormal liver function tests, and elevated blood lead levels. For almost 6 weeks, the patient had been trying to control her diabetes with the help of Jambrulin, an Ayurvedic medicine. The chemical examination of the drug found dangerously high levels of lead. The patient's symptoms improved after she had chelation therapy. However, she developed chronic renal illness as a result of lead exposure. The risks of lead poisoning through Ayurvedic treatments are highlighted in this case study. We should know about the risks of lead poisoning before trying any Ayurvedic therapy (Ciocan et al. 2021).

Karri et al. (2008) documented a 41-year-old man with hypertension who had memory loss, atrophy, and anhedonia. Except for Mahayogaraj-guggul, he was drug-free. He drank this for almost 6 months since it is an ayurvedic drug that helps lower blood pressure. His examination revealed clinical indications of memory loss, significant disorientation, limb weakness with bilateral hypertonic and hyper reflexion, upgoing plantares, and spastic gait. His blood lead level was 161 µg/dL. The parasagittal occipital lobes had anaemia with basophilic stippling, bilateral focal hyperintensity, and similar lesions in the temporal, parietal, and frontal regions. His medical condition was chronic lead encephalopathy. The chelation therapy was administered in combination with stopping his Ayurvedic drug. This helped the client's clinical rehabilitation.

Similarly, these researchers reported a 9-month-old kid dying from lead encephalopathy convulsions after using Ghasard. Ghasard is a kind of traditional medicine. This medicine is provided to prevent constipation in neonates. His parents began supplementing his diet with conventional treatments when he was two months old. After eight months, the baby got drowsy and stopped moving. He also refused bottle feeding, had seizures, and died at nine months old. He started to suffocate and died two days later. The autopsy indicated that the victim had elevated lead amounts in his blood and other tissues. Lead lines were seen on long bone radiographs. All medications given to the youngster contained lead. Ghasard had the most lead by weight, at 1.6% (Karri et al. 2008).

Karri et al. (2008) described a 62-year-old man with arthritis and signs of status epilepticus with lead poisoning. He has been taking Mahayogaraj – guggul for over six years. It is an Ayurvedic drug used to treat arthritis. He wasn't on any other prescription drugs, and he wasn't working with lead. His blood lead level was found to be 89 ng/dL while being monitored for clinical indicators such as anaemia and status epilepticus. His diagnosis was acute and persistent lead encephalopathy. Many chelation procedures and chronic anti-convulsant medication were employed in this case to address the disease. It also caused anoxic brain damage from status epilepticus oppositely (Karri et al. 2008).

Geraldine and Venkatesh (2007) reported a 27-year-old Indian woman who resided in a non-polluted area of Bangalore and was diagnosed with HIV. She'd been utilizing herbal remedies for 13 weeks and complained of stomach aches, nausea, sleeplessness, giddiness, and constipation at the local hospital. IBS and mild hepatomegaly were diagnosed. Further, endoscopic testing confirmed antral gastritis. Her lead toxicity was examined at their facility. Her menstruation history and her husband's sperm count were within normal norms, yet she could not conceive for five years. She's been in the hospital since she refused to participate in follicular research and was advised to do an alternative medicine course. For a year, she was given three unbranded herbal treatments consisting of two types of brown powder and one kind of black pill, all provided randomly. In her interview, she disclosed that she began to experience moderate lead poisoning five weeks into this medicinal therapy. Their institution did a blood test for lead poisoning containing lead and zinc protoporphyrin. The lead levels in the blood and the three herbal drugs were tested using a portable X-ray fluorescence analyzer. She took one teaspoon of each brown powder, two black tablets, and one teaspoon of each white powder once daily.

As a consequence, herbal medications were shown to cause this lead poisoning. After a month of therapy with D-penicillamine (25–35 mg/kg body weight/day), her blood lead and ZPP levels were dramatically decreased, suggesting that she had received successful treatment. The patient's clinical indications indicated lead toxicity; blood lead levels were tested to confirm lead poisoning. As a result of lead poisoning, the haematological and hepatic systems were affected(Geraldine and Venkatesh 2007).

A 45-year-old software expert was poisoned by lead at work, according to Raviraja et al. (2010). He was sent to a Bangalore hospital twice in 10 days due to weakness, vomiting, and stomach pain. Both times the treatment failed to help him. So far, he had used twelve different Ayurvedic remedies, all of which were natural. These were used to relieve stress and maintain health. He bought all drugs without an Ayurvedic physician's prescription. His blood pressure was 120/84 mm Hg, his haemoglobin was 14.2 g/dl, and his BLL was 122.4 g/dl, all indicating lead poisoning. He was also chelated with 250 mg D-penicillamine three times a day for two months, following which he was instructed to discontinue using all ayurvedic drugs. Prescribed were calcium pills, B-complex tablets, and plenty of water. After chelation, two months of medication effectively relieves stomach pain. A year later, his blood lead level was 27.4 ng/dl, and he was no longer in pain. When all twelve ayurvedic products were tested, 75% had lead, arsenic, and mercury levels, several orders of magnitude greater than the daily permissible limits (Raviraja et al. 2010). These researchers also documented a 36-year-old male wing commander from Bangalore, India, who had been diagnosed with lead poisoning. He was admitted to the Air Force Command Hospital in Bangalore due to stomach ache, headache, insomnia, weakness, facial pallor, and joint pain. The study revealed a haemoglobin level of 8 g/dl and significant internal bleeding. The patient had an endoscopy, colonoscopy, and barium meal to establish the origin of internal bleeding. He said he went to a vaidya in Pune, Maharashtra, for psoriasis, a skin condition that affects the hands and feet. According to the local vaidya, Gulkand (Mukta Bhasma, Sapta Ratna, Kumar Kalyan ratna, and Sal Bhaskar ratna) is an efficient arthritic cure. He had to take 2 tablespoons of Gulkand for two months before breakfast and dinner. On examination, the peripheral blood picture exhibited basophilic stippling, suggesting a basophilic stippling infection. Doctors suspected lead poisoning and sent the case to the NRCLPI for a blood lead test, which came back at 115 ng/dl. He was advised to cease taking Gulkand. After chelation therapy with 2 g calcium disodium EDTA in 500 mL saline, the patient's liver and kidney functions and blood calcium and phosphorus levels returned to normal. He had no known risk factors for lead poisoning at the time. The lead content of Gulkand was 11,798 g/g(Raviraja et al. 2010).

Raut et al. (2021) described dual cases of lead encephalopathy because of the intake of ayurvedic medication for a long time. The first case was a female aged 54 years who was taking long-term medication and presented with a confusional state (acute) and disturbances of memory with pain in the abdomen. Magnetic resonance imaging (MRI) of the brain revealed symmetric basal ganglia, signal changes in the cortex, and edema. The presence of lead was in the blood at an elevated level significantly. The second case was a female aged 45 years taking ayurvedic medicine for high blood pressure. She presented with headaches and deterioration in the sensorium rapidly, leading to coma and death. MRI of the brain revealed cerebral edema (diffused), signal changes in basal ganglia and elevated blood level of lead. These two cases have highlighted the requirement for heightened awareness that certain medicines (Ayurvedic) may contain lead potentially at a harmful level, and people using them have the risk of developing toxicity associated with fatality.

Roy et al. (2022) and Jain and Roy (2022) have documented an incidence of toxicity induced by lead in a child suffering from type 1 diabetes mellitus due to *naga Bhasma* (Jain and Roy 2022; Roy et al. 2022). Now it is required to be told in this context that scientific studies reveal that during *naga bhasma* preparation quenching, trichuration along with calcinations of the crude lead is repeatedly done, thereby transforming it into the least toxic lead sulphide, which is nanocrystals. Other organic contents and various nutrient elements that come from the herbs are also used to prepare *naga bhasma*. The shortcomings in preparing *naga bhasma* and its use in an injudicious manner are alarming and may be responsible for the lead toxicity in the child suffering from type 1 diabetes mellitus (Varghese et al. 2022).

3 Lead exposure at the workplace

Lead is a dangerous metal that may cause many health issues, including learning and behavioural issues, lower IQ, and even death. Further exposure to lead may be associated negatively with weight at birth. Lead exposure may occur via many routes, including inhaling lead dust, consuming lead-contaminated food or drink, or exposure to lead-based paint (Dettwiler et al. 2023; Ramadan et al. 2023).

In their research, Ghanwat et al. (2016) evaluated 36 male employees aged 20 to 60 from lead battery plants in Western Maharashtra. The researchers gathered demographic, occupational, and clinical data from healthy male volunteers who did not drink or smoke. Paraesthesia, intermittent stomach discomfort, lack of appetite, nausea, diarrhoea, and constipation were among the symptoms encountered by these employees (Ghanwat et al. 2016). According to the research, the interaction between lead-oxyhaemoglobin and oxygen-free radicals in red blood cells resulted in the production of reactive oxygen species in the circulation. The copper deficit induced by high blood lead levels resulted in increased oxidative damage to red blood cells and reduced superoxide dismutase function (Gwozdzinski et al. 2021). Furthermore, the research discovered that lead exposure impeded heme synthesis and iron absorption enzymes, resulting in a lower heme pool and catalase activity. The researchers used vitamin C supplementation for one month to reduce blood lead levels (Sanders et al. 2009). Although the supplementation reduced lipid peroxidation and nitrite levels, the impact was insignificant. However, antioxidant status markers such as erythrocyte superoxide dismutase and catalase levels increase (Kurutas 2016).

Awasthi et al. (1996) tested pregnant women from Lucknow slums to discover whether lead might be passed to the foetus during pregnancy. The researchers separated 500 pregnant women from 70 slums into four groups depending on gestational age. Personal interviews were done to gather information about age, reproductive history, menstrual cycle, and other possible sources of lead exposure (Awasthi et al. 1996). The researchers determined that 26 women had blood lead levels of more than 20 micrograms per deciliter, averaging 35 micrograms per deciliter. Age, weight, height, haemoglobin levels, or reproductive history did not affect lead exposure (Fadrowski et al. 2010). However, the research found that lead exposure in pregnant women caused nutritional deficiencies and raised the likelihood of infant developmental disorders, particularly when associated with poor nutrition, infectious illnesses, and other poverty-related concerns (Awasthi et al. 1996). Kute et al. (2013) published a case study of a guy who used Sindoor, a traditional cosmetic containing lead, for religious motives. Severe stomach colic, constipation, nausea, vomiting, and other symptoms plagued the 44-year-old guy. A bluish-grey thin line at the gingival tooth edge and lead nephropathy were discovered during the testing. Lead exposure was connected to proximal tubular damage, interstitial nephritis, and chronic kidney disease, all impacting tubular function and vascular reactivity(Al-Naimi et al. 2019). Chelation therapy was part of the patient's treatment (Kute et al. 2013).

Palaniappan et al. (2011) investigated blood lead levels and their consequences in children aged three to seven attending schools in the Chennai area of India. The research was divided into four zones with differing levels of industry and traffic. All 76 youngsters tested positive for blood lead levels over the national average, with half surpassing 10 micrograms per deciliter. The research discovered that higher blood lead levels affected these children's IQ and general cognitive development (Palaniappan et al. 2011). Menezes et al. (2003) described a case study of a 10-year-old child labourer who had joint and epigastric discomfort while working in a battery recycling factory. The youngster has been exposed to lead for a long time owing to poor industrial safety practices. The investigation uncovered the consequences of persistent lead exposure (Menezes et al. 2003).

A total of 107 children from three schools in Mangalore and Dakshin Kannada were tested for lead poisoning by Kuruvilla et al. (2004). The X-ray Fluorescence Analyzer was used to determine the amount of lead in the soil of the schools, playgrounds, and households. They found that 11 of the 107 children, mostly from low-income schools, had 40 g/dl or higher blood lead levels. The assessment found lead spray on the tulsikatte (holy basil platform), peeling paint on playground equipment, yellow paint on the railing of a rice mill, and paint and traditional treatments used in homes(Kuruvilla et al. 2004). Thirty automotive workers in Bijapur were found by Dongre et al. (2011) to have significantly higher lead levels in their blood and urine compared to a control group. The study participants who worked in the automotive industry reported experiencing muscle pain, fatigue, irritation, and decreased haemoglobin and red blood cell counts after prolonged exposure to lead (Dongre et al. 2011). The effects of lead exposure on the health of people who work in the printing industry were the subject of a case study by Ji et al. (2014). Symptoms, including convulsions, diarrhoea, anaemia, colic, nephritic discomfort, and leg pain, were linked to prolonged exposure to lead dust in printing presses. The research highlighted the significance of dust management technologies and the need of fostering appropriate hygiene practises in these settings (Ji et al. 2014). Workers in Calcutta, electrical accumulator firms, were exposed to greater levels of lead due to inadequate protective gear and poor sanitary practices, according to a study by Ghosh et al. (1952). The study highlighted the importance of modern tools, exhaust ventilation, and efficient safety measures in reducing the risks of lead poisoning in the workplace. Goswami (2013)'s study has also linked using Surma, an ore powder, as an eyeliner to an increased risk of lead poisoning in children. The research highlighted that lead may enter the body via many channels, including the eyes and mouth, and identified improper Surma preparation as a critical component in lead poisoning. These studies highlight the need of identifying and controlling lead exposure sources to maintain public health (Goswami 2013).

A major concern for professionals who provide training or work with firearms regularly is exposure to lead. Such professionals include military personnel or people from law enforcement departments. In the study conducted by Schenk et al. (2021) on the military personnel of the Swedish armed forces, it was found that the instructors have more chances of airborne exposure to lead compared to the cadets while training with ammunitions that are led (Schenk et al. 2021). Those instructors who closely supervise the training programme with leaded ammunitions have increased blood lead level (BLL) geometric mean (GM) compared to other instructors. Widyantoro et al. (2021) found that the pipe repair place could expose people to heavy metals like lead exceeding the quality standards (Widyantoro et al. 2021).

4 Lead poisoning and counteracting measures for reducing exposure to lead – critical discussion

Lead, a soft, silvery grey metal, has unique properties that make it essential in various sectors. However, in addition to its positive features, lead seriously threatens human health and the environment (Iqbal et al. 2023). Lead's contradictory nature raises severe concerns about its ubiquitous usage and the possible risks related to its presence in daily things. We will examine the dual nature of lead in this creative debate, emphasizing its numerous uses and the hazards it presents in various circumstances (Cedergreen and Streibig 2005).

Lead's properties, such as corrosion resistance, high density, and low melting point, have made it a sought-after commodity in various sectors. Lead acid batteries, commonly employed in automotive applications, depend on effective electrical energy storage and conversion (Charkiewicz and Backstrand 2020). The jewellery industry favours lead for its malleability, which allows for creating elaborate patterns. Lead's durability and tolerance to the severe maritime environment make it a perfect option for various uses in shipbuilding. Its usage in petrol additives also improves engine performance (Tandon et al. 2001).

While lead has valuable industrial characteristics, its toxicity concerns human health. Lead-based paint, lead-soldered pipes, and polluted air and soil are frequent causes of lead pollution that may harm many organs (Mielke and Egendorf 2023). The customary use of unregulated pharmaceuticals, cosmetic goods, and hand-crafted ceramics adds to the risk of lead exposure. Lead-containing components used in mining, processing, recycling, and disposal may pollute the environment, exacerbating the problem (Abalansa et al. 2021).

Lead contamination is a major problem; lead accidentally enters the food chain via numerous methods (Kumar et al. 2020). Elevated lead levels in cultivated vegetables, cookware, and other food products may be caused by soil contamination, industrial and agricultural pollution, and food processing. Higher soil contaminations around the lead smelters, mines, and vehicular traffic centres have been reported, harming crops planted in such areas. Milk samples from cows grazing near roadways and industrial zones may also have increased lead levels, providing a health concern to humans (Levin et al. 2021).

Plumbing systems polluted with lead contribute to the dispersion of lead in drinking water. The presence of lead is often revealed during the first flush of the plumbing system. Industrial operations such as smelting and cremation and lead-based businesses greatly increase airborne lead concentrations (Jarvis et al. 2018). When lead-based paints on walls and chalking from woodwork and toys are breathed, they become significant sources of lead absorption for newborns. Lead crystal glasses, ceramic glazes, and other glazed food storage, preparation, or serving items may also bring lead into everyday life (Fralick et al. 2016).

A success concerning public health is the removal of lead from gasoline. By eliminating lead from gasoline, several nations have achieved mean (population) blood lead levels (BPb) of 1 μg/dL or less. Such actions have made life safe, enhanced children's intelligence and created enormous economic benefits in nations globally. There has also been a reduction in the incidence of exposure to lead at higher doses by eliminating lead in household paints (Angrand et al. 2022; Eastman and Tortora 2023).

Omeljaniuk et al. (2018) conducted a study to determine lead concentration in the blood and tissue of the placenta of women who suffered from miscarriages. Total 83 women who have experienced miscarriage formed the study group. The control group comprised 35 women either after the child's birth or in the first three months of pregnancy. It has been found that the average concentration of lead in the blood of women who miscarried is more significant when compared to the level of lead in the blood of the control group's women (Omeljaniuk et al. 2018). Similarly, in the placental tissue of women who suffered from miscarriages, the average lead content is greater when compared to the concentration of lead in the placental tissue of the control group's women. Therefore, monitoring the lead level is essential in women planning pregnancy.

Aside from the more well-known causes of lead exposure, many cultural practices unwittingly expose people to lead poisoning (Wani et al. 2015). Surma, a traditional cosmetic ore used as eyeliner, is applied to the conjunctival surface of the eyelids and may be absorbed or ingested if it comes into touch with the mouth (Goswami 2013). Folk medicine and the use of lead in Ayurvedic remedies offer hazards, especially if used over time. Sindoor, a red powder used as a cosmetic and in religious ceremonies, may cause lead poisoning if swallowed incorrectly as a food component (Shah et al. 2017).

Correct diagnosis is a crucial first step in preventing lead poisoning and toxicity. A complete diagnosis requires investigating all potential entry points. The inquiry should focus on the patient's medical history and symptoms. Clinical toxicologists and other medical specialists can aid in making a correct diagnosis and developing an appropriate treatment plan. A major sign of lead toxicity is basophilic stripping. This stripping makes the microscopical visualization of red blood cell specks possible. Lead poisoning can be detected with a thorough blood film evaluation for such symptoms (Samarghandian et al. 2021). Anaemia caused by a lack of iron is linked to lead poisoning. Erythrocyte protoporphyrin (EP) levels in the blood are another method for diagnosing lead poisoning. When blood lead levels are elevated, EP tends to rise a few weeks later. Nonetheless, increased blood lead levels below 35 μg/dL cannot be detected with just the EP level. Lead exposure detection with EP has fallen in use because of its higher detection threshold and because EP levels rise in iron deficiency (Cantor et al. 2019; Sadiku and Rodríguez-Seijo 2022). Lead concentrations in the blood only reflect recent or ongoing exposure to lead, not the total load carried by the body. Indicative only of recent lead exposure, a blood lead level test does not accurately account for lead already present in the body. Non-invasive X-ray fluorescence measurement of lead in bones provides a possible optimal method for gauging total body exposure and load. Foreign objects, such as paint chips containing lead and lodged in the digestive tract, may be visible on an X-ray (Charkiewicz and Backstrand 2020).

Lead poisoning is treated with a combination of dimercaprol and succimer. Given the long-term effects of lead poisoning on children's brain development, the metal's use must be curtailed on a massive scale (Mitra et al. 2017). Chelating salts, such as calcium edentate, the disodium salt of ethylene-diamine-tetraacetic acid (EDTA), are commonly used to treat lead poisoning. These chelating compounds have a high concentration of the solvent they are meant to remove. Because the lead chelating agent is more attracted to lead than calcium, the lead chelate is generated by an exchange reaction. This is then eliminated by the urinary tract, leaving behind only innocuous calcium. As a chelating agent, succimer has been found to minimize BLL and improve cognitive development in children exposed to lead (Lakka et al. 2023). Antioxidants, in particular, are thought to mitigate the harmful effects of substances like lead and its derivatives. Improved biodistribution and bioavailability of poorly soluble medicines may be possible with a novel technology termed nano-encapsulation of antioxidants. Curcumin encapsulated in a pluronic block copolymer was found to have anticancer efficacy on par with free curcumin and a delayed and sustained release profile. These novel approaches can potentially improve treating many human ailments (Niu et al. 2023). N-acetylcysteine (NAC) has been shown in a recent study on a group of lead-exposed employees to lower blood lead levels significantly. Furthermore, it was shown that glutamate dehydrogenase activity increased dramatically in all NAC-treated groups. It was also found that oxidative stress was reduced, and homocysteine levels were normalized after treatment with NAC. As a result, NAC was found to be a viable alternative treatment option for chronic lead toxicity in humans (Kumar and Singh 2023). Various sources of lead poising and its management and prevention measures are presented in Table 1.

Table 1 Various sources of lead poising, It's Management and Prevention

S. N.	Source of lead	Symptoms	Management	Outcome	Ref
1	a sindoor powder consumed for 11 years at 5–10gm	Nephropathy caused by lead poisoning, combined with gingivitis, gout, and high blood pressure	Treatment for lead levels between 50 and 100 mcg/dL (DMSA/Calcium EDTA) may be used	Sindoor lead exposure among Indians	Kute et al. 2013
2	Adult and child use of lead-based glazes	Neurotoxic and Nephrotoxic	Chelation (DMSA/Calcium EDTA).	Promoting Lead free glazes	Estévez-García et al. 2022
3	Romanian Pre-schooler's and School-Aged Children (4-6, 8-11 years) in Industrial and Mining Regions	Neurological dysfunction	preserve human health by limiting or eliminating exposure to heavy metals	After being evacuated from an exposed environment, the health of the youngsters has improved	Nedelescu et al. 2022
4	Necessary minerals ameliorated lead-induced changes in haematological parameters in male Wistar albino rats	There is a decrease in blood haemoglobin and an increase in urine -amino levulinic acid dehydratase (ALAD)	Lead toxicity and speciation may be altered by eating a mineral-rich diet	Lead inhibits -ALAD in leached workers, most likely at zinc-binding sites. -ALAD is required to synthesize porphobilinogen from two -ALA molecules	Dsouza et al. 2022
5	Adult male Wistar rats were studied for lead-induced hematotoxicity	However, the RBC, HGB, HCT, and PLT levels all reduced, although WBC levels increased	TQ, the primary active ingredient in Nigella sativa seeds volatile oil, was effective in treating lead-intoxicated rats	Thymoquinone may be used to treat lead-induced hematotoxicity as well as other poisons and infections	Mabrouk 2021
6	Case report-Traditional Ayurvedic Medicines uses	Acute and chronic Lead encephalopathy	Chelation therapy and discontinuation of ayurvedic medicines	Patient recovered clinically and lesions on MR imaging resolved	Karri et al. 2008
7	Case report- lead-based manufacturing unit	Leg Fatigue & Weakness of Forearm Extensor Muscles Peripheral Neuropathy	D-penicillamine chelation therapy were used to treat his lead poisoning	Chronic exposure to lead requires a series of treatment regimens	Geraldine and Venkatesh 2007
8	Case report-Ayurvedic medicines manufactured using lead based bhasmas	General weakness and significant stomach discomfort are all symptoms of psoriasis	D-penicillamine, and/or Ca-Na2EDTA, and/or environmental intervention, appropriate diet, and enough hydration and education are all necessary components of a comprehensive treatment plan	Discontinue any Ayurvedic medications and drink a lot of water	Raviraja et al. 2010
9	Western Maharashtra battery workers (20-60years)	Some of the side effects include nausea and diarrhoea; constipation; headaches; paresthesia; and myalgia	Vit-C and Iron Intake	Vitamin C does not lower blood lead levels on a monthly basis. Catalase and superoxide dismutases are also enhanced by the supplement	Ghanwat et al. 2016
10	water supply, surma usage, and lead paint	spontaneous abortions, stillbirths, and premature births	Low iron, calcium, or zinc levels during pregnancy may increase intestine absorption and mobilization of bone lead	More research is needed on pregnancy and lead	Awasthi et al. 1996
11	Gasoline	visual-motor, visual-spatial (Drawing task) (Matching task) Peek-a-boo pegboard)	Regulations pertaining to the environment and the workplace are being implemented	Urban Indian children with poor visual-motor integration are more likely to have elevated blood lead levels	Palaniappan et al. 2011
12	Battery-recycling unit.	Osteoarthritis, epigastric pain, and a little rise in ALT and hepatomegaly are all symptoms	Dimercapto succinic acid and penicillamine were the first chelators used in chelation treatment	Small-scale industrial employees must be taught about the hazards of lead poisoning and lead poisoning	Menezes et al. 2003
13	Occupationally and nonoccupationally exposed lead poisoning cases	Constipation, diarrhoea, vomiting, and other symptoms of an upset stomach	Chelation therapy with D-penicillamine	Eating healthful foods, avoiding painted toys and unbranded medications, and taking showers at work may help reduce exposure	Mani et al. 2020

S. N.	Source of lead	Symptoms	Management	Outcome	Ref
14	Lead levels on painted surfaces such as windows, flooring, gates, doors, door frames, walls, shelves, containers, cabinets, playground equipment, etc.	Asymptomatic	Create public awareness	Field Portable XRF instrument	Kuruvilla et al. 2004
15	Exposure to car mechanics (spray painters, radiators, and batteries) India's Bijapur city	Inflammation of the muscles and other symptoms such as itching and moderate tiredness are all signs of an overactive immune system	Create public awareness	For occupational lead exposure screening, a full hemogram, urine ALA and other biomarker activity such PBG and erythrocyte delta-ALAD, and blood pressure readings are helpful	Dongre et al. 2011
16	Bone lead concentration- patella and tibia	Hypertension, heart disease, diabetes mellitus, and cancer	Supporting therapy to minimize symptoms	As occupations were added, they helped to reduce the bone lead and education categories	Ji et al. 2014
17	The environmental air of Two Electrical Factory people	Asymptomatic	Industrial danger may be reduced by using modern scientific methodologies	Scientific ways for preventing lead poisoning are available	Ghosh et al. 1952
18	Traditional eye cosmetics' Surma - Black, brown, orange, grey and white usage by Childrens	Anaemia and convulsions	The use of lead free 'Surma' is suggested. (White)	Decreases in Hb with increasing values of blood lead levels	Goswami 2013
19	The battery recycling and manufacturing people (Western Maharashtra)	nausea, vomiting, diarrhoea, and cramping are all symptoms of irritable bowel syndrome	Chelating with adjacent therapy	Workers in the battery production industry show less indication of serious impairment of liver and renal functions	Kshirsagar et al. 2015
20	Medication, food, cooking utensils, bullet wounds, and water to drink that haven't been labelled	Abdominal discomfort, dizziness, cognitive impairment, and other symptoms may occur	Cuprimine (D-penicillamine, 3-mercapto D-valine) Chelating treatment	No patient had a blue gum line or basophilic stippling. Chelation treatment does not substitute remediation or lead prevention	D'souza et al. 2011
21	Lead poisoning from herbal diabetes medicines	upper abdominal pain, anaemic, icteric, and pyrexial	Calcium ethylenediamine tetra acetate (EDTA) infusion (2.4 g) for 5 days	Diabetic men may have been treated with lead contain an Ayurvedic aphrodisiac may cause severe side effect. Better to avoid	Keen et al. 1994
22	Inhabiting and living	Abdominal pain, bilateral symmetric sensorineural hearing loss with descending configuration, stapedial reflex bilaterally present, speech intelligibility asthenia, paresthesia in upper limbs, progressive hearing loss	Calcium ethylenediamine tetra acetate (EDTA) infusion (2.4 g) for 5 days	Patients recovered clinically and lesions on MR imaging resolved	Kumar and Singh 2023
23	Foreign body	Increased blood pressure, normocytic normochromic and regenerative anemia, transitory headache, reactive anisocoria, asthenia, visual hallucinations, transient aphasia episodes, left pyramidal syndrome, memory impairment and psychomotor retardation, worsened generalized tonic-clonic seizures and altered consciousness, interstitial nephropathy	Chelation therapy with D-penicillamine	Eating healthful foods, avoiding painted toys and unbranded medications, and taking showers at work may help reduce exposure	Lelievre et al. 2020
24	Lead poisoning from medication	Pale conjunctiva, exertional dyspnea, hypochromic microcytic anemia, anisocytosis with basophilic stippling	Traditional Chinese herbal medicine (Qushangjieyu-san powder) - chelation therapy	Patient recovered clinically	Samarghandian et al. 2021

Conclusion and Future Prospects

The paradox of lead displays a complicated interaction between its desirable features and the risks it poses to human health and the environment. The versatile metal is widely used in various sectors, allowing for novel uses and economic advantages. However, the possibility of lead contamination, whether from industrial methods, environmental exposure, or cultural practises, presents serious concerns. As a society, we must balance enjoying the advantages of lead's adaptability and enacting tight laws, safe handling practises, and spreading awareness to reduce exposure dangers. Only then can we properly walk the narrow line between the beauty and danger of lead. Consequently, lead serves no purpose in the body and is hence unnecessary. If lead enters the body, it may stay there indefinitely and cause injury and even death. Lead poisoning accounts for about 0.6 percent of the global disease burden. Because of the toxicity of lead, workers exposed to it experience more severe repercussions than the general population. Various groups, as well as the government and the media, are now striving to increase awareness of lead poisoning by interacting with and educating people who have been exposed to it and the general public about the health dangers connected with it.

Lead poisoning seems to be more common than other heavy metal poisonings. Lead's widespread and historically attested toxicity has led to widespread concern for its safety. It has been adopted globally due to the significance of its physicochemical features. As the industrial revolution began in the seventeenth century, its use skyrocketed, as did its toxicity to humans. Having lead-related industries close to playgrounds increases the danger for children. There is an elevated risk of lead poisoning for those exposed to lead in the workplace. Young children of parents with lead-related occupations should have their blood lead levels monitored often. The available research shows that lead is very hazardous and interferes with virtually every bodily process. Damage to the digestive, neurological, respiratory, reproductive, and other systems results from lead poisoning. To add insult to injury lead inhibits enzyme function. Disabling impairments in the skeleton directly results from lead's interference with the normal DNA transcription process.

Toxic effects can occur at deficient lead concentrations, and the body has no physiologic need for lead. Fortunately, there are methods available today that can reverse the damage and lower lead levels in the body. Chelation therapy, nano-encapsulation, and N-acetylcysteine (NAC) stand out among these. In addition to a variety of antioxidants, they aid in the elimination of lead from the body. The best course of action is to avoid exposure to toxins in the first place, even when effective treatments are available. Parents should also teach their children about the dangers of lead poisoning and how to protect themselves and their families from exposure. Genetic, environmental, and dietary variables all contribute to the fact that not everyone responds the same way to the same treatment.

Abbreviations

BLL: Blood Lead Level

IBS: Irritable Bowel Syndrome

NRCLPI: The National Referral Center for Lead Poisoning Prevention in India

XRF: X-ray fluorescence

ZPP: Zinc Protoporphyrin

Author Contributions

All authors contributed significantly to the work described here. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability

The data supporting this review are from previously reported studies and datasets, which have been cited. The processed data are available from the corresponding authors upon request.

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Journal of Experimental Biology and Agricultural Sciences, June - 2023; Volume – 11(3) page 520 – 533

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Potential benefits and therapeutic applications of "Panchgavya" therapy (Cowpathy) for human and animal health: Current scientific knowledge

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Received – May 23, 2023; Revision – June 25, 2023; Accepted – June 26, 2023
Available Online – June 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(3).520.533

KEYWORDS

Cowpathy

Panchgavya

Cow urine

Milk

Dung

ABSTRACT

Cow's milk, urine, dung, ghee, and curd (together known as "Panchgavya") have incomparable medicinal value in Ayurveda and ancient Indian clinical methods. Panchgavya is also known as Cowpathy in Ayurveda. In India, the cow is revered as a goddess known as "Gaumata" because of its nurturing qualities similar to those of a mother. Almost no adverse effects are associated with using Panchgavya, which is why it is recommended in Ayurveda for treating disorders affecting numerous body systems. Its possible antimicrobial effects have piqued the curiosity of medical researchers and practitioners. Cow milk is widely regarded as a nutritious diet and has been shown to effectively treat various medical conditions, including high body temperature, pain, cancer, diabetes, kidney diseases, and weakness. Milk can prevent the growth of microorganisms, has erotic qualities when combined with the

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Ghee

Curd

Human health and diseases

leaves of medicinal herbs, and the fat in milk has anticancer characteristics. Toned and skim milk, lassi, yoghurt, cottage cheese, and khoa all come from milk and have important medicinal characteristics. Curd (dahi) is recommended as a blood purifier for conditions such as hemorrhoids, piles, and gastrointestinal issues. Ghee made from cows has been shown to boost immunity. It is important to highlight the use of cow dung as an antifungal and for treating malaria and tuberculosis. It has the potential to aid in the development of a populace free from disease, the creation of sustainable energy systems, the fulfilment of all nutritional needs, the elimination of poverty, the promotion of organic farming culture, and the like. Cow urine is a powerful remedy for numerous medical conditions, including but not limited to epileptic convulsions, diabetes, hepatitis, inflammation, fever, and anaemia. The current review article explores how the Panchgavya ingredients can be employed to safeguard human and animal health.

1 Introduction

India is steeped in ancient scientific practices that link social rituals to their scientific underpinnings. The Indians give the cow the names "Gaumata" and "Kamadhenu" because of its nurturing role, similar to that of a mother. Kamadhenu is the name of the sacred cow that is thought to bestow wishes upon their devotees. The healing and preventative effects of Panchgavya are priceless. In the Ayurvedic system of medicine, cow products like milk, ghee, curd, urine and dung are revered for their curative and preventative properties (Arumugam et al. 2019). Ingredients and uses for these items in medicine, agriculture, and other sectors might vary considerably. 'Panch' means five, and 'gavya' means gained from 'Gau,' so together, these two words indicate the five products that may be made from a single cow, hence the name 'Panchgavya. Different 'gavya' have varying curative effects on different ailments. Like allopathy, homeopathy, and naturopathy, "Cowpathy" refers to Panchgavya therapy or treatment. Each 'gavya' has multiple applications, including use as a standalone treatment or in synergy with other medicines. The five products are also versatile since they can be used singly or in tandem with others of synthetic, herbal, or mineral origin (Dhama et al. 2005a; Dhama et al., 2014; Dhama et al. 2015; Arumugam et al. 2019; Bajaj et al. 2022; Sathiyaraj et al. 2022). These five components of Panchgavya are mentioned in Figure 1.

Clinical trials of the therapy have shown promising results in patients with advanced stages of cancer, acquired immunodeficiency syndrome (AIDS), diabetes, tuberculosis, rheumatoid arthritis, leukoderma, flu, asthma, allergies, heart

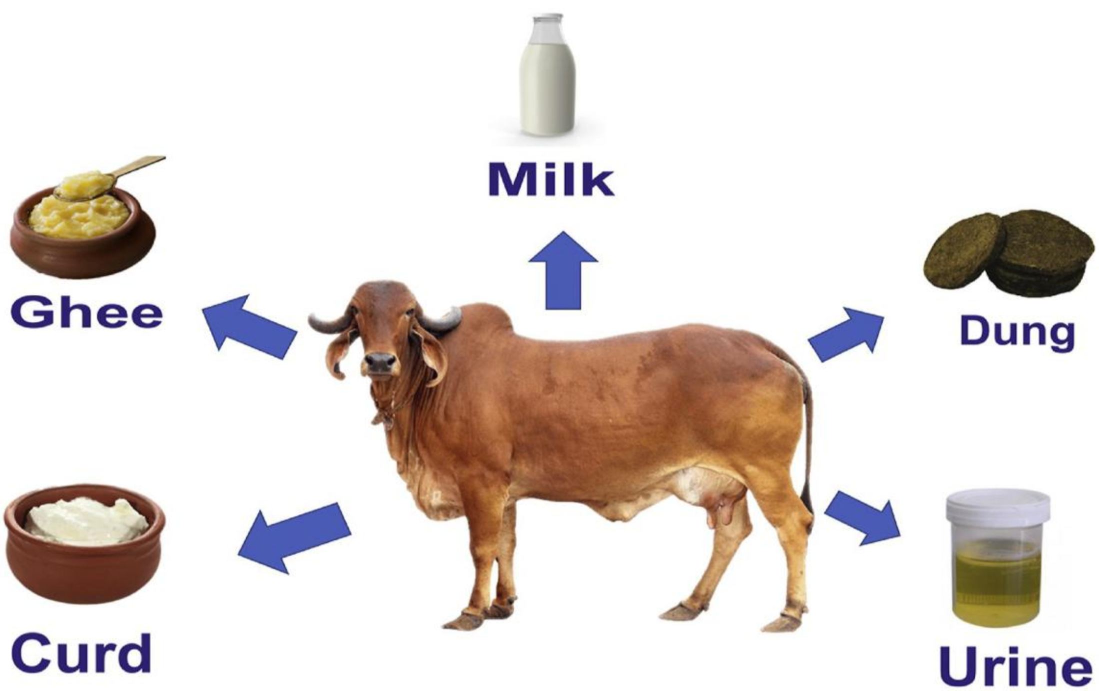

Figure 1 Five elements of "Panchgavya"
Source: Bajaj et al. (2022)

disorders, kidney disorders, healing of wounds, nutritional and digestive disorders, chemical poisoning and other bacterial, viral, and fungal illnesses (Cimmino et al. 2023). As the best organic manure, Panchgavya is essential to sustainable agriculture. Its use guarantees that no artificial inputs will be required, such as fertilizers, herbicides, insecticides, or antibiotics. Panchgavya is the most cost-effective and useful manure on the market. As an organic fertilizer, it helps boost soil fertility, increases earthworm quality, and benefits crop health. Cow manure and dung are valuable energy resources for producing biogas and power (Borgohain et al. 2020; Bajaj et al. 2022). Several recent pandemic infections have occurred, including COVID-19 (coronavirus). Efforts to defend and protect the Indian population from coronaviruses using natural means and Ayurveda are continuing. An investigation into using Panchgavya as a treatment for the coronavirus has recently begun. Panchgavya medication was tested on 19 patients with COVID by Tijare et al. (2020) and Ganesh (2021), who confirmed the preliminary findings. Treatment with Panchgavya and *Gomutra arka* for coronavirus is effective, and those sickened by the virus have been shown to recover from it. They determined that Panchgavya and Gomutra arka treatment is a viable therapeutic option. Products derived from cows contain more minerals, carbolic, succinic, and citric acids, vitamins and hormones. They improve cellular metabolism in prokaryotes in aerobic and anaerobic circumstances, paving the path for synthesizing novel secondary metabolites (such as pharmaceuticals and enzymes) (Raut and Vaidya 2018).

There are a wide variety of additional advantages to using Panchgavya products. Organic farmers can benefit from the urine and dung of cows as high-quality natural manure, and these byproducts can also be turned into vermicompost, biofertilizers, and biopesticides—all of which are excellent and cost-effective agricultural uses that boost soil fertility, increase crop yields, and protect consumers from the potentially harmful effects of chemical fertilizers and pesticides. The nutritional content of cow milk, curd, and ghee is exceptionally high. Alternative and cheaper energy sources, fuel, biogas, and electricity, can be produced from cow dung and urine using Panchgavya products, which can help light every home without any ongoing costs by providing cheap fuel widely available to the masses and solving electricity problems. Since cow manure is resistant to sun radiation and cow ghee can protect the human body from the detrimental effects of radioactive waves, Panchgavya is said to possess the capability to improve the financial status of farmers and landless labour (González et al. 2018; Arumugam et al. 2019; Chandra et al. 2019; Joshi et al. 2022; Rathi and Khan 2023).

Safer and more novel remedies, including herbal medicine, bacteriophage therapy, and others like Cowpathy and dietary immunomodulatory methods, are gaining popularity and should be widely disseminated as a reaction to the growing problem of drug-resistant bacteria and infections (Chandra et al. 2019; Bajaj et al. 2022; Rathi and Khan 2023). This article reviews the panchgavya elements and discusses their medicinal applications and human and animal health benefits.

2 Properties of "Panchgavya"

Panchgavya is a mixture of five different herbs used in Ayurvedic medicine for thousands of years. Chakara Samhita, Chaukambha Sanskrit Pratistana, and other ancient texts detail how Panchgavya can be prepared on its own or in combination with other herbs. *Swapla Panchgavya ghrita*, *Panchgavya ghrita* (which translates to "Panchgavya ghee"), and *Mahapanchgavya ghrita* (which translates to "Panchgavya ghee" with the addition of 18 or 24 herbs) are the many Panchgavya preparations available. Human ailments such as skin disorders, vitiligo, cough, cold, chronic illness, etc., are treated with these varied formulations. Cowpathy, known as Panchgavya chikitsa, therapy, or treatment, employs Panchgavya to heal human illness (Rai et al. 2022). It has been suggested that Panchgavya therapy could be an effective preventative and curative measure for the health of cattle, poultry, and humans. Cow urine, milk, ghee, curd, and dung are the five components that make up the Panchgavya. These items are used either alone or in combination with other plants for therapeutic purposes due to their medicinal characteristics (Ganesh 2021; Anand et al. 2022). Cow products like milk, curd, and ghee have a high nutritional value, and byproducts like urine and dung can be used to generate biogas, fuel, and electricity at a lower cost. Panchgavya is good for humans and is made from indigenous cow breeds. As per the Ayurvedic literature, Panchgavya consists of five items, including cow urine, cow dung, cow milk, curd, and ghee in a ratio of 2:1:6:12:2 (urine, dung, curd, milk, and ghee). Carbolic, succinic, and citric acids; vitamins like A, B, C, D, and E; minerals like iron, copper, zinc, and chromium; and hormones like testosterone and estrogen can all be found in plenty in Panchgavya products (Bajaj et al. 2022). Inducing immunological modulation by boosting cellular and humoral immune responses, up-regulating lymphocyte proliferative activity, and decreasing lymphocyte apoptosis, these products are effective in treating a wide range of human diseases. They combat ageing by halting the production of free radicals and fixing damaged DNA. Studies on the antibacterial, antifungal, anticancer, immunological, pharmacological, and other effects of Panchgavya's five components individually and in combination have been conducted by several researchers (Mahajan et al. 2020; Joshi et al. 2022; Sathiyaraj et al. 2022; Rathi and Khan 2023). Figure 2 depicts the many Panchgavya variants, their therapeutic features, and their usefulness in agricultural contexts.

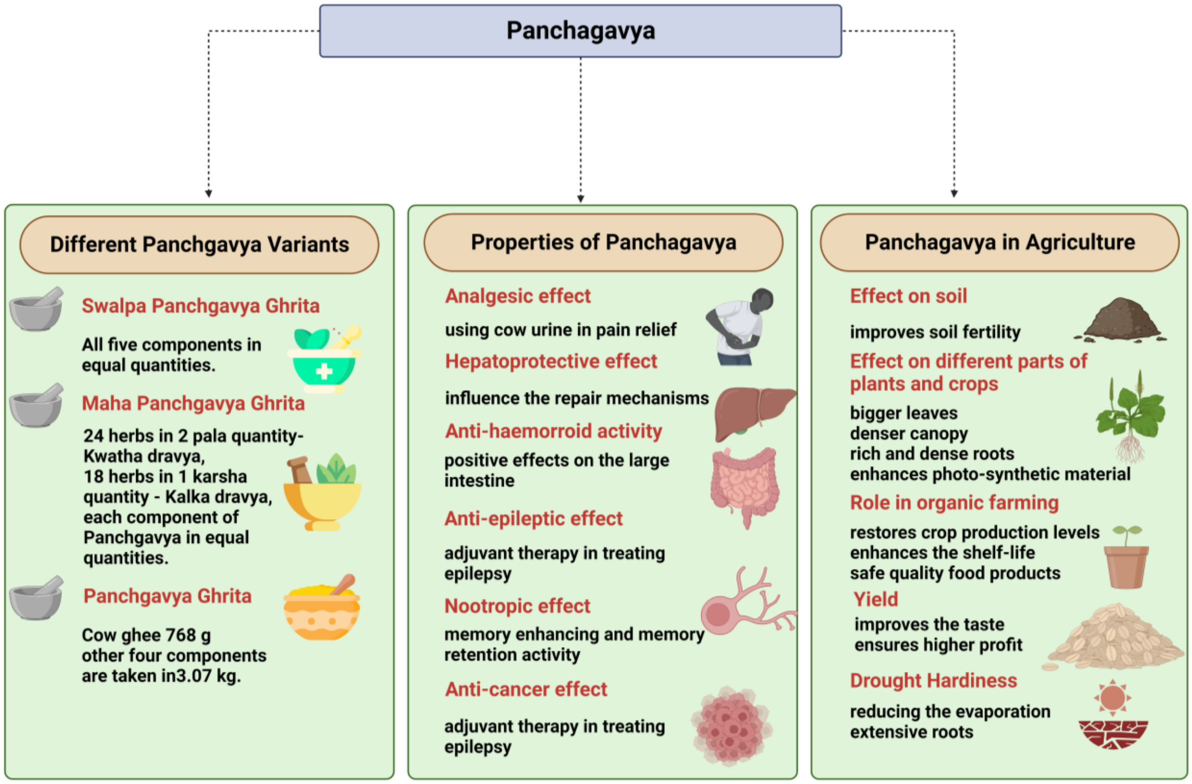

Figure 2 Various variants of Panchagavya, their therapeutic characteristics, and usefulness in agricultural contexts

3 "Panchgavya" therapy

Alternative preventative and therapeutic methods, such as "Panchgavya" treatment (Cowpathy), have been advocated for maintaining healthy cattle and poultry and protecting human health. Multiple illnesses can be treated using Panchgavya products, and the body's natural defences against disease are strengthened. By inhibiting the production of free radicals and speeding up the repair of damaged DNA, cowpathy functions as an anti-ageing agent. It also induces immunomodulation by enhancing antibody-mediated (humoral) and cellular immune responses. This is accomplished by increasing cytokine secretion, lymphoproliferation and macrophage activity and decreasing apoptosis in lymphocytes. Environmental pollution, agrochemical use in agriculture, and the presence of pesticides, fungal toxins, heavy metals, etc., in the food chain, are all reasons why Cowpathy may be beneficial (Tharmaraj et al. 2011; González et al. 2018; Bajaj et al. 2022).

Panchgavya, when appropriately diluted, has been discovered to be a promising growth enhancer of microorganisms; as a result, it increases soil fertility without resorting to chemical fertilizers, and its marked antifungal qualities also make it a useful microbiological growth medium. Panchgavya, when diluted further, shows promise as a simple, naturally generated, and inexpensive bacteriological media with added antifungal action and growth encouragement (Chandra et al. 2019; Borgohain et al. 2020). Although Panchgavya has no direct antibacterial activity, the fermented version of the herb (after 30 days) can be used as a growth stimulator thanks to its improved chemical and microbiological composition (Borgohain et al. 2020). Diseases such as the common cold, allergies, asthma, alopecia, hyperlipidaemia, rheumatoid arthritis, lucorrhoea, leukoderma, cardiovascular disease, high blood pressure, renal disorders, hepatitis, peptic ulcer disease, acid reflux, esophagitis have all been linked to the use of Panchgavya products for both preventative and curative purposes (Dhama et al. 2013; Khan et al. 2015). Figure 3 provides a detailed summary of panchgavya therapy, including its health benefits and therapeutic potential in protecting animal and human health.

4 “Gaumutra”/ Cow urine

"Gaumutra", sometimes known as cow urine, is a non-hazardous liquid byproduct of the dairy industry. Cow urine, or distillation of it, has been shown to have many positive health effects, including increased longevity and improved quality of life for those suffering from life-threatening illnesses (Dhama et al., 2005a; Dhama et al., 2005b; Meena et al. 2019). Cow urine has been used for many medical purposes for centuries, although little scientific evidence

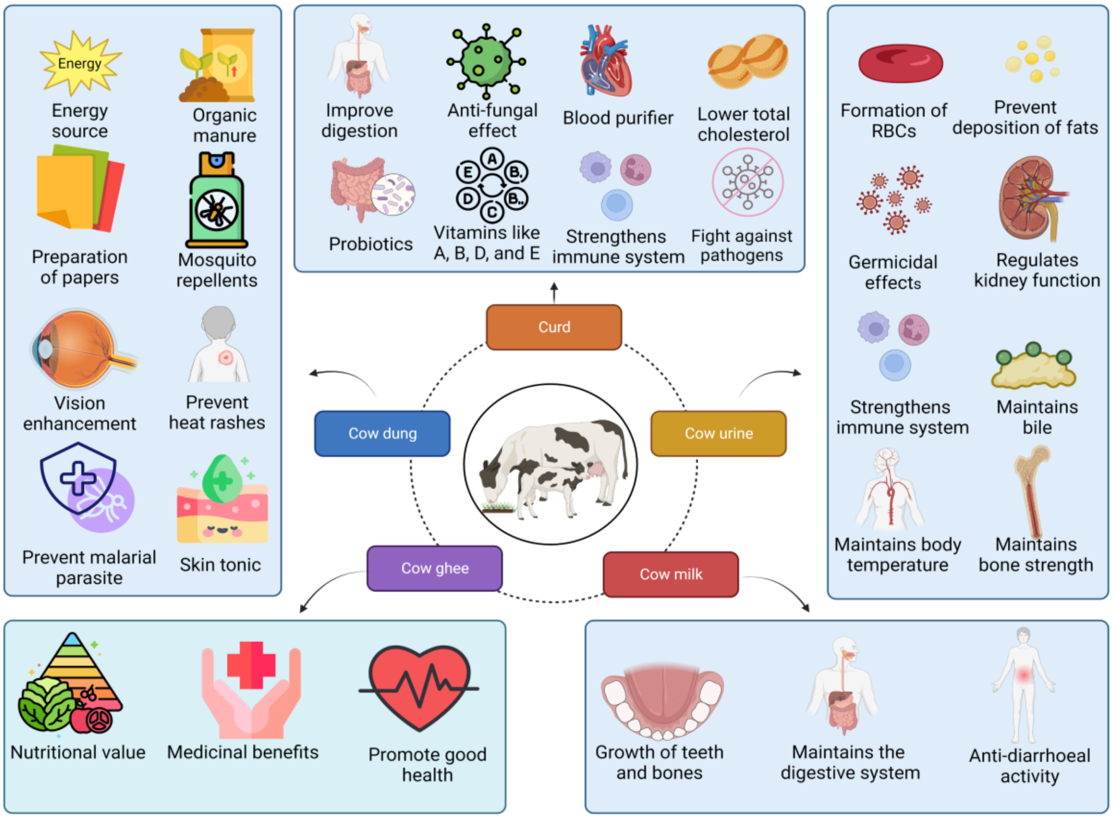

Figure 3 Incorporation of "Panchgavya" to increase productivity in farming and therapeutic outcomes for humans and animals

supports its practice. More research should be done to encourage using time-tested treatments with fewer adverse outcomes. Several formulas in the Ayurvedic medical system call for cow urine. The Ayurvedic texts highlight the many uses of cow urine for health and medicine. Cow urine is a potent nephroprotective agent and diuretic in its own right. Weight loss, digestive problems, edema, and a reverse action against multiple cardiovascular and kidney disorders are all possible benefits. It's also effective against vitiligo, diarrhoea, gastrointestinal infections, jaundice, piles, anaemia, and other skin conditions (Mahajan et al. 2020; Sathiyaraj et al. 2022).

The composition of cow urine consists of 95% water, 2.5% urea, and the remaining 2.5% comprises various components such as enzymes, hormones, salts, and minerals. Cow urine also contains several digestive and immune-boosting enzymes. Cow urine is an excellent source of vitamins A, B, C, D, and E (Khan et al. 2015). The antimicrobial and disease-eradicating properties of copper are well established. The amino acids and cytokines may help boost the immune system. The chemical qualities, potentialities, and ingredients of 'gaumutra' alone are sufficient to correct all imbalances in the body (Dhama et al. 2013; Joshi et al. 2022). Table 1 shows the chemical makeup of cow urine and its therapeutic uses.

Cancer is the most frightening and incurable disease in the world, but "Cow Urine Therapy" demonstrates another, more effective, and less expensive way to prevent and treat this disease. Urinary tract cancer is one of the most common types of cancer in humans, and cow urine has direct implications in this situation, as its use promotes the reduction of medicine doses and, at the same time, functions as a bioenhancer. Common antibacterial, antifungal, and anticancer medications have benefited from "Ark", a bioenhancer that increases their efficacy while decreasing necessary dosage and treatment time. Together, they can stop bacteria from becoming antibiotic-resistant if given simultaneously (Dhama et al. 2013; Khan et al. 2015; Arumugan et al. 2019). Because it contains quinolones and flavoquinolones, cow urine has been discovered to be highly effective against fungus. Multiple fungal agents, including *Claviceps purpurea*, *Fusarium oxysporum*, *Rhizopus oligosporus*, *Alternaria helianthi*, *Aspergillus oryzae*, *Cladosporium* spp., and *Curvularia* spp., are susceptible to the plant's antifungal properties (Chandran 2021; Prakash et al. 2021a; Kamei et al. 2022). Cow urine has excellent value as a bioenhancer and for boosting the effectiveness of medications against infectious pathogens. In addition to ampicillin and isoniazid, clotrimazole and cyanocobalamine have also been linked to enhancement. Bioenhancer activity has been linked to shorter treatment times and lower antibiotic need for tuberculosis patients (Meena et al. 2019; Joshi et al. 2022).

Table 1 Composition of cow urine and respective health advantages

Constituents	Health advantages and therapeutic benefits
Water	Body temperature and fluidity of blood are both kept at a steady state
Urea	Have a bactericidal effect and stimulate the production of more urine
Uric acid	Promote healthy kidney function and aid with heart maintenance
Creatinine	Exert a bactericidal effect
Hippuric acid	Assists the kidneys in flushing out harmful substances
Ammonia	Promotes healthy bile and mucus production; and stimulates the production of red blood cells
Lactose	Suppress anxiety and fortify the cardiovascular system
Carbolic acid	Antiseptic and gangrene preventative
Aurum hydroxide	Boosts immunity, acts as an antibiotic, and is non-toxic
Sodium	Function as an antacid and exhibits blood cleansing property
Nitrogen	Controls blood pressure, maintains normal renal function, and promotes dieresis
Iron	Promotes red blood cell and hemoglobin production.
Calcium	Cleansing the blood and preserving bone density
Copper	Avoid accumulation of fat
Phosphate	Reduce the risk of urinary stone development
Manganese	Antibacterial and gangrene prevention properties
Potassium	Cure inherited rheumatism, improve appetite, and strengthen muscles
Sulphur	Improves bowel regularity and blood purification

Source: Bajaj et al. (2022); Randhawa and Sharma (2015); González et al. (2018); Arumugam et al. (2019); Bajaj et al. (2022)

Cow urine is one of the most effective substances/secretions of animal origin due to its ability to boost immunocompetence and improve general health. The macrophages are stimulated, increasing their bactericidal and engulfing abilities. It enhances the body's innate and adaptive immune responses (Dhama et al. 2013). Cow urine boosts IgG, IgA, and IgM antibody titres in mice and stimulates the Growth of T and B cells. Interleukins 1 and 2 are also secreted at a higher rate. According to its antibacterial and immunologically modifying qualities, cow urine has been used successfully in wound healing in dogs and rats. Lymphocytes are kept alive and not killed off using cow urine as a survival mechanism. It can also fix broken DNA. Indian cow urine is more potent than any other type of urine. Through cow domestication, it may be gotten for free, has curative properties, and is non-toxic (Raut and Vaidya 2018; Bajaj et al. 2022). Cow urine improves vaccinated birds' immune responses, making them more disease-resistant. Along with boosting egg production and quality, it also stimulates lymphocyte proliferation. Positive benefits on body weight increase, haematological profiles, and immunomodulatory effects in layer poultry birds have been documented. Because it inhibits the production of free radicals, an anti-ageing agent, daily use can restore a person's youthful radiance and vitality (Khan et al. 2015). Taking cow urine in the middle of the morning is an excellent prophylactic measure and tonic for minor ailments. A sore throat or cold might be alleviated by gargling with pee. A vaginal douche can aid in the recovery from an infection. Along with its usage as a pest repellent, it has many cosmetic and therapeutic applications. It works well as a footbath for conditions including athlete's foot, ringworm, and other fungal nail and skin infections (Arumugam et al. 2019; Mahajan et al. 2020).

Cow urine concoction (CUC) is a well-liked herbal remedy that is effective against a wide variety of ailments, including as an anticonvulsant, a hypoglycemic, a liver tonic, a fever reducer, a pain reliever, and a blood builder. CUC has been validated for treating conditions brought on by pathogenic bacteria, opportunistic fungi, and helminthes. Adequate focus must be placed on the positive aspects of cow urine, and strategic promotion and support are required in this regard (Kuleep et al. 2013; Bajaj et al. 2022; Joshi et al. 2022). Moreover, cow urine helps control the level of sugar in youngsters who have diabetes in nature. When warm cow urine is provided at daily doses of 1 to 2 ounces, it helps treat liver cirrhosis. Malignant jaundice can be cured by internal uptake of cow urine and antimony sulphide. Interestingly, people who are engaged in cleaning the milk barn with bare feet do not usually suffer from athlete's foot. The urine of cows has also got immunostimulant activities not only in animals but also in plants as well. In the case of sebaceous cysts washing with the cow's urine following incision is suggested. As cow urine acts as a disinfectant and prophylactic agent, it can purify the atmosphere (Khan et al. 2015; Prakash et al. 2021b).

By the use of an excision wound model of Wistar albino rats (both sexes) examination of the activity of wound healing of cow urine has been done. The reference standard used in the study was nitrofurazone. The experiment has revealed a significant ability to heal wounds with cow urine. When cow urine is applied externally for 2 weeks on the damaged area, 50 per cent of wound healing has been noticed. It has also been shown that the efficacy of cow urine compared to nitrofurazone as far as wound healing is concerned is greater (Sanganal et al. 2011).

Recently, it has been suggested that synthesized copper oxide nanoparticles (CuO NPs) (cow urine mediated) can be helpful in therapy against neoplasm. Likewise, CuO NPs and cow urine-mediated palladium and silver oxide nanoparticles show significant antimicrobial activity against several bacterial and fungal strains (Prasad and Kothari, 2021).

5 Cow dung

Various beneficial microorganisms can be found in cow manure, such as Saccharomyces, Lactobacillus, Bacillus, Streptococcus, Candida, etc. Additionally, cow excrement contains a wide variety of cellulose, hemicellulose, vitamins, minerals (like potassium, nitrogen and carbon), mucus, and lignin (Dhama et al. 2005a). Cow dung is utilized to break down municipal and medical garbage because it contains many microorganisms that break down trash. Dried cow dung cakes are used to prepare food in rural areas of India. This reduces reliance on non-renewable energy sources, is entirely eco-friendly, and assures air cleaning by killing the microorganisms in the surrounding environment. Energy from biogas plants is also vital. Methane gas produced from cow manure is used for cooking and power generation. When most cow manure is turned into methane gas, the leftover residue is the best organic manure (Prakash et al. 2021a; Prakash et al. 2021b; Anand et al. 2022).

A cow's manure can generate enough biogas to power a city for a year—the same as burning 6,80,000 metric tons of wood. This would prevent the needless destruction of the environment by preventing the needless destruction of 14 crore trees. Soil, water, and air pollution can all be effectively mitigated using cow dung fertilizers (Chandra et al. 2019).

To keep soil healthy, farmers must use cow manure. Cow manure boosts earthworm populations and increases and controls soil fertility by improving nitrification when combined with the *Eisenia andrei* earthworm species. One of the most significant issues in farming is fungal diseases. Cow dung has been shown to inhibit the Growth of *Fusarium solani*, *Fusarium oxysporum*, and *Sclerotinia sclerotiorum*, three common types of plant-infecting fungi (Chandran 2021; Kamei et al. 2022). Agricultural chemicals like fertilizers, pesticides, weed killers, and antibiotics have been linked to hypersensitivity reactions, immunosuppression, and autoimmune illnesses. The lack of potentially dangerous chemicals in organic farming means the resulting products are in higher demand. Cow dung has a high microbiological count and nutritional value; thus, organic farmers have turned to it as manure. The best alternative to toxic poisons is cow dung, which protects the health of both humans and animals. Cow manure is a source of the fibrous material needed to make paper. Cow dung-based mosquito repellents have recently emerged as a viable alternative to chemical repellents. Cow dung toothpaste has been shown to effectively prevent the spread of oral infections and enhance oral health. Human operations can be made more efficient and less damaging to the natural world by using cow manure (Dhama et al. 2005a; Pal and Patel 2020).

There is no question about the purity of fresh cow dung, but once laid in the ground, changes in its properties become evident. Waste obtained from cows can act as antiseptic and has features of prevention of disease. Microorganisms cause disease; putrefaction as well as fermentation is destroyed by cow dung. The antifungal activity of cow dung is enhanced when combined with cow urine (Kulkarni 2009; Khan et al. 2015). Cow dung and cow urine are widely used in manufacturing formulations of Ayurvedic medicine. Two commonly used preparations are *Sanjeevani vati* and *Punarnava-mandur*. '*Bhasmas*' are still traditionally manufactured by application of heat to cakes of cow dung by fire (Raut and Vaidya 2018).

There is evidence that cow manure can kill bacteria and fungi. It's a skin tonic that helps with conditions including eczema and psoriasis. Crushed neem leaves combined with cow manure effectively treat boils and heat rash. Malaria parasites and *Mycobacterium tuberculosis* can both be eradicated with the use of cow manure. The antifungal activity is seen when tested on Coprophilous yeasts (Dhama et al. 2014; Kamei et al. 2022). The smoke released from burning cow dung irritates the eyes, leading to tears that wash away debris and improve eyesight. *Eupenicillium bovifimosum*, a bacterium found in cow dung, generates chemicals with antifungal action, specifically CK2108A and CK2801B. Cow dung is an applicable model ecosystem for drug degradation and elimination research. The discovery that the basidiomycete strain NRRL6464 can break down lignocellulose and that *Cyathus stercoreus* can degrade drugs like enrofloxacin led to the isolation of these two species of fungi. Immunosuppression, autoimmunity, and hypersensitivity reactions are some of the many adverse effects of biopesticides, making this an urgent problem in countries like India. Because of its rich nutrient content and huge microbial population, cow dung is useful for bioremediating various pesticides (Bajaj et al. 2022; Sathiyaraj et al. 2022; Rathi and Khan 2023).

6 Cow milk

Cow milk was praised for its curative properties in ancient Indian medical texts. Milk is consumed for its medicinal, health-enhancing, and protective benefits (Chandran et al. 2021a; Lejaniya et al. 2021a; Lejaniya et al. 2021b). The milk produced by purebred Indian cows is A2, while the milk produced by hybrid or alien cows is A1. The seven-membered peptide b-casomorphin-7 (BCM-7) is extracted from the *Bos taurus* cow, and more specifically from the HF cow, and is known as the "devil ingredient" in A1 milk (Turck 2013; Chandran et al. 2021b). Beta-casein's digestion produces BCM-7 when histidine replaces a proline residue in the polypeptide chains. The side effects of BCM-7 are numerous. BCM-7 in the bloodstream can lead to schizophrenia and atherosclerosis in persons with leaky gut syndrome (Chandran et al. 2021b). The interaction of BCM-7 with opioid receptors and the inhibition of regular binding and activity of endorphins in breastfeeding infants has been linked to developing type 1 diabetes and autism. Autism spectrum disorder in newborns and schizophrenia in adults is connected to a lack of functioning endorphins. Type 1 diabetes and immune system damage results from BCM-7's activation of opioid receptors. In addition to asthma and heart issues, drinking A1 milk might trigger allergic reactions (Cimmino et al. 2023).

In contrast, the A2 kind of milk produced from the Indian cow breed has been shown to have multiple positive effects on human health and a wide range of therapeutic applications. Cow's milk was traditionally combined with medicines in Ayurveda to improve the therapeutic benefit-to-harm ratio (Dhama et al. 2005a; Dhama et al. 2014; Kaushik et al. 2016). Nutritionally, Indian cow milk scores high, with a breakdown of roughly 4.6% lactose, 4.65% fat, 0.54% minerals, 3.4% proteins, and 86% water. Proteins in cow's milk consist of four different types: alpha-casein (36%), beta-casein (27%), kappa-casein (9%) and peptides (27%). Colloidal casein, which makes up roughly 3% of milk, is present, along with pigments including xanthophyll, carotene, and riboflavin. In addition to being a good source of calcium and phosphorus, milk also has a good amount of important fatty acids. Milk also contains the vitamins A, B2, B3, and K and the phospholipids cephalin, lecithin, and sphingomyelin (Chandran et al. 2021b).

Several health benefits are associated with drinking milk from cows. Infants are fed cow milk as a substitute for breast milk. The development of teeth and bones and regulating cardiac functions rely on it. The low cholesterol fat in milk is crucial for healthy growth in all areas of the body and mind, including the immunological system, nervous system and digestive tract. Lactose is a vital source of fuel. Vitamin A in milk helps you see well, while vitamin K prevents blood clotting too quickly (Pereira 2014; Chandran et al. 2021a; Kamei et al. 2022).

Milk has a wealth of potential health benefits. Milk is effective in treating infant anaemia. Cow milk inhibits the growth of pathogenic gut bacteria while encouraging the spread of good bacteria (Lejaniya et al. 2021a). Enzymes in milk have antibacterial effects (xanthine oxidase, lactoperoxidase, and lysozyme), while peptides (beta-casomorphins, exorphin, and serorphin) have antidiarrheal effects. Patients with gallbladder disease, diabetes, or high cholesterol should drink milk regularly. The cis-isomer of linoleic acid found in milk has been shown to have anticancer effects in animal studies. Cancer growth suppression was seen. Cancers of the skin, colon, and breast are all targets of milk's anticancer properties. The Government of India recognized the importance of increasing indigenous cow breeds to boost the production of A2-type milk, so they established the "Rashtriya Gokul Mission" in 2014 (Sathiyaraj et al. 2022).

7 Cow ghee

Traditional cow ghee has many health benefits and is an excellent source of nutrients because of the way it is made. Butter made from cow milk is heated to a very high temperature until all the moisture evaporates, resulting in ghee. The health benefits of ghee made with milk from an Indian breed cow are greater than those made with the milk of any other kind of cow. Some have speculated that ghee's high fatty acid content makes it a risk factor for dyslipidemia and cardiovascular disease. As a result of myths like this, ghee was primarily avoided in India (Dhama et al. 2014; Kaushik et al. 2016; Patange et al. 2022a). Many scientific researches have been done, and the health benefits of ghee have been re-established, even though Ayurveda has long recommended its use. The antioxidant activity of ghee is enhanced by the presence of linoleic acid (conjugated) in it, and the presence of this acid in conjugated form is responsible for preventing atherogenesis in experimental animals (Chinnadurai et al. 2013; Patange et al. 2022a; Patange et al. 2022b). Traditional preparation of cow ghee is found to be beneficial for therapeutic purposes and the promotion of health. Studies have revealed that docosahexaenoic acid (DHA) is present in greater concentration in ghrita manufactured by the traditional method of Ayurveda. Importantly DHA is beneficial to the health of humans (Joshi 2014; Patange et al. 2022a; Patange et al. 2022b). In therapeutics based on Ayurveda, ghee coadministration with other remedies is done. For target indications development of special formulations based on ghee has been done. Examples include *Adhatoda vasica/ Vasa-ghrita* (for diseases of the respiratory system); *Bacopa monnieri/ Bramhi-ghrita* (for cognitive effects); washed with water hundred times/ *Shatadhauta ghrita* (for ailments of skin) etc. (Raut and Vaidya 2018).

Ghee is an essential component in Ayurvedic medicine, serving as both a delivery mechanism for the active substance and a foundation upon which to build the dose forms. Ayurveda also recommends using ghee in conjunction with other healing methods. To improve the transport and bioavailability of hydrophobic herbs, ghee-based formulations like Ghrita have been developed. To name only a few examples, there is *Brahmi ghrita* for the brain, *Vasa ghrita* for the lungs, *Shatadhauta ghrita* for the skin, *Bhallatakadi ghrita* for wounds, *Kaamdev ghrita* for erection problems, etc. (Dhama et al. 2005a; Sathiyaraj et al. 2022; Rathi and Khan 2023).

The blood-purifying properties of cow's butter are responsible for the cosmetic benefits. Cow ghee's immunostimulant capability in Panchgavya formulations is demonstrated by increased haemagglutination titre, neutrophil adhesion, and delayed-type hypersensitivity (DTH) reactions in rats. Cow ghee, when blended with honey and specific herbs, can treat skin conditions and speed the healing of wounds. *Emblica officinalis*, *Glycyrrhiza glabra*, and cow's ghee are components of the calming Ayurvedic compound known as Panchgavya. The hepatoprotective effect against carbon tetrachloride (CCl_4) poisoning in the rat liver is also observed with *Panchgavya ghrita* (Dhama et al. 2013; Raut and Vaidya 2018).

Cow ghee improves memory, decreases "bad" cholesterol, prevents skin and cardiovascular problems, boosts skin health, keeps digestion going strong, produces energy, cleans the blood, shields the liver, and much more. Several studies have shown that ghee has therapeutic effects, including the ability to reduce inflammation and tumour growth, improve eyesight, and speed up the healing of wounds. It has been used successfully in the treatment of a variety of skin and gastrointestinal disorders due to its immunostimulant, anticholinergic activity, anti-asthmatic impact, and anti-paralytic properties (Arumugam et al. 2019; Mor et al. 2022).

Cow ghee has also demonstrated activity in wound healing potentially. The high content of saturated and unsaturated fatty acids is attributed to the property of healing wounds. The ability to wound heal cow ghee was examined through a study wherein cow ghee is combined with the extract of leaves of *Aegle marmelos*. Evaluation of several parameters, viz., decrease in the wound area, closure and contraction of the wound, and tissue regeneration in the damaged parts, have been done. The combination of cow ghee and leaf extract of *Aegle marmelos* has demonstrated rapid wound healing within eight days (Biyani et al. 2011; Shaikh et al. 2019). Evaluation of the wound-healing activity of cow ghee is also done in combination with *Aloe vera*. 0.5 g of the formulated gel has been applied. It has been found that there is the cessation of wound contraction between 3 weeks and 24 days, an increase in epithelialization, tensile strength is provided, and the formation of collagen has been promoted by such combination therapy (Nandanwar et al. 2010).

Across the globe, a common problem is computer vision syndrome (CVS). This condition is characterized by dryness of the eyes; sensation of burning; redness, and itching. For the treatment, lubricating eye drops are used. But unfortunately, regular use of such eye drops can damage the eyes due to the preservative presence. Interestingly due to the lubricating effect of cow ghee, it can be used for the treatment of CVS without any deleterious effect. Vitamin A in cow ghee helps maintain moisture in the lining (outer) of the eyeball, thereby preventing eye dryness and loss of vision (Mulik et al. 2013).

8 Cow curd/ Dahi

Worldwide, people drink curd—also known as yoghurt and Dahi—because of its high nutritional value and health advantages. To create this product, cow milk is fermented with microorganisms including Acidophilus, Streptococcus, and Lactobacillus. Curd contains a high concentration of probiotics, which are beneficial microorganisms that have been shown to have a variety of health advantages when taken orally (Dhama et al. 2014; Kaushik et al. 2016; Saleena et al. 2022a; Saleena et al. 2022b). Bacteria that produce lactic acid create metabolites, including cyclic dipeptides, phenyl lactic acid, and compounds with antifungal properties. They also have naturally occurring 3-hydroxylated fatty acid and proteinaceous substances. Ayurvedic guidelines for eating curd are conditional on factors like a person's overall health, the surrounding environment, and the weather (Dhama et al. 2005a; Borgohain et al. 2020; Lejaniya et al. 2021a; Lejaniya et al. 2021b; Saleena et al. 2022a; Saleena et al. 2022b).

Curd is a good source of water, vitamins, proteins, and minerals, among other nutrients and micronutrients. Curd's probiotics are excellent for the immunological and digestive systems, and its other vitamins, minerals, and proteins have antimicrobial and antiviral effects. As it inhibits the growth of dangerous germs and encourages the development of beneficial gut flora, it also treats digestive disorders (Kumar et al. 2020). It also has antifungal effects against dandruff from hair and piles. People with *Vata Prakruti* should eat curd with green gram or moong, those with *Pitta Prakruti* should eat it with sugar, and those with *Kapha Prakruti* should eat it with cumin powder. Ayurvedic guidelines suggest that consuming curd with all the necessary safeguards may have considerable health benefits (Dhama et al. 2013; Dhama et al. 2014; Kaushik et al. 2016).

Curd and buttermilk contain many lactic acid bacteria, which produce several antifungal metabolites (Saleena et al. 2022a; Saleena et al. 2022b). Curd has been consumed with either sugar (powdered sugar) or black salt and zira since ancient times. Whey supplemented with salts treats diarrhoea and eliminates parasites in neonatal calves, a practice common in animal medicine. It's being administered to mature animals to boost their output, especially milched buffaloes. Bullocks' work capacity is improved when fed both concentrate and whey (Raut and Vaidya 2018; Kumar et al. 2020). Probiotics have been shown to have positive effects when added to livestock feed. Antibiotic-free illness management may be possible with the use of probiotics. It will also lessen the need for antibiotics in livestock farming. Allergies and antibiotic resistance are just two negative impacts linked to antibiotic residues in milk, eggs, and poultry. Using probiotics, especially in the form of cow curd, will help alleviate these issues. However, much more research is needed to confirm or reaffirm the indigenous cow curd's efficacy as a probiotic in the scientific community (Dhama et al. 2014; Borgohain et al. 2020).

9 Contribution of Panchgavya to Agriculture

9.1 Impact on soil

Soil fertility is enhanced by panchgavya, which encourages the growth and reproduction of microorganisms, increases organic matter and macro and micronutrient levels, and increases plant uptake of nutrients. Application of panchgavya has several effects, such as soil porosity is improved, aggregate stability normalized, soil pH maintained, and the soil's nutrient profile is improved (Jain et al. 2014; Kumar et al. 2020; Prakash et al. 2021a; Prakash et al. 2021b; Kumar et al. 2022). Panchgavya, as confirmed by Bajaj et al. (2022), dramatically affects growth and crop productivity by encouraging beneficial soil bacteria surrounding the roots.

9.2 Impact on the Growth of Plants and Crops

Panchgavya, when sprayed on leaves, causes the plant to produce larger leaves and a denser canopy, as well as more photosynthetic material, allowing for a higher yield of metabolites and photosynthates. It has abundant and high branching, and its roots penetrate deep into the soil, where it can support the largest quantity of fruits as they mature. Moreover, the increased uptake of nutrients and water helps plants and crops maintain their freshness for longer (Tharmaraj et al. 2011; Jain et al. 2014; Pal and Patel 2020; Kumar et al. 2022; Kumari et al. 2022).

9.3 Relevance to organic farming

Synthetic pesticide-free food can be created with the aid of Panchgavya. When a field switches from conventional to organic farming methods within a year, it keeps crop production at the same level or even increases it. It improves the quality and safety of the food produced, extends the storage life of agricultural products, and makes them taste better. The 15-day harvest boost and the ensuing savings in chemical costs for agrarian production mean a healthier bottom line (Dhama et al. 2014; Kaushik et al. 2016; Borgohain et al. 2020; Prakash et al. 2021b; Bajaj et al. 2022; Kumari et al. 2022).

10 Nanotechnology based on the Panchgavya

Nanotechnology is a growing field of study in the field of drug delivery because it promises to provide medication exactly when and where it is needed (Dhama et al. 2013; Dhama et al. 2014; Kaushik et al. 2016; Bajaj et al. 2022). Synthesis of Panchgavya-mediated copper nanoparticles was performed by Arumugam et al. (2019) utilizing Panchgavya filtrate and copper sulphate solution (25 mM) as substrate. Scanning electron microscope (SEM), ultraviolet spectrophotometer, Dynamic light scattering, X-ray fluorescent microscopy, X-ray diffraction analysis, Fourier transmission infrared spectroscopy, high-resolution transmission electron microscope, and so on were all used to characterize copper nanoparticles. They have also been tested for antioxidant and cytotoxic efficacy (Raut and Vaidya 2018; Sathiyaraj et al. 2022; Rathi and Khan 2023). A 1mM $AgNO_3$ solution containing 4 mL of Panchgavya filtrate was used in the synthesis of silver nanoparticles by Govarthanan et al. (2014). Antibiotic-resistant bacteria, including *Aeromonas* sp., *Citrobacter* sp., and *Acinetobacter* sp., were successfully inhibited by the produced nanoparticles, which showed antimicrobial action in a concentration-dependent manner.

Conclusion and future prospects

Cows have an essential role in our culture and our survival. Its offspring and the Panchgavya it yields are versatile and hold great promise for the future of agriculture, human health and nutrition, biofertilizer production, alternative energy generation, and biodiversity preservation. The area's full potential has yet to be realized. Food grain production levels have been attained at a higher cost by using chemical fertilizers, pesticides, and exploiting groundwater, but at the expense of the fertility and health of the soil and the quality of the food produced. Organic farming, with the cow and her offspring among the potential solutions, is the only way to restore equilibrium. Raising cows and their offspring is one key to alleviating poverty and creating potential for long-term economic growth in rural and semi-urban areas.

In addition to the unavailability of branded "Cowpathy" products, few people know the cow's importance to the economy and the therapeutic and scientific benefits of Panchgavya. The country's incredible cattle riches can be better disseminated with the help of future initiatives and measures. Efforts should be made to educate the public about the "virtues of cow" and the "Panchgavya" it provides. Now more than ever, people require research-based knowledge and facts. Most of the tried-and-true methods of cow therapy, Panchgavya, Agnihotra, and milk miracles are dismissed as fantastical tales. That's why it's crucial to incorporate spirituality and wisdom into scientific inquiry. Patents in the United States have been issued due to this fusion, and many more are undoubtedly on the horizon. Panchgavya products have received the highest possible validation from the Indian government by granting U.S. patents. Future research in the livestock sector should be prioritized highly due to the sector's potential impact on poverty reduction. Godhan (the cow and its offspring) has excellent potential as a medical, agricultural, pharmacological, nutritional, environmental, technological, and socio-economic resource, so it's essential to construct research and development institutes to study these aspects. Ayurveda's Panchgavya theory deserves to win over adherents from all walks of life, from traditional households to those with advanced degrees in the hard sciences. Panchgavya has many benefits and uses, but it needs an all-encompassing strategy to get the word out about them. Because of this, we can conclude that Panchgavya therapy or Cowpathy, a modernized form of traditional knowledge, is an approach worth further exploring. So, if we can get the word out about how beneficial cows and Panchgavya are, we can alleviate issues like food grain scarcity, fuel scarcity, housing scarcity, poor health and nutrition, poverty and unemployment, and a lack of alternative energy sources.

With its wide range of biomedical and other therapeutic applications, Panchgavya is a promising treatment for various human and animal disorders. Its use would grow Through scientific confirmation, research funding, clinical trials, commercialization, and public and societal acceptance. The lack of food grains, fuel, shelter, health, nutrition, poverty reduction, employment, and energy, as well as the lack of awareness about the benefits of Panchgavya, can all be addressed by spreading information about its many uses. The health advantages of Panchgavya need to be maximized to effectively combat the myriad maladies and diseases that plague modern society and the dietary shifts accompanying it. However, there is a lack of literature to support the use of Cowpathy in treating animal diseases; thus, much work needs to be done to establish scientific evidence and validation. Scientists, researchers, and physicians must work together to increase public trust in this alternative low-cost therapy. Extensive testing is necessary for every product to ensure the active components are what they claim to be in terms of composition, pharmacological activity, chemical behaviour, toxicity profile, safety, and mechanism of action. Bringing international attention to India's extensive body of traditional culture and literature necessitates public education and the promotion of Panchgavya products.

Acknowledgement

All the authors acknowledge and thank their respective Institutes and Universities.

Author's contribution

All the authors contributed significantly.

Funding

This compilation was written by its authors and required no substantial funding.

Disclosure statement

All authors declare that there exist no commercial or financial relationships that could, in any way, lead to a potential conflict of interest.

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Journal of Experimental Biology and Agricultural Sciences, June - 2023; Volume – 11(3) page 534 – 541

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Impact of the COVID-19 Pandemic on tuberculosis management in India: A Brief Overview

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Received – April 05, 2023; Revision – June 13, 2023; Accepted – June 24, 2023
Available Online – June 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(3).534.541

KEYWORDS

Tuberculosis

COVID-19

Emerging viral infections

Pandemics

ABSTRACT

Chronicles suggests that emerging and re-emerging viral infections disrupting the normal lifestyle of humankind, whether in the form of HIV, Ebola, Influenza, Nepah, or the current SARS-CoV-2 pandemic. Such viral infections disrupt the healthcare system along with the prevention and control of epidemics and pandemics, resulting in an increased burden of such diseases in the post-pandemic period. Tuberculosis (TB) routine services are interfered with by severe lockdowns due to the new COVID-19 virus. This article tried to measure the long-term epidemiological effects of such interruptions on TB prevalence in high-burden countries. The participating facilities performed a comprehensive review based on modifications to the care of TB patients during the COVID-19 pandemic. Retrospectively, clinical factors and household contact information were collected from a literature survey. Researchers looked at numerous strategies over the following five years to see whether they might lessen the effects on TB incidence and death. Present comprehensive literature was collected and analyzed using suitable keywords such as "COVID-19," "Pandemics," "Tuberculosis," and "India" during the current COVID-19 pandemic to investigate the influence of COVID-19 on tuberculosis management. The present article looks at the effects of the breaks in the delivery of TB care in hospital and primary care settings. Lockdown, social isolation, measures to prevent viral transmission, and public health guidelines impacted tuberculosis care. The present study revealed that the COVID-19 pandemic has adversely affected numerous TB prevention, monitoring, and treatment programs. Still, these adverse effects are diminished by the prompt restoration of TB services and the application of particular therapies as soon as restrictions are lifted.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

COVID-19, a respiratory viral disease, was first reported in Wuhan, China, and later spread to almost every country with a slightly different infection rate (Wu and McGoogan 2020). The severity of infection ranges from a common cold to more severe illnesses such as pneumonia reported after the entry of the causative agent SARS-CoV-2 (Chen et al. 2020; Walaza et al. 2020; Budinger et al. 2021). Human-to-human transmission occurs when infectious droplets are inhaled or move into the body through contact with infected surfaces. Supportive care is the most common treatment option, while major illnesses may necessitate a ventilator (Ahn et al. 2020; Dhama et al. 2022).

Of 10 million globally reported tuberculosis (TB) instances, around 2.6 million were reported from India. Further, approximately 0.44 million people die yearly due to this disease. According to the World Health Organization (WHO), one-third of the world's multi-drug-resistant TB infections are found in India (Santosh and Pushp 2022). The worldwide risk of infectious illnesses, especially TB, has long been faced by public health agencies. The World Health Organization's (WHO) "real-life" analyses (surveys and national monitoring programs) provide the majority of information and projections related to global TB epidemiology (Golli et al. 2019). Although nowadays, TB incidence is decreasing, after this also, it remains a serious problem across the world (Crisan-Dabija et al. 2020), particularly in most middle-income and emerging nations, where it is the ninth worldwide leading cause of death. For Eastern European public health, TB has the highest incidence (4 times the average) in the European Union (EU), accounting for a quarter of the overall TB burden in the EU (Small et al. 2010). Various pandemic diseases also significantly affect several aspects of TB endemic. Like, in 1918 influenza pandemic accelerated the decreasing prevalence of tuberculosis in the USA (Noymer 2011). In India, the severity of the TB endemic has increased due to various factors, including a large number of severe forms (MDR or XDR-TB), HIV coinfection, and TB mortality in children. These factors have amplified the intensity of the country's tuberculosis epidemics (Dhamnetiya et al. 2021).

A worldwide health disaster, including TB, has occurred with the COVID-19 pandemic. But national tuberculosis programs, while preserving TB services, actively work to provide an efficient and prompt response to COVID-19 (Tadolini et al. 2020). Although the transmission routes differ, tuberculosis and COVID-19 are transmitted by close personal contact. Because tuberculosis is a prominent transmissible infection in India, public health should continue to focus on surveillance, clinical evaluation, testing, contact tracing, and diagnostic confirmation with supervised/inspected treatment regimes during the COVID-19 pandemic (Lancet 2020). Therefore, the present article looks at the effect of the COVID-19 pandemic on various TB management services (Figure 1).

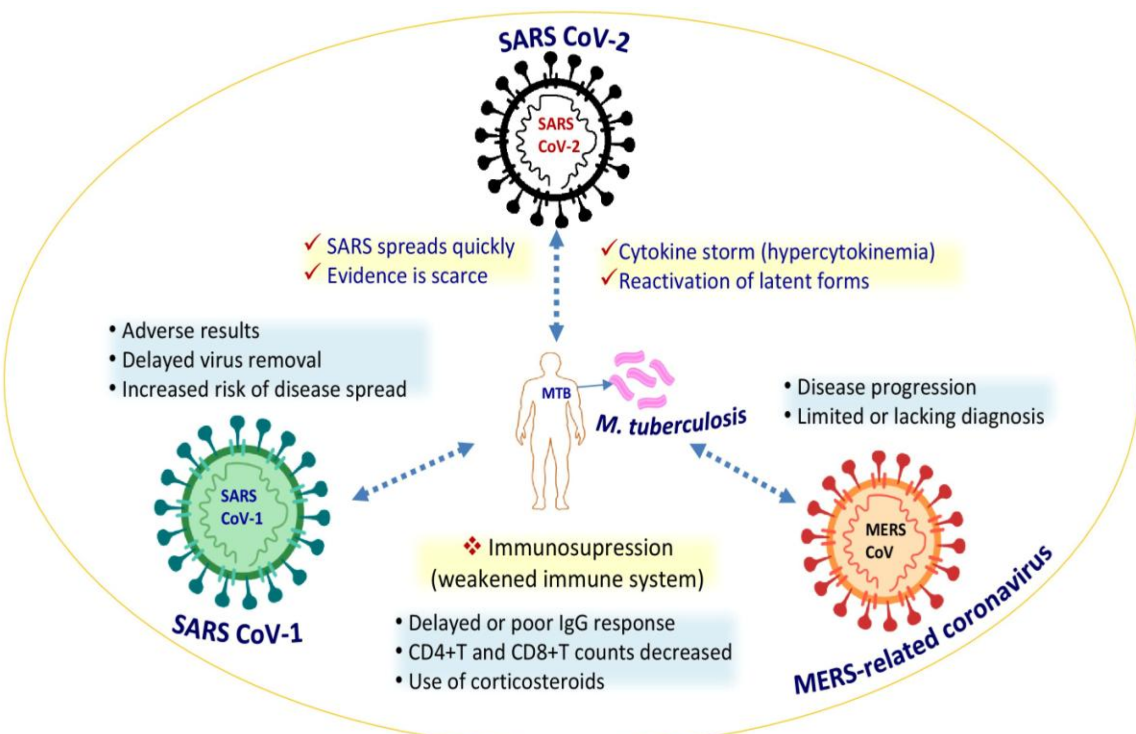

Figure 1 Coronaviruses and MTB: recognized and possible interaction

2 Impact of lockdown

Information regarding the possible connections between COVID-19 and TB in the wake of COVID-19 announcements as an International Public Health Emergency is in scanty, and all the available data were based on assumptions and modelling, then scientific verification (Tadolini et al. 2020; Motta et al. 2020; Migliori et al. 2020; Buonsenso et al. 2021). The novel SARS-CoV-2 virus has resulted in global illness, mortality, and societal consequences. Many countries have used population-wise lockdowns to halt the virus spread and allow their health services to manage it without drug treatments (Anderson et al. 2020). These lockouts significantly impact the transmission of SARS-CoV-2 (Walker et al. 2015; Flaxman et al. 2020; Prem et al. 2020). However, with such comprehensive actions, unexpected repercussions are unavoidable. After the dislocation, under pressure, the negative interruptions in health services (e.g., the spread of infectious illnesses) might endure long in poor and middle-income nations' health systems (Parpia et al. 2016; Arinaminpathy and Dye 2010).

TB incidence and death have substantially declined in recent decades due to continuous advancements in diagnosis, therapy, and prevention. However, COVID-related lockdowns in March 2020 brought the stop TB program to serious attention in several countries (Garg et al. 2022). For example, in India, an 80% reduction in the daily TB notifications was reported after the first weeks of the national lockdown on March 24, 2020 (Zignol et al. 2012). In other nations, such as South Africa, a decline of nearly half in the number of persons tested for tuberculosis was reported during the lockdown (Coker 2004). Such falls may also be attributable in the other part of the world; this might be due to the delay in reporting or decreases in access to diagnosis and treatment; whatever the factors are responsible for this decline but all these have a sustainable effect on the national monitoring and controlling program of TB disease. Missing diagnoses would increase the incidence of transmission while deteriorating treatment outcomes would raise the chance of TB mortality during the lockdown period.

3 Impact of the COVID-19 pandemic on Mycobacterial infection

COVID-19 and tuberculosis (TB) need public awareness and cooperation to effectively prevent, diagnose, and treat. Both diseases have airborne transmission and can be quickly detected (Dheda et al. 2022). Although surveillance on TB and viral infections should be separate because the data related to COVID-19 is still insufficient in the majority of countries, and the specific crucial clinical and immunological data which can be associated the TB with COVID-19 are not yet available (Visca et al. 2021). The primary distinction between these two is that tuberculosis is treatable, but there is no conclusive evidence of effective antiviral treatments for COVID-19. Although Remdesivir, lopinavir, hydroxychloroquine, lopinavir, and interferon repurposed, antiviral drugs had little to no impact on hospitalized patients with Covid-19 as evidenced by overall mortality and length of hospital stay (WHO Solidarity Trial Consortium 2021). For both diseases, research on novel and efficient vaccine candidates is ongoing, and for tuberculosis, various potential candidates are being evaluated to replace the traditional BCG vaccine (Cantini et al. 2020). Currently, mRNA vaccines, RNA-based replicating vector vaccines, and inactivated virus vaccines are in the clinical trial stage for SARS-CoV2 variants and candidates for DNA vaccines are in the pre-clinical trials stage (Narayanan et al., 2022). Proper and regular treatment is the primary method of disease control in the absence of an adult-specific tuberculosis vaccine. Increased tuberculosis transmission is anticipated because so many cases were missed or away from regular treatment during the past two years due to the pandemic effect (Pai et al. 2022).

Detrimental synergism between respiratory viruses (RSV, influenza viruses, and SARS-CoV-2) and bacterial infections elevates the risk of relatable mortality due to chronic lung sickness and immune suppression (Oliva and Terrier 2021). Especially patients with PTB may have a greater risk of these severe infections when compared to non-TB persons. Further, epidemiologic data also imply that patients with TB have a higher risk of viral diseases and are severely associated with pandemics or epidemics (Redford et al. 2014; Prem et al. 2020).

4 Diagnostic Tests

To determine the most appropriate diagnostic tests in most scenarios that apply to Mycobacterium TB and SARS-CoV-2, the WHO has established assured criteria like user-friendly, robust, sensitive, specific, and deliverable to end users. However, regardless of the pathogen, a fundamental shortcoming of all present assays is the difficulty in swiftly determining the pathogen if it is alive and contagious (Mina et al. 2020). The viability of *M. tuberculosis* requires a minimum of six weeks of culture results. Even in these days of cutting-edge technology, information regarding the COVID-19 individuals compared to 'no COVID-19' individuals, the IFN-response to the SARS-CoV-2-unrelated antigens Staphylococcal Enterotoxin B (SEB) and cytomegalovirus (CMV) was compared in whole blood using an innovative experimental method by Petrone et al. (2021), which claims that the SARS-CoV2-specific response may be measured in whole blood and can be seen in both acute and convalescent patients (Murugesan et al. 2021). Both nucleic acid detection and antigens-based tests are available for both diseases, but *M. tuberculosis* is diagnosed using culture-based and smear methods, and SARS-CoV-2 is diagnosed using serology.

Recently, testing people with COVID-19 for TB and vice versa has been the subject of significant debate. The common clinical

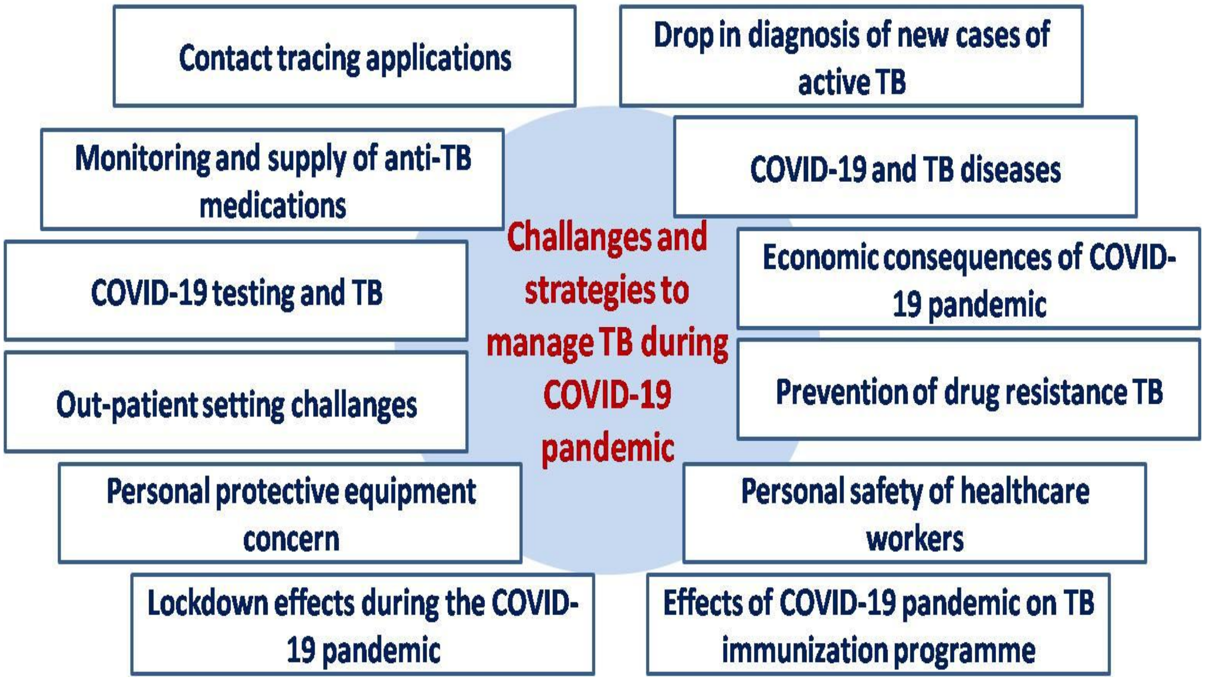

Figure 2 TB management, challenges and strategies during the COVID-19 pandemic

characteristics and manifestations of COVID-19 and TB are fever, breathing deficiency, and coughing. The disease development processes have clear distinctions, and COVID-19 infection develops less quickly than tuberculosis (Visca et al. 2021). Concurrent TB does not exclude the possibility of COVID-19 infection, especially in a TB-endemic country like India (Jain et al. 2020). The European Laboratory Initiative recommended the utilization of the GeneXpert equipment for COVID-19 testing without diminishing its TB usefulness (Simoes et al. 2022). Further, the Truenat™ Beta CoV Test on the Truelab™ workstation has recently been approved for use as a test for COVID-19 by the ICMR (Figure 2).

4.1 Challenges in Diagnose new cases of active tuberculosis

The need to find supplementary strategies to support patients with tuberculosis has been rising with the constraints of confinement and face-to-face reduction. Telemedicine can assist in the management of TB in communities. Admitted Social Health Activist (ASHA), one of the primary members of India's NRHM, would have to visit a TB patient's house to see whether the patient is following treatment (Xu et al. 2017). The continuing lockdown during the COVID-19 epidemic has seriously affected the treatment of TB sufferers, that's why, according to the Nikshay Government of India Central TB Portal (Suárez et al. 2019), the diagnosis of new TB patients has dropped dramatically during the lockdown. The factors associated with this dropdown are the closing of outpatient departments (OPD), lack of access to treatment, and the denial of government or private injection.

5 Anti-TB medication supervision and distribution

The Minister of Health and Family Welfare (MOHFW), Government of India, is attempting to arrange guaranteed diagnosis and treatment of TB patients in a one-month approach, but during the lockdown, many difficulties have been raised in this (Suarez et al. 2019). Monitoring the sickness process of TB patients was a big challenge for health workers to serve TB patients in the era of social alienation and self-isolation due to the lack of medications and restocking the medication at the patient's home. The various countries ' local governments have devised many strategies for establishing outreach services to contact TB patients and send TB medications via mail (Xu et al. 2017). In the case of multidrug-resistant TB, the World Health Organization (WHO) recommends reducing the TB preventive regimen to 1 month per day for persons who have close contact with active TB patients who are treated with rifapentine and isoniazid (https://tbcindia.gov.in). Recently, the government of India advised patients in the ambulatory setting of TB drugs that they should be supplied for one month with TB drugs, as well as a 2-month supply for exceptional conditions, to lessen the patients' requirement to attend clinics and consequently, the danger of disease transmission (https://tbcindia.gov.in).

5.1 Applications for contact tracking

The main factors for the management of both tuberculosis and COVID-19 in society are contact tracing and monitoring. The unexpected increase in the requirement for COVID-19 patient tracking, the government of India's establishment of the ArogyaSetu 'COVID Health Bridge' app for mobile tracking, raises worries about the diversion of the contact monitoring services given for COVID-19 and tuberculosis (Sharma et al. 2020). The Uttar Pradesh Government Ayush Kavach app was also found helpful in tracking COVID-19 individuals. The impact of the COVID-19 pandemic on the inconvenience of essential contact tracking for TB patients should be ignored. The knowledge gained through the TB contact apps should be used to improve the COVID-19 app's functionality. After the completion of the lockdown, an incidence of TB cases may arise, and to avoid this, the effective use of Ni-Kshay Aushadhi, a web-based application that allows the monitoring of all TB patients, including MDR cases, is required (Jain et al. 2020).

5.2 COVID-19 Effect on the TB Vaccine Program

TB protection is provided by the Bacille Calmette-Guerin (BCG) vaccination. Suspension vaccination programs have documented vaccine-preventable deaths connected with diseases and an increased strain on health systems due to the COVID-19 pandemic lockdown (Lobo et al. 2021). In countries with high TB incidence, such as India, South Korea, Indonesia, China and Turkey, the WHO recently suggested extending the newborn neonatal TB BBG vaccine. Evidence indicates that BCG also protects against many viral and non-mycobacterial infections. The idea that BCG vaccination could be used for protection against the adverse effects of coronavirus disease stems from the non-specific effects of BCG, which happen through the induction of trained immunity (Curtis et al. 2020). Phase IV trials are now underway to assess the role of these non-specific vaccines in SARS-CoV-2 infection protection (Lobo et al. 2021).

6 Discussion

The awareness of tuberculosis patients is still the cornerstone of tuberculosis prevention and treatment, which will help diagnose and cure TB patients. Further remote teleconsultation would be helpful in monitoring and treatment of TB patients. The continuation of vital care for TB patients throughout the COVID-19 epidemic should not be ruptured till the demolishing of COVID-19. To avoid the transmission of SARS-CoV-2 in tuberculosis patients a proper hospital care for patients with tuberculosis should be confined to severe cases, as learned from past outbreaks (Visca et al. 2021; Dheda et al. 2022). Since the first discovery of coronavirus in 1960, three human coronaviruses have been known to cause deadly respiratory illnesses. The newest emerging coronavirus (SARS-CoV-2) has resulted in an increasing number of worldwide reported deaths and thousands of new cases confirmed every day, and epidemic diseases like TB also contributed to this pandemic. COVID-19 causes a wide spectrum of host immune responses in asymptomatic patients, from mild cytokine storms to deadly cytokine storms (Dhama et al. 2020). Immunosuppression which has been used to treat COVID-19 can potentially reactivate TB. The gold standard diagnostic testing for COVID-19 includes culture and PCR-based techniques for TB; however, a definitive point-of-care test that can immediately detect if someone is currently infected with TB. New SARS-CoV-2 variants (like omicron) are emerging due to vaccine equity issues, and they are particularly harmful to nations with low vaccination, high poverty, and high tuberculosis rates. More research is required to completely understand COVID-19's abilities to stimulate the reactivation of an existing TB infection. Because COVID-19 and TB have similar signs and symptoms, getting imaging services (chest radiography or computed tomography) may be simpler to uncover evidence of pre-existing TB. There is insufficient information to establish the probable impact of COVID-19 on TB patients' treatment because most of these patients are still receiving treatments in existing series. New SARS-CoV-2 variants like omicron are emerging due to vaccine equity issues, and they are particularly harmful to nations with low vaccination, high poverty, and high tuberculosis rates (Dhama et al. 2022).

Moreover, Crisan-Dabija et al. (2020) suggested that influenza pandemics, seasonal outbreaks, coronavirus epidemics, and other pandemics negatively influence individuals with tuberculosis. A global patient research project is underway to improve the description of communication between the two illnesses (WHO) conducted by the Global Tuberculosis Network and funded by WHO on TB and COVID. These studies mainly describe the features of COVID-19 patients, including diagnostic trials and recommended treatments, and TB patients (current or former). The secondary goal of these types of projects is to (i) evaluate the logistical and organizational viability of a global repository for COVID-19 and TB patients and (ii) explain the clinical outcomes (COVID-19 disease outcomes and TB patients' intermediate and final treatment results). With this global study and others, the GTN has recommended several priority research issues, which included (i) how COVID-19 would affect TB services in the next few years, considering the growing impacts of its third wave?, (ii) is COVID-19 associated with an increased risk of TB illness in those with TB?, (iii) does the BCG vaccine offer COVID-19 protection?, (iv) What additional factors influence mortality in patients with TB and COVID-19?, (v) Is there a difference in treatment for TB & COVID-19 co-infected patients? (Or, to put it another way, what additional services do these patients require?), and (vi) what effect does COVID-19 have on TB mortality?

Compared to COVID-19, tuberculosis care and management have been neglected, while the high infection rates, the emergence of new variants, and the absence of the SARS-CoV-2 vaccine in tuberculosis-endemic countries will continue to feed the global pandemic. The simultaneous epidemics of tuberculosis and COVID-19 exacerbate the vicious cycle of poverty and mortality (Dheda et al. 2022). Disruptions to HIV diagnosis and treatment programs may also contribute to the tuberculosis pandemic in some circumstances (Hogan et al. 2020). Maintaining the most important HIV and tuberculosis prevention programs and medical services could significantly lessen the overall effect of the COVID-19 pandemic (Hogan et al. 2020). Therefore, it is essential to support and refocus HIV and tuberculosis programs.

Conclusion and Future Prospects

The outbreak of COVID-19 and the limitations of lockdowns significantly impact the national and worldwide implementation and monitoring of TB surveillance initiatives. Delays in treating people with TB would aggravate their condition and require more comprehensive management. Individuals with TB may also acquire antibiotic resistance; these patients are susceptible to coronavirus superinfection. It is suggested that an effective and timely response, including monitoring and therapy, should be given to COVID-19 and TB patients. Despite the rapidly rising number of cases, there is still a long way to go before we know to evaluate how the COVID-19 pandemic will affect patients with latent TB and TB sequelae and suggest management in this situation. COVID-19 can strike at any time during a patient's TB journey, with the worst outcomes occurring in individuals with active pulmonary TB disease. According to the research available, death because of TB is significantly affected by various other important factors like malnutrition, diabetes, poverty, HIV coinfection and COVID-19. We need higher-quality prospective studies to address the research's most pressing concerns. Meanwhile, anyone with or without active TB, particularly those with HIV coinfection, must do all possible to prevent the growth of COVID-19 and, when possible, should be vaccinated appropriately.

Competing interest

The authors have no potential conflict of interest to declare.

Acknowledgement

The authors thank Ms Rasanpreet Kaur, Dr Nitin Bhatnagar and Dr Shoor Vir Singh for providing the necessary facilities for data collection and revisions.

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Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Allelopathic effects of the invasive plant Wedelia (*Sphagneticola trilobata* L.) aqueous extract on common beans (*Phaseolus vulgaris* L.)

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Received – April 07, 2023; Revision – June 11, 2023; Accepted – June 20, 2023
Available Online – June 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(3).542.549

KEYWORDS

Sphagneticola trilobata

Allelopathy

Phaseolus vulgaris

Germination

Growth

Seed

Yield

Weeds

ABSTRACT

Sphagneticola trilobata (L.) Pruski is an alien invasive weed with aggressive growth habits, environmental stress tolerance, and the ability to synthesize allelochemicals. However, in many parts of the world, this plant is still recommended for use in composting, phytoremediation, and as an ornamental ground cover in gardens. The present study investigated the allelopathic effect of *S. trilobata* on the seed germination, growth and yield of *Phaseolus vulgaris* L. To analyze the allelopathic effects of *S. trilobata* on *P. vulgaris* seed germination, hundred seeds of *P. vulgaris* were exposed to different concentrations of the aqueous extracts of fresh and dry *S. trilobata* (2.5×10^2, 5.0×10^2 and 7.5×10^2 g/L) in Petri dishes for five days. The impact of *S. trilobata* aqueous extract on the growth and yield of *P. vulgaris* was also studied with seedlings planted in a compost soil mixture. Results of the study suggested negative impacts of *S. trilobata* extracts on *P. vulgaris* seed germination, growth and yield. *P. vulgaris* seed germination was significantly lower in the treatments than in the control ($p<0.05$). Further, *P. vulgaris* plants treated with fresh plant extracts at 5.0×10^2 and 7.5×10^2 g/L concentrations had significantly lower shoot height, growth rate, leaf area, fresh shoot weight, dry shoot weight, root length, pod length and yield ($p<0.05$) than controls. From the results of the study, it can be concluded that *S. trilobata* aqueous extracts have a dose-dependent allelopathic effect on *P. vulgaris* seed germination, growth, and yield and among the dry and fresh plant extracts, fresh plant aqueous extracts have a more significant allelopathic impact. As *S. trilobata* contains water-soluble allelochemical, it should not be used in biofertilizer production, phytoremediation, or as live mulch.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Sphagnaticola trilobata (L.) Pruski, formerly known as *Wedelia trilobata*, is one of the world's top 100 most environmentally destructive invasive alien species (Lowe et al. 2000). *S. trilobata* belongs to the Asteraceae family and is found in Asia, South America, and the Pacific. *S. trilobata* is a creeping and climbing perennial herb that grows up to 30 cm high and 4 m long. This mat-forming plant frequently creates a dense ground cover (Figure 1) and may short-climb trees or other vegetation. *Sphagnaticola* species have antimicrobial, insecticidal, larvicidal and trypanosomicidal properties and are used for medicinal purposes (Huang et al. 2006; Maldini et al. 2009; Toppo et al. 2013) and as a decorative ground cover in many parts of the world (Wagner et al. 1990; Macanawai 2013). It is also used as a cover crop in rubber and tea plantations to control soil erosion in Asia (Nayanakantha 2007). Furthermore, it is used for ground cover in Middle Eastern countries, which helps stabilize the soil and prevent it from moving due to wind. The use of *S. trilobata* as a ground cover is getting increasingly popular in various countries, including the Kingdom of Saudi Arabia (Swaefy and Basuny 2011).

However, *S. trilobata* has become a noxious weed in many countries, covering vast areas in agricultural lands, along coastal areas, rail and roadsides, garbage dumps, open grounds and other disturbed habitats (Wu et al. 2008; Macanawai 2013; Hossen et al. 2020; Gao et al. 2022). It has also been found on the recently developed volcanic islands, atolls, limestone, continental, and even small uninhabited offshore islands in most Pacific Island countries and territories (Wagner et al. 1990). The aggressive growth pattern of *S. trilobata* is attributed to its tolerance to environmental stresses and ability to synthesize allelochemicals (Zeng et al. 1994; Wu et al. 2008; Wang et al. 2012). Allelochemicals have biological effects on other organisms, many of which are still unknown (Waller 2003; Ferguson et al. 2013). Because of its significant impact on plant development and yield reduction in natural and agricultural settings, allelopathy of invasive species has gained increased attention (Waller 2003; Wang et al. 2012; Shahena et al. 2021; Gao et al. 2022). Aqueous plant extract is one of the most commonly utilized ways to assess plant species' allelopathic effects (Zeng et al. 1994; Nie et al. 2004; Ilori et al. 2010; Ullah et al. 2021).

As *S. trilobata* invades agricultural fields and is used as a ground cover, compost production (Setyowati et al. 2014a,b; Hussain et al. 2020; Setyowati et al. 2021) and phytoremediation (Dissanayake et al. 2002), it is a necessity to study its allelopathic effects on cultivated crop varieties. *Phaseolus vulgaris* L. is a popular herbaceous annual crop grown worldwide for its edible beans as dry seed and unripe fruits. It was hypothesized that the allelopathic biomolecules of *S. trilobata* aqueous extract negatively affect the seed germination, growth and yield of *P. vulgaris*. Therefore, the present study was conducted to investigate the allelopathic effect of *S. trilobata* on the seed germination, growth and yield of *P. vulgaris*.

2 Materials and Methods

2.1 Preparation of aqueous extracts from wet and dry plant parts of *S. trilobata*

Aerial parts of *S. trilobata* were collected from the home gardens of the Gampaha district, Western Province, Sri Lanka, from November to December 2020. A voucher specimen of *S. trilobata* was deposited at the Department of Zoology and Environmental Management, University of Kelaniya, Sri Lanka. The flowers, dried yellow leaves, and dead stems were removed, and fresh *S. trilobata* parts were chosen, washed, and cut into 1cm pieces. Seventy-five grams of plant parts were mixed with 100 mL of distilled water and ground well. The resulting solution was filtered and centrifuged, and a stock solution of 7.5×10^2 g/L

Figure 1 *S. trilobata* (a– Densely grown *S. trilobata* in a home garden in Sri Lanka, b- Flower of *S. trilobata*)

of aqueous extract of fresh plant parts was prepared. Fresh pieces of *S. trilobata* were also air-dried for seven days at room temperature (28°C), and a stock solution of 7.5×10^2g/L of aqueous extract was prepared. Another two test solutions were prepared using these stock solutions with concentrations of 2.5×10^2 g/L and 5.0×10^2 g/L.

2.2 Effect of fresh and dry *S. trilobata* aqueous extracts on *P. vulgaris* seed germination

The experiment was conducted using four sets of Petri dishes; three sets were used as treatments and one as a control. Three replications were used for each treatment and control. *P. vulgaris* seeds (Sanjaya variety-bush bean type) were surface sterilized with 5% sodium hypochlorite for 20 minutes before being washed with distilled water several times. A Whatman No.1 filter paper and one hundred *P. vulgaris* seeds were placed on each 70 mm Petri dish. Thirty mL of test solutions (2.5×10^2 g/L, 5.0×10^2 g/L and 7.5×10^2 g/L) and distilled water were added daily into complementary treatments and controls, respectively. The remaining solution added the previous day was removed using an injection cylinder. All Petri dishes were arranged in a completely randomized design. The germination of *P. vulgaris* seeds was recorded upon the emergence of radicle every 24 h for five days.

2.3 Effect of fresh *S. trilobata* aqueous extracts on *P. vulgaris* growth and yield

Four sets of pots filled with the topsoil compost mixture (1:1) were prepared in triplicates to estimate the effect of different *S. trilobata* fresh aqueous extract concentrations (2.5×10^2 g/L, 5.0×10^2 g/L, and 7.5×10^2 g/L) on the growth and yield of *P. vulgaris* plants. Five germinated *P. vulgaris* seeds were planted in each pot (1.98×10^{-2} m²), and pots were arranged in a completely randomized block design. One hundred mL of the test solutions and distilled water were added into three complementary treatments and to the controls on each other day, respectively. As a fertilizer, 100 g of compost was added to each experimental pot once a week. The shoot height of plants was measured, and a digital image (Sony Cyber-shot DSC-H300 20.1 MP, Digital Camera) of the second leaf of each plant was taken weekly. ImageJ software was used to calculate the leaf area in cm^2 (Schneider et al. 2012). Also, pods produced in the plants were harvested, and the lengths of the pods were measured using a thread and meter ruler. The weights of pods were measured using an electric balance (Sartorius CP224S), and the yield of *P. vulgaris* was calculated. All plants were removed after 50 days, and the above and below-ground parts were weighed to calculate each replicate's fresh shoot and root weight. The shoot and root were air-dried in an oven at 70°C for three days before their dry weights were measured. Soil pH (HACH - HQ 40d), organic matter content (Schulte and Hopkins 1996) and total nitrogen (Bremner 1996) in each experimental pot were measured biweekly.

2.4 Statistical analysis

Analysis of variance (ANOVA) was conducted using MINITAB (version 14) software to compare seed germination, growth and yield of *P. vulgaris* exposed to different concentrations of aqueous extracts of *S. trilobata* and the significance of the variation was tested at 0.05 level using Tukey's pairwise comparison. Pearson correlation test was carried out to assess the dose dependency of impacts observed in *P. vulgaris* growth and yield to aqueous extracts of *S. trilobata*.

3 Results and discussion

In this study, the invasive *S. trilobata* had negative allelopathic effects on the seed germination, growth and yield of *P. vulgaris*.

3.1 Effect of fresh and dry *S. trilobata* aqueous extracts on *P. vulgaris* seed germination

Aqueous extracts of fresh and dry *S. trilobata* significantly inhibited the germination of *P. vulgaris* seeds. The number of germinated seeds and the seeds germination ratio in seeds treated with aqueous extracts of *S. trilobata* was substantially lower ($P<0.05$, one-way ANOVA) than in the control (Table 1). These findings suggest the presence of phytotoxic germination inhibitory substances in the aqueous extracts of *S. trilobata*.

Table 1 Effect of *S. trilobata* aqueous extracts on *P. vulgaris* seeds germination and seed germination ratio after five days of exposure

Concentration (g/L)	Fresh plant parts aqueous extract of *S. trilobata*		Dry plant parts aqueous extract of *S. trilobata*	
	Number of germinated seeds	Germination ratio	Number of germinated seeds	Germination ratio
Control	84±0.3^a	100±0.0^a	80±0.7^a	100±0.0^a
2.5×10^2	55±0.3^b	65±0.4^b	53±0.3^b	66±0.4^b
5.0×10^2	31±1.3^c	37±1.6^c	33±0.9^c	41±1.1^c
7.5×10^2	9±0.6^d	10±0.4^d	8±0.3^d	10±0.4^d

Data are the mean of three replicates; ± Standard Error of mean; mean ± SE value followed by the different letter in the same vertical column are significantly different as per Tukey's pairwise significant difference test ($P<0.05$)

The dry and fresh aqueous extract at the concentration of 7.5×10^2 g/L had the highest negative impact on the seed germination of *P. vulgaris*, while the lowest effect was reported at a concentration of 2.5×10^2 g/L. This may be attributed to the higher sensitivity of plants to phytotoxic biomolecules during seed germination and the initial stages of development. Results agree with the findings of Kwembeya et al. (2013), who reported that *Lantana camara* L. negatively affected the germination and growth of Blackjack, *Bidens pilosa* L. when added to the soil under glasshouse conditions. According to Nie et al. (2004), dissolved biomolecules of *S. trilobata* reduced the protective enzyme activities of plant seeds. When exposed to phytotoxic compounds, plants cannot successfully protect the cell membrane from the active oxygen, damaging the membrane and decreasing seed vitality.

In this study, as shown by the results of the Pearson correlation test, the allelopathic effect of *S. trilobata* was dose-dependent, and the number of germinated seeds decreased with increasing concentrations of both types of aqueous extracts. The percentage germination of *P. vulgaris* seeds exposed to aqueous extracts of *S. trilobata* and control gradually increased with time. However, seed germination of *P. vulgaris* in control was always higher than in the treatments. The inhibition of seed germination was higher in seeds treated with an aqueous extract of fresh *S. trilobata* than the dry *S. trilobata.* Drying plant leaves may have reduced the concentrations or effects of allelochemicals in the *S. trilobata*.

The concentration-dependent inhibitory effects of *S. trilobata* extracts on *P. vulgaris* seed germination demonstrated the allelopathic potential of this plant. These results contradict the findings of Krishnan and Rajalakshmi (2021), who exposed cowpea seeds to *S. trilobata* extract (2 – 10 %) and did not find any negative impact on the seed germination. These findings suggest that the phytotoxic effects of *S. trilobata* extracts varied among the tested plants.

3.2 Effect of fresh aqueous extracts of *S. trilobata* on *P. vulgaris* growth and yield

Results of the seed germination study revealed that maximum inhibition in the seed germination was reported in the seed treated with the highest dose (7.5×10^2 g/L) of fresh aqueous extract of *S. trilobata*. Based on its effectiveness, this dose was selected for the pot study under natural conditions, and *P. vulgaris* seedlings were treated with 7.5×10^2 g/L of fresh aqueous extract of *S. trilobata* and found that treated seedlings started to die from the third week and out of 15, only nine plants survived. In contrast, none of the control plants died until the end of the experiment. The shoot heights of the plants treated with the aqueous extracts were significantly lower than that of the control ($p<0.05$). Like seed germination, the fresh extracts at concentrations of 7.5×10^2 g/L and 2.5×10^2 g/L had the strongest and weakest adverse effects on the shoot height of *P. vulgaris*, respectively. A comparison of the *P. vulgaris* plant's height at the end of the experiment is given in Table 2.

The changes in the surface area of the second leaf of *P. vulgaris* treated with aqueous extracts of *S. trilobata* with time are shown in Figure 2. Initially, the size of the second leaf of *P. vulgaris* was comparatively smaller, and later on, it gradually increased until it slowed down in the sixth and seventh weeks. The lowest leaf area was recorded in the plants exposed to 7.5×10^2 g/L of aqueous extract. In contrast, the concentration of 2.5×10^2 g/L had the lowest impact on the leaf area.

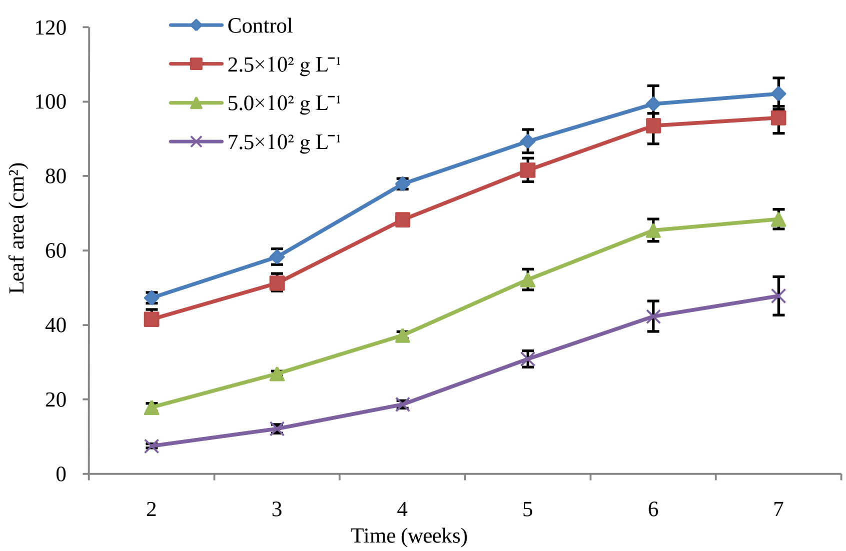

Figure 2 Variation in the surface area (Mean±SEM) of the second leaf of *P. vulgaris* exposed to aqueous extracts of *S. trilobata* with time

Table 2 Effect of fresh aqueous extracts of *S. trilobata* exposure on the *P. vulgaris* plant height

Concentration of *S. trilobata* aqueous extract (g/L)	Height (cm)
Control	73.3±0.9[a]
2.5×10^2	67.7±1.2[b]
5.0×10^2	46.6±1.2[c]
7.5×10^2	37.7±1.3[d]

Data are the mean of three replicates; ± Standard Error of mean; mean ± SE value followed by the different letter in the same vertical column are significantly different as per Tukey's pairwise significant difference test (P<0.05)

Table 3 The second leaf surface area of *P. vulgaris* plants after the seventh week

Concentration of aqueous extract of *S. trilobata* (g/L)	Leaf area (cm²)
Control	102.11±4.23[a]
2.5×10^2	95.70±0.33[a]
5.0×10^2	68.40±2.63[b]
7.5×10^2	47.78±5.16[c]

Data are the mean of three replicates; ± Standard Error of mean; mean ± SE value followed by the different letters in the same vertical column are significantly different as per Tukey's pairwise significant difference test (P<0.05)

Table 4 Effect of fresh aqueous extracts of *S. trilobata* on the various growth parameters of *P. vulgaris* within seven weeks of the experimental period

S. trilobata aqueous extract concentration (g/L)	Fresh shoot weight (g)	Dry shoot weight (g)	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Length of pods (cm)	Yield (g)
Control	8.43±0.27[a]	1.67±0.02[a]	14.7±0.39[a]	0.44±0.02[a]	0.14±0.01[a]	11.5±0.27[a]	20.31±2.17[a]
2.5×10^2	7.86±0.25[a]	1.57±0.03[a]	13.2±0.44[a]	0.41±0.01[a]	0.13±0.01[a]	10.8±0.16[a]	17.71±1.04[a]
5.0×10^2	5.12±0.35[b]	1.17±0.05[b]	11.3±0.50[b]	0.32±0.01[b]	0.11±0.06[b]	8.0±0.13[b]	8.05±0.72[b]
7.5×10^2	3.44±0.36[c]	0.87±0.11[c]	8.5±0.66[c]	0.25±0.02[c]	0.08±0.01[c]	4.6±0.25[c]	1.79±1.20[c]

Data are the mean of three replicates; ± Standard Error of mean; mean ± SE value followed by the different letter in the same vertical column are significantly different as per Tukey's pairwise significant difference test (P<0.05)

Exposure to the higher concentrations of aqueous extracts of *S. trilobata* significantly reduced the leaf area of *P. vulgaris* compared to the control (P<0.05). Although 2.5×10^2 g/L of *S. trilobata* aqueous solution reduced the leaf area compared to the control, this difference is insignificant (Table 3). The allelopathic effect of *S. trilobata* aqueous extract affects plant cell structure, division and elongation, growth regulation systems, metabolism, photosynthesis, mineral ion uptake, amino acid, and nucleic acid synthesis (Cheng and Cheng 2015).

The *P. vulgaris* plants in the control pots began to flower at the beginning of the fourth week. While the plants treated with 2.5×10^2 g/L *S. trilobata* aqueous extract reached the flowering stage at the end of the fourth week and was followed by plants treated with 5.0×10^2 g/L and 7.5×10^2 g/L which were showing flowering at the end of the fifth week. The effect of aqueous extract of fresh *S. trilobata* on growth parameters of *P. vulgaris* plants, such as fresh shoot weight, dry shoot weight, root length, fresh root weight, dry root weight, length of the pod and yield, are presented in Table 4.

All the concentrations of *S. trilobata* aqueous extracts have an inhibitory effect on the *P. vulgaris* shoot growth, which was indicated by lower weights of fresh and dry shoots in the plants treated with *S. trilobata* aqueous extracts compared to the control. Although 2.5×10^2 g/L aqueous extract of *S. trilobata* reduced the fresh and dry shoot weights of *P. vulgaris*, this was not significantly different from the controls. Furthermore, *P. vulgaris* root growth was significantly reduced by the application of the *S. trilobata* aqueous extracts at 5.0×10^2 g/L and 7.5×10^2 g/L concentrations, as evidenced by shorter root lengths and lower fresh and dry root weights compared to controls (P<0.05). The lowest dose of *S. trilobata* aqueous extracts (2.5×10^2 g/L) showed no significant differences from the control plants regarding root length, fresh root weight and dry root weight.

Higher concentrations of *S. trilobata* aqueous extracts (5.0×10^2 and 7.5×10^2 g/L) also reduced the length of the pods and the yield of *P. vulgaris* as compared to the 2.5×10^2 g/L aqueous extract and control plants (P<0.05). No statistically significant difference (P>0.05) was reported in the average yield of *P. vulgaris* seedlings exposed to 2.5×10^2 g/L aqueous extract and control plants. The lower primary productivity due to lower shoot and root development may reduce the yield of plants exposed to higher concentrations of aqueous extracts of *S. trilobata*.

All the measured growth parameters of *P. vulgaris* had a strong significant negative correlation with different concentrations of *S. trilobata* fresh aqueous extracts (P<0.05). The growth parameters of *P. vulgaris* exposed to *S. trilobata* aqueous extracts decreased as the concentrations of aqueous extracts increased (Table 5). Similarly, the aqueous extract of *Wedelia* also significantly inhibited the growth of chickpea (*Cicer arietinum* L.), cowpea (*Vigna unguiculata* L.), and green gram (*V. radiata* L.) seedlings with a more pronounced effect at higher concentrations (Shahena et al. 2021).

Table 5 Correlation between the various concentrations of *S. trilobata* fresh aqueous extracts and various growth parameters of *P. vulgaris* within seven weeks experimental period

Growth parameter	Correlations (r) between concentration and growth parameters*	P value
Shoot height	-0.976	0.024
Leaf area	-0.976	0.024
Fresh shoot weight	-0.975	0.025
Dry shoot weight	-0.978	0.022
Root length	-0.990	0.010
Fresh root weight	-0.984	0.016
Dry root weight	-0.976	0.024
Length of the pod	-0.967	0.033
Yield	-0.980	0.020

*According to the Pearson correlation test, all the growth parameters are significantly (P<0.05) negatively correlated to the concentrations of aqueous extract of fresh S. trilobata.

Table 6 Various physicochemical parameters of soil collected from *P. vulgaris* growing pots

S. trilobata aqueous extract concentration (g/L)	pH	Organic matter content (%)	Total nitrogen (%)
Control	7.06±0.04	6.27±0.10	1.86±0.35
$2.5×10^2$	7.08±0.07	6.56±0.14	1.88±0.15
$5.0×10^2$	7.09±0.02	6.84±0.11	1.84±0.24
$7.5×10^2$	7.11±0.01	7.04±0.29	1.93±0.21

Values in the columns are not significantly different as per Tukey's pairwise significant difference test (P<0.05)

Significantly low seed germination, poor growth and yield were recorded in the *P. vulgaris* plant treated with the *S. trilobata* aqueous extracts; this might be attributed to the presence of the allelopathic biomolecules such as coumarin derivatives, diterpene, sesterpenes and sesquiterpene lactones that are present in *S. trilobata* aqueous extracts (Shahena et al. 2021). Hossen et al. (2020) indicated the presence of two phenolic compounds, i.e. vanillic acid and gallic acid, from the *Wedelia chinensis*(Osbeck) Merr aqueous extract, which are the main constituents of the allelopathic effects. Allelochemicals present in the aqueous extracts of *S. trilobata* could disrupt the photosynthetic activities, mineral absorption, cell division, and energy metabolism of the *P. vulgaris* plants (Pasiecznik 1999; Cheng and Cheng 2015). Due to the disturbance in the plant, as mentioned earlier, weak development of the root system and reduction of the leaf area was reported and this could have a significant effect on the overall growth, fruiting time and yield of the *P. vulgaris*. Pu et al. (2022) have observed a low growth rate and yield of tomatoes in the presence of *S. trilobata*. Similar results were observed when rice (*Oryza sativa* L.) was fertilized with green manure manufactured using *W. trilobata* (Nie et al. 2004). Extracts of *W. chinensis* also affected the shoot growth of lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), and rapeseed (*Brassica napus* L.) (Hossen et al. 2020).

3.3 Physicochemical parameters of soil

There were no significant changes in the various physicochemical parameters such as pH, total nitrogen and organic matter content of the soil collected from *P. vulgaris* growing pots (Table 6).

According to the results, soil quality has not changed by adding different concentrations of *S. trilobata* extracts. This suggests that the toxic effects of *S. trilobata* extracts are mainly because of the allelochemicals in the extract.

Conclusion

This study indicates the presence of water-soluble phytotoxic substances in the aqueous extracts of *S. trilobata*, which show allelopathic effects on the seed germination, root and shoot development and final yield of *P. vulgaris.* Fresh plant aqueous extracts had a more significant allelopathic effect than the dry plant extracts of *S. trilobata*. As *S. trilobata* contains water-soluble allelochemicals, it should not be recommended in biofertilizer production, phytoremediation, or live mulch.

Conflicts of Interest

The authors declare no conflict of interest.

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Journal of Experimental Biology and Agricultural Sciences, June - 2023; Volume – 11(3) page 550 – 562

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Agro-morphological characterization of selected varieties of vegetable cowpea [*Vigna unguiculata* (L.) Walp.] in Burkina Faso

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Received – February 23, 2023; Revision – May 27, 2023; Accepted – June 14, 2023
Available Online – June 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(3).550.562

KEYWORDS

Vegetable cowpea

Variety

Agro-morphological characterization

Burkina Faso

ABSTRACT

Vegetable Cowpea (*Vigna unguiculata*) is one of the neglected legumes in Burkina Faso, and as a result, its genetic diversity remains poorly known. The main aim of this study was to know its genetic variability through an agro-morphological characterization. Twenty vegetable cowpea varieties were evaluated at the Kamboinsé Environmental, Agricultural and Training Research Center following a three-replication Fischer block design under rainfed conditions. Fifteen quantitative and nine qualitative variables were collected and subjected to various statistical analyses. Analysis of variance was significant for the variables 50% flowering, vegetable cowpea date, number of pods obtained per plant, number of seeds per pod, fresh pod weight, fresh pod yield, pod length, plant height, seed length and chlorophyll content. Strong correlations were also reported between the various variables. The observed diversity is structured in three morphological groups viz., Group 1 consists of individuals with early flowering, high chlorophyll content and the number of pods obtained per plant. Group 2 brings together the varieties of average agronomic performance for pod length, the number of pods per plant, number of days at 95% maturity, fresh pod weight, yield of fresh pods and group 3 of varieties with long pods, early green date, high pod weight and good fresh pod yield. Among the tested varieties, the varieties IT83S-872 (30 pods), IT84S-2246 (27 pods), Baguette (25 pods), IT83S-818 (26 pods), and IT85F-2682 (24 pods) stood out for their high pod production. In addition, the varieties of vegetable cowpea baguette, baguette grimpant, Telma, and IT83S-911 showed the best performance in terms of early

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

vegetable cowpea date stage, longest pods, highest pod weight and best yield of fresh pods. The high genetic variability level within the tested varieties could be exploited in future green cowpea breeding programmes.

1 Introduction

Cowpea (*Vigna unguiculata*), a seed legume, is commonly grown and used as a source of diet plant protein in developing countries (Ibrahim et al. 2010). About 10% of this cowpea is cultivated for its fresh pods in East Asia and Africa (Boukar et al. 2015). In Burkina Faso, cowpea is an important staple food and much prized by the local populations. Indeed, it occupies the first place in seed legumes grown in the country and fourth in the national food crops after sorghum (1.929.835 tons), maize (1.700.127 tons) and millet (1.189.079 tons) (DSS/DGESS/MAAH 2019). However, this production only concerns dry-seed cowpea, as Common bean production is poorly developed in Burkina Faso. Vegetable cowpea has been cultivated for their long, soft, succulent and immature tender pods with fewer seeds and ripening late over a long period (Pandey et al. 2006). Like the vegetable cowpea (*Phaseolus vulgaris* L.), the vegetable cowpea is also eaten at the fresh immature pod stage. As a result, it is a subsistence food during the lean season and a source of monetary income by selling pods. Thus, it is an excellent alternative vegetable that is more resilient than bean vegetables and other crops (Peksen and Peksen 2012). However, there is little research on this variant of cowpea in Burkina Faso (Udealor 2002; Ano 2006) because generally, the majority of research on cowpeas is more focused on cowpea with dry seeds considering the green cowpea as a "neglected legume" (Ndukwe et al. 2012). Although there are varieties of vegetable cowpea in the germplasm of the Kamboinsé experimental station, Burkina Faso, very little work has been done on improving its adaptation and production conditions in the country. Nevertheless, some studies have already been undertaken on resistance to Cowpea Aphid-Borne Mosaic Virus and evaluating agronomic and forage performance (Nanama et al. 2020; Coulibaly et al. 2020). Thus, research on vegetable cowpea would allow us to know this variety better and to develop interesting and adapted varieties with agronomic and nutritional performances meeting the expectations of producers and consumers. With this in mind, this study was initiated with the general objective of knowing the agro-morphological variability of a collection of vegetable cowpea varieties to select promising varieties for use in a varietal selection program in Burkina Faso. The specific objectives of this study are (i) to establish the level of variability of the varieties studied through the measured or observed traits; (ii) to establish the relationship between the various characters, and (iii) to identify varieties of agronomic and nutritional interest.

2 Materials and methods

2.1 Experimental site

The study was done at the cowpea breeding unit of the Agricultural Research and Formation Center (CREAF), Kamboinsé, Burkina Faso, from July to September 2019. The center is located at 12°28' North latitude, 1°32' West longitude and 296 m altitude on the Ouagadougou-Kongoussi axis. During the study periods (2019-2020 agricultural season), cumulative rainfall was 782.5 mm, spread over 8 months, and August was the wettest month (MétéoInera 2019). Due to their topographical location, Kamboinsé soils are increasingly poor in organic matter and very sensitive to erosion (Zougmore et al. 2004).

2.2 Plant material

Twenty (20) vegetable cowpea varieties obtained from China and Taiwan constituted the plant material used in this study. Some specific characteristics of the selected varieties are mentioned in Table 1.

Table 1 Specific characteristics of interest of the twenty (20) varieties of vegetable cowpea used

N°	Variety	Color Seeds	Texture	N°	Variety	Color Seeds	Texture
1	IT83S-872	Cream	Smooth	11	IT83S-911	Red	Smooth
2	Ex-Iseke	Cream	Smooth	12	Niébé baguette grimpant	Red	Smooth
3	TZA 2344	Cream	Smooth	13	IT85F-2805-5	Cream	Smooth
4	RW-CP-2	Cream	Smooth	14	RW-CP-5	Red	Smooth
5	IT83S-818	White	Wrinkled	15	TUMAINI	Cream	Smooth
6	UG-CP-8	Cream	Smooth	16	LBR7	Red	Smooth
7	UG-CP-6	Cream (spotted black)	Smooth	17	Baguette	Cream	Smooth
8	IT84S-2246	Cream	Smooth	18	IT85F-867-5	Red	Smooth
9	UG-CP-3	Black	Smooth	19	Telma	Red	Smooth
10	IT85F-2682	Cream	Smooth	20	IT86F-2089-5	Red	Smooth

2.3 Methods

2.3.1 Experimental setup

This study was carried out in a randomized complete block design with three (03) replications. Each repetition comprised twenty (20) elementary plots of each corresponding variety. On each elementary plot, a variety was sown on four (04) lines of 3 m, with a spacing of 0.8 m between the lines to the line and 0.4 m between clusters, i.e. 8 clusters per line. This arrangement gives a plant population of 64 plants per elementary plot. The area of the elementary plot was 7.2 m^2 (3 m x 2.4 m), the spacing between elementary plots was 1 m, and the spacing between replicates was two meters. The total area of the trial was 871 m^2 (67 m x 13 m).

2.3.2 Conduct of the study

The soil was prepared through motorized flat ploughing followed by harrowing. Compost (organova: organic matter>30%) at a rate of 2.5 t/ha and NPK fertilizer (14-23-14-6S-1B) at a rate of 100 kg per hectare were applied to the plot before ploughing as a bottom dressing. Manual striping was done according to the spacing to obtain the seed lines. Semi-seeding was done manually on the lines on July 22, 2019, at two seeds per packet.

Maintenance operations consisted of weeding, application of NPK fertilizer (14-23-14-6S-1B), and insecticide applications. Two weeding seasons were made; the first was fourteen (14) days after sowing, during which fertilizer was applied, and the other thirty-five (35) days after sowing. Similarly, two insecticide application seasons were made. The first application was carried out at the time of flower bud formation, while the second was at the time of pod formation. Treatments were done with Deltacal 12.5 EC insecticide (12.5 g Deltametrine Concentrated Emulsion) with the recommendation of two (2) ml per liter of water.

2.3.3 Data collection

The different variables were collected according to the recommendations in the cowpea descriptors (IBPGR 1983; UPOV 2009). Sixteen (16) plants from the central rows were selected to measure quantitative and qualitative variables' observations. The studied quantitative variables are plant height, date of 50% flowering, the number of days corresponding to the ripening of fresh pods, number of pods obtained per plant, the weight of fresh pods in kilograms, date of 95% dry maturity expressed after sowing (DAS), number of seeds per pod, length and width of seeds, the weight of one hundred (100) seeds in grams, seed weight expressed in kilograms, the number of seeds per pod, the fresh pod yield calculated from the formula Yield = [(Fresh pod weight x 62500 plants/ha) ÷ (Number of plants per unit plot x 1000)] and the seed yield calculated from the formula Yield = [(Seed weight x 62500 plants/ha) ÷ (Number of plants per unit plot x 1000)]. The studied qualitative characteristics are Plant type or stem habit (Creeping, Erect, Semi-erect), Leaf texture (Membranous, leathery), Leaf color (Dark green, Light green), Leaf shape (Hastate, Sub-hastate, Globular and Sub-globular), Flower color (White, Purple), Pod position on peduncles (Erect, curved), green pod shape, pod color, seed color (Cream, Black, White, Red) and seed size as described by Ebong (1970).

2.3.4 Statistical analysis of the collected data

An analysis of variance (ANOVA) and Pearson's correlation test were performed to determine the varieties' discriminating characteristics and the relationships between the variables. An estimate of the degrees of association between the different quantitative traits studied was made through a Principal Component Analysis (PCA). Thus, using the STATISTICA software, well-represented and poorly correlated variables were analyzed in hierarchical ascending classification (HAC) to structure the studied varieties. The different groups from the hierarchical ascending classification (CAH) were characterized by discriminant factor analysis (DFA). The ANOVA, PCA, and DFA were performed with XLSTAT 2016 software.

3 Results

3.1 Variation in quality characteristics of vegetable cowpea

The results of the qualitative characteristics analysis showed the existence of significant variability (Table 2). Thus, four modalities, namely erect, semi-erect, climbing, and creeping, were observed in terms of plant habit type (Figure 1). Thirty-five percent (35%) of the varieties studied showed a semi-erect habit corresponding to most of the collection. On the other hand, the upright varieties (30% of the collection) are characterized by an erect main stem from which secondary branches emerge. While 30% of varieties are creeping, and the rest 5% are climbing type.

Flower color is highly variable among the studied varieties and is classified into two broad categories (Figure 2). Among the studied varieties, about 15% of the varieties produced white flowers, while the rest 85% had purple flowers.

Further, in the case of pod positions, two types of pod positions on the peduncle were observed (Figure 3). In most varieties (85%), the pods are curved at 30-60° while the rest (15%) are erect. Regarding pod shape, 90% of the varieties had linear pods, and only 10% had curved pods (Figure 4).

The selected variants were also identified based on the predominance of pod color, and it found that 70% of the pods are dark green color while the rest 30% are light green pods color characteristics (Figure 5).

Table 2 Variation in quality characteristics in the selected cowpea varieties

Parameters	Modalities	Number of variety	%
Wearing of the stem	Erected	6	30
	Semi-dry	7	35
	Climbing	1	5
	Rampant	6	30
Leaf shape	Hasté	1	5
	Subhasted	8	40
	Subglobular	8	40
	Globular	3	15
Leaf texture	Membrane machine	8	40
	Tough	12	60
Color of the flower	White	3	15
	Violet	17	85
Position of the pod on the stalk	Curved	17	85
	Dressed	3	15
Color fresh pods	Light green	10	50
	Dark green	10	50
Fresh pod shape	Linear	2	10
	Curved	18	90
Color of the seeds	Cream (mottled black)	1	5
	Cream	10	50
	Black	1	5
	White	1	5
	Red	7	35
Seed size	Great	1	5
	Average	18	90
	Small	1	5

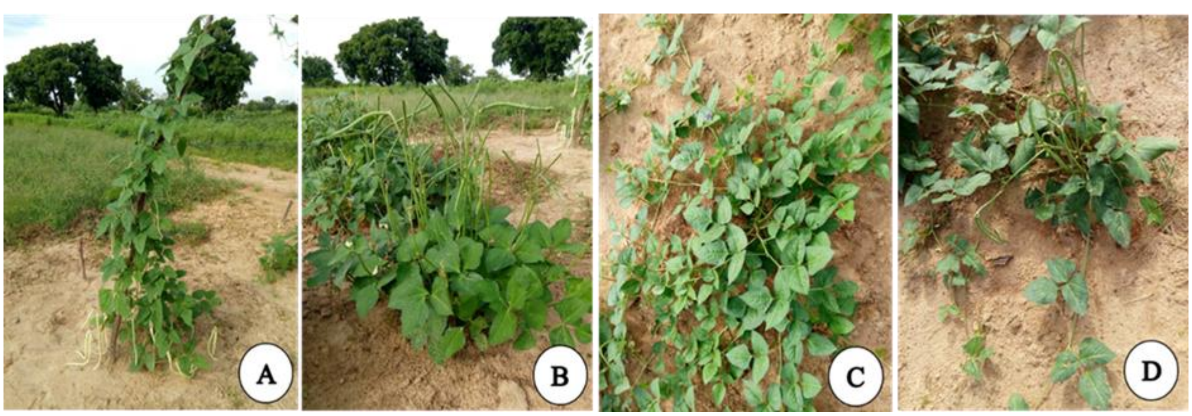

Figure 1 Different types of bearing observed in the varieties studied A: Climbing type, B: Erect type, C: Creeping type, and D: Semi-erect type.

Figure 2 Flower color A: Violet color, B: White color

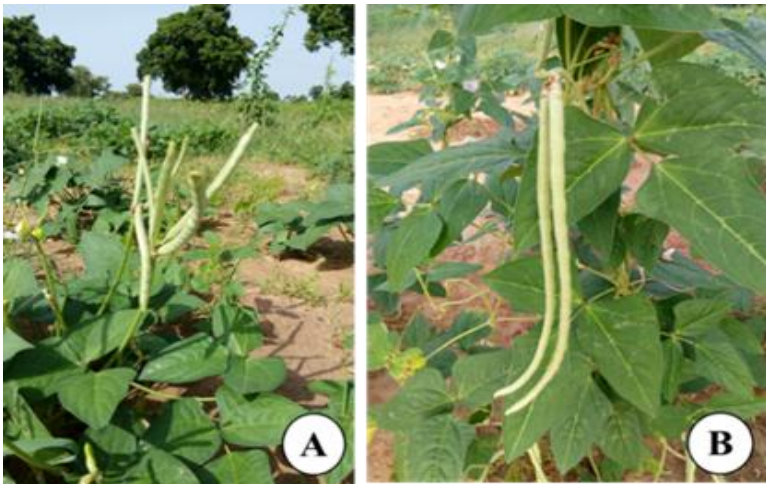

Figure 3 Position of pods on stalk A: upright position. B: curved position

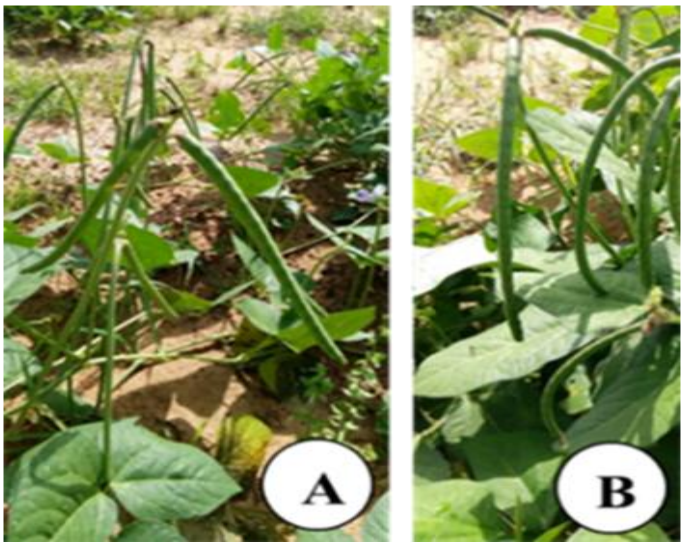

Figure 4 Pod shape. A: linear pods. B: curved pods

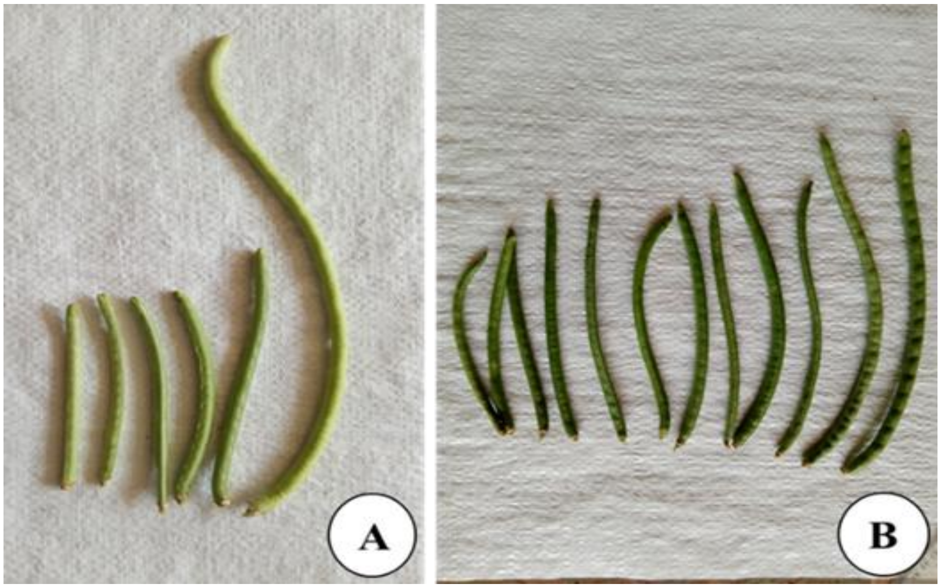

Figure 5 Fresh Pod color A: Light green. B: Dark green

Table 3 Results of analysis of variance of phenological parameters of 20 vegetable cowpea varieties.

Variables	Minimum	Maximum	Average	CV (%)	P-Value (5%)
Plant height (cm)	31.67	265	113.92±34.6	58	0.000**
50% Flowering (DAS)	45	51	47±1.37	5	0.000**
Date vegetable cowpea (DAS)	54	57	55±0.79	2	0.005*
95%Maturity (DAS)	65	73	70±1.96	4	0.006*

*Analysis of variance significant at the 5% level; **Analysis of variance highly significant at the 1% level; CV: Coefficient of variation; P-value - Probability value at 5%; DAS - Day After Sowing

3.2 Variation in quantitative characteristics

3.2.1 Phenological stages

The analysis of variance (Table 3) shows that plant height and date 50% flowering discriminate significantly between varieties at the 1% threshold and the vegetable cowpea date and date 95% maturity significantly from varieties at the 5% threshold. Thus, the height of the plants varied from 31.66 cm (IT86F-2089-5) to 265 cm (baguette grimpant), with an average of 113.917 cm. With an average number of days to 50% flowering of 47 days, the varieties Ex-Iseke; IT85F-2682; IT83S-911 and UG-CP-8 (45 days to 50% flowering) were the first to flower, and varieties UG-CP-6 and TZA 2344 bloomed at 50 JAS. On average, vegetable cowpea varieties reached their vegetable cowpea dates and 95% maturity at 55 JAS and 70 JAS. Thus, the baguette grimpant vegetable cowpea varieties, IT83S-872, IT84S-2246, Telma, IT85F-2805-5, IT83S-911, LBR7, IT85F-867-5, UG-CP-8 reached their vegetable cowpea date as early as the 54th JAS and the TZA 2344 variety at the 57th JAS. Vegetable cowpea varieties IT83S-911 and UG-CP-8 were the earliest (64 and 65 days to maturity) compared with cowpea varieties RW-CP-5, TZA -2344 and baguette grimpant (72 days to maturity). The coefficient of variation was high (CV > 30%) for plant height (58%) but low (CV < 30%) for 50% flowering (CV= 5%), vegetable cowpea date (CV= 2%) and 95% maturity (CV= 4%).

3.2.2 Yields and their components in fresh pods and seeds

The analysis of variance for the parameters of fresh pod yield and its components (Table 4), showed that all the variables discriminate very highly between varieties at the 1% level except for the variable fresh pod yield, which discriminates significantly between varieties at the 5% level.

The average pod length was reported to be 17.07 cm (Figure 6), while the number of pods per plant varied from 9 to 30 pods, with an overall average of 18 pods. Among the tested 20 varieties, the cowpea variety Baguette climber produced the longest pods (28.88 cm), followed by Telma (23.11 cm) and Baguette (21.72 cm). In contrast, the shortest pods (12.51 cm) were recorded in IT83S-818. The highest number of pods (30) was produced by IT83S-872, followed by IT84S-2246 (27), and the lowest number was observed in variety TZA 2344 (9 pods).

Table 4 Results of analysis of variance of pod and seed variables of 20 vegetable cowpea varieties

Variables	Minimum	Maximum	Average	CV (%)	P-Value (5%)
Pod length (cm)	12.51	28.88	17.07±0.78	23	0.000**
Number of pods/plants	9	30	18±3.78	41	0.000**
Weight of fresh pods (kg)	0.01	0.54	0.29±0.11	67	0.000**
Fresh pod yield (t/ha)	0.72	13.16	3.94±2.51	99	0.002*
Number of Seeds/Pod	14	59	29±7.2	43	0.000**
Weight Seeds (kg)	0.08	0.48	0.25±0.21	52	0.008*
Weight 100 Seeds (g)	11.03	19.5	15.1±0.99	16	0.000**
Grain yield (t/ha)	1.67	5.1	3.05±1.12	51	0.056ns

**Analysis of variance highly significant at 1%; *Analysis of variance significant at 5%; ns - Analysis of variance not significant at the 5% level; CV - Coefficient of variation; P-value - Probability value at 5%.

Figure 6 Variability in pod length of vegetable cowpea

For the tested varieties, the average pod yield (t/ha) was 3.94 tons/hectare, with 0.30 kg as the average fresh pod weight. The maximum pod yield was recorded in the baguette variety (13.16 t/ha), followed by Ex-Iseke (8.56 t/ha) and climbing baguette (7.12 t/ha). The variety TZA 2344 produced the lowest yield of fresh pods (0.72 t/ha). While the baguette variety had the highest pod weight (0.54 kg), while the lowest was recorded in the TZA 2344 variety (0.06 kg). Apart from pod length with a 23% coefficient of variation, the other variables had a coefficient of variation greater than 30%. Analysis of variance of seed length, seed width, number of seeds per pod and weight of 100 seeds was highly significant (p = 0.0001) and significant (P = 0.008) for seed weight (Table 4). The average number of seeds per pod ranged from 14 to 59, with 0.25 g as the average seed weight per elementary plot. The high number of seeds per pod was recorded in varieties IT83S-818, with 59 seeds per pod, while the lowest number of seeds was reported for the variety RW-CP-5 (14 seeds per pod). Variety IT83S-872 stood out with the highest seed weight with a value of 0.47 kg, and RW-CP-5 obtained the lowest seed weight of 0.07 kg. Regarding the weight of 100 seeds, the average weight was 15.10g, and it was reported highest (19.5 and 18.067 g) for the varieties UG-CP-6 and RW-CP-5, respectively, while for the varieties UG-CP-8, IT86F-2089-5 lowest 100-seed weights were recorded with 11.03 g and 11.43 g respectively. Coefficients of variation were high for the number of seeds per pod (CV = 43%), seed weight (CV = 52%) and seed yield (CV = 51%).

3.3 Relationships between quantitative Characteristics

The relationships between the studied traits revealed various correlations (Table 5). Strong and positive correlations were obtained between fresh pod yield, pod length (r = 0.37) and fresh pod weight (r = 0.51). Further, a negative correlation was observed between fresh pod yield and the number of days to 50% flowering (r = -0.37). Also, the number of days to 50 % flowering was strongly and positively correlated with the date of vegetable cowpea (r = 0.7)

Table 5 Correlation matrix between the different variables studied

Variables	PL	PH	50%Flo	DNV	95%Mat	NGP	NGG	PGr	PGFr	RoWGr	CCP	Rdt Gr
PL	1											
PH	0.562**	1										
50%Flo	-0.183	-0.049	1									
DNV	-0.322*	-0.159	0.669**	1								
95%Mat	0.079	0.039	0.445**	0.238	1							
NGP	-0.222	-0.346*	-0.459**	-0.289	-0.136	1						
NGG	-0.166	0.091	-0.472**	-0.192	-0.071	0.413**	1					
PGr	-0.126	-0.16	-0.298**	-0.234	0.043	0.243	0.399*	1				
PGFr	0.455**	0.11	-0.488**	-0.421*	-0.106	0.303*	0.152	0.397*	1			
RoWGr	0.366*	0.318*	-0.369*	-0.186	-0.048	0.302*	0.341*	-0.197	0.511**	1		
CCP	0.117	0.362*	-0.091	-0.165	0.056	0.089	0.11	-0.196	0.113	0.358*	1	
Rdt Gr	-0.052	0.246	-0.393*	-0.247	0.051	0.418*	0.734**	0.191	0,055	0.505**	0.268*	1

*significant, **highly significant, PL - Pod length (cm), PH - Plant height (cm), 50 % Flo - 50 % Flowering (JAS), DNV - Green cowpea date (JAS), 95 % Mat - 95 % Maturity (JAS), NGP - number of seedlings pods, NGG - number of seeds per pod, PGr (kg) - seed weight, PGFr (kg) - fresh pod weight, PCG (g) - hundred seed weight, RdtGFr - fresh pod yield(t/ha), RdtGr - grain yield (t/ha)

Table 6 Eigen values and contribution of the characters expressed by the first two (02) axes of the principal component analysis

Main Components	F1	F2
Eigen value	3.785	2.917
Total variance (%)	25.233	19.448
Total cumulative variance (%)	25.233	44.68
Characters defining the axes and their eigenvalues		
LG	-0.145	0.49
HP	-0.113	0.401
LGr	-0.121	0.392
LaGr	-0.118	0.227
50%Flo	0.415	0.061
SPAD	-0.182	0.392
DNV	0.338	-0.103
95%Mat	0.116	0.063
NGP	-0.31	-0.308
NGG	-0.335	-0.247
Weight Gr	-0.188	-0.17
PGFr	-0.341	0.146
RoWGr	-0.326	0.117
Weight 100 Gr	-0.177	0.18
Rdt Gr	-0.328	-0.129

LG - pod length (cm), HP - plant height (cm), LGr - Grain length (mm), LaGr - Grain width (mm), 50% Flo - 50% Flowering (JAS), SPAD - Chlorophyll content, DNV - Vegetable cowpea date (JAS), 95% Mat - 95% Maturity (JAS), NGP - Number of pods plants, NGG - number of seeds per pod, PGr (kg) - seed weight, PGFr (kg) - fresh pod weight, PCG (g) - hundred seed weight, RdtGFr - fresh pod yield(t/ha), RdtGr - seed yield (t/ha), F1 and F2 are axes and the number of days to 95 % maturity (r = 0.45). However, the number of days to 50% flowering was negatively correlated with fresh pod weight (r = -0.49) and the number of pods per plant (r = -0.46). The strong correlation between vegetable cowpea date and fresh pod weight is negative (r = -0.42). In addition, a positive and strong correlation was obtained between seed yield and the number of seeds per pod (r = 0.73). A strong and positive correlation was observed between the number of seeds per pod and seed weight (r = 0.4).

3.4 Structuring diversity

Principal component analysis (PCA) yields two axes (F1 and F2), explaining 25.23% and 19.44% of the total variability, respectively. The first two components, which absorb 44.68% of the variance, were selected to analyze the agro-morphological variability of the varieties, with an eigenvalue ranging from 2.92 to 3.79 (Table 6). Axis 1 positively associates the number of days to 50% flowering (r = 0.42), vegetable cowpea date (r = 0.34), while it negatively associates the number of pods obtained per plant (r = -0.31), number of seeds per pod (r = -0.34), the weight of fresh pods (r = -0.34), the yield of fresh pods (r = -0.33) and the yield of seeds (r = -0.33). This axis can be defined as the vegetable cowpea cycle and fresh pod yield axis. Axis 2 positively associates pod length (r = 0.49), plant height (r = 0.40) and seed length (r = 0.39). It can be defined as the axis of plant, pod and seed size. F1 and F2 are the two axes of the principal component analysis benchmark.

3.5 Organization of the diversity of vegetable cowpea varieties

The hierarchical ascending classification (HAC) carried out based on the quantitative discriminant variables made it possible to divide the varieties studied into three (3) distinct groups (Figure 7). Among these, Group 1 consisting of three (3) individuals, includes varieties IT84S-2246, IT83S-872 and IT83S-818 while group 2 consisting of five (5) individuals including the varieties TZA 2344, RW-CP-5, Tumaini, UG-CP-5 and RW-CP-2 and the group 3 is made up of twelve (12) individuals and is composed of the varieties Baguette, Ex-Iseke, IT83S-911, Niébé baguette grimpant, IT83S-867-5, IT85F-2682, IT85F-2805-5, IT86F-2089-5, LBR7, Telma, UG-CP-3 and UG-CP-8.

3.6 Characteristics of the groups formed by the HAC

The results of the differentiations factor analysis (Figure 8) were used to characterize the three groups based on traits related to the vegetable cowpea cycle (the date at 50% flowering of plants and vegetable cowpea date), pod yield and its components and plant size. Group 1 includes varieties characterized by early flowering (46 days) and the high number of pods obtained per plant (28 pods). Group 2 includes varieties with average agronomic performance for pod length, number of pods obtained per plant, date at 95% maturity of the pods, fresh pod weight and fresh pod yield. Group 3 includes the best-performing varieties. These varieties are characterized by a high pod length (28.88 cm), an early vegetable cowpea date (55 JAS), a high pod weight (0.54 kg) and a high fresh pod yield (13.16 t/ha).

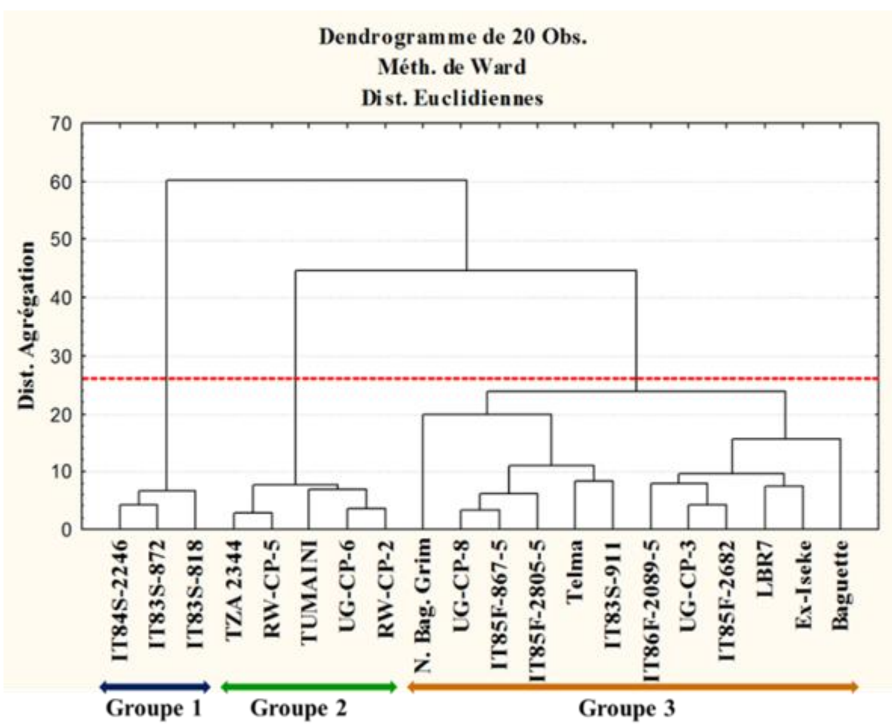

Figure 7 Dendrogram from the hierarchical ascending classification of twenty (20) vegetable cowpea varieties

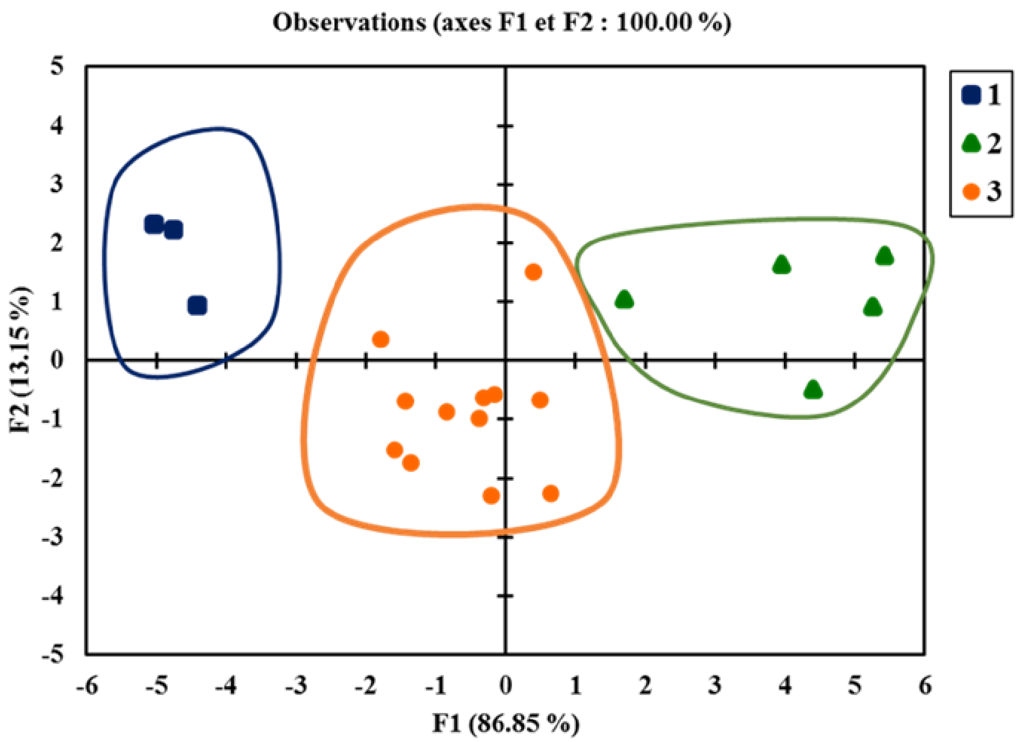

Figure 8 Representation in the ½ plane of the SFM of the variety groups from the HAC

4 Discussion

The results of analyses highlighted a high variability within the studied varieties at the qualitative and quantitative levels. Indeed, the high values of the coefficient of variation for several characters and the number of modalities per qualitative character would indicate a high heterogeneity within the studied material (Boyé et al. 2016). This heterogeneity between varieties is apparent in the principal component analysis with its two components to reflect the diversity within cowpea varieties. The principal component analysis makes it possible to retain the relevant parameters for the realization of a study and thus save time (Abe et al. 2015). This variability resulted in the existence of several discriminating traits and provided opportunities for selection.

The high proportions of the qualitative variables were obtained in the different plant organs' color, shape and type. However, a difference was observed in flower color compared to those observed by Cobbinah et al. (2011), who obtained white-purple flowers in their work in Ghana. This difference is explained by low variability within the material tested for this character. Since the pigmentation of flowers, pods and seed coats in cowpeas is expressed by a single gene, the relationship between flower color and other traits may be useful in selecting important or economic traits (Egbadzor et al. 2014). The type of semi-erect habit within the studied material indicates that these varieties can be retained in a breeding program. Indeed, according to Animasaun et al. (2015), erect plants with the advantage of being less attacked by rodents could also be harvested using mechanical harvesters. Plant habit types are also important in the choice of seeding spacing and the choice of cropping system. Hall et al. (1997) state that climbing and erect plant types are used in monoculture or intercropping production systems. Pod color is an important characteristic in consumer choice (Manju 2006). Indeed, of the observed pod colors, there is a general preference for greener pods in vegetable cowpea (Peksen and Peksen 2012). The dark green color of the pods could therefore have high photosynthetic activity of the plant. Thus, the varieties with green-dark pods may offer the preferred color to the producers and consumers.

Lovely and Radhadevi (2017) observed significant differences in quantitative variables with vegetable cowpea varieties in Kerala, India. The mean flowering cycle (47 JAS) and that of vegetable cowpea date (55 JAS) show that the studied varieties have an early cycle. This early cycle indicates that these varieties could be grown in Burkina Faso, as it is a criterion of choice. Indeed, according to Doumbia et al. (2013), early-flowering varieties are a solution to adapt to the effects of climate change. In addition, according to Vural et al. (2000), the harvest date of vegetable cowpea pods is between 5 and 9 weeks, depending on the environment. Therefore, the average date for harvesting fresh pods would be reached from the seventh (07) week after sowing for semi-early varieties. These flowering cycles and vegetable cowpea stages of the studied varieties differ from those obtained on vegetable cowpea varieties

(Coulibaly et al. 2020). These differences in the number of flowering days and vegetable cowpea dates between varieties would depend on their genotypes. According to Pandey et al. (2006), the difference in the vegetable cowpea cycle is due to the growing environment or the genetic makeup of the varieties. Harvest time is very important to avoid the presence of fiber in fresh pods, which consumers do not appreciate (Peksen 2004). Pandey et al. (2006) indicate that the optimal harvest stage is also important for the vegetable market. Therefore, the pod harvesting period is an important parameter for selecting high-yielding vegetable cowpea cultivars (Kutty et al. 2003). Indeed, this harvest period could be predicted from the dates of flowering or maturity of the pods (Cobbinah et al. 2011), and the strong and positive correlation between flowering and vegetable cowpea date reveals this. Thus, plants that reach their flowering date and vegetable cowpea date very early are precocious. Coulibaly (2018) also mentioned a similar correlation between flowering date, and vegetable cowpea date with varieties grown in Burkina Faso. The mean value of 70 JAS of the 95 % pod maturity date indicates that all the study varieties are early maturing. Early cycle varieties have a maturity between 67 and 70 days, and those with a long cycle have a maturity between 74 and 77 days (N'gbesso et al. 2013). These results are close to those of Coulibaly (2018), who in his work on 12 varieties of vegetable cowpea, observed a maturity between 60 and 70 days. The earliness of vegetable cowpea varieties could help to cope with the constraints of scarce rainfall at the end of the season.

Differences in varietal performance related to fresh pod yield and yield components are thought to be explained by the growing environment or by the genetic makeup of the varieties. These differences are reflected in the structuring into three groups of varieties given by the hierarchical ascending classification. Indeed, according to Pandey et al. (2006), the yield in fresh pods is the determining variable for selecting a particular variety for marketing. Thus, Telma (5.37 t/ha), IT85F-2805-5 (6.03 t/ha), baguette grimpant (7.12 t/ha), Ex-Iseke (8.56 t/ha), and Baguette (13.16 t/ha), were distinguished by their high pod yield. The results of the present study are superior to those of Nwofia (2012), who found fresh pod yield which ranged from 4.5 to 9.57 t/ha in cultivars IT 93k -915, IT86F- 2062-5 and IT81D-1228-14 on vegetable cowpea cultivars in Nigeria. In addition, for Peksen and Peksen (2012), the most important yield components for vegetable cowpea are pod length, pod count per plant, and pod weight. Pandey et al. (2006) identified pod length as one of the key factors in selecting vegetable cowpea varieties. According to Kutty et al. (2003), the number of pods per plant exerts the most important direct effect on fresh pod yield. These effects are reflected in the strong positive correlations between fresh pod yield and pod length, number of pods per plant and fresh pod weight. These correlations corroborate with Peksen (2004), who found that fresh pod yield was correlated with pod length, number of pods obtained per plant and average pod weight. Thus, direct selection is possible for different selected traits. For this purpose, the varieties baguette grimpant, Telma, Baguette, IT83S-911, IT83S-872, IT84S-2246, IT83S-818, IT85F-2682, and LBR7 respond better to the agronomic characteristics expected from vegetable cowpea. Thus, varieties from group 3 of the hierarchical ascending classification with long pods, early vegetable cowpea date, high pod weight and high fresh pod yield in the cross with individuals from group 1 have early flowering, and the high number of pods obtained per plant would allow the creation of early and high yielding varieties. To create vegetable cowpea varieties with high fresh pod yield, it is important to consider pod harvesting time, pod length, number of pods obtained per plant and average pod weight.

The number of seeds per pod, ranging from 14 to 59 seeds, is higher than that obtained by Egbadzor et al. (2014), which varied from 9 to 21 seeds. According to Boyé et al. (2016), the variability observed within varieties concerning the number of seeds per pod reflects their ability to mobilize assimilates to reserve structures. Concerning the weight of 100 seeds, Khan et al. (2010) showed that the variability obtained between varieties depends on genotype and climatic factors. According to Khan et al. (2010) a very significant variation was reported in the 24 cowpea genotypes, and in this, the climatic adaptation factor could also play a role in the increase of seed weight. As a result, vegetable cowpea varieties with the highest number of seeds per pod and weight of 100 seeds could be produced as both seed cowpea and vegetable cowpea. The strong negative correlations between flowering date, the number of pods obtained per plant and fresh pod weight mean that late flowering plants have fewer pods per plant and a lower pod weight, as confirmed in the varieties TZA 2344, RW-CP-2, RW-CP-5, TUMAINI.

Conclusion

The agro-morphological characterization study revealed the existence of significant variability within the studied varieties, which could be exploited and used in a cowpea breeding program for traits of interest. The current study showed that out of all the studied 20 varieties, 12 varieties were identified for their morphological and agronomic performance, which could constitute a base of varieties of interest. The flowering cycle and date of vegetable cowpea, pod length, the number of pods obtained per plant, pod weight and the yield of fresh pods were identified as expected traits of interest for vegetable cowpea in the climatic and economic context of Burkina Faso. Among the tested varieties, IT83S-872 (30), IT84S-2246 (27), Baguette (25), IT83S-818 (26), and IT85F-2682 (24) were thus identified for their high number of pods per plant. The varieties Baguette, baguette grimpant, Telma, and IT83S-911, which reached their vegetable cowpea stage early, have the longest pods and a high fresh pod yield. These varieties are, therefore, potential parents that meet growers' selection criteria. After genetic improvement and multi-local testing, they could be recommended for the extension. Strong correlations were reported between various quantitative traits, which could facilitate the selection of traits of interest to producers and consumers by reducing the number of parameters to be collected. In addition, this study made it possible to classify the varieties into three groups according to their performance.

Declaration of competing interest

The authors expressed no conflicts of interest in this study.

Acknowledgements

We are grateful to the researchers of the CREAF Plant Genetics and Biotechnology Laboratory for their support with equipment and to the members of the Plant Genetics and Improvement team (UJKZ) for their contribution to the manuscript.

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Journal of Experimental Biology and Agricultural Sciences, June - 2023; Volume – 11(3) page 563 – 571

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Isolation and characterization of chlorpyrifos-degrading bacteria in tea-growing soils

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Received – April 13, 2023; Revision – June 18, 2023; Accepted – June 25, 2023
Available Online – June 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(3).563.571

KEYWORDS

CPF

Degradation

E. adhaerens VNN3

M. populi CNN2

Remediation

ABSTRACT

The excess use of pesticides in the agricultural sector has caused environmental pollution and affected the complete ecosystem. Among the various commonly used pesticides, chlorpyrifos (CPF) is widely used against multiple agrarian pests due to its effectiveness and higher insecticidal activities. However, along with its beneficial usage, CPF has various residual effects on the environment, causing multiple negative impacts on aquatic organisms and human health. Consequently, methods for eliminating CPF in the background are essential. Among the currently available approaches to CPF remediation, biological methods using microorganisms are eco-friendly and cost-effective. Therefore, this study was conducted to isolate and characterize chlorpyrifos-degrading bacteria from the tea-growing soil of Vietnam. For this, soil samples were collected from the 20 tea-growing areas of Vietnam. From the collected samples, three bacterial strains viz., *Methylobacterium populi* CNN2, *Ensifer adhaerens* VNN3, and *Acinetobacter pittii* CNN4 have been isolated by using streak plate method and identified based on 16S rRNA gene analysis. The study results showed that under laboratory conditions, *E. adhaerens* VNN3 had the highest CPF degradation ability and was followed by the strain *M. populi* CNN2. In liquid medium, CPF concentration (100 mg/L) was reduced by 95.2% and 81.4% by *E.adhaerens* VNN3 and *M. populi* CNN2, respectively, after 72 h. Further, under *in-vitro* conditions, the concentration of CPF was reduced from 500 mg/kg to 112 ± 1.73 (77.6%) and 197 ± 2.08 mg/kg (60.6%) by *E. adhaerens* VNN3 and *M. populi* CNN2, respectively. Based on the obtained results, it can be concluded that *E. adhaerens* VNN3 and *M. populi* CNN2 can be used for CPF-contaminated agricultural soil remediation.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI]
(http://www.horizonpublisherindia.in/).

1 Introduction

Organophosphorus (CPF) pesticides have been widely used in agriculture to control pests and insects. However, their extensive usage has caused serious environmental pollution and ecological risks (Huang et al. 2021). Chlorpyrifos (o,o-diethyl-o-3,5,6-trichloro-2-pyridinyl phosphorothionate) (CPF) is an organophosphate pesticide that has been widely used for pest control (Bose et al. 2021). It has been reported that the half-life of CPF in the soil was 60 - 120 days, depending on the soil types and environmental conditions (Elshikh et al. 2022; Huang et al. 2021). Further, 3,5,6-trichloro-2-pyridinol (TCP), a major metabolite of CPF, is also persistent in the environment with a half-life of 65 – 350 days in soil (Bose et al. 2021). Due to the wide application of CPF in agriculture, CPF and its residues have been detected in surface water, groundwater, soils, sediments, crops and even human breast milk (Huang et al. 2021; Oltramare et al. 2022; Sishu et al. 2022; Takayasu et al. 2017). The CPF concentration range from 239 to 675.4 µg/kg was determined in agricultural soils (Bhandari et al. 2020; Tan et al. 2020). The adverse effects of CPF on organisms and human health have also been well-documented (Jin et al. 2015; Huang et al. 2021; Cheng et al. 2023). Bioaccumulation of CPF affects the human endocrine and cardiovascular systems (Bose et al. 2021); furthermore, CPF and its metabolites can disrupt normal pregnancy, induce the occurrence of obesity and breast cancer (Blanco et al. 2020; Hazarika et al. 2020; Moyano et al. 2020). Underexposure to CPF, oxidative stress and disruption of neurotransmitter metabolism were observed in zebrafish (Sud et al. 2020). In addition, the genotoxicity effects of CPF have also been recorded in various aquatic organisms (Huang et al. 2020). Due to the ecological risks that CPF poses to the environment, the elimination of CPF is necessary. Current methods such as ultrasonication, photocatalytic degradation, and biochar adsorption have been used for CPF removal; however, these physicochemical methods are costly and technically challenging (Soltani-Nezhad et al. 2020; Huang et al. 2021). Biological methods using microorganisms that degrade organic compounds and complex xenobiotics are considered environmental-friendly and efficient approaches to detoxify CPF (Bose et al. 2021; Elshikh et al. 2022; Hadibarata et al. 2023). The CPF-degrading potential of various bacterial (*Pseudomonas*, *Stenotrophomonas*, *Bacillus*, *Klebsiella*) and fungal species (*Aspergillus terreus*, *Trichoderma harzianum*, *Ganoderma* sp. JAS4) have been well documented (Ishag et al. 2016; Khalid et al. 2018; Bose et al. 2021). Phosphoric triester hydrolases, lignolytic laccases, and carboxypeptidase (CPD) are some enzymes involved in the microbial degradation of CPF (Bose et al. 2021; Huang et al. 2021). Biodegradation of CPF is of great importance in eliminating CPF and reducing its toxicity in the environment; therefore, studies on microbial CPF degradation are highly significant. The present study aimed to isolate and characterize CPF-degrading bacteria in tea-growing soils and evaluate the potential of CPF degradation by the isolated strains.

2 Materials and methods

2.1 Isolation of CPF-degrading bacteria

The isolation of CPF-degrading bacteria was performed as per the method of Hossain et al. (2015) and Elshikh et al. (2022) with some modifications. For the bacterial isolation, 20 soil samples were collected from a depth of 20 cm of the tea-growing areas of Phu Thinh commune, Dai Tu district, Thai Nguyen province and Anh Son commune, Hung Son district, Nghe An province of Vietnam. Each soil sample was prepared by properly mixing the five diagonally taken from the selected field. The bacterial isolation was performed using the mineral salt medium (MSM) containing (g/L) K_2HPO_4 (1.8), NH_4Cl (4.0), $MgSO_4.7H_2O$ (0.5), $FeSO_4.7H_2O$ (0.1) and trace solution (1 ml/L). From this, a 5 g soil sample was added to 95 mL of the mineral salt medium (MSM) containing 50 mg/L CPF and the cultured flasks were incubated at 28 - 30^{o}C in an incubator for five days. After the incubation period completion, 5 mL was taken out and reinoculated in the fresh MSM medium containing CPF at 50 mg/L. The procedure was repeated three times, and the culture was diluted at a concentration of 10^{-3} to 10^{-5}. 50 µl of the diluted culture was spread on agar plates containing MSM medium supplemented with 100 mg/L CPF. The plates were incubated for five days at 28 - 30^{o}C, and the isolated strains were purified by streaking on the agar plates supplemented with 100 mg/L CPF.

2.2 Growth of the isolated strains and microbial density determination

The growth characteristics of the isolated strains were determined by using LB medium containing (g/L): peptone (10), yeast extract (5), NaCl (5) and 100 mg/L of CPF. After 24, 48, 72 and 96 h, the measurement of optical density (OD) at 620 nm was carried out. The bacterial density was determined by using agar plates and sample dilution at 10^{-7} - 10^{-9} concentrations.

2.3 Identification of the isolated strains using 16S rRNA analysis

According to Ausubel et al. (1994), DNA isolation was carried out. The 16S rRNA gene amplification was carried out by using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (3'-TACGGTTACCTTGTTACCGACTT-5'). The PCR was performed with a total volume of 25 µl per sample having total DNA (1 µl), Pr16F (1 µl), Pr16R (1 µl), 10 mM dNTPs (2 µl), Taq polymerase (0.25 µl), Buffer Taq polymerase 10 X (5 µl) and deionized water. The carried out thermal cycle was as follows: (1) initial denaturation at 94^{o}C for 3 min, (2) denaturation at 94^{o}C for 1 min, (3) annealing at 55^{o}C for 1 min, (4) extension at 72^{o}C for 2 min. Steps 2-4 were repeated in 30 cycles, and the final extension was conducted at 72^{o}C for 7 min. The PCR product was quality-checked via agarose gel electrophoresis (0.8% agarose) at 100V for

30 minutes. The 16S rRNA gene sequences were submitted to the Genbank database.

2.4 Evaluation of CPF degradation by the isolated bacterial strains

The isolates were cultured in MSM medium supplemented with 100 mg/L CPF until the OD_{620} value of 0.6 was reached. After 24, 48 and 72 h, the CPF concentration of the samples was determined, and the CPF degradation ability of the isolated strains in the cultured medium was determined.

The CPF degradation capability of the isolated strains was also evaluated under *in-vitro* conditions. For this, 20 soil substrates were collected, and 500 mg/kg CPF was added. The soil substrate was inoculated with selected isolated strains in MSM solution (OD_{620} ~ 0.6) (10 mL/kg soil sample). The samples were incubated at 30^0C for 10 days. The degradation of the residual CPF in soil samples after 3, 7 and 10 days were determined.

2.5 Determination of CPF concentration

CPF in liquid samples was extracted by using petroleum ether for 1 minute. The supernatant containing CPF was measured at 294 nm by spectrophotometric method. The CPF samples were shaken with 5 mL of acetonitrile and filtered using a 0.22 µm syringe filter. CPF concentration was measured using HPLC at 300 nm (Elshikh et al. 2022). The column used was the XDB-C18 (5 Mm, 4.6x250 mm). The mobile phase was a mixture of acetonitrile and buffer containing acetic acid and water. The retention time of CPF was 9.5 min. The residual CPF concentration in soil samples was determined by GC/MS (EPA 1996).

2.6 Statistical analysis

Data were presented as the mean values of three replicates ± standard deviation using Microsoft Excel version 2108.

3 Results and discussion

3.1 Isolation of CPF-degrading bacteria

From the collected 20 soil samples, a total of 51 bacterial strains were isolated, and among these, four CPF-degrading bacterial strains were isolated, namely CNN1, CNN2, VNN3 and CNN4. Figure 1 shows the colonies of the four isolated strains. Figure 2 shows the morphologies of the bacterial cells under an optical microscope with 100x magnification, respectively. All four strains had rod shape, single stand cells. CNN1 and CNN4 were approximately 0.5 x 1.0 µm, whereas CNN2 and VNN3 were 0.8 x 1.0 µm and 1.0 x 1.5 µm in size, respectively. The colonies of CNN1 were round, glossy, ivory-yellow, and non-pigmented (diameter: 1 - 3 mm). The colonies of CNN2 were pale pink, uniformly round, smooth, round, convex, glossy and non-pigmented (diameter: 0.5 mm). The colonies of VNN3 were white, round, small, regular, smooth surfaces, and non-pigmented

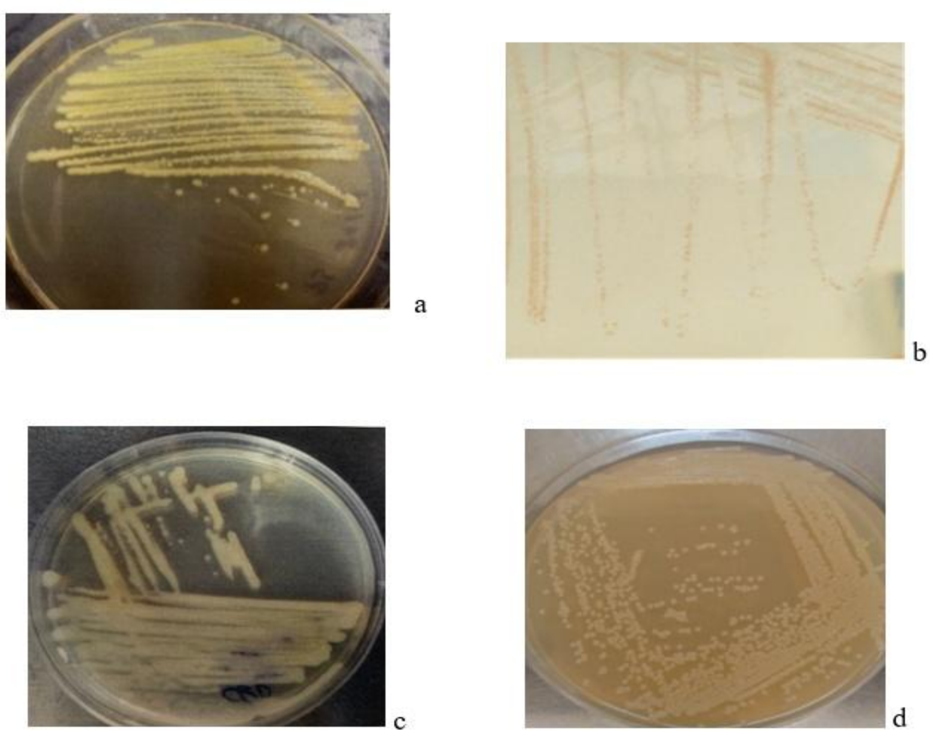

Figure 1 Colonies of the strains (a) CNN1, (b) CNN2, (c) VNN3 and (d) CNN4

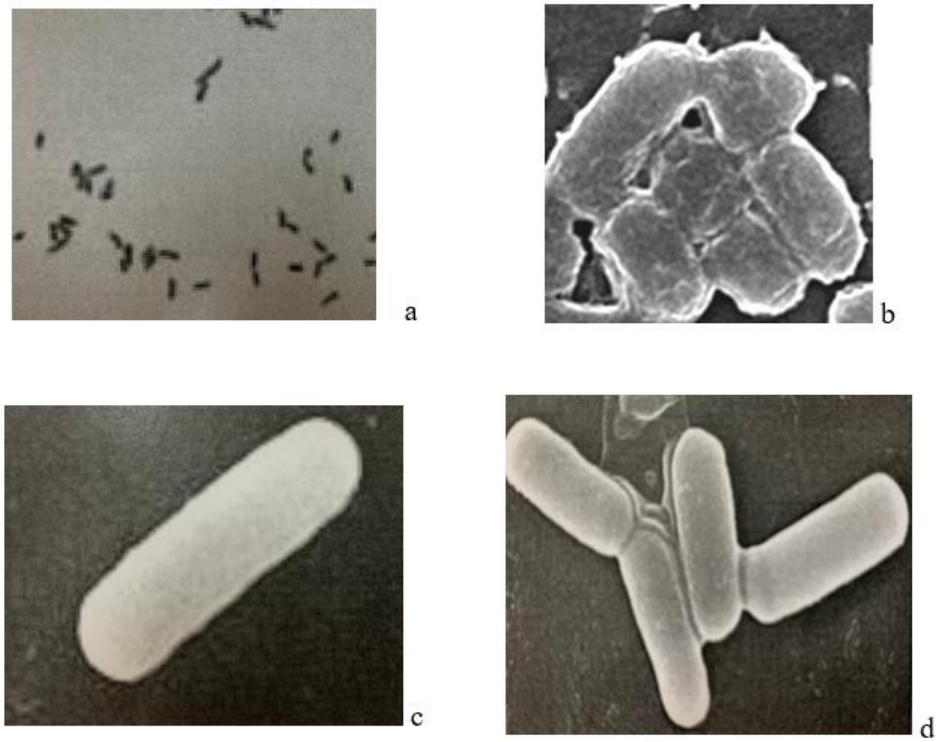

Figure 2 Morphological characteristics of the strains (a) CNN1,(b) CNN2,(c) VNN3 and(d) CNN4

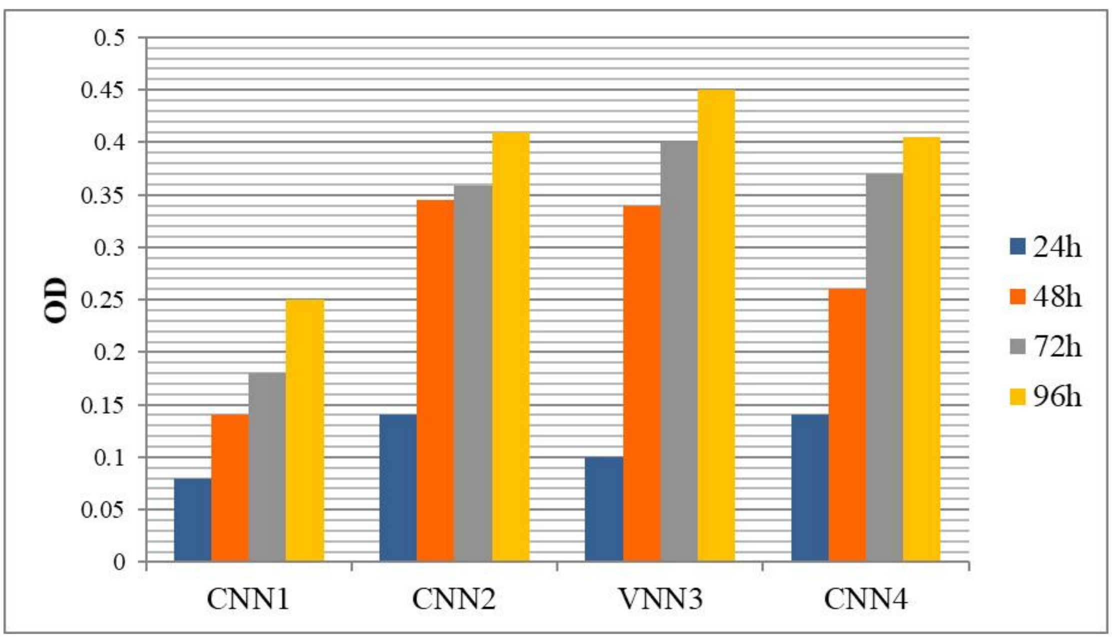

Figure 3 OD_{620} of the four isolated strains growing in CPF-supplemented LB medium

(diameter: 1.5 mm). The colonies of CNN4 were ivory white, round and small, smooth surface and non-pigmentated (diameter: 1.5 mm). In a previous study, Asamba et al. (2022) found that colonies of six CPF-degrading bacterial strains isolated from contaminated soils in Kenya were rod-shaped and pigmented.

The isolates were cultured on LB medium supplemented with the corresponding CPF substrate (100 mg/L) to determine the best growth time. The results from the measurement of OD_{620} of the four isolates are shown in Figure 3. The bacterial density of the four strains is shown in Table 1.

Table 1 Bacterial density of the four strains (CFU/mL)

Strains	Bacterial density (CFU/mL)			
	24 h	48 h	72 h	96 h
CNN1	$3.5x10^{7}$	$2.1x10^{8}$	$3.8x10^{8}$	$2.3x10^{7}$
CNN2	$2.6x10^{8}$	$3.2x10^{9}$	$8.8x10^{9}$	$2.8x10^{8}$
VNN3	$3.9x10^{8}$	$4.8x10^{9}$	$9.2x10^{9}$	$4.4x10^{8}$
CNN4	$4.3x10^{8}$	$5.8x10^{9}$	$9.0x10^{9}$	$5.7x10^{8}$

Table 2 Similarities of the 16S rRNA genes of CNN2, VNN3 and CNN4 with sequences published in GenBank

Strain	Code	Name	Similarity (%)
CNN2	AP014809.1	*Methylobacterium populi*	99.80
	MF171057.1	*Methylobacterium populi* H1.3	99.80
	MK850375.1	*Methylorubrum thiocyanatum* TA1	99.80
	AB900974.1	*Methylobacterium rhodesianum* P-15S	99.80
	GU294335.1	*Methylobacterium zatmanii* 6012	99.80
	GQ281065.1	*Methylobacterium zatmanii* NBCS25	99.80
	CP039546.1	*Methylorubrum populi* YC-XJ5	99.71
	MN094857.1	*Methylorubrum* sp.TH1	99.71
	MG190781.1	*Methylorubrum pseudosasae* IMB16-188	99.71
VNN3	KX507144.1	*Ensifer adhaerens*JS1020	98.11
	KY992904.1	*Ensifer* sp.PZS_S05	98.11
	KY660614.1	*Ensifer adhaerens* PZG_S19	98.11
	KY660602.1	*Ensifer adhaerens* PZG_S11	98.11
	KY660583.1	*Ensifer adhaerens* PZS_S05	98.11
	KX673850.1	*Ensifer* sp.P16P1	98.01
CNN4	CP033530.1	*Acinetobacter pittii* 2014S07-126	99.24
	CP040903.1	*Acinetobacter pittii* AP007	99.15
	MN049561.1	*Acinetobacter calcoaceticus* GCPIR7	99.15
	LC485224.1	*Acinetobacter pittii* SN6-2	99.15
	MK954115.1	*Acinetobacter* sp. A4	99.15
	MK834827.1	*Acinetobacter calcoaceticus* Bi	99.15
	MH890513.1	*Acinetobacter* sp. Ap6	99.15

The results showed that after 96 h, the OD_{620} of the strain VNN3 was highest (0.45), followed by CNN2 and CNN4 (0.41). The bacterial density after 96 h for these three strains was also high (2.8 – $5.6x10^{8}$ CFU/mL). Among the four strains, CNN1 had the lowest OD_{620} value, and it was reported at 0.14 after 48 h and 0.25 after 96 h. Based on the results, the strains CNN2, VNN3 and CNN4 were selected for further experiment and analysis. The results based on the OD_{620} value are comparable to those recorded by Asamba et al. (2022) who reported that OD_{600} value of the six isolated CPF degrading bacterial strains was from 0.05 to 0.25 after five days. The values of bacterial density in the present study were lower than the previous research, which reported that the density of the bacterial CPF-degrading strain *Hortaea* sp. B15 was from $0.5x10^{16}$ CFU/mL to $4.5x10^{16}$ CFU/mL after 30h of incubation (Hadibarata et al. 2023). These authors found that the highest bacterial count of 3.8 x 10^{16} CFU/mL after 20 h enhanced the 91% CPF degradation (Hadibarata et al. 2023).

3.2 Identification of selected strains using 16S rRNA gene analysis

Isolated strains CNN2, VNN3, and CNN4 were identified using 16S rRNA gene analysis. The similarities of the 16S rRNA genes of the strains CNN2, VNN3 and CNN4 with sequences published on GenBank are shown in Table 2.

From the obtained results, it can be concluded that the three isolated strains were identified as *Methylobacterium populi*(CNN2), *Ensifer adhaerens*(VNN3) and *Acinetobacter pittii*(CNN4). Bacterial members of the genera *Methylobacterium*, *Ensifer* and *Acinetobacter* have been reported to be able to degrade CPF (Zhao et al. 2014; Li et al. 2020; McDonald et al. 2021). The strain *Acinetobacter* sp. $MemCl_4$ isolated from agricultural soil in India has been demonstrated to degrade 98% of CPF within 144 h of incubation (Pailan et al. 2016). The removal rate of CPF was found to be 89.5% to 91.1% by the bacterium *Hortaea* sp. B15 within 30 h of incubation (Hadibarata et al. 2023). In another study, 23% of CPF can be removed by *Enterobacter* sp. SWLC2 after 30 minutes (Jha et al. 2022). It has been reported that *M. populi* YC-XJ1 possesses important genes that encode enzymes responsible for degrading various exogenous compounds, including CPF (Li et al. 2020).

3.3 CPF degradation in liquid medium by the isolated bacterial strains

The isolated strains *M. populi* CNN2, *E. adhaerens* VNN3, and *A. pittii* CNN4 were selected to test their CPF degradation ability by culturing the strains in MSM medium supplemented with 100 mg/L CPF. The CPF concentration in control (without inoculation) and inoculated sample with the strain *M. populi* CNN2, *E. adhaerens* VNN3 and *A. pittii* CNN4 after 24, 48 and 72h are shown in Figure 4. The study results showed that the strain *M. populi* CNN2, *E. adhaerens* VNN3 and *A. pittii* CNN4 could degrade CPF. *E. adhaerens* VNN3 showed the highest CPF degradation rate among the three strains, followed by *M. populi* CNN2 and *A. pittii* CNN4. After 24 hrs, the CPF concentration decreased by 41.1% and 34.2% by the strain *E. adhaerens* VNN3 and *M. populi* CNN2, respectively. After 72 hrs, the CPF concentrations in the samples inoculated with the *E. adhaerens* VNN3, *M. populi* CNN2, and *A. pittii* CNN4 were 4.95 ± 0.02, 19.06 ± 0.03 and 39.96 ± 0.01 mg/L, respectively; whereas the CPF concentration in the control sample was 94.34 ± 0.98 mg/L. The decrease of CPF concentration by the strain *E. adhaerens* VNN3 and *M. populi* CNN2 after 72 hrs reached about 95.2% and 81.4%, respectively.

The ability of CPF degrading by the selected three strains in this study was higher than reported in the previous studies. Zhao et al. (2014) reported a 60.2% CPF degradation ability of the bacterial strain *Acinetobacter calcoaceticus* D10 isolated from the chives' rhizosphere after 18 days. In another study, the strain *Methylobacterium populi* YC-XJ1 isolated from desert soil has a capacity of CPF degrading by 27.3% after 72 h incubation (Li et al. 2020). Furthermore, Elshikh et al. (2022) reported that the strains *Bacillus cereus* CP6 and *Klebsiella pneumoniae* CP19 degraded about 70% CPF at 200 – 300 mg/L concentrations after 10 days.

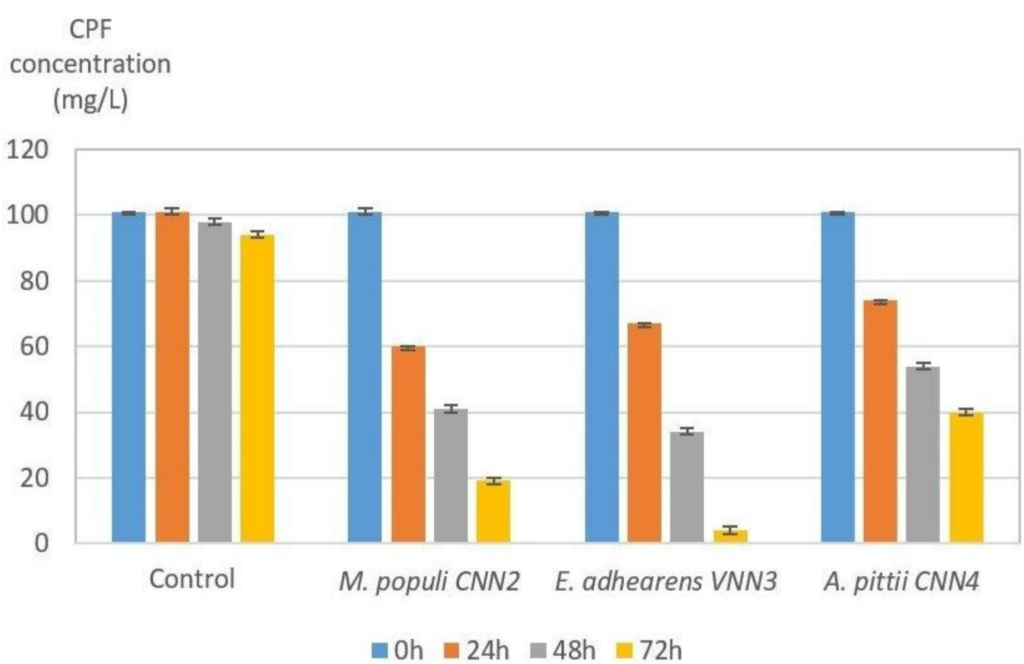

Figure 4 Changes in the CPF concentrations with different time duration by the selected strains

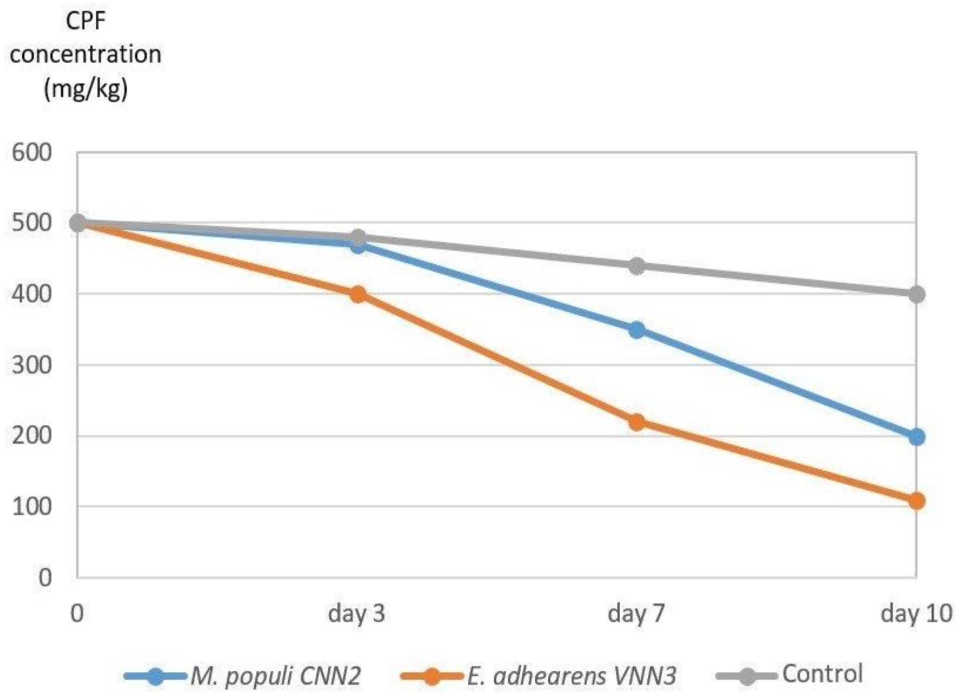

Figure 5 CPF concentration in the soil samples after inoculation of the strain *M. populi* CNN2 and *E. adhaerens* VNN3

3.4 CPF degradation ability in soil under in-vitro conditions by selected isolated strains

The changes in CPF concentration in the soil samples inoculated with the best results given bacterial strain *M. populi* CNN2 and *E. adhaerens* VNN3 after 3, 7 and 10 days of incubation are shown in Figure 5.

The results showed that the CPF concentration gradually decreased up to 3 days of incubation and sharply decreased after 10 days. The strain *E. adhaerens* VNN3 exhibited more vital CPF degradation ability than the strain *M. populi* CNN2. After 7 days, the CPF concentration was reduced from 500 mg/kg to 225 ± 1.73 and 349 ± 0.59 mg/kg by the strain *E. adhaerens* VNN3 and *M. populi* CNN2, respectively, whereas in the control sample, this reduction in the CPF concentration was reported only 438 ± 2.65 mg/kg. After 10 days, the CPF concentration were reduced by 77.6 and 60.6% (equivalent to 112 ± 1.73 and 197 ± 2.08 mg/kg, respectively) by the strain *E. adhaerens* VNN3 and *M. populi* CNN2, respectively. The results of the current study are superior to the findings of Li et al. (2020) those, who reported only a 27.3% reduction in initial CPF concentration after 3 days by the strain *M. populi* YC-XJ1 isolated from desert soil. In another study, the strains *Achromobacter xylosoxidans* (JCp4) and *Ochrobactrum* sp. (FCp1) isolated from pesticide-contaminated soil could degrade CPF concentration by 40 and 60% after 7 and 10 days of incubation, respectively (initial CPF concentration of 200 mg/kg) (Akbar and Sultan 2016). As a result, compared to other reports, the results in this study indicate that the strain *E. adhaerens* VNN3 and *M. populi* CNN2 has a higher potential of CPF-bioremediation from the CPF contaminant agricultural sites.

Conclusion

Among 20 soil samples from tea-growing sites in Vietnam, four bacterial strains that could degrade CPF were isolated. Among them, three potential strains *M. populi* CNN2, *E. adhaerens* VNN3, and *A. pittii* CNN4, have been selected for mass culturing and to identify their bioremediation potential. Among the selected bacterial strains, *E. adhaerens* VNN3 showed the highest CPF degradation ability in both cultured MSM medium and in-vitro soil experiments, followed by the strain *M. populi* CNN2. The study highlights the potential application of the isolated strains *E. adhaerens* VNN3 and *M. populi* CNN2 in CPF treatment and soil bioremediation in agricultural areas, contributing to pollution elimination and sustainable development.

Conflict of interest

The authors of this article declare no competing financial and personal interest.

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Journal of Experimental Biology and Agricultural Sciences, June - 2023; Volume – 11(3) page 572 – 580

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

The Impact of Elicitation on Potato (*Solanum tuberosum* L.) Production, Enzymatic and Antioxidant Activity in Nuevo León, Mexico

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Received – March 14, 2023; Revision – June 03, 2023; Accepted – June 27, 2023
Available Online – June 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(3).572.580

KEYWORDS

Defense responses

Elicitors

Fianna

Physiological

Tuber

ABSTRACT

Many compounds available in the market act as elicitors and can be incorporated into agronomic management. But the focus is on elicitors frequently used for the induction of different responses related to the systemic resistance of plants to increase the production of bioactive metabolites, biomass accumulation, and yield. For that case, this work aimed to evaluate the effects of three elicitors on potato crops under field conditions. The potato cultivar "Fianna" was used, and a completely randomized design with four treatments and four repetitions. The effect of three elicitors at a dose of 2.5 g. L^{-1} for Activane®, 2.5 ml. L^{-1} for Micobiol® and 2.5 g. L^{-1} for Stemicol® was evaluated on growth, yield, enzymatic and antioxidant activity. Generally, the elicitors had a positive effect on the enzymes and antioxidant capacity of the potato plant. It was concluded that the application of elicitors Stemicol® (T4) had the most significant result on the number of tubers and weight per plant at harvest while allowing a more substantial number of tubers to be obtained. In comparison, Activane® (T2) influenced the growth variables of stem length and number of leaves per plant.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

The potato (*Solanum tuberosum* L.) occupies the fifth place of the crops with the highest production worldwide, followed by corn, rice, wheat and beans. In the world, 385 million tons of potatoes are produced, with China as the leading producer, followed by India, the United States, Russia, and Germany (INTAGRI 2017). Potato cultivation in Mexico is important for everything it generates because it is an economical food, a source of low-cost energy to the human diet and a reasonable basis for feeding the population. Though Mexico occupies the 31st place in the world production of this vital tuber, around 68,000 hectares of land are cultivated in Mexico, from which a total of 1.8 million tons are obtained with a production value of $11,300 million (SIAP 2022). Potato is grown in 23 states of the Mexican Republic, and among these, Sonora is the primary potato-producing state at the national level with 24.5% of the total production, followed by Sinaloa with 17%, Puebla with 9.85%, Veracruz with 8.30% and there are other important producing states such as the State of Mexico, Nuevo León, Chihuahua, and Baja California Sur (Haro 2019; SIAP 2022).

The use of pesticides in potato production to prevent and eliminate insect pests, pathogens, and weeds, is closely related to the degree of knowledge and responsibility in the application procedures, which generates the risk of intoxication of producers and deterioration of the environment. However, some insects in the population are naturally resistant to certain types of chemicals, making it difficult for them to control (Mandal et al. 2009: Niks et al. 2021). Recently, much focus has been directed to biological products capable of inducing defences response or induced systemic resistance in plants (Nasir et al. 2014). Elicitors are molecules capable of stimulating any defence in the plant. These elicitors are prepared to promote different modes of plant defence such as Systemic acquired resistance (SAR) (related to salicylic acid and PR proteins), Induced Systemic Resistance (ISR) (activated by bacterial strains of saprophytic rhizobacteria) and localized acquired resistance (LAR) (triggered by the plant hypersensitive response and phytoalexins production) (Choudhary et al. 2007; INTAGRI 2017).

Using elicitors has shown a high priority as an alternative to producing bioactive compounds, enzymes and secondary metabolites for growth, development, and defence in different crop cultivars (Serrano-Cervantes et al. 2016; Cham et al. 2022). Enzymes are protein molecules that speed up the chemical reactions in the plant, reducing the activation energy so that the roots can absorb nutrients more quickly and easily (Ramírez and Aceves 2014). Previously, only a few studies have reported using elicitors to improve growth and production and trigger different plant defences (Mandal et al. 2013; Garcia and Zavala-Garcia 2018: Cham et al. 2021; Cham et al. 2022). Research carried out in butterhead lettuce showed that the effect of elicitation depended on several factors, including the time of application and type of elicitor, and that the main effect was observed on polyphenols and carotenoid concentration (Moreno-Escamilla et al. 2020; Giri and Giri 2022). Very few research has been carried out on the effect of elicitors on the production of potatoes to increase their vigour, metabolites, and yield and activate disease resistance pathways in plants, acting against a wide range of pathogens. Therefore, the objective of our research focuses on evaluating the effects of three major elicitors (Activane®, Micobiol® and Stemicol®) on the growth, yield, and production of bioactive compounds and polyphenol oxidase defence enzyme as a new alternative that permits their use in potato crop production.

2 Materials and Methods

2.1 Geographic Location and Plant Material

This research was carried out at the Autonomous University of Nuevo León (UANL) experimental station in Marín. The study area is located at 25° 53' North latitude and 100° 03' West longitude; the average annual rainfall was of 573 mm, an average annual temperature of 22° C, and an elevation of 375 above sea level (García et al. 2018). The plant material used was the "Fianna" Cultivar from southeastern Mexico, Perote Veracruz. The planting was carried out on April 21, 2020, in polyethene bags of 20 cm (height) by 20 cm (width) with a substrate consisting of a mixture of 2/3 parts of soil from the region and 1/3 part of sheep manure.

2.2 Description of the experimental design and treatments

This experiment was established under a completely randomized design with four treatments and four repetitions. The four treatments evaluated are Treatment T1: (Control) with plants without application of elicitors; T2 (Activane®), T3 (Micobiol®) and T4 (Stemicol®) with application of elicitors (Figure 1). We realized four applications of the elicitors, first on planting day and then on days 21, 43 and 65, respectively. The said elicitors were prepared in solution and sprayed on the leaves of the plants at a dose of 2.5 g. L^{-1} for each selected elicitor.

2.3 Evaluation of Variables

2.3.1 Agronomic Variables

The evaluation of the variables of interest, such as height, number of stems per plant, length of stems per plant and number of leaves, was carried out at different dates of plant development. Once the fruits were harvested, they were counted and weighed to calculate the gram (g) yield and number of tubers per plant.

The studied variables were evaluated in the following ways: the height of the plant was measured from the base of the plant to the

Figure 1 Potato (*Solanum tuberosum* L.) cultivar "Fianna" with four applications of elicitors at 21 days after planting A) T1, (Control) with plants without application of elicitors; B) T2, (Activane®), C) T3, (Micobiol®) and D) T4, (Stemicol®) with application of elicitors

apex, and the number of leaves and the number of stems per plant were counted for each of these variables; this was followed by the estimation of the number of tubers per plant, and the count was made for all the harvested plants and the average weight per tuber was measured in grams. Data collections were carried out on days one, 21, 43 and 65 days after transplantation, randomly taking data from each experimental unit from the plants.

2.3.2 Enzyme Obtention

The leave samples collected from each treatment were used to evaluate enzymatic and antioxidant activity in the potato plant at 21, 43 and 65 days, respectively. Cham et al. (2021) procedure was used to obtain the leave extracts. For this, a total of 1.0 g of leaves from each sample collected was weighed on an electronic balance, followed by homogenization with 5 mL of 0.1 M phosphate buffer, and the pH was 7.0. The samples were centrifugated at 5000 x g for 5 min at 4ºC, and the supernatant was collected and placed in 1.5 mL Eppendorf tubes wrapped in aluminium foil and stored at -20 ºC for further use.

2.3.3 evaluation of polyphenol oxidase enzymatic activity

The activity of polyphenol oxidase (PPO) (EC 1.14.18.1 or EC 1.10.3.2) was determined by a change in colour as evidence of a chemical reaction, using a phosphate buffer pH 7.0 and 500 ppm gallic acid as substrates. Subsequently, the sample was incubated at 40 ºC for 2 min in a laboratory water bath and the final sample mixture at 40 ºC for 1 hour. The absorbance was recorded at 420 nm for the reaction; a UV spectrophotometer was used to measure the absorbance. The polyphenol oxidase activity was expressed in units per g^{-1} of fresh tissue (U g^{-1}fw) (Gasull and Becerra 2006; Cham et al. 2021).

2.3.4 Inhibition of the DPPH radical

The inhibitory activity of the DPPH (2,2-diphenyl1-picrylhydrazil) radical was determined using the methodology established by Cham et al. (2021), with some modifications, for which 100 µL of solution was taken of extract (100 µg/mL of ethanol) and transferred to a 96-well microplate, where they were mixed with an equal volume of DPPH reagent (300 µmol). The solution is homogenized for 10 sec and incubated for 30 min at room temperature in the dark. Subsequently, the absorbance was measured at 517 nm in a spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The inhibition percentage was calculated using the following equation:

$$\text{DPPH Inhibition (\%)} = [1\text{-Abs(S)/Abs (0)}] \times 100$$

Abs(S) is the absorbance of the antioxidant at 30 min whereas Abs (0) is the absorbance of the control at time 0 min

The antioxidant capacity of DPPH in samples was expressed as the mill equivalent of gallic acid per g^{-1} of fresh tissue (mEqGA g^{-1} fw) (Cham et al. 2021).

2.4 Statistical Analysis

To perform the statical analysis with the data obtained, we carried out the analysis of variance (ANOVA) to determine the statistical differences, and tests were determined by comparison of means by Tukey ($P \leq 0.05$). The analyses were conducted using the statistical software SPSS (Statistical Package for the Social Sciences), IBM.

3 Results and Discussion

This research work was carried out at the experimental station of the Autonomous University of Nuevo León (UANL) in Marín

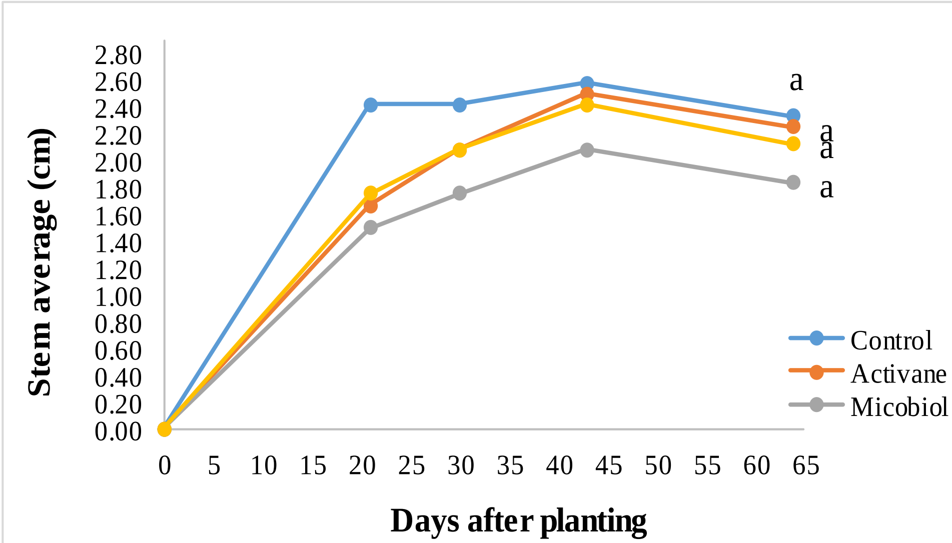

Figure 2 Comparison of the number of stems during the four applications of elicitors in crop development

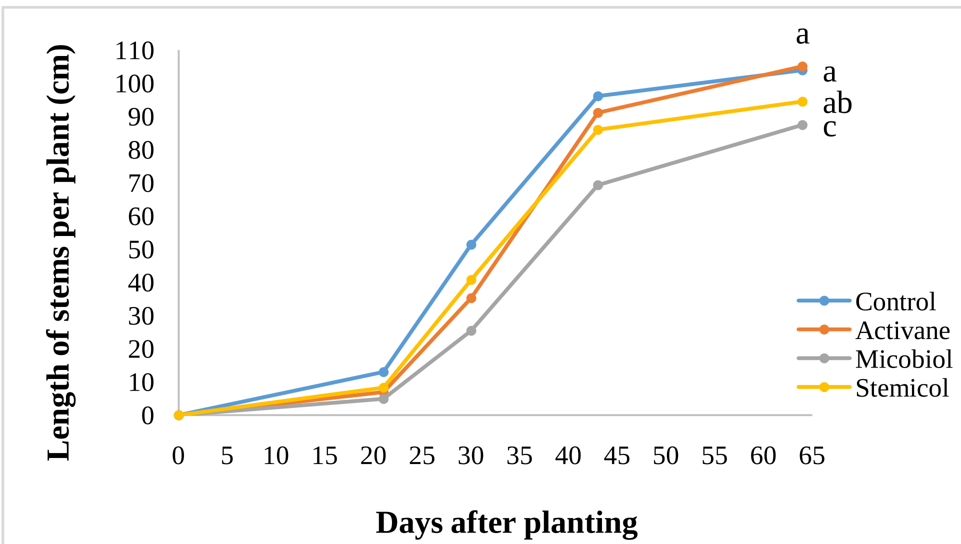

Figure 3 Comparison of mean length of stems during the four applications of study elicitors

during the period from April 2020 to January 2021, to evaluate the effect of elicitors on the growth, development and production of the potato crop, cultivar "Fianna". The results of the study are described in the subsequent section of the study.

3.1 Number of stems per plant

The application of the elicitors did not show any significant difference between the various treatments (Sig.=0.05), and the highest average stem was reported from the control, and these results were followed by the T2, T4 and T3 treatments (Figure 2).

These results contradict the findings of Contreras-Liza et al. (2017), who observed significant differences between the doses and their interaction with the cultivars for the number of stems per plant at 30 days. Likewise, salicylic acid (SA) treatments between 0.2 and 0.4 mM significantly affected the number of potato stems per plant. On the other hand, Jerez-Mompié et al. (2017) reported the average number of stems per plant was slightly higher in the treated plants, presenting significant differences between the treatments evaluated.

3.2 Stem length per plant

The stem length showed a significant difference between treatments, and among the tested treatments, Activane® (T2) has the highest stem length, followed by the T1, T4 and T3 treatments (Figure 3).

According to the results obtained by Larqué-Saavedra et al. (2010), salicylic acid (SA) positively affected the length of potato stems and increased by 43% with a concentration of 1.0 mM and 18% with 0.01 mM. In addition, García et al. (2018) reported an increase in plant height from natural elicitors in plants inoculated with *Fusarium*. This height increase was only observed for the plants treated with elicitors. Jerez-Mompié et al. (2017) also reported that the height of the potato plants had a rise in growth, and there was a strong correlation between stem number and leaf area in those treatments where QuitoMax® was applied twice during the crop cycle (Lemaga and Caesar 1990).

3.3 Number of leaves per plant

The numbers of leaves have shown highly significant differences in the elicitor treatment, and the highest leaves number were recorded from the treatment Activane® (T2). For this reason, the statistical analysis showed a significant positive effect of these elicitors on the number of leaves (Figure 4).

Based on the result obtained from the number of leaves evaluated, treatment Activane® (T2) tends to have great potential to increase potato plant biomass. According to the results obtained by Jerez Mompié et al. (2017), the leaf surface was higher in the plants sprayed with QuitoMax, but without significant differences between treatments, although more elevated numbers of leaves were observed in treated plants with respect to the control. While the finding of Vallad and Goodman (2004) did not show any significant difference between various doses in the number of leaves per plant.

3.4 Crop Yield

3.4.1 Harvested tubers per plant

The harvested number of tubers in this investigation showed significant differences (Sig.=0.05) between the treatment Stemicol® (T4) and the rest, but there was no significant difference between T2, T3 and T1(Figure 5).

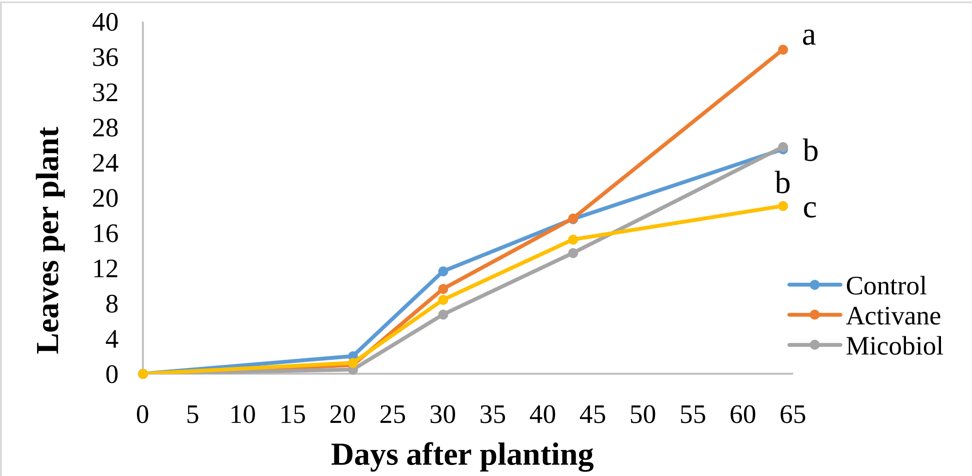

Figure 4 Comparison of means of the number of leaves during the four study applications

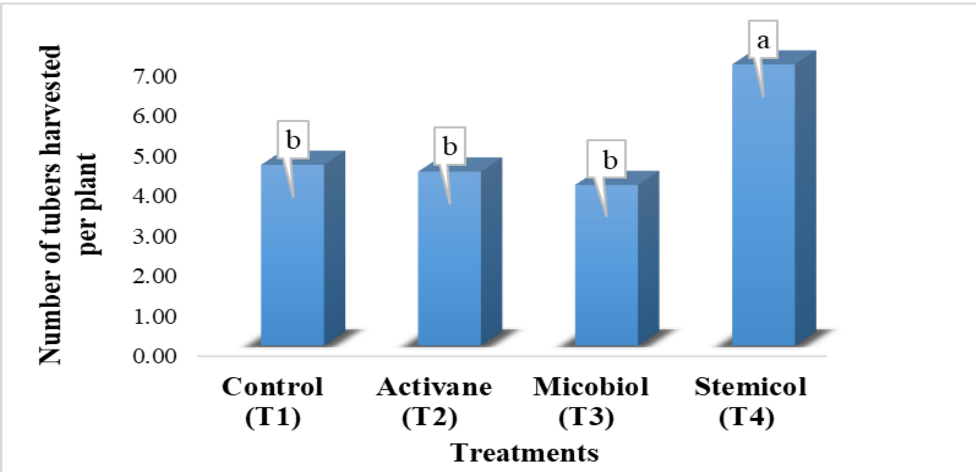

Figure 5 Effect of the elicitors application on the number of tubers per plant at harvest

The Activane® (T2) and Micobiol® (T3) did not show any significant differences in the application in the total production of potato tubers with the control; however, the elicitors Stemicol® (T4) showed a significant difference. The results obtained by Jerez-Mompié et al. (2017) suggested that the number of tubers per plant increased compared to the control when the bio-stimulant Pectimorf® was applied under different environmental conditions. On the other hand, Fu et al. (2022) reported the positive effects of the elicitor Riclinoctaose (RiOc) in the induction of different responses related to the SA-mediated pathway as well as changes in root metabolism and transcription for more significant plant growth and production.

These results from the findings of Jerez-Mompié et al. (2017) reported that the average weight of potato yield per plant of two years was higher with the application of the elicitor QuitoMax ® in potato crop production. Other results showed a contradictory finding, where the control showed the most increased total production, implying that elicitor application affects crop production. The potato plants that received the application of AS and glucosamine were the ones with the lowest production (28.6 and 15.8 % less than the control, respectively), which may be attributed to the fact that the two elicitors promote greater allocation of resources to the defence of the plant than to the production (Burgos-Avila et al. 2021).

3.4.2 Tuber production (grams per plant)

The harvested tuber weight per plant showed significant differences between various treatments, and the highest tuber weight was reported in the plant treated with Stemicol® (T4) and Activane® (T2) (Figure 6).

3.4.3 Average weight per tuber (gram)

The average weight per tuber in this investigation showed the effect of the elicitors; amongst the tested treatments, the Activane® (T2) treatment has the highest average weight per tuber. While the untreated plants have the lowest average tuber weight (Figure 7).

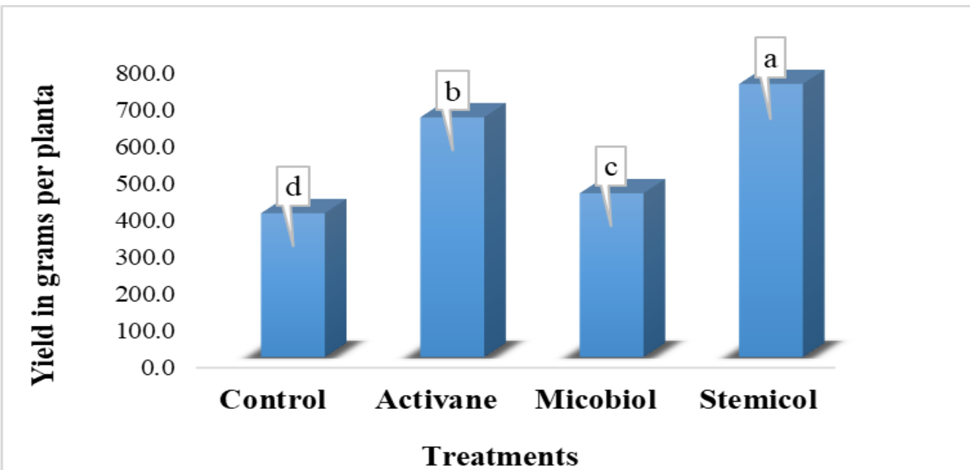

Figure 6 Effect of the elicitors application on harvested tuber weight per plant of the production during the crop cycle

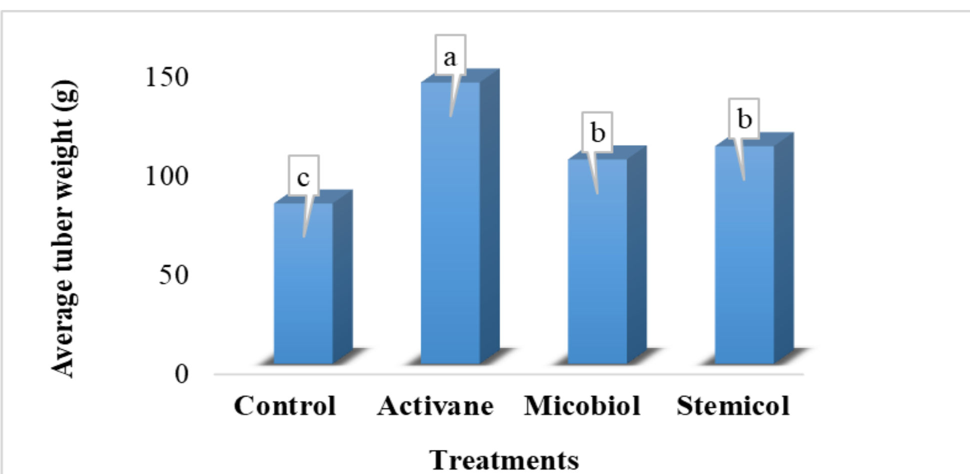

Figure 7 Effect of the elicitors application on total tuber weight obtained during the crop cycle

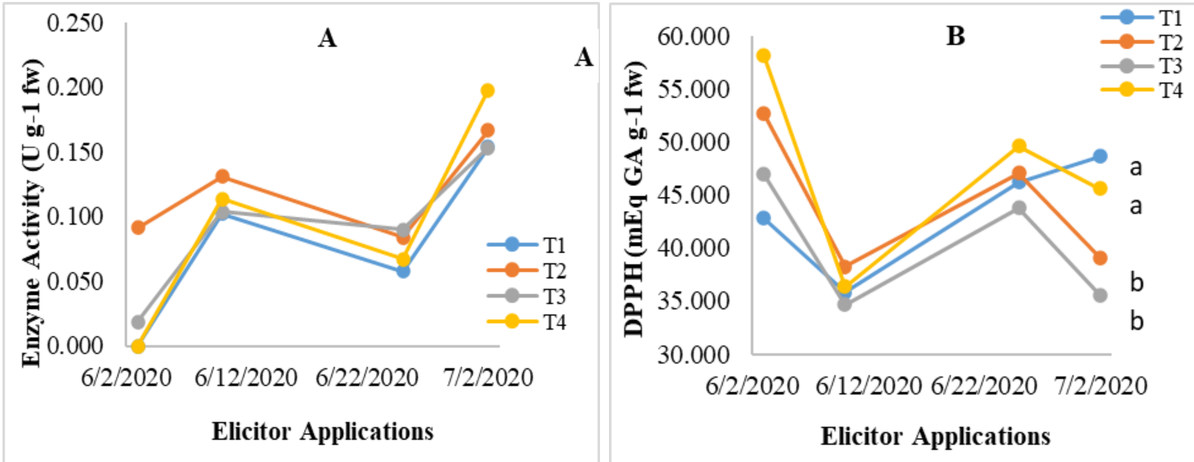

Figure 8 Comparison of means tests for the a) enzymatic activity of polyphenol-oxidase and b) DPPH antioxidant in potato leaves (*S. tuberosum* L.) of the "Fianna" cultivar as a result of elicitation.

The results obtained by Vallad and Goodman (2004) in different salicylic acid (SA) doses in potato crops did not significantly affect tuber weights, so they can be considered favourable for field application. It has been assumed that the plant uses part of the energy in the systemic resistance, and there could be problems of decreased production due to overdosage of SA Results suggest that the elicitors Activane®, Micobiol® and Stemicol® have the potential to increase potato tuber weight per gram in plant treated.

3.5 Enzymatic activity of polyphenol-oxidase and DPPH antioxidant activity

The evaluation of the analysis of variance showed a significant difference (p=0.000) for the enzymatic activity of polyphenol-oxidase in the leaves. However, the comparison of means of the samplings indicated that treatment Stemicol® (T4) had the highest concentration of polyphenols, and treatment T1 had the lowest concentration of polyphenols. The interaction between the treatments and the samples was insignificant (p=0.804), so the difference between the samples was similar in all the treatments (Figure 8(a).

On the other hand, the analysis of variance of DPPH antioxidant activity at 65 days showed significant differences (P=0.489) between the treatments in the leaves. However, the treatment T3 resulted in a lower amount of DPPH. The interaction between treatments and samples was insignificant (p=0.483), so the difference between samples was similar in all treatments (Figure 8b).

The polyphenol-oxidase (PPOs) in vegetables is vital for growth and resistance, and enzymatic activity showed a significant difference between the treatments, with the treatment with Stemicol® (T4) showing more significant enzymatic activity in different applications stages than the other treatments, followed by the Activane® (T2), and Micobiol® (T3), while the control had less enzyme activity. Similar results obtained by Cham et al. (2021) reported an increase in PPO activity with a reduction in pathogen incidence because of elicitation in tomato production under greenhouse conditions. Rodríguez-Guzmán et al. (2019) reported an increase in polyphenol oxidase (PPO) activity because of the application of elicitor chitosan compared to the negative control. Likewise, Soliva et al. (2000) reported the ability of polyphenol-oxidase to catalyze the oxidation of phenolic compounds to quinones (antimicrobial), which are toxic to pathogens. In addition, polyphenol-oxidase is also reported to play a vital role in plant cell lignification and promoting plant resistance against phytopathogens (Chen et al. 2014).

Furthermore, Mejía-Lotero et al. (2018) evaluated the antioxidant and antimicrobial activity in *Tropaeolum tuberosum* and *Ullucus tuberosus*, where a high percentage of antioxidant activity was found in all parts of the plants (leaves, stem, and tuber), mainly in plants treated with elicitors. On the other hand, Cham et al. (2021) reported a significant difference ($P \leq 0.05$) between the treatments in the tomato "Saladette" Cultivar elicitors Activane® (T2) and Micobiol® (T3) with the highest of the DPPH scavenging activity in tomato fruits. In addition, Fu et al. (2022) observed an improved effect of elicitors Riclinoctaose (RiOc) on efficiency in production and allocation of defence and growth-related metabolites in potato plants.

Conclusions

The application of elicitors positively affected the number of leaves per plant, tuber production, and enzyme activities. Elicitors applied to the leaves of potato plants increased yield compared to control plants; the elicitor Stemicol® (T4) with the most significant result on the number of tubers and weight per plant at harvest while allowing a more substantial number of tubers to be obtained. Similarly, the growth variables were favoured with the applications of these elicitors, specifically Activane® (T2).

Acknowledgement

I want to express my special thanks to all the personnel of the laboratories of Plant Biotechnology (Marín unit) and the Plant Tissue Culture of the FAUANL as well as my advisers who gave me the golden opportunity to do this wonderful project. Special thanks to Conacyt for the financial support and to LIDA de México, de SA de CV for the donation of the elicitors.

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Journal of Experimental Biology and Agricultural Sciences, June - 2023; Volume – 11(3) page 581 – 592

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Impact of *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* Oral Infusions on Cognitive Memory Decline in Mild Cognitive Impairment

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Received – March 07, 2023; Revision – May 29, 2023; Accepted – June 07, 2023
Available Online – June 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(3).581.592

KEYWORDS

Learning

Memory

Bacillus subtilis

Staphylococcus aureus

Escherichia coli

Pseudomonas aeruginosa

Cognitive decline

ABSTRACT

Synaptic plasticity is a result of changes in the neuronal circuits which may result in the formation of protein-dependent (long-term memory (LTM) formation) and protein-independent (short-term memory (STM) formation) memories. This STM formation is based on existing proteins, but LTM formation depends on RNA and protein synthesis within the neuronal cells. This RNA and protein synthesis may depend on stimulus exposure like odour, taste, and other environmental stimuli. The present study is aimed to show the impact of oral bacterial infusions on cognitive memory formation through pre and post-infusive behavioural analysis. The results of the study revealed that oral infusions of *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* result in impaired cognitive learning and memory formation. This impaired cognitive memory formation is shown with the help of two-step (pre and post-infusive) behavioural analysis. Pre-infusive behavioural study shows no decline in cognitive learning and memory formation before oral microbial infusions in a serene habituated environment. After oral microbial infusions, a post-infusive behavioural analysis may reveal a memory decline in the treated group. Comparative two-step behavioural analysis indicates that *P. aeruginosa* infusions strongly impact cognitive memory decline compared to the other three groups. This cognitive memory decline may happen due to the production of primary/secondary metabolites within the animal gut and their transportation to the CNS through the blood-brain barrier. The outcome of the present study states that poor oral hygiene plays a significant role in cognitive memory decline concerning mild cognitive impairment (MCI).

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

In brain cognition, long-term memory (LTM) formation plays a pivotal role in the survival of any organism (Kandel 2001; Bisaz et al. 2014). This LTM is formed by continuous exposure to any environmental and physiological stimulus for a limited/unlimited period. LTM formation is based on the RNA-dependent protein synthesis mechanism and is initiated by releasing neurotransmitters from the presynaptic neuron into the synaptic cleft. Released neurotransmitters may bind with their respective receptors present on the surface of postsynaptic neurons at the synaptic cleft (Davis and Squire 1984; Schafe and LeDoux 2000; Jones et al. 2001; Scharf et al. 2002; Abraham and Robins 2005; Igaz et al. 2006; Fonseca et al. 2006; Abraham and Williams 2008; Alberini 2008; Alberini 2009; Smart and Paoletti 2012; Evans et al. 2021; Lin et al. 2021). As a result of binding of neurotransmitters with the specific postsynaptic membrane receptors may result in the upregulation of cyclic adenosine monophosphate (cAMP). Increased level of cAMP further activates several downstream molecules like protein kinase A (PKA), enzyme-regulated kinase – 1/2 (ERK– 1/2), cAMP response element binding protein - 1 (CREB-1), Immediate early genes (IEG's), and postsynaptic density (PSD) proteins (Jones et al. 2001; Mohamed et al. 2005; Abraham and Williams 2008; Ganesh et al. 2010; Besnard et al. 2011; Ganesh et al. 2012; Philips et al. 2013; Nelson et al. 2013; Miyashita et al. 2018; Mukilan et al. 2018a, 2018b; Wang et al. 2019; Sharma and Singh 2020; Mukilan 2022). The Collective expression of these molecules will result in the formation of LTM in a stress-free environment (Ganesh et al. 2010; Besnard et al. 2011; Ganesh et al. 2012; Nelson et al. 2013; Miyashita et al. 2018; Mukilan et al. 2018a, 2018b; Wang et al. 2019; Sharma and Singh 2020; Karen et al. 2021; Mukilan 2022).

Recent reports show that gut microbiota (GM) is essential for the synthesis of brain precursor neurotransmitter components, which will affect the synthesis and concentration of related neurotransmitter production in the brain (Gao et al. 2018; Ticinesi et al. 2018; Gao et al. 2019; Jameson et al. 2020; Chen et al. 2021; Dicks 2022). It is also reported that intestinal precursor neurotransmitter transportation pathways are involved either directly or indirectly with the cognitive functions of the brain (Ticinesi et al. 2018; Caspani and Swann 2019; Jameson et al. 2020; Chen et al. 2021; Dicks 2022). Neurotransmitters like serotonin (5-HT), dopamine (DA), and norepinephrine (NE) are converted from amino acid precursors, and it is released from presynaptic neuron into the synaptic cleft and binds with postsynaptic neuronal receptors (Yano et al. 2015; Agus et al. 2018; Luqman et al. 2018; Strandwitz 2018; van de Wouw et al. 2018; Sadaqat et al. 2021; Chen et al. 2021; Dicks 2022). One of the precursor molecules, short-chain fatty acids (SCFA), regulates this process. These SCFA molecules act as a crucial molecules for the signal transport between gut microorganisms and the brain (Franco-Robles and López 2015; van de Wouw et al. 2018; Heyck and Ibarra 2019; Hu et al. 2020; Markowiak-Kopeć and Ślízewska 2020; Verhaar et al. 2022; Neto et al. 2023). Recent studies showed gut microorganisms have an impact on the gut-brain axis (GBA) through the hypothalamic-pituitary-adrenal (HPA) axis (Murciano-Brea et al. 2021; Rosin et al. 2021). Some of the gut bacterial species may also regulate neurotransmitters like 5-HT, DA, noradrenaline (NA), and γ-aminobutyric acid (GABA) (Chen et al. 2017; Wong et al. 2018; O'Donnell et al. 2020).

In a normal/non-infected state, synthesized precursor neurochemical substances are transported from the enteric nervous system (ENS) to the central nervous system (CNS) through the vagus nerve for the synthesis of neurotransmitters in the brain (Cryan and Dinan 2012; Bauer et al. 2016; Baj et al. 2019; Giuffré et al. 2020; Orr et al. 2020; Sadaqat et al. 2021). Any imbalance in GM may result in impaired neurochemical signal transmission to the CNS and further results in the altered expression of 5-hydroxytryptamine (5-HT) receptor subunits in the hippocampus, which may lead to impaired cognition (Ganesh et al. 2010; Zhang and Stackman Jr 2015; Mukilan et al. 2018a; Bo et al. 2020; Liu et al. 2021; Roux et al. 2021; Li et al. 2022). The present study has made an initial attempt to show the impact of *P. aeruginosa*, *B. subtilis*, *S. aureus*, and *E. coli* oral infusions in cognitive memory formation with the help of reward-based learning based on pre and post-infusive training strategies in the naïve goldfish *Carassius auratus.*

2 Materials and Methods

2.1 Experimental Animals and Apparatus

Naïve goldfish (*Carassius auratus*) with a body length of 6.5 – 8 cm and 6 – 15 g weight were purchased from a local aquarium. Purchased fish were housed in rectangular tanks (serving as a home tank) having a length of 42 inches, breadth of 30 inches, and height of 21 inches. Fish were housed in groups of 8 (n = 8/group) at the home tank with sufficient continuous aeration at a standard temperature of 28 ± 2° C and 12 hours of light and dark cycle for maintaining a regular circadian rhythm. Commercially available round food pellets were purchased from the local market and used as a food source for the experimental fish. Fish were given enough food pellets twice daily to maintain their energy budget (6 g/fish). The home tank was minimized to debris free environment by cleaning and replacing the water available in the home tank on alternative days. Experimental protocols followed as per the institutional ethical guidelines of Sri Ramakrishna Institutions, Coimbatore.

2.2 Experimental Apparatus

An experimental glass tank of 42 X 30 X 21 inches was selected for the *C. auratus* experiment. The experimental glass tank was divided into three compartments: one central chamber (CC) and two feeding chambers (one on the right side of CC and another on the left side). The central chamber was 30 X 30 X 21 inches and the feeding chamber (FC) was 6 X 30 X 21 inches.

2.3 Oral Infusion Mixture Preparation and its Infusion

All four isolates *P. aeruginosa*, *B. subtilis*, *S. aureus*, *E. coli* were availed from PSG Institute of Medical Sciences & Research, Coimbatore. All microorganisms were streaked on nutrient agar for their pure isolation. After confirming purity, colonies were selected from quadrant-streaked plates overnight in four 5 ml nutrient broth test tubes. The grown overnight culture was used to prepare an infusion mixture with saline/PBS for the oral route infusions in the *C. auratus* experimental groups. The formulated infused blend was orally infused into the experimental animal with 500 ml as a single dose. After infusions, experimental animals were maintained in a separate tank for their recovery and habituation for 24 hrs.

2.4 Behavioural Study

Behavioural analysis was carried out for all formulated five groups of experimental animals, i.e. Control (CON), *P. aeruginosa* infused group (T_1), *B. subtilis* infused group (T_2), *S. aureus* infused group (T_3), and *E. coli* infused group (T_4) through pre and post infusive behavioural studies.

2.4.1 Pre-infusive Behavioural studies

To examine the role of *P. aeruginosa*, *B. subtilis*, *S. aureus* and *E. coli* infusions on cognitive learning and memory formation, uninfused animal groups (n = 8/group) underwent behavioural analysis through exploration, learning and memory behavioural paradigms. Pre-infusive studies showed the normal behavioural responses of experimental groups in a habituated/ stress-free environment concerning positive/negative reward mechanisms.

2.4.1.1 Exploration

After habituation, animals were allowed to explore the experimental setup for three days to avoid stress during behavioural analysis.

2.4.1.2 Training

Animals were trained in an experimental setup with two feeding chambers. In each feeding chamber, one blue and red colored light was placed. The blue-light chamber acted as a positive reward place with a food pellet, and the red-light chamber acted as a negative reward place without a food pellet. Animals were introduced in the centre chamber at 0 minutes of the training phase. Further, animal movements were watched to know the time to reach the feeding chambers after introduction in the central chamber to know the learning state of each group. The positive reward was confirmed by the intake of food pellets by the animal.

2.4.1.3 Testing

Testing was done after three days of training, and this interval time was given to all experimental groups after pre and post-infusive training for proper memory consolidation in the brain. Both pre-infused and post-infused animal groups were tested for retrieving learned information through the reward mechanism used during the training paradigm.

2.4.2 Post-infusive Behavioural studies

After 24 hrs of infusions, the animal groups were trained to the positive/negative reward mechanism. Different experimental groups were tested for the retrieval of memory formation after 72 hrs of training in the experimental setup.

2.5 Data analysis

Behavioural scores of all experimental groups (exploration, training and testing) are plotted with KyPlot (version 1.0) for graphical representation.

3 Results

3.1 Effect of pre-infusive behavioural studies on cognitive memory formation

3.1.1 Exploration

Exploration data showed that, initially, all groups of animals spent more time in the CC during the exploration period of three days. On Day 1, animals spent more time in the central chamber due to the new environment/stress while the group 2 and 3 spent less time in either feeding chamber, but group 1 still needs to be exposed to the FC. Later on days 2 & 3, the time spent on CC is reduced, and a visit to both feeding chambers is increased due to the increased exploration skills acquired by the animal in the CC. These exploratory behavioural scores were plotted as a graph (Figure 1).

3.1.2 Training

Followed by the exploration studies, training was carried out for all experimental groups with the help of positive/negative reward mechanisms through red/blue visual cues. The training was given between days 4 - 6. During the period of training, animals came to know about food rewards-based learning by their entry into both of the feeding chambers. From their exploratory behaviour, animals learned about their visit to FC with blue color light having a reward and FC with red color light not having a reward. The behavioural scores of training showed that group 3 animals had more visits to the right chamber than groups 2 and 1 (Figure 2).

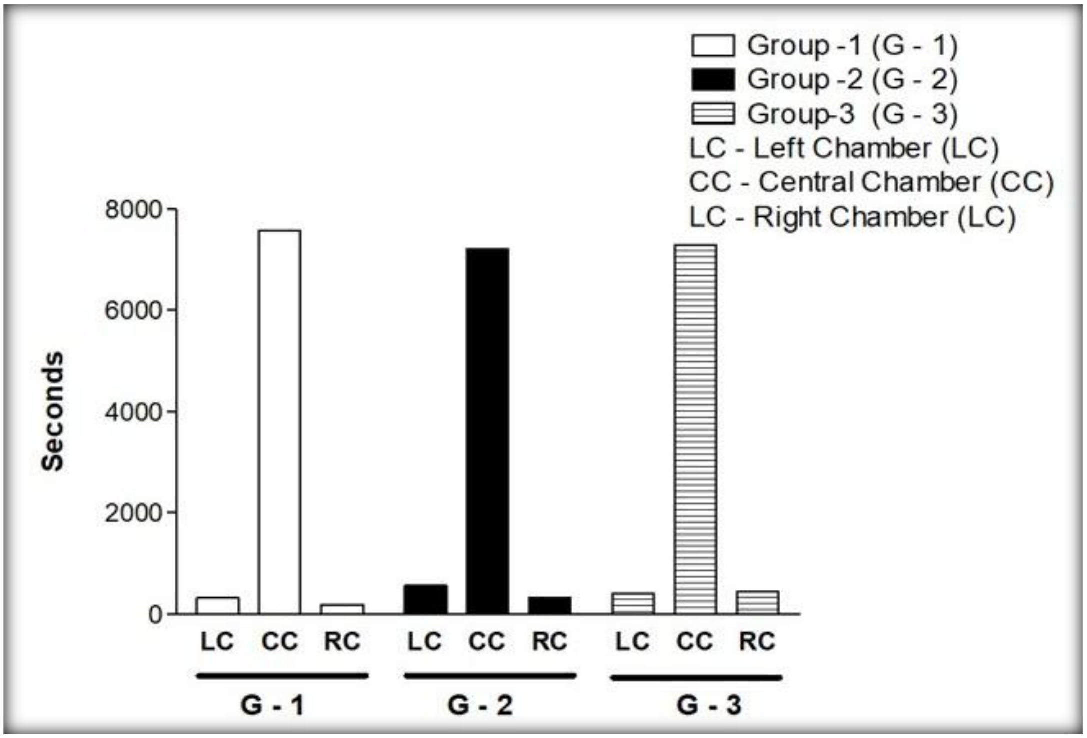

Figure 1 Exploration phase of pre-infusive analysis showed that animals were active and explored all the regions of the experimental setup in a stress-free manner

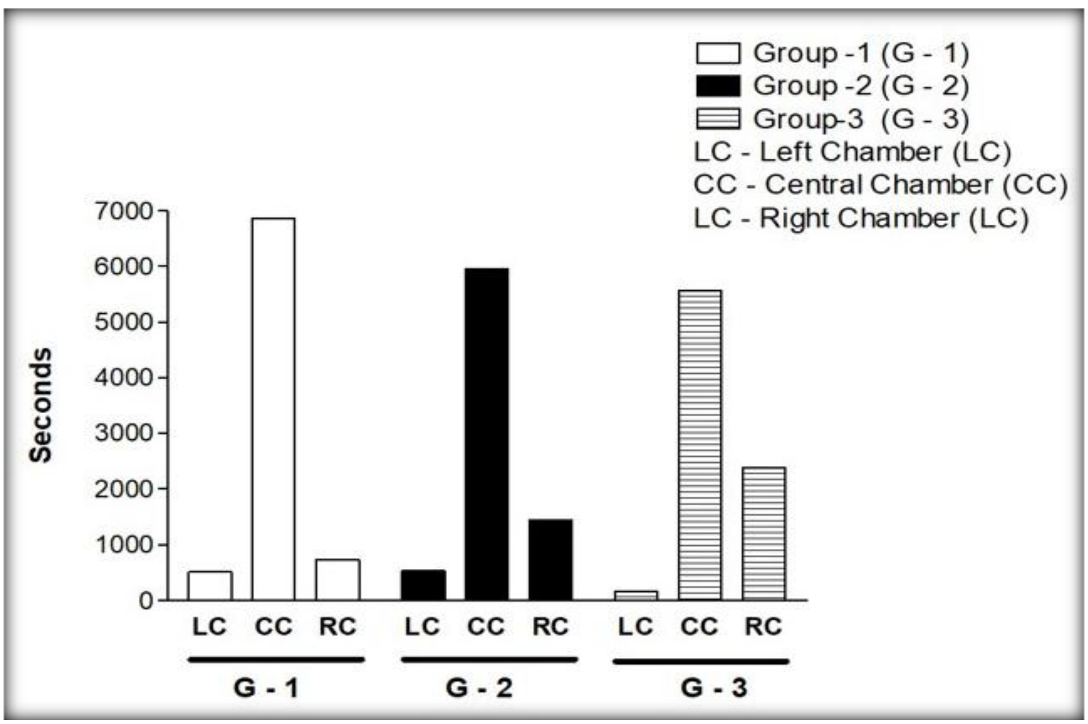

Figure 2 The Training phase of pre-infusive analysis showed that animals learned the task on subsequent experimental days (Days 4 – 6). The number of attempts was increased from the first day of training till the third day. Time spent in the central, right, and left chamber is given in the bar diagram, which shows an increased response in reward-based learning, and experimental animals learned about reward-based colour cues.

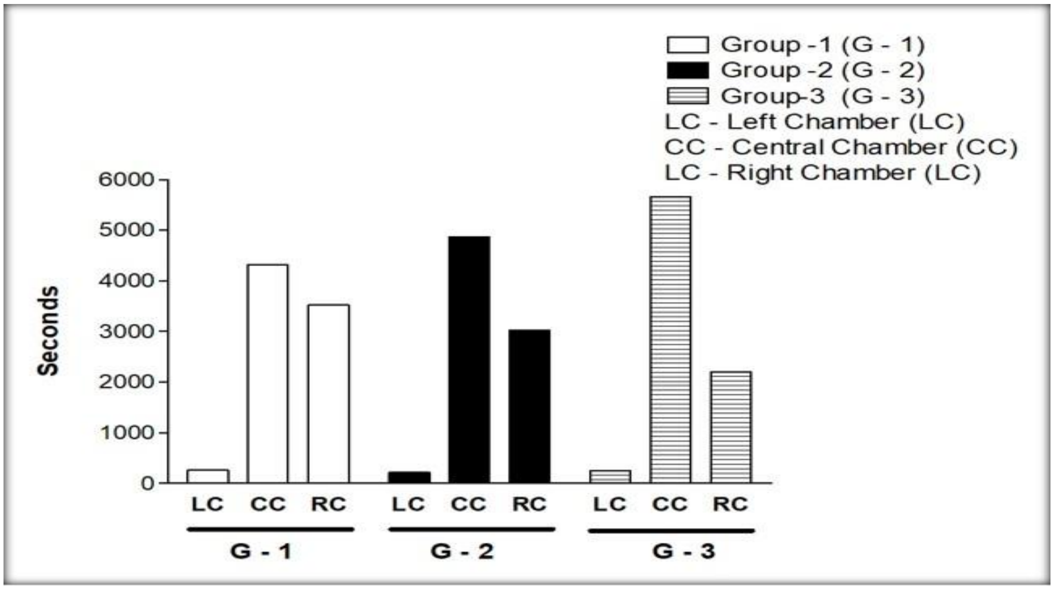

Figure 3 The testing phase of pre-infusive analysis showed that animals learned about the task and retrieved the learned information. The number of correct choices was high compared number of wrong choices.

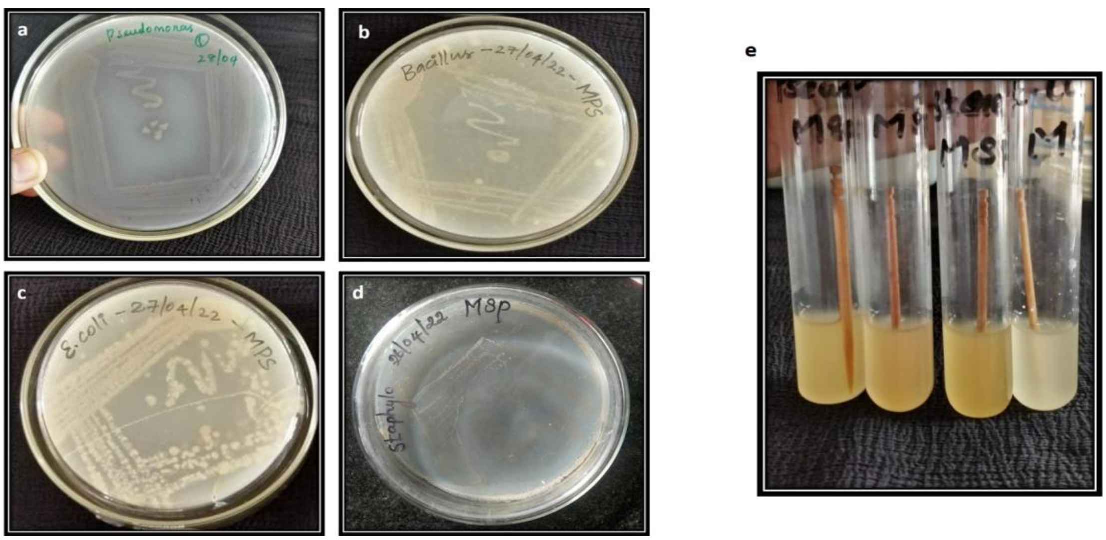

Figure 4 Representative photographs showing the quality of pure culture used in the study. All four microorganisms *P. aeruginosa*, *B. subtilis*, *S. aureus*, and *E. coli* were grown in the nutrient agar medium to check the purity of the culture with the help of the quadrant streaking method.

3.1.3 Testing

After three days of intervals, memory was tested for the three groups between days 10 - 12, with the same experimental setup having red and blue visual cues. Behavioural scores showed that memory retrieval was responsible for making the correct choice. Blotted behavioural scores showed that all trained individuals have an increased number of correct and fewer incorrect visits. It showed that stored information was retrieved from the brain during training. The behavioural scores of testing's showed that there was a greater number of correct responses in all the groups. It was also reported that group - 1 retrieved stored information efficiently through neuronal plasticity development to groups 2 and 3 (Figure 3).

3.2 Impact of oral infusions on cognitive memory formation

Pure culture of all four microorganisms (*P. aeruginosa*, *B. subtilis*, *S. aureus* and *E. coli*) were grown overnight in 5 ml of nutrient broth and mixed with saline/PBS in an appropriate ratio, and these were infused into the oral passage of the animal with an oral gauge (Figure 4). After oral infusions, animals were given three days

(Days 13 - 15) for proper movement of infusion towards the gut of the experimental animals. From Day 16, post-infusive behavioural analysis was done with the help of training and retention behavioural scores.

To understand the impact of infusions on cognitive memory formation, infused animal groups were trained in the experimental setup to understand whether infusions impact cognitive memory formation. During post-infusive behavioural training, there was an enhanced impact on correct decision-making in all experimental groups between 16 - 19 days. After training, testing was done at three different time intervals (Days 23 - 26). Observed behavioural scores showed a gradual decrease in making correct decisions (Figure –5 a, b). After 17 days of oral infusion, post-infusion analysis

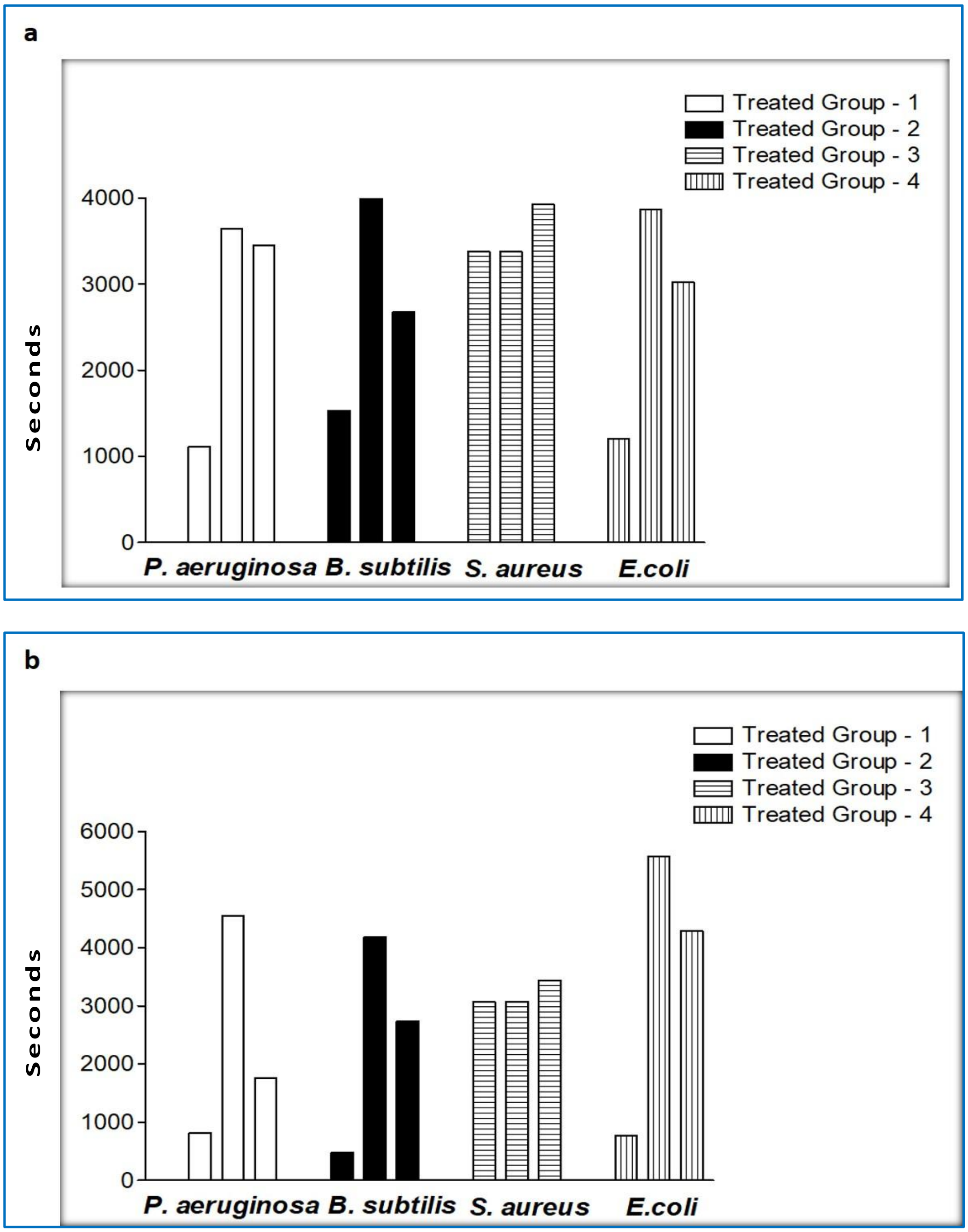

Figure 5 Post-infusive analysis showed that oral infusions did not impair its homeostasis mechanism. Scores of the training phase (5 a) provoked no hindrance in the learning abilities compared to the testing phase (5 b).

was done again to check whether these microorganisms have a more prolonged impact on cognitive memory formation. Surprisingly, obtained results showed that there might be a recovery in the testing phase, which offers a gradual increase in memory formation after pre-infusive analysis (Figure 6 a, b). Obtained results strongly concluded that cognitive decline might happen due to the infused microorganisms' production of primary or secondary metabolite for a limited time in the experimental groups. This study reports that increased *P. aeruginosa*, *B. subtilis*, *S. aureus*, and *E. coli* in the gut impact brain cognition.

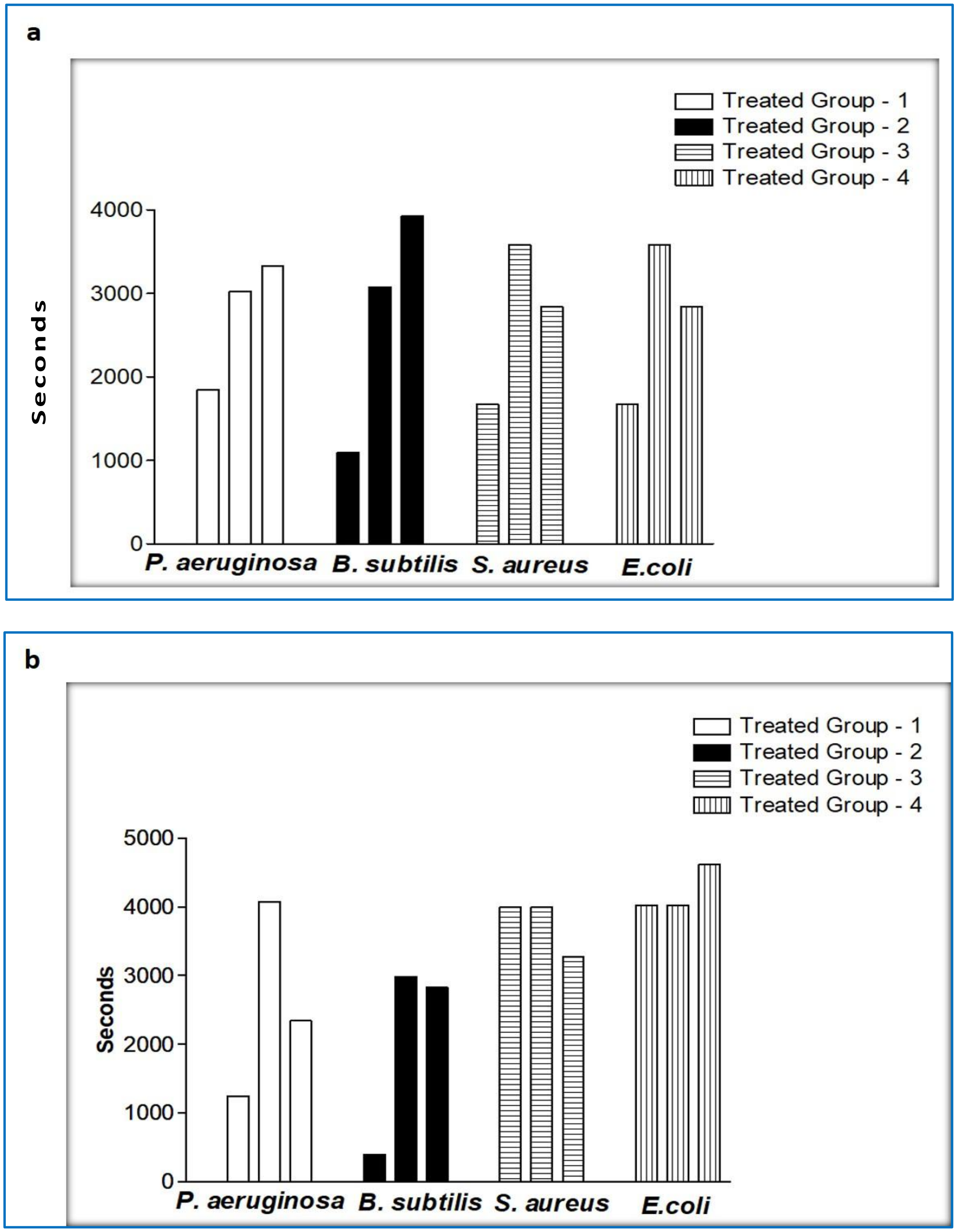

Figure 6 Post-infusive analysis showed that oral infusions do not have a prolonged impact on memory formation. Scores of the training phase (Fig. 6 a) proved that microbial colonization does not impact cognitive learning and memory formation. Obtained scores state that *P. aeruginosa*, *B. subtilis*, *S. aureus*, and *E. coli* oral infusions may impact cognitive memory formation for a short period.

4 Discussion

In brain cognition, oral hygiene, and the GBA will play a major role in the formation of LTM through the enteric nervous system (ENS) by its interconnection (Ribeiro et al. 2012; Martande et al. 2014; Shoemark and Allens 2015; Orr et al. 2020; Sadaqat et al. 2021). The ENS transports gut-secreted precursor chemical components to the central nervous system (CNS) for the synthesis of neurotransmitters like 5-HT, DA, NE, etc. (Strandwitz 2018; van de Wouw et al. 2018; Agus et al. 2018; Sadaqat et al. 2021; Dicks 2022). Synthesized neurotransmitters are released into the synaptic cleft by the presynaptic neuron, and it binds to the specific receptors of the postsynaptic neuron and resulting in the activation of molecules like adenyl cyclase (AC), extracellular regulated kinase-1/2 (ERK-1/2), protein kinase A (PKA), cAMP response element binding protein (CREB), immediate early gene (IEG) cascade and postsynaptic density proteins (PSD). Apart from these molecules, some microRNAs (miR) like miR-132/148a may also critically regulate LTM formation through the activation of down-stream molecules (Ganesh et al. 2010; Ganesh et al. 2012; Mukilan et al. 2018a, 2018b; Mukilan 2022). These signalling molecules will be expressed in a required balanced state during stimulus exposure in the development of LTM through extracellular regulated kinase neuronal signalling pathway (ERK-NSP) (Ganesh et al. 2010; Ganesh et al. 2012; Mukilan et al. 2018a, 2018b; Rajan 2021; Mukilan 2022). Formed LTM may be affected by environmental pollution, lifestyle, microbial colonization, pathogenic infection, etc (Byzitter et al. 2012; Zhang et al., 2020; Vinay et al. 2021; Da et al. 2023).

Recently, it was reported that pathogenic bacterial infections might contribute to neurodegenerative disorders, and these reports showed that there is an interlink between oral/gut dysbiosis and cognitive decline (Balin et al. 1998; Gérard et al. 2006; Lahner et al. 2009; Huang et al. 2018; McGee et al. 2018; Lotz et al. 2021; Gao et al. 2020; Orr et al. 2020; Da et al. 2023). Other than gut dysbiosis, increased pathogenic bacterial infections in the oral cavity may also lead to reduced cognitive memory formation in neurodegenerative disorders like AD, PD, and mild cognitive impairment (MCI) (Balin et al. 1998; Gérard et al. 2006; Lahner et al. 2009; Jahn 2013; Huang et al. 2018; McGee et al. 2018; Lotz et al. 2021; Ryder 2022; Da et al. 2023). Compared to PD, and MCI, decreased learning and memory formation were more prevalent in AD patients. This may happen due to high colonization of *Porphyromonas gingivalis* and other periodontitic bacteria in the oral cavity (Murman 2015; Dominy et al. 2019; Olsen 2021; Seymour and Zhang 2022; Cammann et al. 2023; Liu et al. 2023). Increased *P. gingivalis* further travelled from the oral cavity to the gut, resulting in gut dysbiosis. As a result of gut dysbiosis, a reduced amount of neurotransmitter precursor chemical components were transported from ENS to CNS (Dominy et al. 2019; Chen et al. 2021; Narengaowa et al. 2021; Cammann et al. 2023). Transported precursor components were responsible for synthesizing and releasing fewer neurotransmitters in the synaptic cleft. Later on, decreased amount of neurotransmitter released results in reduced expression of neuronal signalling molecules involved in LTM formation (Ganesh et al. 2012; Mukilan et al. 2018a, 2018b; Rajan 2021; Vinay et al. 2021; Mukilan 2022). For the first time, it was shown that other than periodontic microorganisms, *P. aeruginosa*, *B. subtilis*, *S. aureus*, and *E. coli* may also involve in the formation of impaired cognitive memory. Future studies may address the role of specific microbial components responsible for short-term memory decline in mild cognitive impairment (MCI).

Conclusion

The present study showed the impact of *P. aeruginosa*, *B. subtilis*, *S. aureus,* and E. *coli* oral infusions on cognitive memory formation. The study's outcome proved that poor oral hygiene caused by microbial colonization plays a significant role in cognitive memory decline in MCI. Experimental results demonstrated that proper oral hygiene is important in cognitive memory development, supported by the pre-infusive analysis. Compared to the pre-infusive analysis, the post-infusive analysis showed that oral microbial infections result in cognitive memory decline. From the post-infusive analysis, it is validated that poor oral hygiene results in cognitive memory impairment. This impaired cognitive memory may develop due to the transport of microbial primary or secondary metabolites from the gut to the brain through the blood-brain barrier. Thus, the current research finding stated the impact of oral and gut dysbiosis was pivotal for cognitive memory decline in MCI.

Acknowledgements

Author thanks the reviewers for the revision comments and suggestions for improving the manuscript. MM sincerely thank the Indian Institute of Technology, Kharagpur, India, for the Institutional Post-Doctoral Fellowship. This research work is also supported by the SEED MONEY grant sanctioned to MM by the Sri Ramakrishna College of Arts & Science (SRCAS), Coimbatore. Department of Biotechnology, Sri Ramakrishna College of Arts & Science (SRCAS), Coimbatore was supported by the DST-FIST PG College Level – A Program (SR/FST/COLLEGE-/2022/1203).

Author Contributions

MM contributed to developing the research idea and its design, prosecution, manuscript preparation, and manuscript revision. The author agrees with the data presented in this research manuscript.

Conflicts of Interest

The author declares no conflict of interest to express.

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Journal of Experimental Biology and Agricultural Sciences, June - 2023; Volume – 11(3) page 593 – 597

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Ovarian Gene Transcriptional Responses To Antidepressant Drugs (Imipramine And Fluoxetine) In Female Wistar Rats

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Received – December 03, 2022; Revision – March 30, 2023; Accepted – May 03, 2023
Available Online – June 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(3).593.597

KEYWORDS

Imipramine

Fluoxetine

Gene

RT-PCR

Bcl-2

Rats

ABSTRACT

This study was designed to investigate ovarian gene transcriptional responses to selected antidepressant drugs (imipramine and fluoxetine) in female rats. Fifteen female rats (120 – 140 g) were used for this study. Imipramine (0.71 mg/kg) and fluoxetine (0.57 mg/kg) were given orally for 50 days. The method of RT-PCR was employed to investigate the expressions of FSH-R, p53 and Bcl-2 genes. Graphics were generated as mean +/- SEM using GraphPad Prism version 8.0. Results of the study revealed that the FSH-R, p53 and Bcl-2 expressions were significantly ($p<0.05$) up-regulated in the imipramine-treated rats relative to their controls. Conclusively, it can be suggested that imipramine induced follicular growth and apoptosis in female Wistar rats.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI]
(http://www.horizonpublisherindia.in/).

1 Introduction

Antidepressants are a group of drugs used to cure depression, anxiety, pain, and to treat addictions (Jennings, 2018). Their side effects are xerostomia, obesity, sexual impairment (Healy et al. 2018) and emotional disorder (Sansone and Sansone 2010). Taking these drugs can lead to a higher chance of suicide thought by people of different age categories. Stoppage of antidepressant medication may lead to discontinuation syndrome (Gabriel and Sharma 2017).

Some research that has been carried out previously revealed the efficacy of antidepressant agents in adults (Barth et al. 2016), while some other researchers have given contrary results (Jakobsen et al. 2020); however, the evidence of their usefulness in children and adolescents has not been proven (Cipriani et al. 2016). In Nigeria, the most often prescribed antidepressant agents are twenty-one in number, and they give better results than a placebo for short-term treatment of mature patients suffering from depressive ailment (Cipriani et al. 2018). Investigation concerning the efficacy of antidepressant agents is carried out on people suffering from severe symptoms; this group of people show lower responses to placebo, which indicates that the outcome cannot be generalized to the whole people suffering from this ailment (Cipriani et al. 2018).

The effects of antidepressant drugs on rats' neurogenic regions (Nasrallah et al. 2010), trained rats to discriminate centrally given isoproterenol (Alicia and James 2002), sexually induced side effects (Dimitry et al. 2017), rats prenatally stressed (Jordan et al. 2014), mice hippocampus (Filiou et al. 2014), genetics of mice (Kazuko et al. 2013), pregnant mice (Rahn et al. 2019), DNA damage (Eduardo et al. 2022) as well as on cognition and cardiovascular system (Ali, 2022) have been well studied. But, as a result of limited information obtained from the literature concerning the effects of antidepressant drugs (imipramine and fluoxetine) on ovarian gene expression in female rats, this research intends to bridge this gap.

2 Materials and Methods

2.1 Experimental Animals

Fifteen female rodents of weight range 120 – 140 g raised in the Animal Holding of ABUAD were used in the current study. These rodents were accommodated in a conducive laboratory atmosphere with an unlimited feed and water supply for two weeks before starting the experiments. The Helsinki Declaration on animal experimentation was used for animal experiments.

2.2 Drugs

Antidepressant drugs Imipramine (Dellwich Healthcare Ltd) and fluoxetine (MedreichPlc, UK) were purchased from Danax Pharmacy, Ibadan, Nigeria. Among these, imipramine (25 mg) and fluoxetine (20 mg) were liquefied in l0 ml of distilled water to produce concentrations of 2.5 mg/ml and 2.0 mg/ml, respectively. The dosages of the antidepressant drugs considered in this research were as per the suggestions of the manufacturing industries.

2.3 Experimental Design

Fifteen matured female rats (five per group) used in this study received the oral doses of the antidepressant drugs and distilled water (control) for 50 days as per the predefined group as follows (i) Group I rodents (control group) were given 5 mL/100 g of water (distilled), (ii) Group II rodents were given 0.71 mg/kg of imipramine, and (iii) Group III rodents were given 0.57 mg/kg of fluoxetine.

On the next day after the last treatment (day 51), the rodents were euthanized by overdosing with diethyl ether; ovaries were harvested with the fatty tissue removed and quickly transferred into TRIzol reagent (ThermoFisher Scientific) for total isolation of RNA.

2.4 Isolation of RNA

RNA was isolated from whole tissues as described by Omotuyi et al. (2018). In summary, the ovaries were homogenized in TRI reagent at cold 4 °C. Partitioning Total RNA in chloroform was done by centrifuging at 15,000 rpm for 15 minutes. The supernatant containing RNA was removed from the solution with isopropanol of the same volume. Ethanol (70%) was used to wash the extracted RNA twice, which was then dehydrated for 5 minutes before being re-suspended in the buffer.

2.5 Conversion of cDNA

Spectrophotometer was used to determine the purity and quantity of total RNA at an absorbance of A_{260}/A_{280}, as described by Omotuyi et al. (2018).

2.6 Polymerase chain reaction (PCR)/Electrophoresis

FSHR, p53 and Bcl-2 genes were amplified by PCR targeting primers highlighted in the table below. A software called Primer3 was used to design the primers. The PCR amplification process was carried out as described by Omotuyi et al. (2018).

Amplification products were Electrophoresis in agarose gel (1.5%) using 0.5X TBE (Tris-borate EDTA, JHD chemicals, China) containing ethidium bromide at 100V for 60 minutes. The gel was visualized with UV light with a photo documentation system fitted with a camera. Gel images were analyzed using the keynote platform described by Omotuyi et al. (2020), and Image J software was used to quantify them. Graph-pad prism version 8.0 was used to plot the graphs as average +/- SEM.

Table 1 List of used primers

Primers	Sequence	Product length	Annealing temperature
FSHR	F:ATTCTTGGGCACGGGATCTG R:TGGTGAGCACAAACCTCAGTT	140	55.09 °C
P53	F: TCTCCAGATTCGGCAGCAAG R: GGCCCGTCAGAGCTTTCAT	126	55.10 °C
BCL-2	F:GCGTCAACAGGGAGATGTCA R:TTCCACAAAGGCATCCCAGC	119	55.47 °C

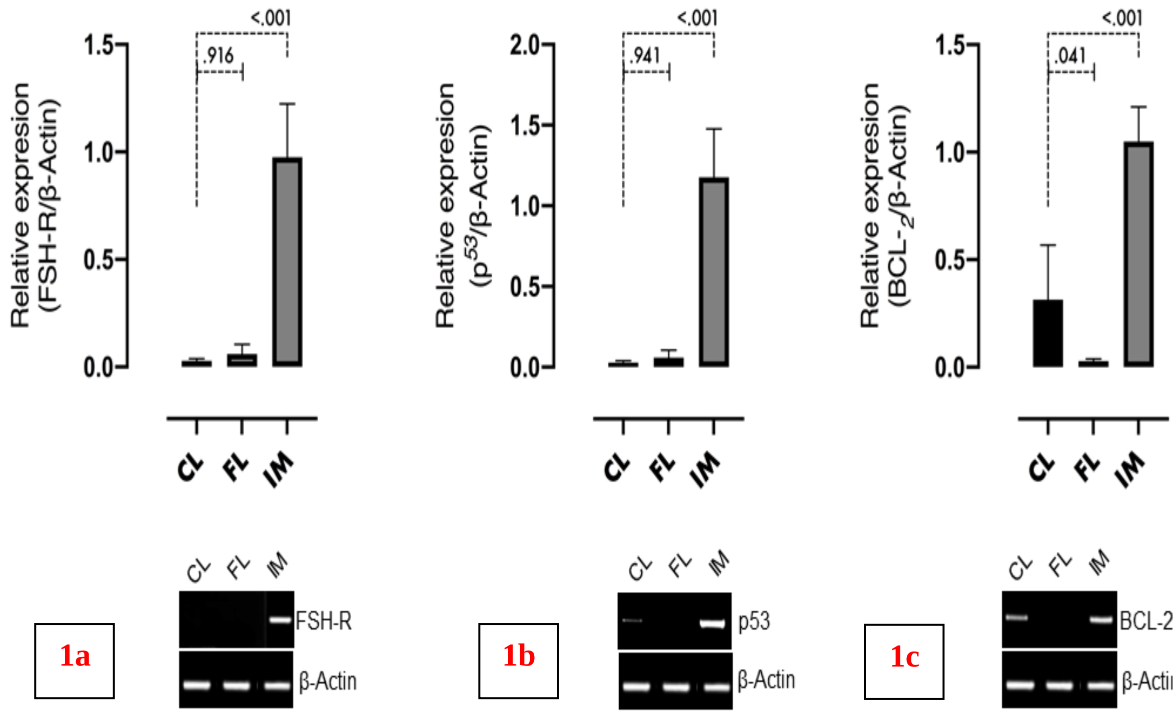

Figure 1 Comparative expression of (1a) FSH-R, (1b) p53, (1c) Bcl-2 in the ovary of rats treated with fluoxetine (FL) and imipramine (IM) 1a as well as gel image expression patterns of FSH-R and β-actin for fluoxetine (FL) and imipramine (IM) treated rats (β-actin served as the internal control). The band density (image J) was plotted as a bar graph (n=5 $p<0.05$).

3 Results

Results presented in Figure 1a revealed that FSH-R expression was significantly up-regulated ($p<0.05$) in the imipramine-treated rats relative to the control. Furthermore, the results presented in Figure 1b suggested that the expression of p53 was significantly up-regulated ($p<0.05$) in the imipramine-treated rats relative to the control. Similarly, figure 1c also suggested that Bcl-2 expression was up-regulated significantly ($p<0.05$) in the imipramine-treated rats relative to the control.

In addition, results presented in Figures 1a and 1b revealed that FSH-R and p53 expressions were nonsignificantly ($p>0.05$) up-regulated in the fluoxetine-treated rats compared to the control. In contrast, Bcl-2 expression was significantly ($p<0.05$) down-regulated in the fluoxetine-treated rats as compared to the control (Figure 1c).

4 Discussion

The FSH-R expression was significantly up-regulated in the imipramine-treated rats, which probably indicates that the imipramine induced follicular growth and these results were corroborated with the findings of Dewailly et al. (2016) and Jamnongji and Hammes (2006) while the findings of Jue et al. (2014) in Pacific Oyster treated rats are contrary to the findings of the present study. Similarly, the p53 expression was significantly up-regulated in the imipramine-treated rats, which suggests that imipramine induced apoptosis and these results are validated by the assertion of Fridman and Lowe (2003). Ukwade et al. (2020) reported a similar effect in the *Byrsocarpus coccineus* treated ovarian cancer cell line. In addition, Bcl-2 expression was also significantly up-regulated in the imipramine-treated rats, which probably indicates that imipramine stimulated or induced apoptosis in ovarian tissue, and these results were corroborated by the assertions of Boise et al. (1993), Oltvai et al. (1993), and Choudhuri et al. (2002) while the findings of Majid et al. (2019) in *Olea europaea* are contrary to the present study.

Unlike imipramine, the expression of FSH-R was insignificantly down-regulated in the fluoxetine-treated rats, which probably indicates that fluoxetine inhibits follicle growth, and these results corroborated with the findings of Dewailly et al. (2016), Jamnongji and Hammes (2006), and Seyedeh-Roza et al. (2021). Similarly, the Bcl-2 expression was significantly down-regulated in the fluoxetine-treated rats, which probably indicates that fluoxetine prevented apoptosis in ovarian tissue, and these findings are supported by the previous study of Boise et al. (1993), Oltvai et al. (1993), Ebrahim et al. (2016), and Choudhuri et al. (2002) while contrary to the result reported by Mohammad et al. (2022) in minocycline treated rats. Further, the expression of p53 was nonsignificantly up-regulated in the fluoxetine-treated rats, suggesting that fluoxetine induced apoptosis. Similar results were reported by Fridman and Lowe (2003), while the findings of Nori-Garavand et al. (2020) in selenium-treated mice contradict the results of this study.

Conclusively, it can be suggested that imipramine induced follicular growth and apoptosis in female rats, while fluoxetine probably inhibited follicular growth and apoptosis in female rats.

Conflict of Interest

There is an absence of conflicting interests in this research work.

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Journal of Experimental Biology and Agricultural Sciences, June - 2023; Volume – 11(3) page 598 – 611

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Effect of Transpiration on the Monocot Ornamental Plants Leave Anatomy

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Received – January 27, 2023; Revision – May 12, 2023; Accepted – June 27, 2023
Available Online – June 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(3).598.611

KEYWORDS

Transpiration

Leaf anatomy thickness

Palisade

Spongy

Shrinkage

ABSTRACT

Transpiration refers to the loss of water from leaves, and increased levels can lead to changes in leaf morphology and anatomy, affecting the total thickness. This study aims to determine the effect of transpiration on leaf anatomy, particularly thickness, in six types of monocots ornamental plants, namely *Rhoeo discolor* (L'Her.) Hance ex Walp., *Hymenocallis littoralis* (Jacq.) Salisb., *Cordyline fruticosa* (L.) A. Chev., *Chlorophytum laxum* R. Br, *Dracaena reflexa* Lam, and *Aglaonema commutatum* Schott. The study procedures were conducted using a Factorial Completely Randomized Design (Factorial CRD) with an experimental approach. The first factor was the type of plant, while the second was the condition before and after transpiration. The data obtained were analyzed using ANOVA, followed by LSD and Pearson correlation tests. The results showed that the plant type factor significantly affected the thickness of leaf tissues. The conditions before and after transpiration also significantly impacted all leaf tissues except for the lower epidermis. Furthermore, this finding was supported by the positive correlation between the thickness shrinkage of the upper epidermis-mesophyll and transpiration. The results also revealed that the mesophyll of *R. discolor*, *C. laxum*, *D. reflexa*, and *A. commutatum* differentiated into palisade and spongy layers, but there was no differentiation in the other two species. The transpiration rate was observed to change along with the specific anatomical structure of the leaf tissues. The lowest rate was found in *R. discolor* with thicker hypodermis tissue, while the highest was in *C. laxum* with thinner mesophyll.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

1 Introduction

Urban vegetation plays a crucial role in reducing the adverse effects of heat on the environment (Hsieh 2017; Rahman et al. 2020). Among the various vegetation types, ornamental plants are often found in urban areas due to their aesthetic appeal, which can positively impact people's minds. Furthermore, these plants produce oxygen-rich air and absorb CO_2, thereby improving air quality (Widyastuti 2018). These processes are greatly influenced by various physiological activities, such as transpiration (Williams et al. 2021; Thom et al. 2022), which is essential for growth, as it helps to regulate leaf and environmental temperatures (Salisbury and Ross 1995; Sundberg 1985; Gao et al. 2020). However, excessive transpiration can lead to issues such as wilting, drought, and death (Beckman, 1964), which diminishes the beauty of ornamental plants (Toscano et al. 2019) and change leaf anatomy (Boughalleb et al. 2014). This indicates that leaf anatomy can serve as a crucial indicator for assessing the water requirement of plants (Carr 2013) and determining the level of resistance to drought conditions.

Water loss from leaf tissue has been identified as the primary factor leading to changes in its thickness, as reported by Bachman (Burquez 1987). In a more recent study, Ningsih and Daningsih (2022) investigated the effect of transpiration on six monocot ornamental plants. The results showed alterations in the total leaf thickness of various species, including *Rhoeo discolor* (L'Her.) Hance ex Walp., *Hymenocallis littoralis H. littoralis* (Jacq.) Salisb., *Cordyline fruticosa* (L.) A. Chev., *Chlorophytum laxum* R. Br, *Dracaena reflexa* Lam, and *Aglaonema commutatum* Schott. Kutlu et al. (2009) also reported a similar finding in *Ctenanthe setos*, where the mesophyll thickness decreased under drought conditions. However, contradictory results have been reported by several studies. Such as Burnett et al. (2005) found that the leaf tissue of *Salvia splendens* remained unaffected by water shortage conditions. This suggests that different plants often develop distinct leaf anatomical adaptations to water shortages (Boughalleb et al. 2014). Meidner (1975) reported that the water from the leaves was sourced from the epidermis around the stomata, considering the proximity of the epidermal cells to the substomata cavity. However, Fahn (1995) and Onoda et al. (2015) reported that the epidermis consistently exhibited higher density and rigidity to form a compact layer without intercellular spaces. Epidermal cell walls tend to be thick, accounting for 10-20% of the total volume of leaf cells (Crang et al., 2018). The cuticle, which exists in the outer and inner layers of the epidermis, plays a vital role in preventing water loss (Wullschlenger and Oosterhius 1989). It primarily consists of cutin, an insoluble substance that acts as a barrier to water passage (Beck 2010). As an alternative adaptive mechanism, the epidermis can also thicken its walls to prevent water loss (Nawazish et al. 2006; Hameed et al. 2012; Boughalleb et al. 2014).

According to Taiz et al. (2018), most vapour leaving the leaves originates from the mesophyll tissue. This finding is consistent with Kutlu et al. (2009) and Kapchina-Toteva et al. (2014), who reported that water shortage could be affected by the mesophyll cell shrinking and reducing the number of layers. The flexibility of this tissue and the absence of cuticles can also increase the rate of water loss (Onoda et al. 2015; Crang et al. 2018). The flexibility of the mesophyll is attributed to its elastic properties, which allow it to adjust its thickness. This property is also closely related to the plant cell wall, which regulates thickness. In drought conditions, a decrease in cell size often leads to an increase in elasticity, as Martinez et al. (2007) and Xing et al. (2022) reported.

Several studies have shown that the mesophyll tissue in some plants is differentiated into two layers: palisade and spongy (Hidayat and Niksololihin 1995). The palisades contain a higher number of chloroplasts compared to the spongy cells. Therefore, previous studies have inferred that they have a higher water content due to their association with photosynthesis (Outlaw et al. 1976; Cutler et al. 2007). Further, Boughalleb et al. (2014) and Han et al. (2016) also suggested that mesophyll often experiences shrinkage under drought conditions. According to Canny et al. (2012) and Xing et al. (2022), water loss is more pronounced in the spongy part compared to the palisade.

Ningsih and Daningsih (2022) conducted a study on six types of monocot ornamental plants, and the results showed an association between leaf thickness and transpiration, but the role of shrinking tissues was unknown. The anatomical arrangement and thickness of the six types of plants provided by Daningsih et al. (2022) served as a foundation for further studies. Therefore, this study aims to examine the effect of transpiration on the leaves' anatomy.

2 Materials and Method

2.1 Time and Place

This study was conducted in a simple plant house at the Biology Education Laboratory, Faculty of Teacher Training and Education, Tanjungpura University, Indonesia, from February to September 2022.

2.2 Tools and Materials

The tools used in this study included Olympus-CX 21 microscope, C-1 objective micrometer, Optilab Advance SN: MTN004210791, vials, Euromax rotary microtome, thermometer, lux meter AS803, GM816 anemometer, HTC-2 hygrometer, measuring cup, Ohaus analytical balance, Nikon D5200 DLSR camera, *ImageJ* software, and *Image Raster 3* software. Meanwhile, the materials were vaseline, FAA solution, alcohol, safranin 2%, tert-Butyl alcohol, paraffin oil, fast green 0.5%, xylol, paraffin, Haupt's adhesive, formalin 3%, and distilled water. A total of six selected monocot ornamental plants samples were utilized for slide preparation, including Boat Lily (*Rhoeo discolor*), Spider Lily (*Hymenocallis littoralis*), Cabbage Palm (*Cordyline fruticosa*), Bichetti Grass (*Chlorophytum laxum*), Song of India (*Dracaena reflexa*), and Philippine Evergreen (*Aglaonema commutatum*).

2.3 Method and Research Design

This study used a quantitative experimental method with a Completely Randomized Design Factorial (CRD-Factorial) approach. Furthermore, it determined the effect of various treatments on the thickness of leaf tissue. The first factor was the types of ornamental plants, while the second was the condition before and after transpiration. The combination factor was the interaction of species type and conditions with three replications. In this study, the sample population consisted of 36 plant samples.

2.4 Plant Preparation

The plants used in this study had the same size and almost the same number of leaves. The samples were transferred to a homogeneous planting medium consisting of burnt soil and poor sand in a ratio of 2:1, which were placed in polybags measuring 30 x 35 cm. The plants were watered daily, and the NPK fertilizer was given once every two weeks. After two weeks of adaptation, samples were taken from the planting medium for further analysis.

2.5 Transpiration Measurement

Transpiration rate were measured using modified gravimetric method refered to Ningsih and Daningsih (2020). Meanwhile, the leaf area was measured using the *ImageJ* program proposed by Reinking (2007). The transpiration rate was calculated using the formula below:

$$\text{Transpiration Rate} = \frac{\text{initial weight (gr)} - \text{final weight (gr)}}{\text{time (hours)x leaf area(cm}^2\text{)}}$$

2.6 Leaf Transverse Incision Preparations

Transverse leaf incisions were made using a modified Johansen (1940) paraffin method. The modification was made in several steps, including the thickness of the incision (Sass 1951), the treatment of the paraffin tape after cutting, the adhesive preparations (Berlyn and Miksche 1976), and the use of fabric softener (containing *Diethylester Dimethyl Ammonium Chloride*) on hard paraffin blocks (Orchard et al. 2008). Samples were taken in the basal position under two conditions: before and after transpiration. Although the leaves selected for the pre and post-transpiration measurements differed, they were close to each other. It was assumed the leaves were of the same age and had received comparable amounts of nutrients, light, and water.

During the investigation, the leaf samples were sliced into 1 x 1 cm sections using a razor, following the direction of the main veins, without including them. The monocot samples were taken at 9 AM (Jakarta time) before transpiration, followed by sampling after measurements. The leaves obtained before and after transpiration were soaked in FAA fixative solution for 24 hours. The process was continued with preparation, which consisted of washing, clearing, infiltration, and embedding. Subsequently, the samples were cut into a thickness of 12 µm using a microtome. The incisions were glued to the slide using Haupt's adhesive, placed in an oven at 38^{o}C for 12 hours, and stained using safranin-fast green double stain.

2.7 Measurement of leaf anatomical thickness

Leaf anatomy was observed under an Olympus C-X 21 microscope with 100x magnification, and the images observed were captured with Optilab Advance. Furthermore, the leaf anatomy thickness was measured using Image Raster 3 Software, focusing on the upper epidermis and hypodermis, mesophyll (palisade & spongy), lower epidermis, and total thickness.

2.8 Measurement of External Factor

The external factors of this study were measured using various tools. For example, a thermometer measured the temperature, while air humidity was assessed using the HTC-2 hygrometer. Furthermore, the wind speed was calculated using the GM816 anemometer, and light intensity was measured with the AS803 lux meter.

2.9 Statistical Analysis

Data on the thickness of leaf tissue anatomy were analyzed using an ANOVA test, which determined the effect of the treatment. Subsequently, further analysis was carried out using the Least Significance Different (LSD) test at the 5% level to determine the difference among all treatments. The process was then continued with the Pearson correlation test to determine the relationship between the transpiration rate and the difference in leaf tissue anatomy before and after transpiration. The classification of the correlation coefficient was carried out based on the method proposed by Amruddin et al. (2022).

3 Result

3.1 Transpiration Rate of Six Monocot Ornamental Plants

The transpiration rate data obtained in this study were subjected to normality and homogeneity tests. The results showed that the data had normal distribution ($p>0.05$) and homogeneity ($p>0.05$). Based on the ANOVA results, the transpiration rate differed significantly from one plant to another ($p<0.05$). The highest rate was obtained

Table 1 The average difference in the transpiration rates of six types of monocot ornamental plants

Plant Types	Average of Transpiration Rate (gr.cm^{-2}hour^{-1})
Bichetii Grass (*C. laxum*)	0.00963[a]
Philippine Evergreen (*A. commutatum*)	0.00484[b]
Cabbage Palm (*C. fruticosa*)	0.00374[bc]
Spider Lily (*H. littoralis*)	0.00262[cd]
Song of India (*D. reflexa*)	0.00259[d]
Boat Lily (*R. discolor*)	0.00187[d]

Note: The difference in letters behind the numbers shows a significant difference in the LSD test (α=0.05).

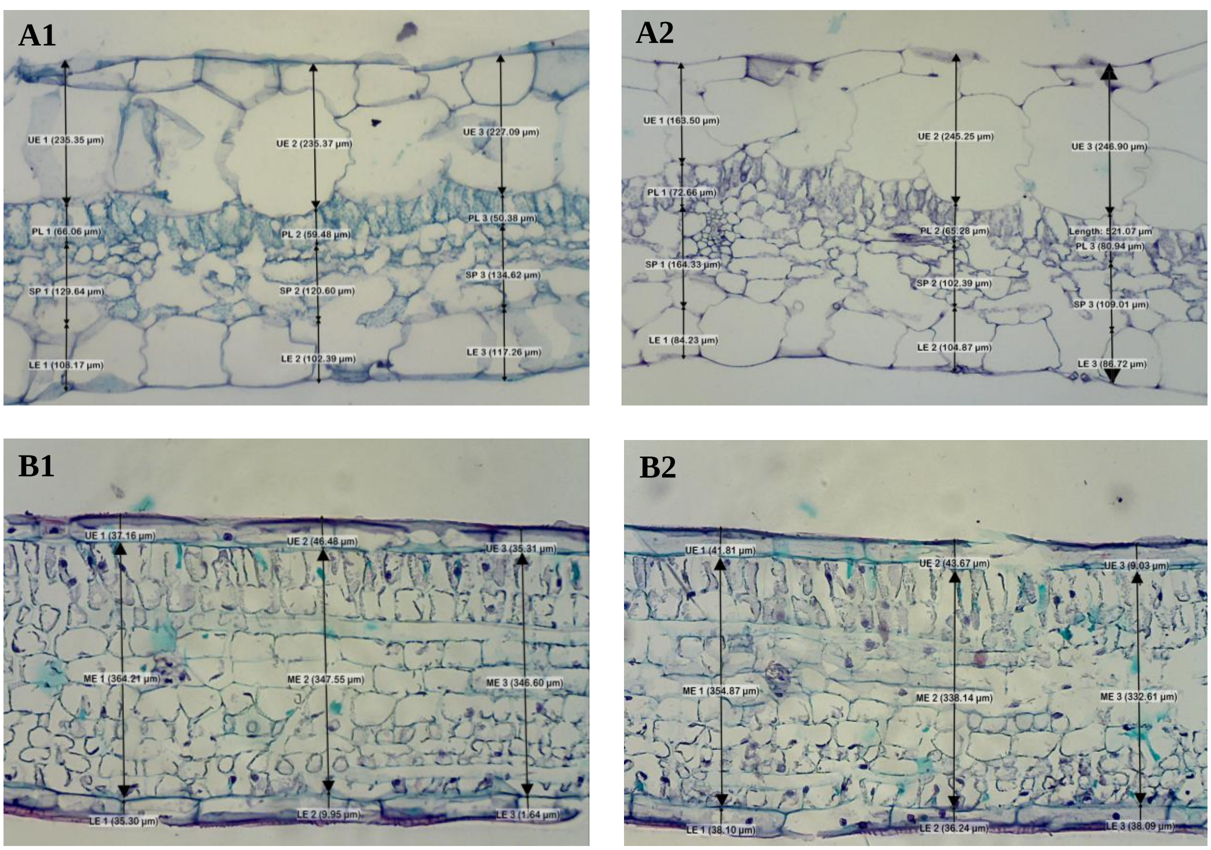
in the Bichetii Grass (*C. laxum*), while the lowest was found in Boat Lily (*R. discolor*). The results of various plants' transpiration rates recorded in this study are presented in Table 1.

Based on Table 1, the transpiration rates of Bichetii Grass were significantly different from other plants. Meanwhile, the Philippine Evergreen and Cabbage Palm had no significant difference. Similarly, Cabbage Palm and Spider Lily do not show any significant difference. The results showed that Spider Lily exhibited high similarity with the song of India and Boat Lily. These measurements were carried out at an average temperature of 35^{o}C, with air humidity level, wind velocity, and light intensity of 49%, 0.367 m.s^{-1}, and 103.5901 watts.m^{-2}, respectively.

3.2 Leaf Anatomy of Six Monocot Ornamental Plants

The leaf anatomy of six types of monocot ornamental plants consisted of the upper epidermis and hypodermis, mesophyll (palisade and spongy), and lower epidermis, as shown in Figure 1.

The current study documented that the upper and lower epidermis of the six monocot ornamental plants consisted of one-layer tissue with a rectangular and tight arrangement. However, the Boat Lily had an additional tissue under the upper epidermis, known as the hypodermis. The observation showed that the upper epidermis tended to be thicker than the lower epidermis. In some plants, such as Spider Lily and Cabbage Palm, these tissues were almost the same



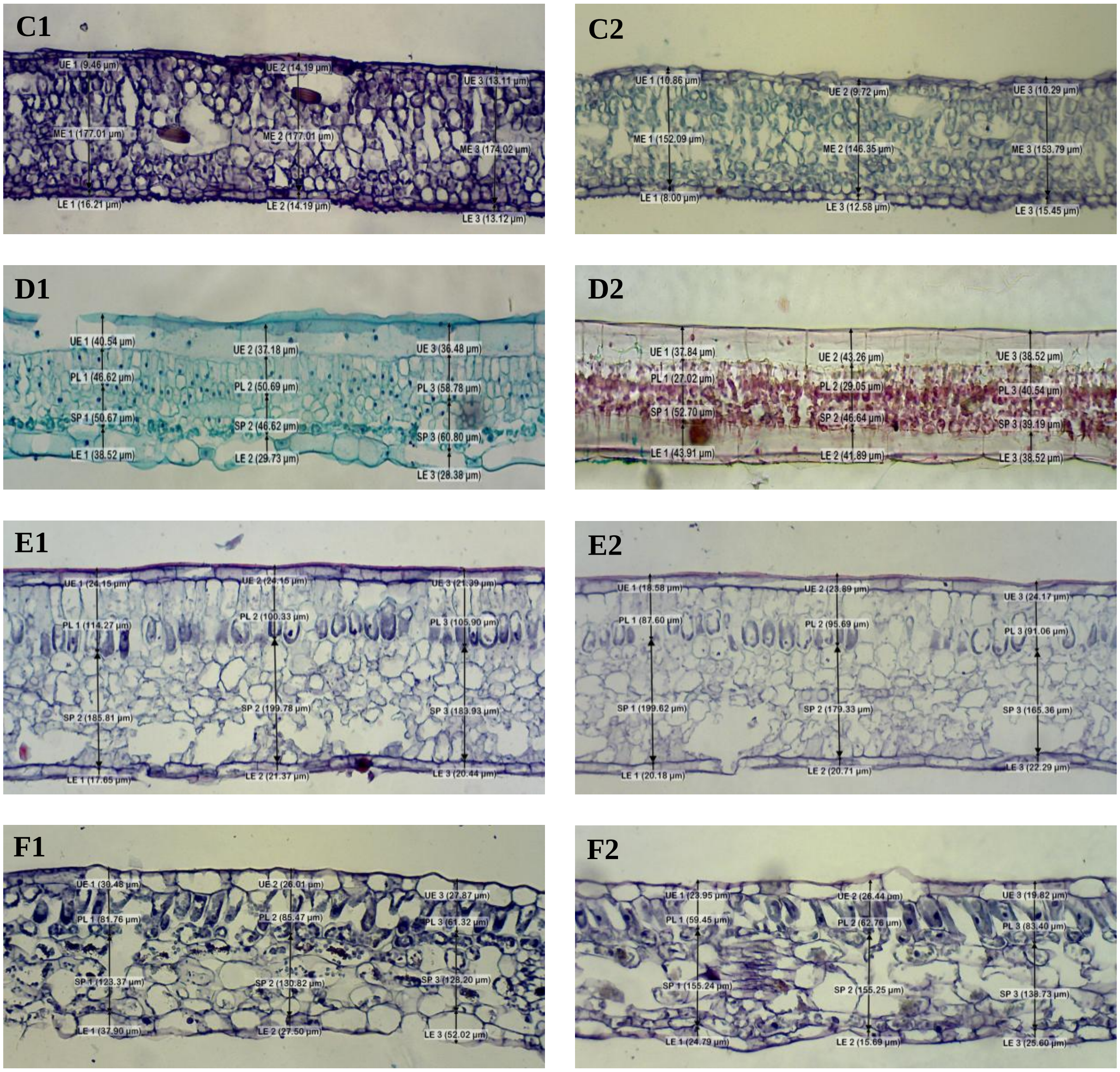


Figure 1 Leaf anatomy of six types of monocot ornamental plants, before transpiration (1), after transpiration (2), Boat Lily (*R. discolor*) (A), Spider Lily (*H. littoralis*) (B), Cabbage Palm (*C. fruticosa*) (C), Bichetii Grass (*C. fruticosa*) (D), Song of India (*D. reflexa*) (E), Philippine Evergreen (*A. commutatum*) (F).

size. This study also found that the mesophyll in Spider Lily and Cabbage Palm leaves were similar (homogeneous) in terms of the palisade and spongy segment. The undifferentiated mesophyll was due to the chlorenchymatous cells containing several chloroplasts. Meanwhile, the four other plants' mesophyll showed significant structure, shape, and arrangement differences. The palisade leaves of Boat Lily and Bichetii Grass were composed of one dense layer with a tubular shape. In Philippine Evergreen, one loosely arranged layer was observed. The palisade of Song of India was composed of two layers, which tended to be dense. The spongy tissue of Song of India, Boat Lily, and Philippine Evergreen showed larger and denser intercellular spaces compared to Bichetii Grass.

3.3 ANOVA Test Results for Leaf Tissue Anatomical Thickness

The normality test showed that all data were normally distributed ($p>0.05$). However, data on the total, mesophyll, and palisade thickness ($p<0.05$) were not homogeneous. The Factorial-CRD results using the ANOVA test showed that the plant type factor had a significant effect ($p<0.05$) on all leaf tissue. Furthermore, the condition factor had a significant effect ($p<0.05$), except in the lower epidermis. The results showed that the interaction between plant species and conditions has a significant impact ($p<0.05$) on the upper epidermis. All the leaf tissue responded differently after transpiration except the lower epidermis. Before and after

Table 2 ANOVA results of leaf thickness of six monocot ornamental plants

Factor	Average of leaf thickness (µm)					
	TT	UE	LE	ME	PL	SP
Plant Types	*	*	*	*	*	*
Boat Lily (*R. discolor*)	535.171[a]	234.087[a]	118.800[a]	182.204[d]	58.762[b]	123.425[c]
Spider Lily (*H. littoralis*)	431.266[b]	39.154[c]	38.594[b]	343.518[a]	-	-
Cabbage Palm (*C. fruticosa*)	182.152[e]	14.254[e]	12.527[c]	155.371[e]	-	-
Bichetii Grass (*C. laxum*)	174.900[e]	47.276[b]	39.274[b]	88.455[f]	39.337[c]	47.835[d]
Song of India (*D. reflexa*)	321.342[c]	24.892[d]	17.841[c]	278.609[b]	97.694[a]	180.916[a]
Philippine Evergreen (*A. commutatum*)	255.211[d]	28.662[d]	27.114[bc]	199.435[c]	61.279[b]	138.156[b]
Condition	*	*	ns	*	*	*
BeforeTanspirasi (BT)	327.727[a]	66.658[a]	42.810	218.290[a]	68.097[a]	129.341[a]
After Transpirasi (AT)	305.620[b]	62.784[b]	41.933	200.903[b]	60.439[b]	116.469[b]
Combination of plant types and condition	ns	*	ns	ns	ns	ns
BT- Boat Lily (*R. discolor*)	552.259	243.047[a]	119.360	189.887	61.159	128.692
AT- Boat Lily (*R. discolor*)	518.047	225.127[b]	118.399	174.521	56.364	118.157
BT- Spider Lily (*H. littoralis*)	437.076	38.811[e]	39.965	358.299	-	-
AT- Spider Lily (*H. littoralis*)	425.457	39.497[d]	37.223	348.737	-	-
BT- Cabbage Palm (*C. fruticosa*)	189.564	14.360[g]	13.516	161.688	-	-
AT- Cabbage Palm (*C.fruticosa*)	174.739	14.148[g]	11.537	149.053	-	-
BT- Bichetii Grass (*C. laxum*)	181.514	48.114[c]	38.855	94.754	42.679	52.087
AT- Bichetii Grass (*C. laxum*)	165.286	46.437[c]	39.693	82.155	35.996	43.583
BT- Song of India (*D. reflexa*)	342.839	26.778[ef]	26.692	297.589	105.762	191.826
AT- Song of India (*D. reflexa*)	299.846	23.006[f]	27.536	259.630	89.626	170.004
BT- Philippine Evergreen (*A. commutatum*)	263.078	28.837[e]	18.472	207.546	62.788	144.758
AT- Philippine Evergreen (*A. commutatum*)	247.348	28.488[e]	17.209	191.323	59.770	131.553

Note: *= Significant on (α=0.05), ns= Non-significant, TT= Total Thickness, UE= Upper Epidermis, LE= Lower Epidermis, ME= Mesophyll, PL= Palisade, SP= Spongy, BT= Before Transpiration, and AT= After Transpiration. The difference in letters behind the numbers indicates a significant difference in the LSD test (α=0,05).

transpiration, the interaction factor between species and condition only influenced the upper epidermis, as shown in Table 2.

3.4 Correlation Between Anatomical Shrinkage Thickness of Leave and the Transpiration Rate

This study examined the shrinkage in leaf thickness, encompassing the upper epidermis, mesophyll, palisade, spongy tissue, and total anatomical thickness of the six monocot ornamental plants. Table 3 presents a range of results, varying from no correlation to strong correlation.

The result showed that each plant had different anatomical thicknesses due to the varying response to transpiration. This served as a criterion for selecting plants more resistant to drought. Furthermore, the correlation coefficient showed a mixed relationship between the shrinkage of leaf anatomical thickness and the transpiration rate. This indicated the tendency that the response was caused by plant genetic characteristics rather than transpiration.

3.5 The Potential Relationship of Transpiration Rate and Thickness Shrinkage of Leaf Tissue

The data explained in the previous section were used for mapping the relationship between the shrinkage of anatomical tissue thickness and the transpiration rate. Table 4 shows the pattern of specific anatomical shrinkage with the transpiration rate (from high to low).

Table 3 Correlation between the shrinkage of leaf tissue anatomical thickness and the transpiration rate on six types of monocot ornamental plants

Plant Types	Transpiration	Leaf Anatomical Thickness Correlation Coefficient Value				
		TT	UE	ME	PL	SP
Boat Lily (*R. discolor*)	2.00929×10^{-3}	0.711	0.935	0.596	0.005	0.680
		strong	very strong	moderate	uncorrelated	strong
Spider Lily (*H. littoralis*)	2.62796×10^{-3}	0.166	0.613	0.993	-	-
		very weak	strong	very strong		
Cabbage Palm (*C. fruticosa*)	3.74401×10^{-3}	0.924	0.672	0.936	-	-
		very strong	strong	very strong		
Bichetti Grass (*C. laxum*)	9.63219×10^{-3}	0.999	0.695	0.998	0.705	0.932
		very strong	strong	very strong	strong	very strong
Song of India (*D. reflexa*)	2.59619×10^{-3}	0.999	0.256	0.806	0.875	0.245
		very strong	very strong	strong	strong	Very weak
Philippine Evergreen (*A. commutatum*)	4.84362×10^{-3}	0.949	0.458	0.942	0.057	
		very strong	weak	very strong	uncorrelated	very strong

Note: TT= Total Thickness, UE= Upper Epidermis, ME= Mesophyll, PL= Palisade, and SP= Spongy.

Table 4 Potential relationship between transpiration rate and shrinkage of leaf tissue thickness on six types of monocot ornamental plants.

Transpiration Rate	High →			Low
Leaf Tissue Shrinkage	SP (I)	SP (I)	SP (II)	SP (I)
	PL (I)	PL (II)	PL (I)	PL (II)
The thickness of UE&LE	Medium	Thin	Thin	Thick
Species	*Chlorophytum laxum*	*Aglaonema commutatum*	*Dracaena reflexa*	*Rhoeo discolor*

Note: PL=Palisade, SP= Spongy, UE= Upper Epidermis, LE=Lower Epidermis. I= very strong-strong, II= uncorrelation-moderate.

Based on Table 4, the palisade's and spongy tissue's thickness impacted leaf shrinkage due to transpiration. The results showed that the transpiration rate increased following a decrease in the thickness of the spongy and palisade tissue, as observed in Bichetti Grass. In this study, Bichetti Grass strongly correlated with the shrinkage of the upper epidermis, palisade, and spongy. This characteristic indicated that its tissue could not withstand the transpiration rate, leading to massive water loss. Based on these findings, it was concluded that the species cannot live in water shortage conditions. However, lower transpiration occurred when only one of the tissues experienced high shrinkage, such as in the Philippine Evergreen and Song of India. The transpiration rate was also lower when the upper and lower epidermis was thick, such as in Boat Lily.

Discussion

Transpiration involves the process of releasing water from leaves into the air in the form of vapour. In plants, this process was often controlled by stomata cells and was aimed at achieving energy (Hopkins and Huner 2009; Kocchar and Gujral 2020). Furthermore, the plants with the highest and lowest transpiration rates among the six types of monocot ornamental plants had the same sequence pattern as the findings of Ningsih and Daningsih (2022). The highest rate was found in Bichetti Grass, while the Boat Lily had the lowest (Table 1). The four other plants showed varying veining patterns compared to Ningsih and Daningsih (2022). This difference was influenced by internal and external factors (Salisbury and Rose 1995). The internal factors included plant variations (Wang et al. 2022) and leaf thickness (Giulani et al. 2013), while the external were temperature (Yang et al. 2012; Sugiarto et al. 2020), wind speed (Kuiper 1961; Schymanski and Or 2015), humidity (Tullus et al. 2012), and light intensity (Park et al. 2020).

Ningsih and Daningsih (2022) measured the transpiration rate at a temperature of 32°C, with 20.30 watts.m^{-2} light intensity, 42% humidity, and 1 m.s^{-1} wind speed. Furthermore, the external factors' value was lower than the current study, except for the wind speeds. The transpiration rate obtained in this study was lower than that of Ningsih and Daningsih (2022). This difference was caused by the high temperature, which led to the closure of the stomata (Schulze et al. 1973). This finding was consistent with Slot and Winter (2017) that the stomata often closed at an average temperature of 30^{o}C. Lugassi et al. (2015) also reported that high light intensity could lead to the closure of the organ, thereby causing a decrease in transpiration. This phenomenon occurs in citrus plants which are expressed with *Arabidopsis* hexokinase. The closing of the stomata has long been known as a plant's adaptation strategy to reduce excessive transpiration (Agurla et al. 2018). Therefore, it could be assumed that the transpiration rate depended on the environmental conditions. In this study, Bhicetti Grass had the highest rate, followed by Philippine Evergreen, Cabbage Palm, Spider Lily, Song of India, and Boat Lily.

Several studies reported transpiration's association with leaf anatomy (Giulani et al. 2013; Da Costa and Daningsih 2022; Daningsih et al. 2022; Ningsih and Daningsih 2022). According to Cutler et al. (2007), the anatomical structure of leaves was generally the same, consisting of the epidermis, mesophyll, and vascular tissue. However, each species had distinctive features despite belonging to the same group (Budel et al. 2017). These variations were dominantly influenced by genetics (Olsen et al. 2013), leaf position, and leaf age (Xie and Luo 2003). Environmental factors (Cassola et al. 2019), including light intensity (Coble and Cavelari 2017), temperature (Von Caemmerer and Evans 2014), humidity, and water content affecting stomata (Fanourakis et al. 2013) have been reported to also contribute to these variations. The differences in leaf anatomical structure could be used as a basis for species identification (Mabel et al. 2014; Budel et al. 2017; Ozcan et al. 2015; Bahadur et al. 2018; Chatri et al. 2020; Da Costa Santos et al. 2020), as well as an essential indicator for determining plant adaptation to different habitats (Carr 2013; Bertel et al. 2016; Oguchi et al. 2018).

The six types of monocot ornamental plants showed varying leaf characteristics, consistent with Wang et al. (2022), that each species had distinctive features. According to Wang et al. (2022) the 6 test plants were unique, as exemplified by Boat Lily with hypodermis tissue. The observation results showed that the mesophyll of Boat Lily, Bichtti Grass, Song of India, and Philippine Evergreen differentiated into palisades and spongy layers. Meanwhile, mesophyll was not differentiated in the Spider Lily and Cabbage Palm, as shown in Figure 1. The variation in leaf features was likely influenced by the genetics of each species (Coneva and Chitwood 2018).

Six monocot ornamental plants' upper and lower epidermis comprised a single layer of dense tissue. They were typically rectangular, except for the upper epidermis of Boat Lily, which was equipped with a hypodermis (Figure 1). The result presented in Table 2 indicated that the samples' upper epidermis was thicker than the lower epidermis. According to Tihurua et al. (2020), a thick epidermis could reduce the transpiration rate, thereby preventing changes in thickness. Boughalleb et al. (2014) also found that the thickness in the A*stragalus gombiformis* Pomel plant did not change due to water outflow (Fahn 1995; Becraft 1999; Cutler et al. 2007; Kutschera 2008; Crang et al. 2018).

This study showed a significant difference in the epidermal thickness of six monocot ornamental plants between the pre and post-transpiration conditions (Table 2). Based on the results, there were no changes in the thickness of the upper epidermis of Cabbage Palm, Bichetti Grass, Song of India, and Philippine Evergreen due to the rigidity of the cells. However, the differences in this parameter and the effects of transpiration were more visible in Boat Lily and Cabbage Palm. These variations were indicated by the combined LSD shown in Table 2. The difference in the thickness of the upper epidermis was probably caused by the different adaptations in some plants, specifically Cabbage Palms, which experienced thickening due to transpiration. The observation results showed that the Boat Lily experienced shrinkage due to the reduction in the thickness of the hypodermis.

The hypodermis was typically a colourless tissue with the primary function of storing water and was located in the upper (adaxial) or lower (abaxial) epidermis (Das 1999; Nurida et al. 2012). It was often found in plants inhabiting aqueous or halophyte habitats (Cutler 2007; Rashid et al. 2020; Tihurua et al. 2020), as well as minimal water areas (xerophytes) (Cutler 2007; Griffiths and Male 2017; Hafiz et al. 2013). Although the hypodermis served as a storage area, its water content could still diffuse out, leading to a decrease in thickness. In this current study, the thickness of the hypodermis decreased along with a reduction in that of the upper epidermis caused by transpiration (Table 2). Kutlu et al. (2009) found that the hypodermis, located under the upper epidermis of *Ctenanthe setosa*, had the flexibility to reduce its size under minimal water conditions, leading to the curling of the leaves in some cases.

The measurement of the upper epidermis thickness of the Boat Lily was not carried out separately in this study. Future studies should perform separate measurements of the upper epidermis and hypodermis tissue to determine the reduction level. The upper epidermis of the Boat Lily did not change significantly after transpiration, except for the hypodermis layer. Furthermore, the hypodermis experienced a reduction in size due to transpiration. Under these conditions, the shrinkage could lead to a decrease in the upper epidermis of Bichetti Grass. The bottom epidermal tissue of the six types of monocot ornamental plants was visually thin compared to the upper epidermis. The results also showed non-significant differences between the conditions before and after transpiration, as shown in Table 2. The absence of differences revealed that the epidermal tissue was composed of rigid and stiff cells. Therefore, the lower epidermis only experienced a slight reduction and did not differ much between both conditions. Several studies have also reported that this tissue layer could protect the component above it (Beck 2010).

Table 2 shows some significant changes in the mesophyll of the six monocot ornamental plants before and after transpiration. Galmes et al. (2013) stated that changes in mesophyll were some of the strategies of plants to adapt to water availability. Based on morphology, the leaves of Bichetii Grass were curved after exposure to high temperatures and light with a lack of water. This indicated that the plant could not adapt well to water drought conditions. According to Boughalleb et al. (2014) and Han et al. (2016), mesophyll could experience shrinkage due to the occurrence of transpiration. Zhang et al. (2014) and Vastag et al. (2020), changes in the thickness of the tissue were caused mainly by the accumulation of water. Consequently, the mesophyll became thicker compared to others (Table 2). The tissue also had an elastic ability that caused changes in its size due to the cellular component and a shift in the balance between the evaporation rate and water supply (Canny and Huang 2005; Xing et al. 2022). A previous study reported that a size reduction could also occur due to decreased intercellular space (Canny and Huang 2005; Kutlu et al. 2009).

Four monocot ornamental plants, including Boat Lily, Bichetti Grass, song of India, and Philippine evergreen had mesophylls that differentiated into palisades and spongy tissues. Meanwhile, the other two plants, spider lily and Cabbage Palm, were undifferentiated. Monocots commonly had undifferentiated mesophylls, and this was consistent with the finding of El-Gawad and El-Amier (2017) on three species, including *Arundo donax*, *Pennisetum setaceum*, and *Saccharum Spontaneum*. However, some of the plants could have differentiated mesophylls palisade. According to Sumardi and Wulandari (2010), *Musa* spp., a monocot plant, had a leaf anatomy consisting of palisade and spongy tissues with numerous chloroplasts. Similar results were also obtained by Kocyigit et al. (2023) in *Allium sphaeronixum*. Cutler et al. (2007) highlighted that undifferentiated mesophyll was commonly found in the monocot species, with few exceptions.

The results showed a relationship between the size or the number of mesophylls layer and the transpiration rate (Table 3). The Boat Lily had the lowest transpiration rate among the six plants due to its unique anatomical structure. The hypodermis, located between the upper epidermis and the palisade, was only found in Boat Lily, suggesting that this extra layer was the cause of the low rate (Figure 1). The results showed that the thicker the mesophylls, the lower the level of transpiration, as observed in undifferentiated and differentiated plants. The spider lily had the thickest mesophylls (343.518 µm, Table 2) and showed the lowest rate.

Species with differentiated mesophylls consisting of two palisade layers, including Bichetti Grass and Song of India showed lower transpiration rates than those with one layer, such as Philippine evergreen, as shown in Figure 1. Palisade was usually composed of compact and dense cells and appeared as the densest part of the mesophyll tissue on visual observation (Cutler et al. 2007). Several studies have shown that this tissue's thickness could help prevent water loss (Ashton and Berlyn 1992; Oliveira et al. 2018), leading to resistance to environmental changes. In this study, the palisade tissue in the Song of India was composed of two thicker layers, leading to lower transpiration rates (Figure 1; Table 1). Meanwhile, its looseness in the Philippine evergreen caused a higher transpiration rate than India's song. These phenomena indicated that thick palisade tissue played a role in resisting evaporation, but transpiration could also occur due to the spacing between the palisade cells, as shown in Figure 1 and Table 1. This was evidenced by the significant shrinkage experienced after transpiration in four types of monocot ornamental plants. According to Kapchina-Toteva et al. (2014), environmental differences could affect the number of palisade layers. In this study, the four monocot ornamental plants did not show any change in the number of palisade layers because they grew in the same environment.

Four of the six monocot ornamental plant types showed irregular spongy tissue forms (Figure 1). Spongy tissue had a wide extracellular space, accommodating more H_2O and CO_2 (Xing et al. 2022), which played an important role in the physiological processes in leaves (Salisbury and Ross 1995). According to Canny et al. (2012), it experienced higher water loss levels than Palisades. Furthermore, Kapchina-Toteva et al. (2014) found a reduction in spongy thickness in *Lamium album* L. plants, which were grown under different conditions, but the structure did not change. In this study, the spongy thickness of the four types of monocot ornamental plants before and after transpiration showed a significant decrease, indicating the loss of water (Table 2). Canny et al. (2012) stated that the shrinkage of this tissue could occur due to a reduction in the spaces between the cells.

Bichetti Grass visually had the thinnest spongy thickness, with denser inter-cell spaces compared to Boat Lily, song of India, and Philippine evergreen (Figure 1). Although the spaces between the spongy cells in Bichetti Grass were denser, the transpiration rate was significantly higher than in other plants (Table 1). Bichetti Grass contained water in its palisade tissue and spongy cells. Consequently, the water discharge from the leaves caused shrinkage of the two tissues, as indicated by the total mesophyll thickness, which was thinner than other species. Bichetti Grass had the thinnest total thickness compared to other plants (Table 2).

According to Buckley et al. (2015), reducing the leaves' total thickness could increase the hydraulic conductivity outside the xylem. Similar results were reported by Rahman et al. (2020) in species with thin leaves and simple shapes. The shape indicated the presence of higher water loss compared to species with thicker or more compound leaves. This study also found a similar condition, specifically in Bichetti Grass, where the plants had a high transpiration rate due to thinner leaves. A total of types of monocot ornamental plants, namely spider lily and Cabbage Palm, had mesophyll that was not differentiated into palisades and spongy (Figure 1). Spider lily have thicker and denser mesophyll than Cabbage Palm, which has dense but thin tissue. Consequently, the transpiration rate was lower than Cabbage Palm, as shown in Figure 1. According to Bosabalidis and Kofidis (2002), denser mesophyll cells could reduce the transpiration rate.

The leaf anatomy thickness changes significantly impacted by reducing the total thickness (Figure 1; Table 2). According to Osakabe et al. (2013), a reduction in leaf thickness indicated the loss of water from the cells, leading to a decrease in turgor pressure, softening, and a decrease in size. The correlation test results showed a mixed relationship between shrinkage in leaf anatomical thickness and transpiration (Table 3).

Each plant had various leaf anatomical characteristics and could adapt to different environmental habitats (Bertel et al. 2016). Plants with thick leaves could adapt better in environments exposed to sunlight than compared to others (Zwieniecki and Boyce 2014). Based on previous studies, plants with narrow and thick leaves, such as Boat Lily adapted to their habitat by forming a thicker epidermis. The leaves were also equipped with a hypodermis below the upper epidermis, leading to low transpiration rates. This indicated that the Boat Lily was more drought-resistant. Furthermore, the presence of the hypodermis played a role in storing water when the Boat Lily experienced drought. Boat Lily and Song of India also had the potential to resist environmental changes, as indicated by the two layers of palisade and low transpiration rate.

Conclusion

Transpiration caused leaf anatomy shrinkage in six monocot ornamental plants, specifically in the mesophyll tissue. The upper epidermis generally did not experience changes, but shrinkage occurred in the hypodermis of Boat Lily. The results showed that transpiration increased along with shrinkage of the palisade and spongy, such as in Bichetti Grass. However, lower rates were observed when only one of the tissues between the palisades or spongy experienced reduction, as shown in the song of India, Philippine evergreen, and Boat Lily, which experienced a decrease in the hypodermis. Among the six monocotyledon ornamental plants, the leaves of Boat Lily were more adaptive and resistant to growing in sunlight and water-deficient conditions than Bichetti Grass.

Acknowledgement

The authors are grateful to the Faculty of Teacher Training and Education, Tanjungpura University, for providing financial support for this study.

Conflict of Interest

The authors declare that there is no conflict of interest in this study.

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Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Pluronic F-127 hydrogel for stem cell research: a bibliometric analysis using Scopus database

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Received – May 28, 2023; Revision – June 25, 2023; Accepted – April 29, 2023
Available Online – June 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(3).612.624

KEYWORDS

Pluronic F-127

Tissue engineering

Thermoresponsive hydrogel

Mesenchymal stem cells

Encapsulation

Regenerative medicine

ABSTRACT

Stem cell research holds immense promise in regenerative medicine. However, the successful utilization of stem cells relies on their inherent properties and the appropriate support matrix that provides an optimal environment for growth and differentiation. Optimizing their delivery and retention at the target site is crucial to enhance stem cell-based therapies' effectiveness. In recent years, hydrogels have emerged as a popular choice for culturing and delivering stem cells due to their unique properties, including biocompatibility, tunable physical and chemical characteristics, and mimicking the native extracellular matrix. Among the various hydrogels available, Pluronic F-127 (PF-127) has gained significant attention in stem cell research. This paper aims to study the publication trends of research that discuss the utilization of PF-127 hydrogel for stem cell research. The analysis is based on data extracted from the Scopus database using bibliometric methods. The results revealed the publication trends, collaboration patterns among authors and institutions, research areas, influential journals, funding agencies, and thematic connections in this field. By understanding the current state of research and identifying key areas of focus, this analysis provides valuable insights for researchers and practitioners interested in harnessing the potential of PF-127 hydrogel in regenerative medicine and tissue engineering.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Stem cell research holds immense potential in regenerative medicine (Sharun et al. 2020; Bist et al. 2021; Sharun et al. 2021; Peer et al. 2022; Sharun et al. 2022a; Sivanarayanan et al. 2023). However, the successful utilization of stem cells relies not only on their inherent properties but also on the appropriate support matrix that provides an optimal environment for their growth and differentiation (Bist et al. 2021; Peer et al. 2022; Sivanarayanan et al. 2023). In recent years, hydrogels have emerged as a popular choice for culturing and delivering stem cells due to their unique properties, including biocompatibility, tunable physical and chemical characteristics, and ability to mimic the native extracellular matrix (Tsou et al. 2016; Mantha et al. 2019). Among the various hydrogels available, PF-127 has gained significant attention in stem cell research (Sharun et al. 2023).

PF-127 is a thermosensitive and biocompatible hydrogel that exhibits a sol-gel transition at physiological temperatures, making it suitable for various biomedical applications (Sharun et al. 2023). Its unique thermoresponsive behaviour allows for easy gelation and subsequent encapsulation of stem cells, providing them with a supportive 3D microenvironment (Tsou et al. 2016; Mantha et al. 2019). Additionally, PF-127 hydrogel possesses excellent biodegradability and controllable release properties, making it an ideal candidate for controlled drug delivery and tissue engineering (Diniz et al. 2015). The use of PF-127 hydrogel in stem cell research offers numerous advantages. Its non-toxic nature and high-water content facilitate cell viability, proliferation, and differentiation. The hydrogel's porous structure facilitates efficient nutrient and oxygen exchange, promoting cell survival and functionality (Youn et al. 2021; García-Couce et al. 2022). Moreover, PF-127 can be easily modified to incorporate bioactive molecules, growth factors, and therapeutic agents, enhancing the bio functionality of the hydrogel and promoting specific cellular responses (Zhou et al. 2013; Kim et al. 2014).

It is necessary to explore the recent advancements and promising outcomes achieved using PF-127 hydrogel in regenerative medicine (Sharun et al. 2023). By understanding the capabilities and limitations of PF-127 hydrogel for stem cell research, we can unlock its full potential in harnessing the power of stem cells for therapeutic interventions. This knowledge will contribute to developing novel strategies for improving stem cell-based therapies and advancing regenerative medicine, bringing us closer to realizing effective clinical applications.

This paper aims to study the publication trends of research that discuss the utilization of PF-127 hydrogel for stem cell research. We will also address the challenges and future directions in the field, such as improving long-term cell viability, achieving controlled release kinetics, and enhancing tissue regeneration.

2 Materials and Methods

2.1 Data extraction

The data extraction was performed on June 21, 2023, using the Scopus database (Elsevier B.V) to identify the works of literature that use PF-127 in stem cell research. The search strategy aimed to collect the journal articles (research articles) data published to date while certain publication types such as reviews, editorials, conference papers, book chapters, letters, books, notes, short surveys, retracted papers, erratum, and reports are excluded (Sharun et al. 2022a; Sharun et al. 2022b). Articles published in any language were included in the analysis. The following search query was employed: TITLE-ABS-KEY [(poloxamer 407 OR "Pluronic F-127" OR pf-127 OR pluronic-f-127 OR "Pluronic™ F-127" OR "Plutonic F127" OR pf127 OR "Pluronic® F-127" OR "Pluronic® F127") AND ("stem cell" * OR "stromal cell" *) and (LIMIT-TO (DOCTYPE, "ar")) AND (LIMIT-TO (SRCTYPE, "j")].

2.2 Analysis of variables

The extracted data file was further analyzed using R Studio and the bibliometrix package, a powerful open-source tool specifically designed for science mapping analysis within the R statistical programming language (Aria and Cuccurullo 2017). The extracted data were further analyzed, and interpretation was made based on the findings.

2.3 Visualization

The data was visualized using VOS viewer software (version 1.6.17) (van Eck and Waltman 2010). In addition, Biblioshiny, a web application framework that integrates with the R programming language, was also used for creating interactive and dynamic bibliographic data visualization tools (Aria and Cuccurullo 2017).

3 Results and Discussion

3.1 Overview of publication trends

The data presented encompasses the timespan from 2010 to 2020 and includes information from 17 documents. The annual growth rate for these documents is 0%, indicating a stable publication output over the specified period. The average age of the papers is 7.29 years, suggesting that the data primarily includes relatively recent research. On average, each document has been cited 33.86 times, indicating moderate impact and recognition within the scholarly community. Regarding document contents, the data includes 496 keywords identified as "Keywords Plus" and 63 author-provided keywords. The data involves 98 authors, with no single-authored documents among the 17 analyzed. The collaboration among authors is evident, with an average of 7.14 co-authors per document, indicating a high level of cooperation in the field. Additionally, 28.57% of the co-authorships are international, reflecting a global perspective and collaboration within the research community. The document types included in the dataset are solely articles, indicating a focus on original research and scientific analysis.

3.2 Research areas

The subject areas in the Scopus database that have been published on PF-127 hydrogel in stem cell research vary in terms of the number of documents (Figure 1). Engineering appears to be the most prominent subject area, with 11 articles indicating a significant focus on the engineering aspects of using PF-127 hydrogel in stem cell research. The fields of Biochemistry, Genetics, Molecular Biology, Materials Science, and Medicine are equally represented with seven documents each, highlighting the interdisciplinary nature of this research topic. Additionally, Pharmacology, Toxicology, and Pharmaceutics have contributed three articles, indicating the interest in exploring the potential therapeutic applications of PF-127 hydrogel. Chemical Engineering has published two articles focusing on this hydrogel's engineering and manufacturing aspects. Lastly, Physics and Astronomy have contributed 1 document. This data reflects the multidisciplinary nature of PF-127 hydrogel research in stem cell studies involving various scientific disciplines and their collaborative efforts.

3.3 Analysis of keywords

The WordCloud illustrating the top 50 most frequent Keywords Plus is given in Figure 2a. Poloxamer (38), article (16), hydrogel (13), rats (13), cell survival (12), mesenchymal stromal cells (12), nonhuman (12), animal (11), animals (11), cell differentiation (11), chemistry (11), hydrogels (11), male (11), mesenchymal stem cell (11), and mesenchymal stroma cell (11) were some of the most frequently used Keywords Plus identified in this study. Similarly, Figure 2b presents the WordCloud of the top 50 authors' keywords used in their publications. Hydrogel (3), cell therapy (2), alginate/poloxamer systems (1), biocompatibility/soft tissue (1), bioprinting (1), biphilic (1), bmscs (1), bone (1), bone graft (1), and bone marrow mesenchymal stem cell (1) were some of the most frequently used authors keywords identified in this study.

3.4 Identification of top institutes and countries

Sun Yat-Sen University is the leading affiliation in productivity, with four publications dedicated to this subject. Université Paris Descartes follows closely behind with three publications, demonstrating its significant contributions to the scientific community's understanding of the intersection between PF-127 hydrogel and stem cell research. Several affiliations, including the Royal College of Surgeons in Ireland, Universidad de Santiago de Compostela, Saarland University Medical Center, Technical Institute of Physics and Chemistry, Universidade de Santiago de

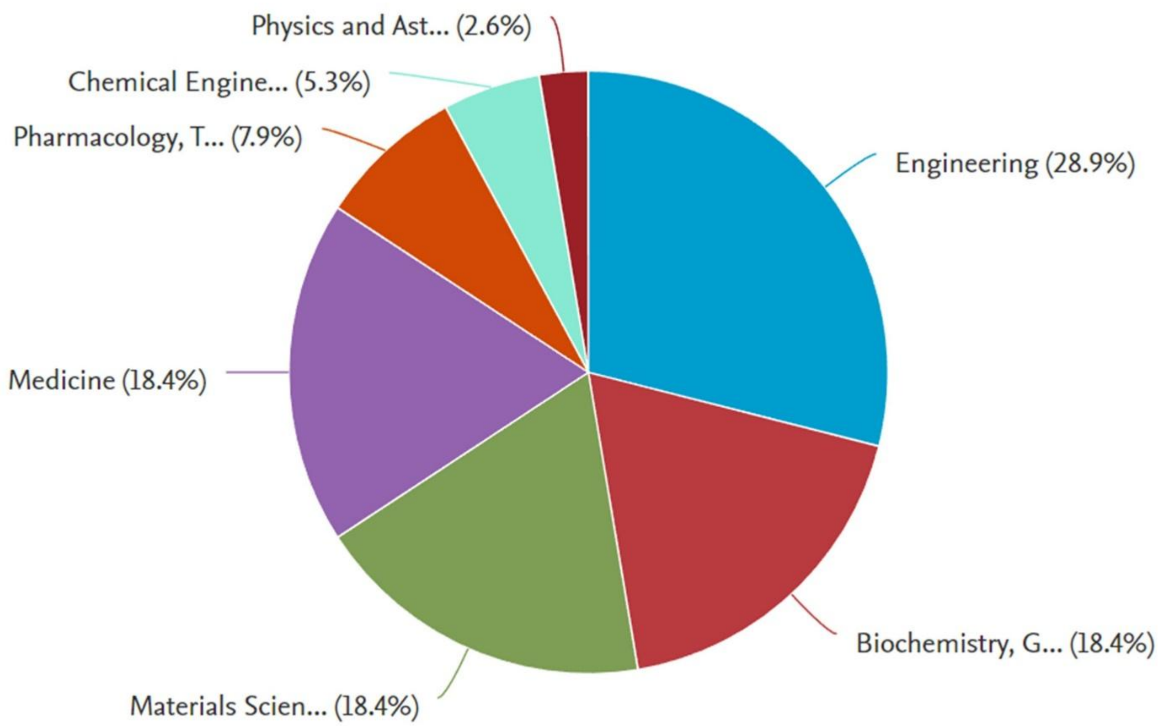

Figure 1 Pie chart illustrating the subject areas under which articles on Pluronic F-127 hydrogel for stem cell research are classified in the Scopus database.

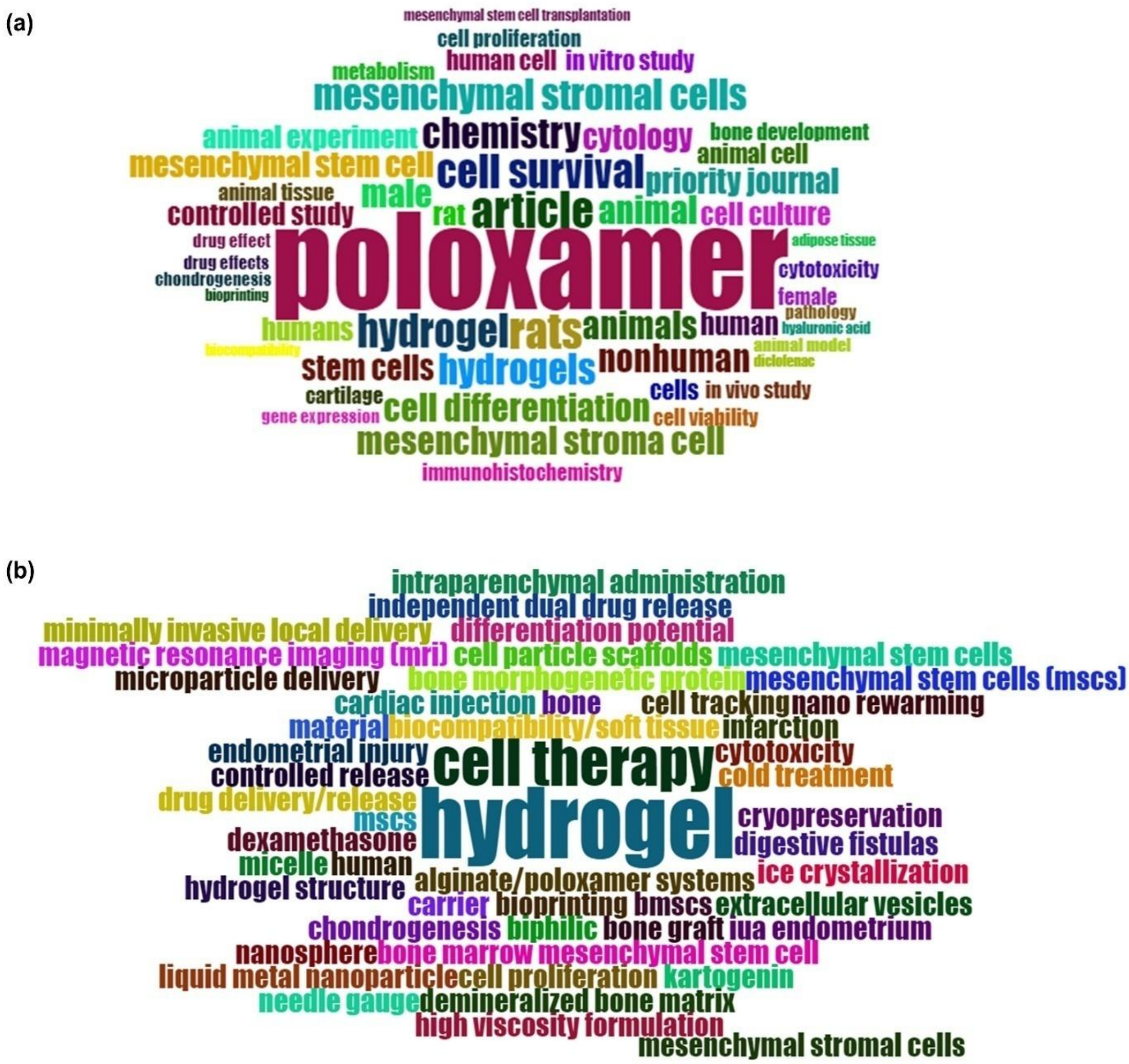

Figure 2 (a) Word Cloud illustrating the top 50 most frequent Keywords Plus (b) and 50 most frequent words by authors keywords.

Compostela, Trinity College Dublin, University of Iowa, and the University of Southern California, have all made notable contributions with two publications each. These affiliations represent a diverse range of research institutions from different countries, indicating the global interest in exploring the potential of PF-127 hydrogel in stem cell research. The collective efforts of these affiliations have enriched the scientific literature and furthered our understanding of the applications and implications of this hydrogel in the context of stem cells.

Among the countries, China is the most prolific, with 12 publications dedicated to this topic (Figure 3). China's strong presence in the field of PF-127 hydrogel research demonstrates its commitment to advancing stem cell studies and exploring the potential applications of this hydrogel in regenerative medicine. France and South Korea are closely behind China, contributing significantly with nine publications each. These countries have shown a substantial interest in the intersection of stem cell research and PF-127 hydrogel, contributing valuable insights to the scientific community. The United States, a leading hub of scientific research, has also made notable contributions with seven publications. Ireland and Spain demonstrate a commendable presence in the field, with five publications each indicating their active involvement in stem cell research involving PF-127 hydrogel. Other countries such as Belgium, Germany, the United Kingdom, Brazil, and Mexico have also made contributions with smaller numbers of publications. The collective efforts of these countries reflect the global interest in exploring the potential of PF-127 hydrogel in stem cell research and highlight the collaborative nature of scientific exploration in this field.

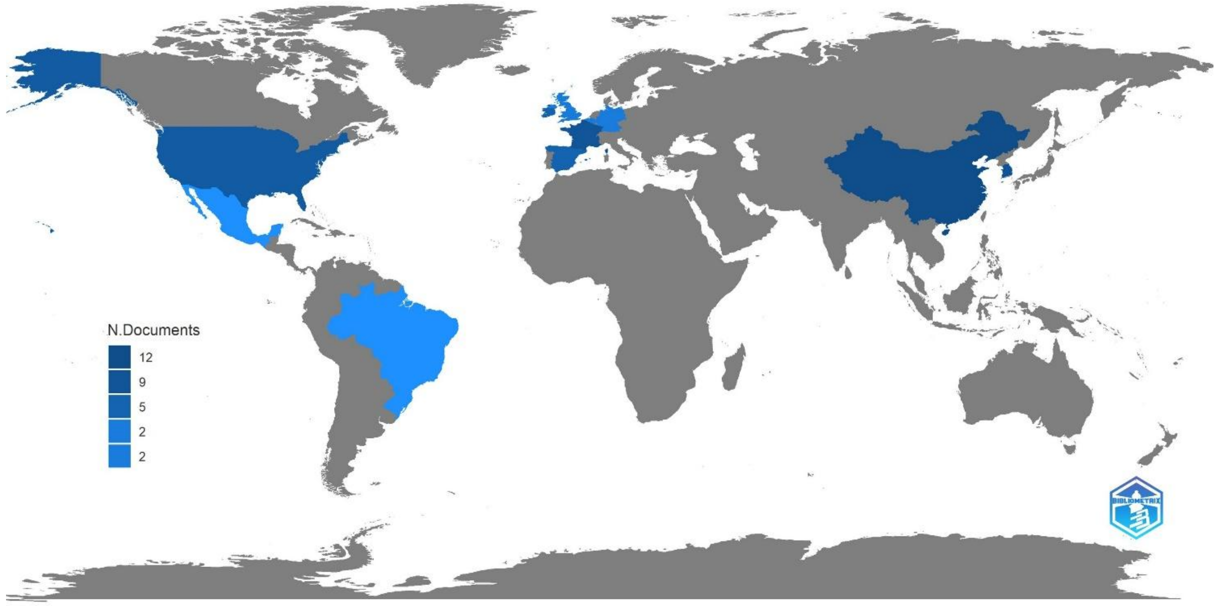

Figure 3 Map representing Country Scientific Production on Pluronic F-127 hydrogel for stem cell research across the globe.

3.5 Most cited countries

The data highlights the most cited countries and their corresponding total citations (TC) and average article citations. The United States (USA) leads the list with a total citation count of 172, indicating a high impact and recognition of research originating from the country. The average article citation for the USA is 57.30, suggesting that publications from the US receive a significant number of citations on average. Korea follows closely behind with a total citation count of 129 and an average article citation of 32.20, indicating a strong impact and influence of Korean research within the scholarly community. With a total citation count of 76 and an average article citation of 38.00, China demonstrates significant recognition and impact in the field. France has a total citation count of 49, with an average article citation of 49.00, reflecting a high average citation rate for publications originating from the country. Germany, the United Kingdom, Spain, and Ireland have lower total citation counts and average article citations, indicating a relatively lesser impact in citation numbers. Nevertheless, it is important to note that these values can vary depending on the specific research field and the time frame considered.

3.6 Identification of influential journals

The data provided showcases the most relevant journals publishing articles on PF-127 hydrogel in stem cell research and the corresponding number of published articles. Acta Biomaterialia, International Journal of Pharmaceutics, Chinese Journal of Tissue Engineering Research, and Journal of Biomedical Materials Research - Part B Applied Biomaterials emerge as the leading journals in this field, each publishing two articles on PF-127 hydrogel and its applications in stem cell research. These journals have established themselves as prominent platforms for disseminating research. Additionally, ACS Nano, Journal of Controlled Release, Cell Transplantation, CIOS Clinics in Orthopedic Surgery, Journal of Biomedical Materials Research - Part A, Stem Cell Research and Therapy, Journal of Materials Science: Materials in Medicine, West China Journal of Stomatology, and Tissue Engineering - Part C: Methods are also recognized as contributors to this field, each publishing one article on PF-127 hydrogel and its role in stem cell research. These journals serve as important outlets for researchers to share their findings and advancements, highlighting the diverse range of journals contributing to the body of knowledge in this area. Collectively, these publications contribute to the growing understanding of the applications and potential of PF-127 hydrogel in stem cell research.

Bradford's law is a bibliometric concept that suggests that scientific literature can be divided into zones based on the frequency of publication in certain journals. Zone 1, according to Bradford's law, represents the core journals that publish a significant portion of the articles in a particular field. In the given data, three journals, namely Acta Biomaterialia, Chinese Journal of Tissue Engineering Research, and International Journal of Pharmaceutics belong to zone 1 of Bradford's law. Overall, including Acta Biomaterialia, Chinese Journal of Tissue Engineering Research, and International Journal of Pharmaceutics in zone 1 of Bradford's law indicates their significance as core journals in their respective fields. These journals serve as important platforms for researchers to publish their findings related to PF-127 hydrogel and its applications, contributing to advancing knowledge in biomaterials, tissue engineering, and pharmaceutical sciences.

3.7 Funding agencies

The data provided highlights the sponsorship of articles by various funding agencies. Among the mentioned agencies, most articles were sponsored by the National Natural Science Foundation of China, with three publications. The European Regional Development Fund and the National Research Foundation of Korea follow closely, each sponsoring two articles.

Several other funding agencies have sponsored one article each, including the Beijing Municipal Science and Technology Commission, Engineering and Physical Sciences Research Council, Fundación Pedro Barrié de la Maza, Institut National de la Santé et de la Recherche Médicale, Korea Institute of Science and Technology, Centre National de la Recherche Scientifique, Ministerio de Economía y Competitividad, National Key Research and Development Program of China, Ministerio de Educación, Cultura y Deporte, Ministry of Education, Science and Technology, Ministry of Health and Welfare, Ministry of Knowledge Economy, National Institute of Dental and Craniofacial Research, Instituto de Salud Carlos III, National Institutes of Health, Natural Science Foundation of Guangdong Province, Sun Yat-sen University, Université Paris Descartes, and Xunta de Galicia. This diverse range of funding agencies reflects the international nature of the research and the importance of financial support from various organizations in advancing scientific knowledge and innovation.

3.8 Thematic map

In bibliometric analysis, a thematic map containing development degree and relevance degree provides valuable insights into the distribution and characteristics of research topics within a specific field (Figure 4). The development degree indicates the research activity and output level in a particular research area or region. It reflects the quantity and growth of publications related to specific themes. A higher development degree suggests greater research output in that area. On the other hand, the relevance degree in a thematic map indicates the degree of interconnectedness or similarity between different research topics or areas. It helps identify clusters or groups of closely related research themes. A higher relevance degree indicates stronger thematic connections or shared characteristics among the topics.

The thematic map on PF-127 hydrogel in stem cell research is inserted in Figure 4. Researchers can gain a comprehensive understanding of the research landscape by considering both the development degree and relevance degree in a thematic map. They can identify hotspots of research activity, detect emerging trends, and uncover areas of high significance and potential for collaboration. This information can guide decision-making, resource allocation, and strategic planning in bibliometric analysis and research evaluation.

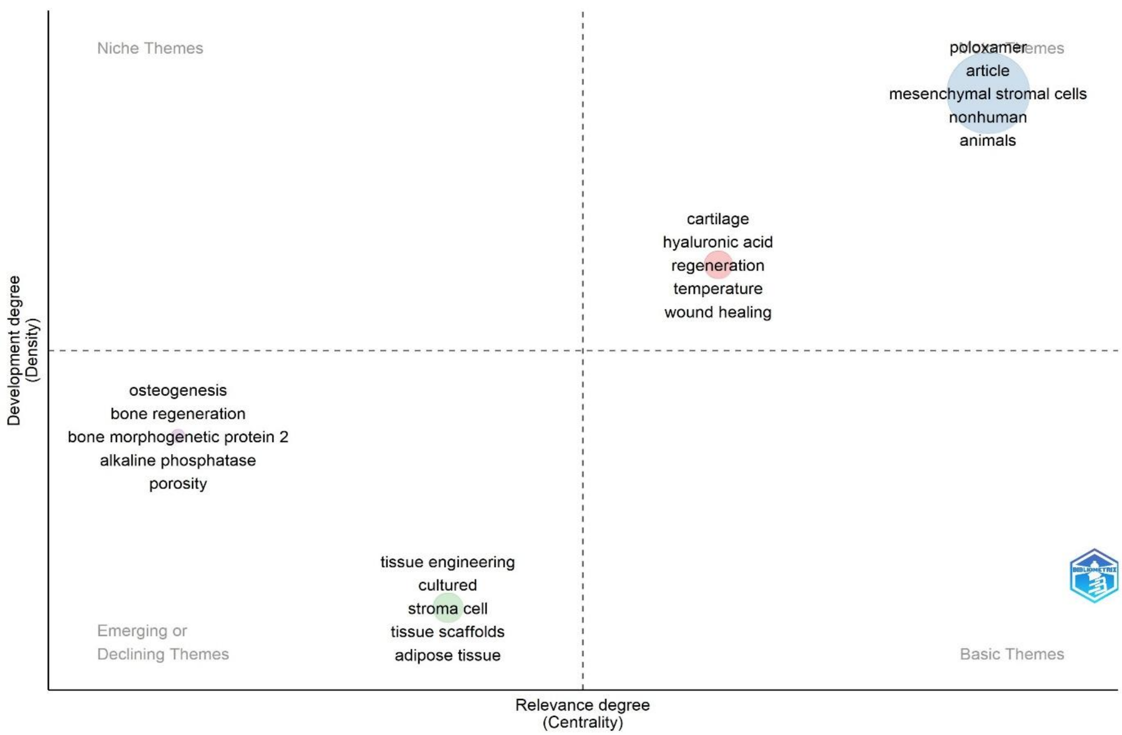

Figure 4 The thematic map on Pluronic F-127 hydrogel in stem cell research.

3.9 Network visualization map

The map indicates the connections between authors. Specifically, authors with at least one document were selected for inclusion in the map. Although 111 authors met this threshold, some were not connected; therefore, the most extensive set of connected items constituting 16 authors were illustrated (Figure 5a). Similarly, 4 countries of the 11 countries that met the threshold were illustrated since they formed the largest network (Fig. 5b).

3.10 Research on PF-127 hydrogel for stem cell research

The salient findings of the 17 papers published on the use of PF-127 hydrogel for stem cell research are presented in Table 1. Diniz et al. (2015) investigated the potential of dental pulp stem cells (DPSCs) to undergo differentiation into bone and fat tissues when encapsulated in a PF127 hydrogel scaffold. The viability, proliferation, and differentiation of DPSCs were assessed, and the results revealed that PF-127 hydrogel is a promising and safe scaffold for encapsulating DPSCs. These findings suggest that PF-127 hydrogel can be considered a potential candidate for delivering DPSCs in tissue engineering applications. Similarly, Kang et al. (2016) study describes the synthesis of F127/COS/KGNDCF nanospheres, which are composed of outer cross-linked PEO chains of dicarboxylate PF127 and chitosan oligosaccharide (COS) conjugated with kartogenin (KGN) and inner PPO chains of diclofenac (DCF) loaded PF127. Furthermore, the F127/COS/KGNDCF nanospheres were found to promote chondrogenic differentiation of human BMSCs, with enhanced effects observed upon cold shock treatment.

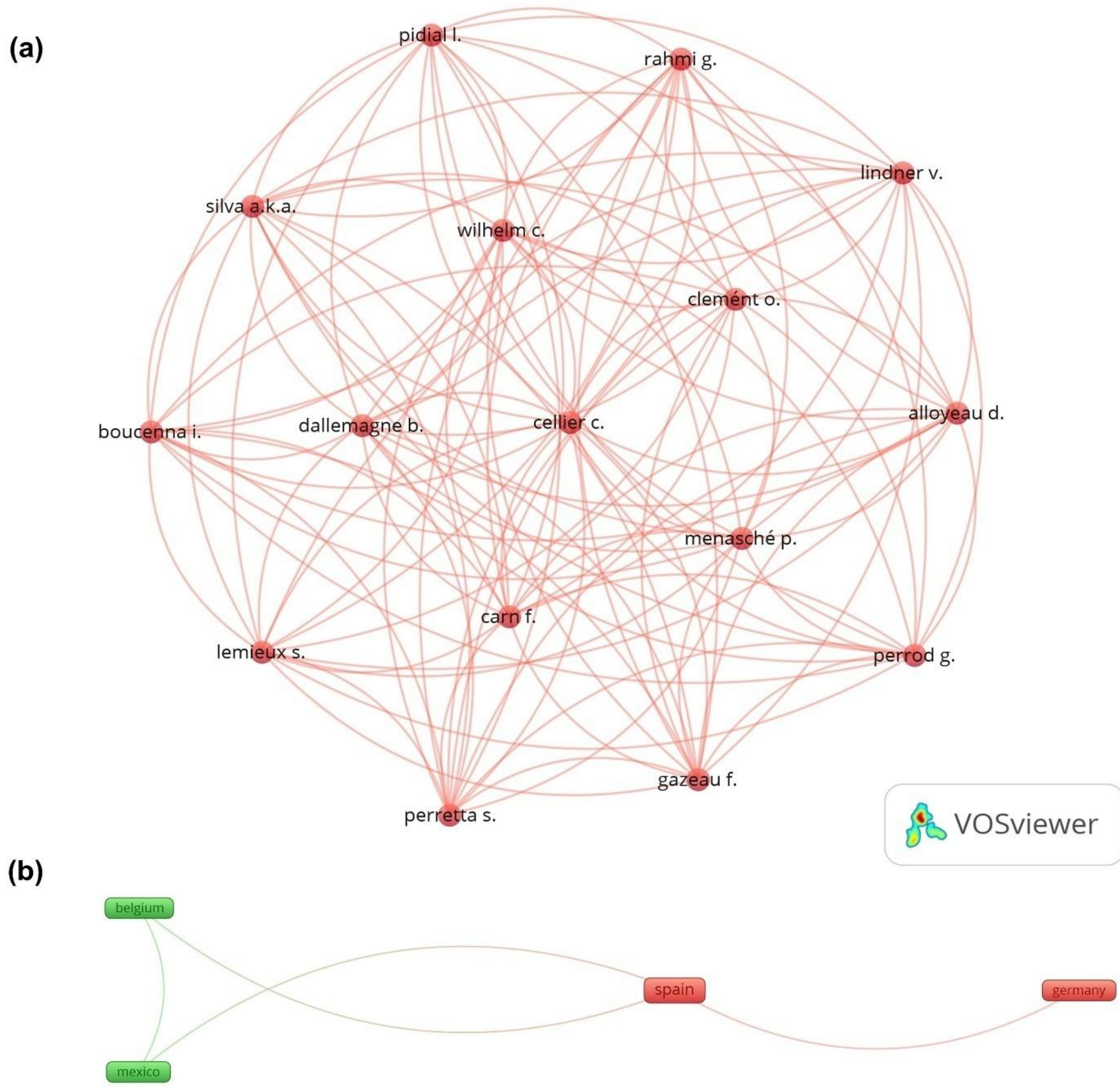

Figure 5 (a) The network visualization map indicating the connecting link between authors and (b) countries.

Table 1 Characteristics of the research articles that use Pluronic F-127 hydrogel in stem cell research.

Paper	DOI	Total Citations	TC per Year	Normalized TC
Diniz IM et al. Pluronic F-127 hydrogel as a promising scaffold for encapsulation of dental-derived mesenchymal stem cells. J Mater Sci Mater Med. 2015 Mar;26(3):153. PMID: 25773231; PMCID: PMC4477746.	10.1007/s10856-015-5493-4	124	13.78	1.65
Kang ML et al. Thermoresponsive nanospheres with independent dual drug release profiles for the treatment of osteoarthritis. Acta Biomater. 2016 Jul 15;39:65-78. PMID: 27155347.	10.1016/j.actbio.2016.05.005	79	9.88	1.84
Silva AKA, et al. Thermoresponsive Gel Embedded with Adipose Stem-Cell-Derived Extracellular Vesicles Promotes Esophageal Fistula Healing in a Thermo-Actuated Delivery Strategy. ACS Nano. 2018 Oct 23;12(10):9800-9814. PMID: 30231208.	10.1021/acsnano.8b00117	49	8.17	1.69
Yang H, et al. Vitamin C plus hydrogel facilitates bone marrow stromal cell-mediated endometrium regeneration in rats. Stem Cell Res Ther. 2017 Nov 21;8(1):267. PMID: 29157289; PMCID: PMC5697119.	10.1186/s13287-017-0718-8	46	6.57	1.89
Hou Y, et al. Soft liquid metal nanoparticles achieve reduced crystal nucleation and ultrarapid rewarming for human bone marrow stromal cell and blood vessel cryopreservation. Acta Biomater. 2020 Jan 15;102:403-415. PMID: 31734413.	10.1016/j.actbio.2019.11.023	30	7.50	1.00
Seol D, et al. Biocompatibility and preclinical feasibility tests of a temperature-sensitive hydrogel for the purpose of surgical wound pain control and cartilage repair. J Biomed Mater Res B ApplBiomater. 2013 Nov;101(8):1508-15. PMID: 24591226.	10.1002/jbm.b.32981	27	2.45	1.86
Díaz-Rodríguez P, et al. Effective genetic modification and differentiation of hMSCs upon controlled release of rAAV vectors using alginate/poloxamer composite systems. Int J Pharm. 2015 Dec 30;496(2):614-26. PMID: 26556623.	10.1016/j.ijpharm.2015.11.008	26	2.89	0.35
Bae SE, et al. Effect of temporally controlled release of dexamethasone on in vivo chondrogenic differentiation of mesenchymal stromal cells. J Control Release. 2010 Apr 2;143(1):23-30. PMID: 20056124.	10.1016/j.jconrel.2009.12.024	25	1.79	1.00
Gettler BC, et al. Formation of Adipose Stromal Vascular Fraction Cell-Laden Spheroids Using a Three-Dimensional Bioprinter and Superhydrophobic Surfaces. Tissue Eng Part C Methods. 2017 Sep;23(9):516-524. PMID: 28665236.	10.1089/ten.tec.2017.0056	21	3.00	0.86
Kim TH, et al. Bone morphogenetic proteins-immobilized polydioxanone porous particles as an artificial bone graft. J Biomed Mater Res A. 2014 May;102(5):1264-74. PMID: 23703875.	10.1002/jbm.a.34803	14	1.40	1.12
Lee JH, et al. The effect of poloxamer 407-based hydrogel on the osteoinductivity of demineralized bone matrix. Clin Orthop Surg. 2014 Dec;6(4):455-61. PMID: 25436071; PMCID: PMC4233226.	10.4055/cios.2014.6.4.455	11	1.10	0.88
Qutachi O, et al. Improved delivery of PLGA microparticles and microparticle-cell scaffolds in clinical needle gauges using modified viscosity formulations. Int J Pharm. 2018 Jul 30;546(1-2):272-278. PMID: 29753905.	10.1016/j.ijpharm.2018.05.025	9	1.50	0.31
Argibay B, et al. Easy and Efficient Cell Tagging with Block Copolymer-Based Contrast Agents for Sensitive MRI Detection in Vivo. Cell Transplant. 2016 Oct;25(10):1787-1800. PMID: 27093950.	10.3727/096368916X691303	7	0.88	0.16
Curley CJ, et al. An in vitro investigation to assess procedure parameters for injecting therapeutic hydrogels into the myocardium. J Biomed Mater Res B ApplBiomater. 2017 Nov;105(8):2618-2629. doi: 10.1002/jbm.b.33802. Epub 2016 Oct 20. PMID: 27764526.	10.1002/jbm.b.33802	6	0.86	0.25
Zhou N, et al. [Experimental study on transplantation of bone morphogenetic protein-2 gene transfected bone mesenchymal stem cells compounded with Pluronic F-127 for promoting bone regeneration in rabbit mandibular distraction]. Hua Xi Kou Qiang Yi Xue Za Zhi. 2013 Jun;31(3):247-52. Chinese. PMID: 23841294.	NA	2	0.18	0.14
Dong C, Xin J. Effect of kartogenin/pluronic f127 micelles on osteogenic differentiation of bone marrow mesenchymal stem cells. Chin J Tissue Eng Res. 2021;25(34):5473-7.	10.12307/2021.241	0	0.00	0.00
Yan L, et al. Hydrogel combined with bone marrow mesenchymal stem cells in the treatment of damaged endometrium in rats. Chin J Tissue Eng Res. 2022;26(31):4940-5.	10.12307/2022.776	0	0.00	0.00

Silva et al. (2018) explore the potential therapeutic application of allogenic extracellular vesicles (EVs) derived from ASCs for fistula healing. A local minimally invasive delivery strategy was employed, where the EVs were administered in a porcine fistula model using PF-127 gel. The gel was injected locally at 4 °C and gelled at body temperature, effectively retaining the EVs in the entire fistula tract. The results demonstrate the successful induction of a therapeutic effect in the swine fistula model through ASC-EV delivery within the PF-127 gel, highlighting its potential as a local minimally invasive approach. Yang et al. (2017) investigated the feasibility of using a combination of hydrogel PF-127, Vitamin C (Vc), and a mixture of BMSCs to enhance endometrial regeneration in a rat model of intrauterine adhesion (IUA) caused by mechanical damage. In vitro experiments showed that Vc improved the survival and health of PF-127-encapsulated BMSCs, counteracting the cytotoxic effects of PF-127 and promoting cell survival and growth. Therefore, a cell therapy approach combining a biomaterial scaffold, BMSCs, and Vc as modulatory factors were found to promote damaged IUA endometrium restoration.

Hou et al. (2020) reported that the PF-127-liquid metal nanoparticles (PLM NPs) demonstrated excellent characteristics, including uniform particle size, high photothermal conversion efficiency (52%), stable photothermal performance, and low cytotoxicity. The viability of human BMSCs after cryopreservation using PLM NPs reached 78±3%, three times higher than conventional warming methods (25±6%). Seol et al. (2013) investigated the safety and efficacy of a novel PF127 and hyaluronic acid-based hydrogel (HG) for therapeutic delivery. Standard in vitro cytotoxicity and drug release tests were performed, and *in-vivo* biocompatibility tests were conducted in a rat model. Additionally, the effectiveness of the HG as a stem cell carrier in a rat cartilage defect model was determined. The HG exhibited comparable levels of viability and biocompatibility to those reported for PF127 or hyaluronic acid individually. Stem cells encapsulated within the hydrogel remained in their original position and promoted cartilage regeneration in experimental defects.

Díaz-Rodríguez et al. (2015) developed hydrogel structures using alginate (AlgPH155) and poloxamer PF127 for encapsulating and releasing recombinant adeno-associated viral (rAAV) vectors. The different hydrogel systems exhibited high transduction efficiencies and gene expression levels in human mesenchymal stem cells, comparable to direct vector application. AlgPH155+PF127 demonstrated the most favourable results. Furthermore, no negative impact was observed on cell viability or chondrogenic differentiation potential. These findings suggest the potential of AlgPH155+PF127 hydrogel for the effective and safe delivery of rAAV vectors in stem cell applications. Bae et al. (2010) examined how the release of dexamethasone (Dex) from two groups of poly(lactic-co-glycolic acid) (PLGA) microspheres influenced the chondrogenic differentiation of MSCs in an in vivo setting. Each group of microspheres was incorporated into a composite hydrogel comprising hyaluronic acid (HA) and PF127, along with rabbit MSCs. The release profiles of Dex from each group of microspheres exhibited significant differences over time. The composite hydrogel underwent a sol-gel transition, forming a gel at body temperature. The hydrogel-containing nanoparticle microspheres (NPMS) demonstrated better in vitro cell viability than those with plain microspheres (PMS).

Gettler et al. (2017) utilized three-dimensional bioprinting technology, and this study successfully generated SVF cell-laden spheroids embedded in a collagen I biomatrix. The findings demonstrate that a biphilic surface can create a uniform and viable SVF-laden spheroids. By incorporating automation through a 3D bioprinter, the process becomes high throughput and suitable for point-of-care clinical applications. Additionally, using a water-soluble hydrophilic spot and the phase transition properties of PF-127 enable minimally disruptive spheroid removal, facilitating manipulation for various applications. These collagen-based SVF spheroids offer a potential strategy to enhance the therapeutic efficacy of cellular infusions in regenerative medicine by improving cellular localization and retention.

According to Kim et al. (2014) BMPs were immobilized onto polydioxanone (PDO)/PF127 porous particles to create a bone graft. Heparin and BMPs (BMP-2 and BMP-7, single or dual) were sequentially bound to the porous particles. The BMPs were successfully immobilized onto the particle surfaces via heparin binding and exhibited sustained release for up to 21 days, regardless of the BMP type. The BMP-immobilized particles effectively promoted in vitro osteogenesis of BMSCs. These BMP-immobilized PDO/PF-127 porous particles, whether containing BMP-2 alone or a combination of BMP-2 and BMP-7, hold promise as a bone graft option for clinical applications involving delayed or insufficient bone healing.

Further, Lee et al. (2014) suggested that demineralized bone matrix (DBM) possesses osteoinductivity that relies on an appropriate carrier for clinical use. This study evaluated the impact of using a poloxamer 407-based hydrogel as a carrier for DBM compared to sterile water. The findings indicate that the poloxamer 407-based hydrogel demonstrates potential as a DBM carrier, as it promotes ectopic bone formation. However, it negatively affects the osteoblastic differentiation in rat abdominal ectopic bone and MSCs.

Qutachi et al. (2018) investigated the impact of needle diameter on the delivery yield, and a modified viscosity formulation was developed to enhance microparticle delivery through clinically relevant needle diameters. A biocompatible formulation consisting of 0.25% PF-127 and 0.25% carboxymethyl cellulose was identified as optimal, leading to a 520% increase in delivery payload across needle gauges 21-30G. This optimized formulation was utilized to improve the delivery yield of PLGA microparticles and PLGA-cell scaffolds that support viable MSCs. Notably, this study presents the first in vitro delivery of the PLGA-cell scaffold system.

Argibay et al. (2016) suggested that superparamagnetic iron oxide nanoparticles (MNPs) are widely used with MRI to track stem cells. However, commercial MNPs often require transfection agents and lengthy incubation periods for adequate cell labelling and subsequent in vivo cell detection. This study synthesized MNPs coated with PF-127 and tetronic 908 and was evaluated as contrast agents for MRI-based cell detection for MSCs and a mice-derived multipotent neural progenitor cell line (C17.2). These MNPs offer several advantages, including easy preparation, efficient cell labelling, and high sensitivity for in vivo cell detection. Curley et al. (2017) focus on optimizing parameters to effectively retain cell-loaded hydrogels after intramyocardial injection while maintaining cell viability. The research investigates the impact of mechanical factors on hydrogel retention and compares different needle designs. Results indicate that smaller-diameter needles have greater hydrogel retention. When human mesenchymal stem cells (hMSCs) embedded in fibrin hydrogel were injected using helical and 26G bevel needles, no significant difference in cell viability was observed at 48 hours. However, the helical group exhibited lower metabolic activity and decreased cell viability compared to the 26G bevel group over time. These findings emphasize the importance of considering biological and mechanical factors in localized stem cell delivery for myocardial regeneration. Zhou et al. (2013) evaluated the effectiveness of transplanting BMSCs transfected with the BMP-2 gene, combined with PF-127, for promoting bone regeneration in the mandibular distraction osteogenesis (DO) rabbit model. The findings indicate that the transplantation of BMP-2 gene transfected BMSCs combined with PF-127 effectively enhances bone regeneration in rabbit mandibular DO.

Chen and Jiang (2021) investigate the effect of Kartogenin/PF-127 micelles on the directional osteogenic differentiation of BMSCs. Kartogenin is known for its ability to activate the Smad4/Smad5 pathway and promote bone differentiation. However, its drug effect is limited. The study found that the Kartogenin/PF-127 micelles enhanced the osteogenic differentiation of BMSCs. Similarly, Yan et al. (2022) investigated the effect of combining hydrogel with BMSCs on endometrial reproduction in rats. The study results indicate that combining PF-127 hydrogel with BMSCs is feasible for treating endometrial injury. This finding provides new insights and potential strategies for endometrial repair.

Conclusion and Future Prospects

In conclusion, PF-127 hydrogel has shown great potential in various biomedical applications, particularly in regenerative medicine. Several studies have investigated its effectiveness as a scaffold material for encapsulating different types of stem cells, including DPSCs, BMSCs, and ASCs. The findings from these studies consistently indicate that PF-127 hydrogel provides a favourable environment for cell viability, proliferation, and differentiation. It has been demonstrated to support the differentiation of DPSCs into bone and fat tissues, BMSCs into chondrogenic and osteogenic lineages, and MSCs into various lineages such as osteoblasts and adipocytes.

Moreover, the hydrogel has been successfully utilized as a carrier for delivering therapeutic factors and gene vectors, enhancing their effectiveness in promoting tissue regeneration and healing. The thermoresponsive nature of PF-127 hydrogel, which undergoes sol-gel transition upon reaching body temperature, enables minimally invasive and localized delivery of cells and therapeutic agents. This property has been exploited in studies involving the local administration of hydrogel-encapsulated stem cells or extracellular vesicles, demonstrating its potential to improve therapeutic outcomes in tissue repair and regeneration models. Furthermore, PF-127 hydrogel has been combined with other biomaterials, such as alginate, hyaluronic acid, and PLGA, to enhance its properties and functionality. These composite hydrogels have shown improved cell viability, sustained release of therapeutic agents, and enhanced osteogenic or chondrogenic differentiation.

In addition to its applications in tissue engineering, PF-127 hydrogel has been investigated for its use in cell tracking and imaging studies. This advancement can significantly contribute to stem cell therapy by allowing non-invasive cell fate and distribution monitoring. Stem cells have garnered significant attention in regenerative medicine due to their remarkable potential to differentiate into different cell types and promote tissue regeneration. However, efficient and controlled delivery of stem cells to the target site is crucial for successful clinical translation. PF-127 hydrogel provides an ideal platform for stem cell encapsulation, offering numerous advantages that make it a favourable choice for researchers and clinicians.

One of the key features of PF-127 hydrogel is its thermoreversible property. This hydrogel transitions from a liquid state at low temperatures to a gel state at body temperature. This unique characteristic allows for easy handling and minimally invasive delivery. The liquid form facilitates cell encapsulation and homogeneous distribution, while the gel form provides a three-dimensional (3D) scaffold that supports cell proliferation and differentiation. The ability to encapsulate stem cells within the hydrogel matrix ensures their spatial confinement and protection from harsh external environments during transplantation.

Furthermore, PF-127 hydrogel exhibits excellent biocompatibility and biodegradability. It has been extensively studied and proven to be non-toxic to cells and tissues, minimizing the risk of adverse reactions or inflammation. The hydrogel gradually degrades over time, allowing for the release of encapsulated stem cells and their secreted factors, which play a crucial role in tissue regeneration. This controlled release mechanism ensures sustained and localized delivery of stem cells, maximizing their therapeutic potential. The tunable mechanical properties of PF-127 hydrogel make it highly versatile for different tissue engineering applications. By adjusting the concentration of the hydrogel, the stiffness and elasticity can be modulated to match target tissue requirements. The ability of PF-127 hydrogel to mimic the native tissue properties enhances cell adhesion, migration, and integration into the host tissue, facilitating successful tissue regeneration. Moreover, PF-127 hydrogel can be easily functionalized and modified to incorporate bioactive molecules, growth factors, or extracellular matrix components. These modifications can further enhance the stem cell response and guide specific cellular behaviours. The hydrogel can release growth factors in a controlled manner, promoting stem cell differentiation towards desired lineages and facilitating tissue-specific regeneration. Additionally, incorporating bioactive molecules or ECM components can provide signalling cues that mimic the natural tissue microenvironment and improve the overall efficacy of stem cell therapy.

Overall, the studies reviewed demonstrate the promising potential of PF-127 hydrogel as a scaffold material in various stem cell-based applications. Its biocompatibility, thermoresponsive behaviour, and ability to support cell viability, proliferation, and differentiation make it an attractive choice for regenerative medicine applications and drug delivery. However, further research and optimization are still required to exploit its capabilities fully and address potential limitations in different clinical scenarios. Further research and development efforts are needed to optimize the formulation, design, and application of PF-127 hydrogel-based systems to harness the full therapeutic potential of stem cells in regenerative medicine. Overall, PF-127 hydrogel represents a valuable tool in stem cell research, offering a supportive and customizable microenvironment for stem cell growth, differentiation, and delivery. Its versatile properties and compatibility with various cell types make it a promising candidate for future biomedical applications.

Ethical approval

Not applicable.

Data statement

The authors confirm that the data supporting the findings of this study are available within the article.

Funding

No substantial funding is to be stated.

Declaration of Interest

All authors declare that there exist no commercial or financial relationships that could, in any way, lead to a potential conflict of interest.

Authors' contribution

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication

Acknowledgments

The authors thank the Director, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, India,and the All-India Network Program on Diagnostic Imaging and Management of Surgical Conditions in Animals (AINP-DIMSCA) for providing the necessary research facilities to carry out this work.

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