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PESTICIDAL REPERCUSSIONS OF DIFFERENT PLANTS OIL ON LARVAE AND ADULT of *Tribolium confusum* JAQUELIN DU VAL

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ABSTRACT

Continues use of synthetic insecticides has resulted in resistance towards insecticides. Therefore, present study was conducted to evaluate the biocontrol potential of three essential oil fumigation viz., *Zea mays*, *Zingiber officinale*, and *Allium sativum* against flour beetle *Tribolium confusum*. For this purpose, fourth larval instars and adults were treated with a different concentration of selected essential oils. Results of present study revealed that after 24 and 48 hours, fourth larval instars were more susceptible to the used plants oil fumigation as compared to the adults. Among the tested oils, fumigation by ginger oil showed highest mortality rate on the fourth larval instars and adults after 24 and 48 hours and it was followed by the fumigation by garlic essential oil. Therefore, from these results, it can be conclude that essential oils fumigation could be use for the biocontrol of flour beetle *Tribolium confusum*.

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1 Introduction

Mycoplasmal diseases are the infectious diseases caused by smallest wall less prokaryotes known as mycoplasma (Nicholet, 1996; Kumar et al., 2011a). It is now an emerging threat and transboundary epidemiological disease posing a worldwide regulation on small ruminant productions and hence huge economic constraints for farmers and small ruminant rearing countries (Thiaucourt & Bolske, 1996; Ruffin, 2001; Kumar et al., 2011a; Chakraborty et al., 2014; Prats-van der Ham et al., 2015). The pathogens of these diseases Stored grain pests are the major problem for stored grain and their commercial products throughout the world because they reduce the quantity and quality of grains. Storage of grains is an integral part of the post-harvest system since the food material passes on its way from field to the consumers. About 5-15% of the total weight of all cereals, oilseeds and pulses is lost after the harvest (Anonymous, 1989). The estimated annual losses by insects and rodents in transit or during storage are about 10% in North America and 30% in Africa and Asia. In India, the annual loss due to insect pests is about 26% (Anonymous, 2015). However, the food grains account for 20-25% damage by storage insect pests (Rajashekar et al., 2010).

Mostly post harvest losses are because of unsuitable traditional or modern storage facilities. These losses constitute a major problem that is due to infestation by a great horde of insect pests of stored products. The cryptic, hideous creatures caused quantitative and qualitative losses (Upadhyay & Ahmed, 2011). Most probably, it occurred due to unavailability of proper storage facilities especially in the rural regions. This unavoidable damage of grains is due to stored grain insect's pests, which contaminate during storage, shipping and transportation. These insect pests considered a serious constraint in all parts of the globe especially in the 3rd world and developing countries (Talukder et al., 2004; Doby et al., 2008). The infestation by these stored grain pests always leads to depreciation in weight and quality of the stored products (Rayhan et al., 2014). In addition, heavy infestation might induce a great change of fungal and other dangerous microflora infestation (Koul et al., 2008; Rajashekar et al., 2012).

Confusion beetles (*Tribolium confusum*) are the members of the darkling beetle's order *Coleoptera*, family *Tenebrionidae*. Its name is due to its confusion with the rusty red flour beetle *T. castaneum* Herbst, *T. destructor* and *T. ferrugineum* Fab. Currently it is a worldwide insect pest of great economic significance in stored product. It is the most common pest of flourmills, cereal products, groundnuts, cacao, spices, dried figs and dates, copra, dried yam, palm kernels, various nuts, oilseeds and cottonseed. Its rapid development and readiness to breed in the laboratory have made it a popular tool in physiological and genetic studies (Nenaah, 2014).

The documented and authenticated facts about the scare of pesticide residues in storage commodities and the awareness of the public demand has made it urgent for the FDA to embrace and advocate more emphasis for the public health. Therefore, many researchers have been attracted towards the novel, promising potentials of the green pesticides that contribute in reducing insect pest populations (Imran et al., 2015; Lal et al., 2017). Recently various researches have been conducted on the use of plant extracts and their byproducts against harmful storage insect pests and result of these studies proved that these extract might have many active components that works as a biopesticides many. These researches proved that plant based pesticides might be suitable alternatives for the biocontrol of many insect pests (Nawrot & Harmatha, 1994; Adam et al., 1998; Kabera, 2004). These plants extract are rich in enormous compounds and active ingredients that show anti-feedant, hideous, sterilization and lethal deleterious or malformation effects on a multitude of insect pests (Isman, 2006).

The desired lethal toxicity of insecticides on stored grain pests may be manifested as contact or through fumigation action; albeit the effective toxicity of most essential oils has been sharpened lately and gained encouraging appraisals for the applied usage of plant products as potential pesticides on a variety of stored grain pests (Golob et al., 1999; Weaver & Subramanyam, 2000). Many essential oils including ginger oil (*Zingiber officinale*); garlic oil (*Allium sativum*); corn oil (*Zea mays*); soybean oil (*Glycine max*); castor oil (*Ricinus communis*); sunflower oil (*Helianthus annuus*); cardamom oil (*cardamomum*) have been successfully used as control agents against variety of stored grains pests. These essential oils were used to evaluate their residual, repellent and fumigation efficacy on immature and adults of different grain pests. Many encouraging results were reported hailing the pivotal role of plant extracts and byproducts as urgent factors in the arena of combating the stored grain pests (Prakash & Rao, 1997). Information regarding the use of essential oil fumigation against the confused flour beetles is in scarcity. Therefore, the present study has been undertaken to investigate the efficacy of selected plant oils viz., corn, ginger and garlic at different concentrations against the fourth instar larvae and adults of the confused flour beetles.

2 Materials and Methods

2.1 Extraction of oil

The corn, ginger, and garlic essential oils were extracted by following below given method.

2.1.1 Corn oil using Soxhlet apparatus

Corn oil was extracted from ground corn seed using ethanol as the solvent. Optimal conditions were a solvent-to-solids ratio of 4 mL/g corn, an ethanol concentration of 100%, 30 min of extraction time, and a temperature of 50°C. A three-stage extraction was carried out, where the same corn was exposed to fresh ethanol. Moisture was absorbed linearly by ethanol from the corn in successive stages, which, in turn, decreased oil yield and increased nonoil components in the extract.

2.1.2 Ginger oil

Ginger oil was extracted by Soxhlet extraction, with the optimized operational condition. The operational conditions include an optimized sample (Ginger corm), temperature (70°C to 80°C), extraction time (1 to 1.30 hr), and ratio of ginger to solvent (50 gm ginger : 200 ml Ethanol).

2.1.3 Garlic oil

The extraction of garlic oil was carried out with a Soxhlet extractor using n-hexane (boiling point of 40°C – 60°C) for six hours. The oils were obtained after the solvent was removed under reduced temperature and pressure and refluxing at 70 C, to remove any excess solvent used for the oil extracted. The extracted garlic oil was stored in refrigerator freezer at 2°C for subsequent physicochemical analyses (Warra et al., 2011).

2.2 Sample collection

Larvae and adults were carefully reared under laboratory conditions at the department of biological Sciences, insects have been maintained for many generations in incubators under

darkness conditions at 27±3°C and 65±5% R.H. Experimental animals were fed with a mixture of whole-wheat flour and brewer's yeast 4:1. Fourth instars and ten-days post-emerging adults were used for all experiments.

2.3 Fumigant toxicity test

To determine the fumigant toxicity of the selected three plant oils on the fourth larval stage and adults of *T. confusum*, the Whatman filter papers No.1 cut into circles with 2-cm diameter pieces then impregnated with oil at 25, 50, 100, 200, and 400 µL/L concentrations. The impregnated filter papers were attached to the undersurface of the screw cap of a glass vial (1L). The caps screwed tightly onto a vial containing 10 insects. Each concentration were replicated four times and kept in darkness in the incubators at 27±3°C and 65±5% R.H. The percentage mortality was determined after 24 and 48 hrs post-treatment by counting the number of completely dead insects.

3 Results

The data obtained from both fourth instar larvae and adults were statistically analyzed to reveal the efficacy of the three plant oils on the confused flour beetle (CFB).

3.1 Effect of Essential oils on 4th Larval Instars of *T. confusum*

3.1.1 The effect of corn oil fumigation on 4th Larval Instars of the *T. confusum*

The treatment of 4th larval instars of *T. confusum* with corn oil at different concentration and the corresponding recorded percentage mortality is shown in Table 1. The results of study revealed that the percentage mortality increased with increasing the

Table 1 Effect of various essential oils fumigation on mortality percentage of 4th larval instars of CFB

Dose (µL/L)	Mortality Percentage					
	Corn Oil		Ginger Oil		Garlic Oil	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
25	30	30	32	45	32	55
50	32	32	35	52	37	62
100	37	45	37	54	40	67
200	47	47	42	55	47	69
400	52	57	52	62	50	85
^a Control	10	10	10	10	10	10
slope	0.49	0.65	0.43	0.71	0.54	1.09
(Chi) ²	0.03	0.27	0.004	0.33	1.93	1.96
LC ₅₀	301	147.9	391.9	16.47	288.9	28.96

(^a) Corrected with Abbot's formula, (1925)

concentrations ($\mu\text{L/L}$ air) of oil and duration from 24 hrs to 48 hrs as indicated in Probit analysis (Figure 1A). The calculated slope value of the Probit analysis curve was 0.49 and 0.65 after 24 and 48 hr respectively. Result presented in figure (1A) indicated that as time increased from 24 to 48 hrs the corn oil is more sensitive in controlling the 4th instars larvae. Non-significant differences were reported between the various doses of oil and mortality percentages ($P < 0.05$) as shown by Chi2 (0.03 & 0.27). The lethal concentration at which 50% of the population responded (LC50) was recorded 301 ppm for 24 hrs and 147.9 ppm for 48 hrs. This indicated that corn oil was not much effective in controlling the 4th instars larvae of the (CFB) as time increased from 24 to 48 hrs.

3.1. 2 Effect of Ginger oil fumigation on 4th Larval Instars of the *T. confusum*

Effect of different concentrations of ginger oil on percentage mortality of 4th larval instars of *T. confusum* has been represented in Table 1. Like maize, percentage mortality increased with the increasing ginger oil concentration (ppm) and exposure time from 24 hrs to 48 hrs. The calculated slope value from the curve of Probit analysis was 0.43 and 0.71 after 24 and 48 hrs (Figure 1B). Further, result of present study suggested that the ginger oil was more effective in controlling the 4th instar larvae of the (CFB) and this effectiveness increased with increasing time from 24 to 48 hrs. The differences between various doses of essential oil and mortality percentages were not significant ($P < 0.05$) as shown by Chi2 and it was reported 0.004 & 0.33 after 24 and 48 hrs respectively, as compared to control. The concentration at which 50% population responded (LC50) was 391.9 ppm for 24 hrs and 16.47 ppm for 48 hrs. This indicated that ginger oil was highly effective in controlling the 4th instars larvae of the (CFB) flour beetle as time increased from 24 to 48 hrs.

3.1.3 Effect of Garlic oil fumigation on 4th Larval Instars of the *T. confusum*

The treatment of the 4th larval instars of *T. confusum* with garlic oil at different concentration and the corresponding recorded percentage mortality is shown in Table 1. The results showed that the percentage mortality has been increased with the increasing essential oil concentration (ppm) and exposure time from 24 and 48 hrs post-treatment as indicated by Probit analysis (Figure 1C). The calculated slope value from the curve of Probit analysis was 0.54 and 1.09 after 24

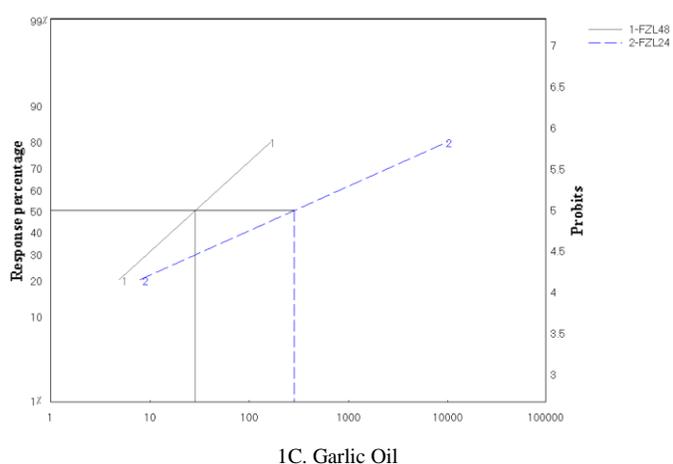
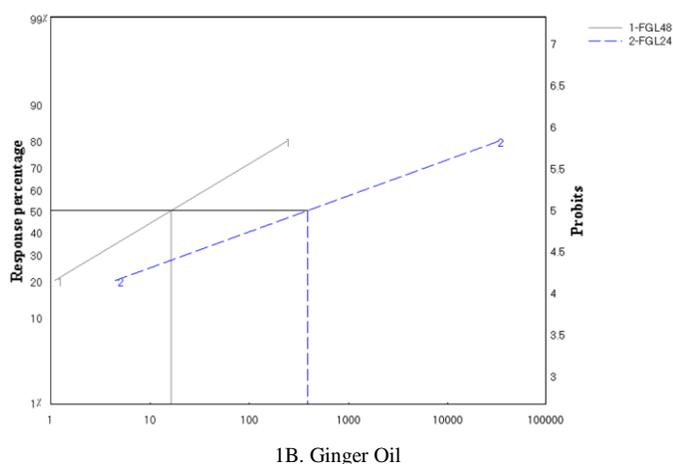
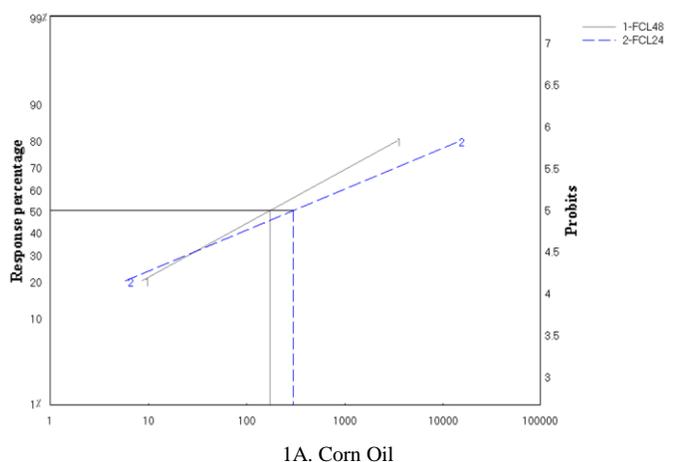


Figure 1 Probit analysis showing the relationship between the fumigation mortality percentage and the doses of select oil on 4th larval instar of the (CFB); FCL (Fumigant effect of corn oil on the 4th instars Larvae)

Table 2 Effect of various essential oils fumigation on mortality percentage of CFB adults

Dose (μ L/L)	Mortality Percentage					
	Corn Oil		Ginger Oil		Garlic Oil	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
25	30	32	31	32	30	34
50	31	34	33	34	31	38
100	33	36	35	41	32	40
200	34	37	50	52	42	57
400	38	40	90	98	80	90
^a Control	10	10	10	10	10	10
slope	0.9	0.53	0.98	1.56	1.13	1.38
(Chi) ²	0.13	0.79	11.35	16.4	2.71	0.32
LC ₅₀	185	77.8	118.45	64.4	89.8	50.1

(^a) Corrected with Abbot's formula, (1925)

and 48 hr respectively. The differences between doses of oil and the mortality percentages were significant at $P < 0.05$ level of probability as shown by Chi2 and it was reported 1.93 after 24hrs and 1.96 after 48 hrs in comparison to the control. The concentration at which 50% of the population responded (LC50) was recorded 288.9 ppm for 24 hrs and 28.96 ppm for 48 hrs. This indicated that garlic oil have significant effect on the 4th instars larval mortality.

3.2 Effect of various essential oil on Adult's stage

3.2.1 Effect of Corn oil fumigation on Adult stage

Mortality percentages in treatment containing adults of *T. confusum* along with different concentrations of corn essential oil are presented in Table 2. Like other treatments, here also percentage mortality increased with the increasing the doses (ppm) of corn oil and time duration from 24 hrs to 48 hrs post-treatment as indicated by probit analysis (Figure 2A). The calculated slope value from the curve of probit analysis was 0.49 and 0.53 after 24 and 48 hrs respectively. Further, it was reported that corn oil was not that much effective in controlling the CFB adults. The differences between doses of oil and the mortality percentages were not significant as shown by Chi2. The concentration at which 50% of the population responded (LC50) was recorded 185 ppm for 24 hrs and 77.8 ppm for 48 hrs.

3.2.2 Effect of Ginger oil fumigation on Adult stage:

Effect of ginger essential oil on the adult mortality of *T. confusum* has been represented in Table 2. Result of study revealed that

percentage of mortality increased with increasing the concentration of essential oil (ppm) and time duration (Figure 2B). The calculated slope value from the curve of probit analysis was 0.98 and 1.56 after 24 and 48 hrs respectively. This indicated that the ginger oil was quite effective in controlling the CFB Adult. The differences between doses of oil and the mortality percentages were not significant at $P < 0.05$ level of probability as shown by Chi2 and this value was reported 11.35 & 16.4 after 24 and 48 hrs respectively in comparison to the control. The concentration at which 50% population responded (LC50) was recorded 118.45 ppm for 24 hrs and 64.4 ppm for 48 hrs exposure. These results suggested that ginger oil have significant effect on CFB adults mortality and among the tested essential oil, this is highly effective one.

3.2.2 Effect of Garlic oil fumigation on Adult stage:

Results presented in table 2 revealed a significant effect of garlic essential oil on *T. confusum* adult mortality. Further, mortality percentage increased with increasing essential oil concentrations (ppm) and exposure time from 24hrs to 48hrs. The calculated slope value from the curve of probit analysis was 1.13 and 1.38 after 24 and 48 hr respectively (Figure 2C). This indicated that the garlic oil was highly effective in controlling CFB adults and this effectiveness increased from 24 to 48 hr. The differences between doses of oil and the mortality percentages were significant at ($P < 0.05$) level of probability as shown by Chi2 and it was reported 2.71 after 24 hrs of exposure and 0.32 for 48hrs of exposure, in comparison with the control. The concentration at which 50% of the population responded (LC50) was recorded 89.8 ppm after 24 hrs and 50.1 ppm after 48 hr. This indicated that

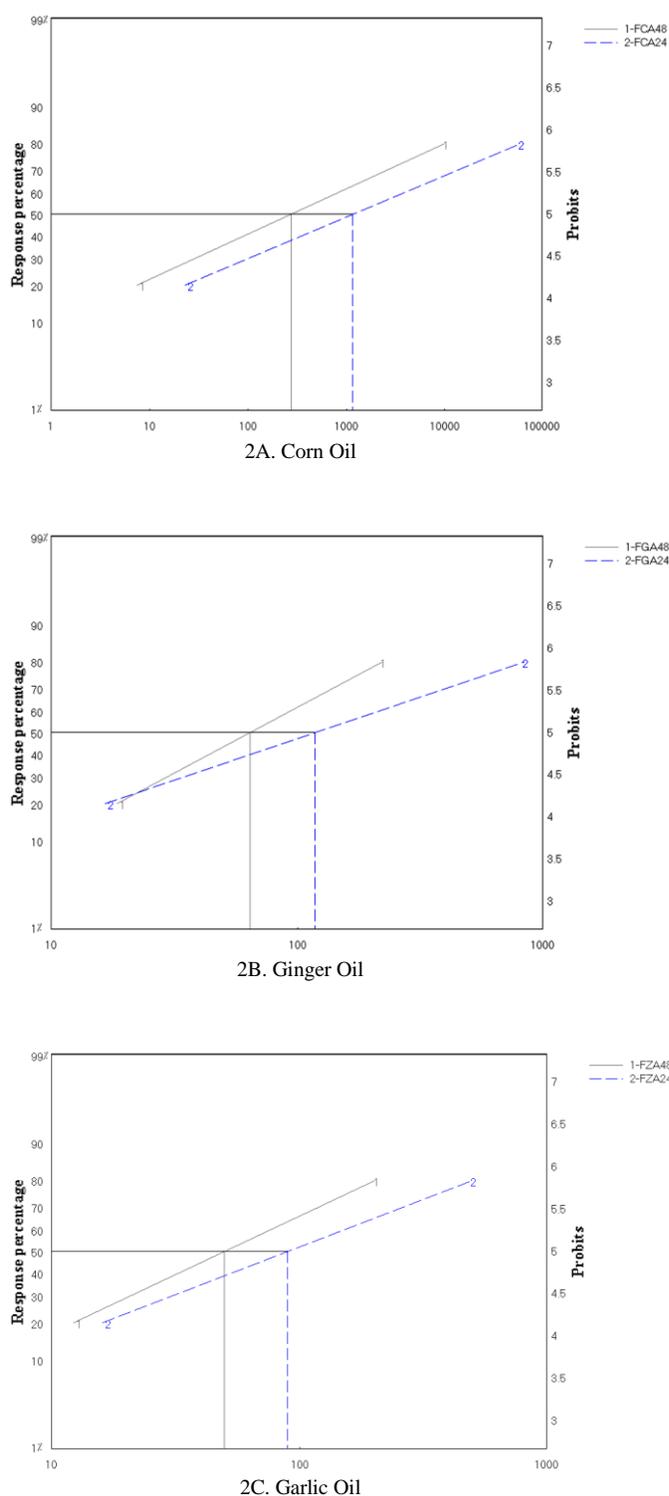


Figure 2 Probit analysis showing the relationship between the fumigation mortality percentage and doses of selected oil on adults of CFB; FCA (Fumigant effect of Corn oil on the adults)

garlic oil was quite effective in controlling the flour beetle and this effectiveness increased with the increasing exposure time increased from 24 to 48 hrs.

4 Discussion

Results of study revealed significant effect of used essential oil fumigation on 4th Larval Instars and adult of *T.confusum*. Further, among these three essential oils, ginger and garlic essential have great CFB management potential and it reported highest on the highest concentration (400ppm) of both essential oils. The lethal effect of garlic acid on 4th instar larvae was 52% after 24 hrs exposure at 400 ppm concentration and this increased when the exposure time was increased to 48 hrs. While in case of adults, ginger oil gave 90% mortality after 24 hrs and it increased to 98% after 48 hrs of exposure. Results of present study are in agreement with the findings of Mikhael (2011), those who have reported significant inhibitory effect of ginger oil against *T. castaneum* larvae. Similarly, Talukder & Khanam (2011) also reported that ginger extracts were most effective as a fumigant agent against *S. oryzae*. Lethal effect of ginger essential oil was followed by the essential oil of garlic and corn essential oils. Corn oil was found least effective with 52% and 57% 4th instar larval mortality while it was reported only 38% and 40% for adults after 24 and 48 hrs respectively. These results are in agreement with the findings of Kim et al. (2012) and Wang et al. (2014) who found that the fumigant activities of *Cinnamom umcassia* and *Allium sativum* seemed to be stronger on 6th instars larvae than on adults of the darkling beetle, *Alphitobius diaperinus*.

From the result of study, it can be conclude that plant-based biopesticide can be used as an alternate of commercial chemical insecticides. These biopesticides are not only ecofriendly but also have significant biocontrol potential. Further studies are required for mass scale application of these plant-based biopesticide.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this review paper.

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