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

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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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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

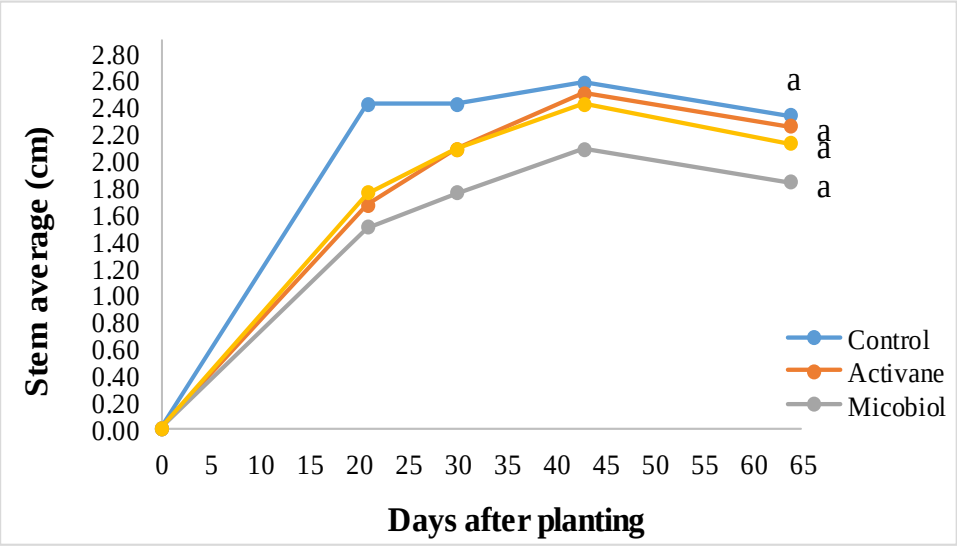

Figure 2 Comparison of the number of stems during the four applications of elicitors in crop development

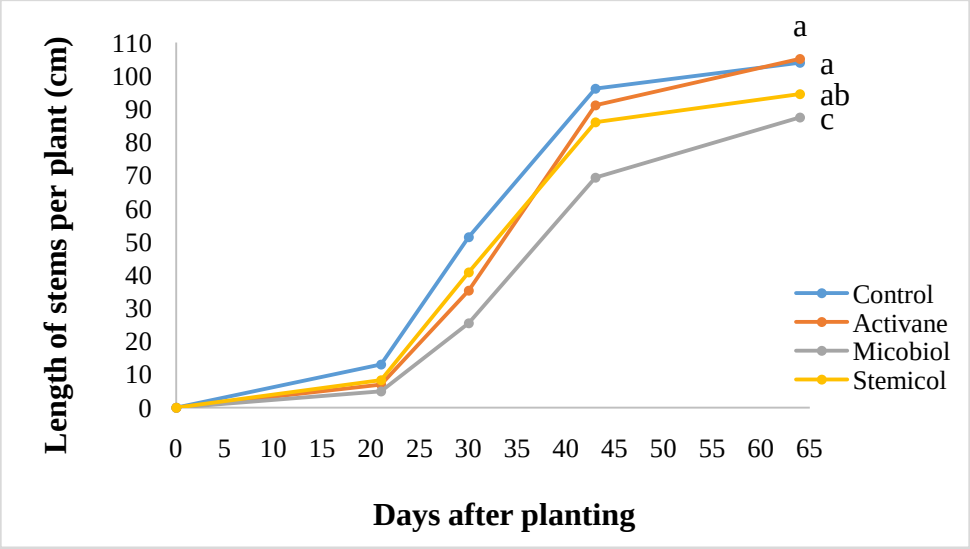

Figure 3 Comparison of mean length of stems during the four applications of study elicitors

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).

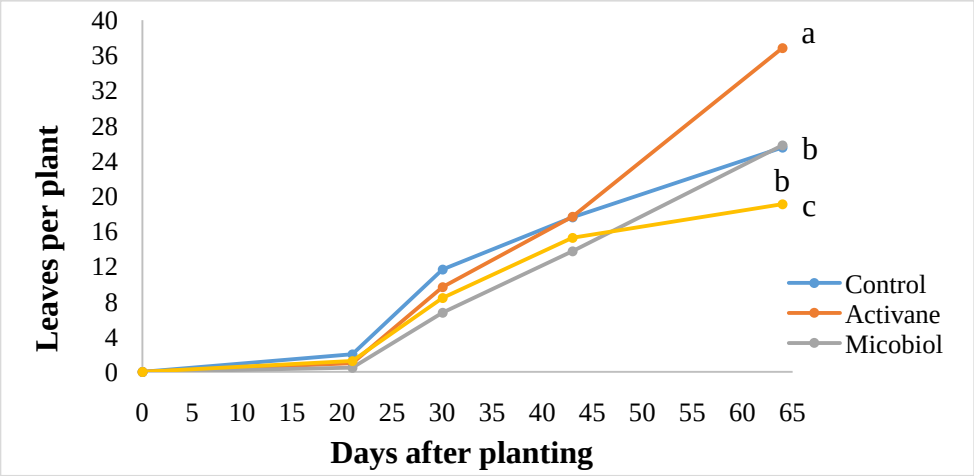

Figure 4 Comparison of means of the number of leaves during the four study applications

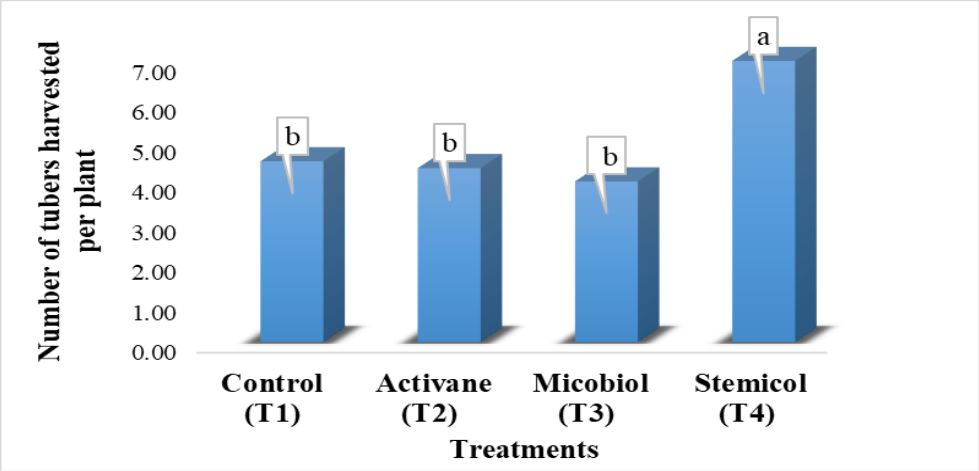

Figure 5 Effect of the elicitors application on the number of tubers per plant at harvest

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).

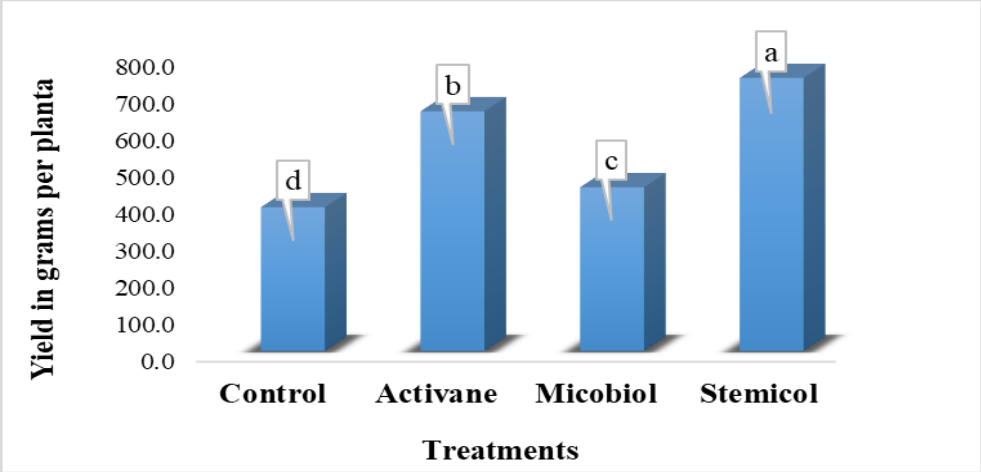

Figure 6 Effect of the elicitors application on harvested tuber weight per plant of the production during the crop cycle

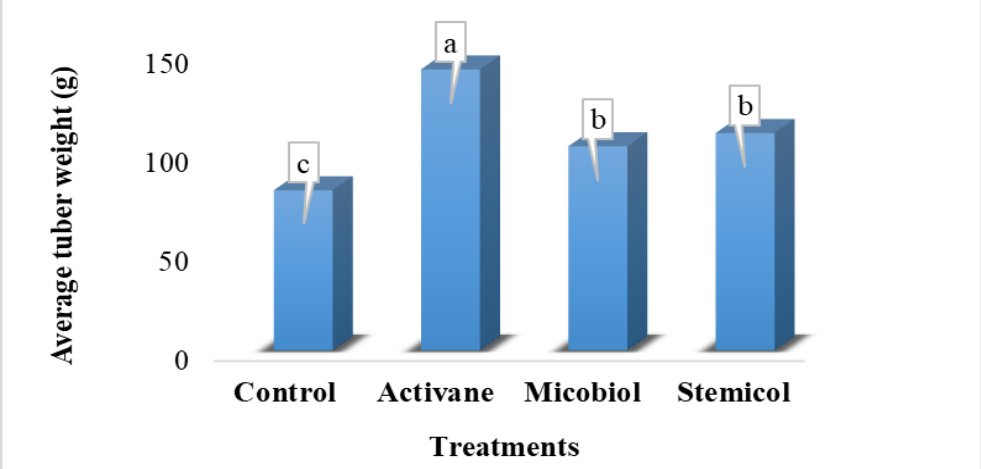

Figure 7 Effect of the elicitors application on total tuber weight obtained during the crop cycle

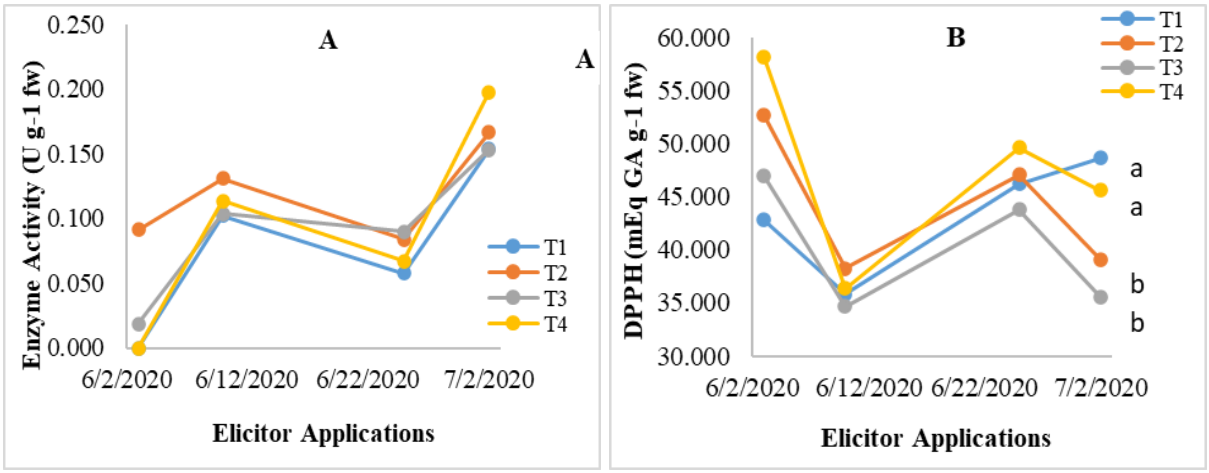

Figure 8 Comparison of means tests for the a) enzymatic activity of polyphenol-oxidase and b) DPPH antioxidant in potato leaves (*S. tuberosum* L.) of the "Fianna" cultivar as a result of elicitation.

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≤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).

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