Colegio de Postgraduados

Biostimulants and insecticides for a biorational management of Serrano pepper (*Capsicum annuum* L.) in protected conditions in macrotunnel

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ABSTRACT

Objective: to evaluate the effect of two biostimulants and two insecticides on the biorational management of Serrano pepper cultivation under protected macrotunnel conditions.

Design/Methodology/Approach: three biostimulant treatments were evaluated 1: T22® (*Trichoderma harzianum* strain T22); 2: Mix® (*Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma asperellum*, *Trichoderma koningii*); and 3: control (tap water), applied at doses of 0.5% (w/v). In addition, three insecticide treatments were evaluated 1: Movento® (Spirotetramat) at a dose of 250 mL ha^{-1}; 2: the bioinsecticide *Isaria javanica* strain 304 at a dose 1 L ha^{-1}, and 3: control (tap water). The experimental design was a factorial of treatments in randomized complete blocks. The response variables for the biostimulants were weight, equatorial diameter and polar diameter of the fruit, as well as the total weight of 20 fruits. For insecticides, a single application of each product was made. An aphid sampling was made before insecticides application, then four more afterwards at 3, 5, 7 and 14 days after application (daa).

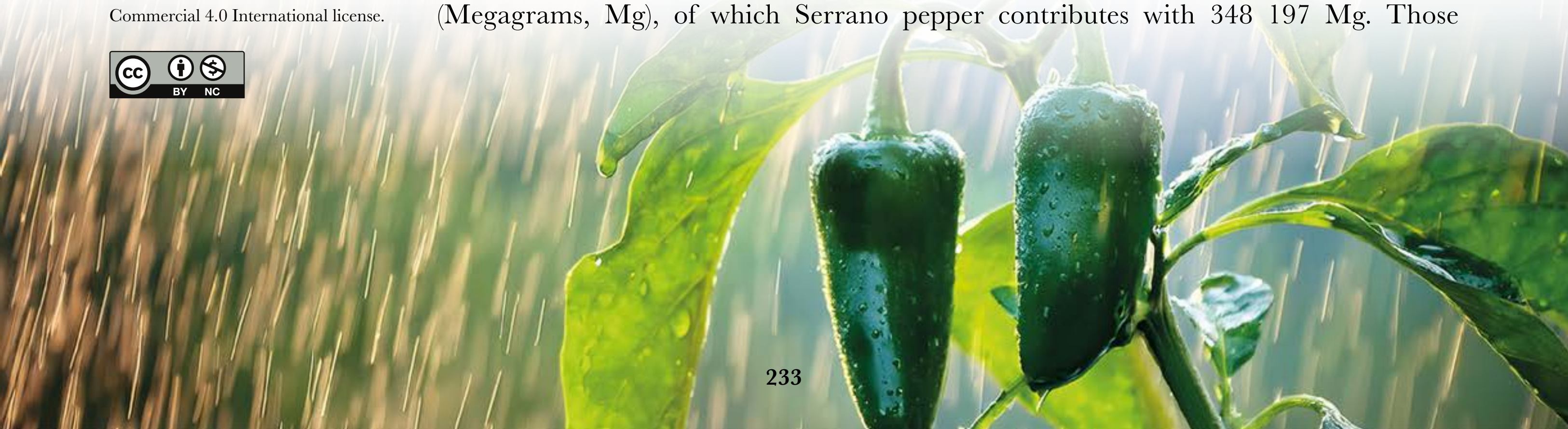
Results: results demonstrated a greater potential of the biostimulant formulated with *T. harzianum* strain T22 to increase the weight and size of Serrano pepper fruits. Also, the insecticide based on spirotetramat was more effective for aphid control; whereas the fungus *I. javanica* strain 304 was efficient at 7 and 14 daa, with mortality percentages less than 60%.

Findings/Conclusions: the interactions of biostimulants and insecticides had beneficial effects on weight and size of Serrano pepper fruits, as well as on aphid pest control.

Keywords: vegetables, *Trichoderma harzianum*, spirotetramat, *Isaria javanica*, aphids.

INTRODUCTION

In Mexico, vegetables compose approximately 20% of national agriculture production. Among vegetables, green peppers account for the highest production 3 069 865 tons (Megagrams, Mg), of which Serrano pepper contributes with 348 197 Mg. Those



Mexican states with the highest Serrano pepper production are Sinaloa, San Luis Potosí, Michoacán, Veracruz and Guanajuato (SIAP, 2023). The Serrano pepper is an important crop in Mexico as it is consumed fresh; also, it is a source of economic resources through labor in farms. In addition, its cultivation generates foreign exchange in the agrifood exports sector (Ramírez-Seañez *et al.*, 2023). Most of this pepper is grown on lands in the open and very little under protected conditions. This generates a considerable demand for inputs, such as fertilizers and commercial insecticides (Barrón-Contreras *et al.*, 2022; Díaz-José *et al.*, 2023).

Currently, production systems that depend heavily on chemical inputs are characterized by increasing production costs and contributing to the problems of environmental pollution and decreased soil fertility (Silveira-Gramont *et al.*, 2018; Esquivel-Valenzuela *et al.*, 2019) (Galindo *et al.*, 2020; Guerrero & Guevara, 2021). Therefore, production systems nowadays should consist of ecological or bio-rational approaches, which reduce the excessive use of conventional fertilizers and insecticides, while increasing efficiency in plant nutrition and plant protection against pests. One of these strategies is the use of plant biostimulants and biological or new generation insecticides, which are used in the biorational management of crops (Murillo-Cuevas *et al.*, 2020).

The term biorational refers to certain types of components, mostly biological. But, if they are synthetic, then should be structurally similar and functionally identical to those that are biological. These have advantages in terms of risks of contamination or pest resistance, while offering acceptable efficiency in pest control or plant nutrition (Murillo-Cuevas *et al.*, 2023). Biorational products include microorganisms, plant extracts, basic substances, as well as non-pesticide products such as bio-stimulants, biological performance enhancers, plant health promoters and soil conditioners (Matyjaszczyk, 2018). Their use in plant production varies, some act directly against pests, are highly selective and their efficiency is similar to that of chemical products, while others act mainly on plants, increasing their tolerance to various biotic and abiotic factors (Shankar y Tripathi, 2021).

Biostimulants formulated with beneficial microorganisms, such as fungi and bacteria promoting plant growth, act on the solubilization of minerals and nitrogen fixation, as well as through physiological and genetic mechanisms that stimulate the production of phytohormones, enzymes, lipopolysaccharides and organic compounds (Caulier *et al.*, 2019; Lephatsi *et al.*, 2021). In addition, these biostimulants improve tolerance to biotic and abiotic stresses, as they activate genes in plant defense system to produce phenols, enzymes, amino acids, and organic acids (Backer *et al.*, 2018; Vocciante *et al.*, 2022). Fungi of the genus *Trichoderma* increase the height, stem diameter, aboveground biomass, and root volume of habanero pepper plants; as well as yield in fruit number and fruit weight (Cristóbal-Alejo *et al.*, 2021). Likewise, formulations of *Bacillus* bacteria increase weight and size of habanero pepper fruits (Adame-García *et al.*, 2021; Mejía-Bautista *et al.*, 2022).

Bioinsecticides are products developed from entomopathogenic fungi such as *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea*, among others, which are used in pest management strategies, since they are environmentally compatible. These products offer economic benefits to farmers, while contributing to the protection of the environment and consumer health (Pacheco *et al.*, 2019). In artisanal productions in rural communities, it is reported that bioinsecticides control pests in lettuce, onion, cabbage, potato, and coriander crops, causing mortalities of more than 80% within 72 hours, compared to control (González-Maldonado and García-Gutierrez, 2012). Compatibility and efficiency of *B. bassiana* and *M. anisopliae* in the control of whiteflies in lettuce is also demonstrated, in addition to the fact that *B. bassiana* and *M. anisopliae* independently control aphids in cabbage leaves (Silva *et al.*, 2020).

Likewise, within the biorational management of pests, state-of-the-art insecticides formulated with active ingredients belonging to new chemical families, such as Spirotetramat and Spiromesifen, are included. These compounds have a low incidence of cross-resistance with conventional insecticides, so they are recommended as part of strategies to prevent the development of resistance in pest populations (Cortez-Mondaca *et al.*, 2018; Díaz *et al.*, 2019).

The objective of this study was to evaluate the effect of two biostimulants and two insecticides on the biorational management of Serrano pepper cultivation under protected macrotunnel conditions.

MATERIALS AND METHODS

This research was established in the vegetable production area of the Instituto Tecnológico de Úrsulo Galván, located in the municipality of Úrsulo Galván, in the central coastal region of the state of Veracruz, Mexico (19° 24' 43.12" N; 96° 21' 32.66" W). The experiment was implemented in a 3×30 m macrotunnel greenhouse (90 m^2), covered with an anti-aphid mesh. A drip irrigation system was used and two seedbeds with white and black plastic mulching were established. Plant arrangement consisted of one plant every 25 cm in staggered (triangular) planting, which allowed a total of 120 plants per bed and 240 per macrotunnel. During the crop cycle, an average temperature of 28.4 °C and an average relative humidity of 73.5% were recorded.

Serrano pepper seed (*Capsicum annuum* L.), variety chister-522 was used, which was provided by INIFAP Campo Experimental Cotaxtla. For seedling production, seeds were previously inoculated with mycorrhiza (*Rhizophagus intraradices*) supplied by INIFAP, then sown in polyethylene trays. The seedlings were transplanted to a soil with the following characteristics pH 6.81, electrical conductivity (EC) 81.3 μS, bulk density (DAP) 1.3 g mL^{-1}, sand content 31.64%, silt 31.16%, clay 37.98%, organic matter (M.O.) 3.11%, potassium (K) 0.18 Cmol kg^{-1}, calcium (Ca) 12.04 Cmol kg^{-1}, magnesium (mg) 3.83 Cmol kg^{-1}, total nitrogen (N) 0.11% and available phosphorus (P) 11.7 mg L^{-1}.

The culture management consisted of the application of humic acids (12% humic and 12% fulvic) 15 days after transplantation. Subsequently, application was made every 30 days until the end of the production cycle. A conventional chemical fertilization (100: 60: 60, N: P: K) was applied during the complete production cycle (at 20, 50, 90 and 120 ddt). Also, every 20 days, foliar applications of micronutrients were made with commercial products (13% N, 9% P_2O_5, 12% K_2O, 0.17% Ca, 0.05% Cu, 0.01% Co, 0.07% B, 0.04% S, 0.08% Fe, 0.5% Mn, 0.18% Mg, 0.03% Mo, 0.20% Zn; diluents and conditioners, 64.31%). In addition, at the beginning of flowering and every 20 days afterwards, foliar applications of a commercial product with calcium and boron were made (9% Ca, 2% B, 4% Zn; diluents and conditioners, 85%).

Three treatments were evaluated 1: T22® (*Trichoderma harzianum* strain T22); 2: Mix® (*T. harzianum*, *T. viride*, *T. asperellum*, *T. koningii*); and 3: control (tap water), applied at doses of 0.5% (w/ v), as biostimulants. Also, other three treatments 1: Movento®, at a dose of 250 mL ha^{-1}; 2: the bioinsecticide *Isaria javanica* strain 304® at a dose of 1L ha^{-1}, and 3: control (tap water) were evaluated as insecticides.

Biostimulants were applied monthly to the soil using the drench technique. The solution was directed to the neck of the plant, from the moment of transplantation. For evaluation, fruits were collected in two harvest cuts, at 113 and 123 ddt. In each treatment and replicate, 20 fruits per plant were randomly selected to measure the response variables, individual fruit weight (g), equatorial diameter (cm), polar diameter (cm) of the fruit, and total weight of 20 fruits (g).

The evaluation of insecticides was done at 63 ddt, when the presence of the pest was detected *Myzus persicae* (Hemiptera: Aphididae), commonly known as green aphid, which has spread throughout the crop. A single dose of each insecticide was applied using a 20 L electric sprayer (HYUNDAI®). To avoid cross-contamination between treatments, 2 m high screens between experimental blocks were used as physical barriers. Aphid population monitoring was done before insecticide application and subsequently at 3, 5, 7 and 14 days after application (daa). In each experimental block, the two central plants were sampled, and two leaves per plant were selected (one in the upper part and the other in the middle part). The total number of live aphids was counted on each leaf, recording treatment and repetition, on each evaluation date.

A factorial design was used in randomized complete blocks. In each bed of the macrotunnel, six large blocks of 18 plants were established to distribute the biostimulant treatments, obtaining two replications per bed. Within each large block, insecticide treatments were distributed in small blocks of six plants per treatment, obtaining six replicates per seedbed.

The normality of the data was assessed with the Shapiro-Wilk goodness-of-fit test. Data were transformed to $\sqrt{+0.5}$ to be normalized in order to perform an analysis of variance with interaction. In the cases where significant differences were identified ($p \leq 0.05$), a Tukey mean comparison test was applied with the original means of the data. The InfoStat© version 2020 software was used for data analysis.

RESULTS AND DISCUSSION

The biostimulants applied had a positive effect on the weight and dimensions of the Serrano pepper fruit in the two harvest cuts (Table 1). In the first harvest, both treatments with biostimulants significantly increased ($p=0.0001$) the average weight of the fruit compared to the control. However, the size of the fruit (polar diameter and equatorial diameter) and the total weight of 20 fruits showed a significant increase ($p=0.0001$) only in the treatment with the T22® biostimulant. In particular, the T22® biostimulant increased the average fruit weight by 36% compared to the control fruits, while the Mix® biostimulant achieved an increase equivalent to 6.8% (Table 1).

Table 1. Effect of biostimulants on Serrano pepper fruits produced in protected macrotunnel conditions.

Treatments	Weight (g)	Eq. D (cm)	Polar D. (cm)	Weight of 20 fruits (g)
Cutting 1				
T22®	2.36±0.02^{a}	0.80±0.01^{a}	5.42±0.07^{a}	46.50±0.80^{a}
Mix®	1.62±0.03^{b}	0.65±0.01^{b}	4.98±0.09^{b}	34.04±1.00^{b}
Control	1.51±0.04^{c}	0.66±0.02^{b}	4.98±0.11^{b}	35.44±0.96^{b}
C.V. (%)	14.56	6.89	12.63	8.14
Cutting 2				
T22®	2.04±0.02^{a}	0.72±0.01^{a}	5.47±0.07^{a}	39.63±0.80^{a}
Mix®	1.97±0.03^{b}	0.72±0.02^{a}	5.41±0.06^{a}	38.34±0.87^{a}
Control	1.78±0.02^{c}	0.68±0.01^{b}	5.18±0.07^{b}	32.28±0.95^{b}
C.V. (%)	14.83	7.73	11.74	8.78

D: diameter. Means with common letters are not significantly different ($p>0.05$). Values are presented in mean plus standard error, $\overline{X}\pm$S.E.

T22® increased the equatorial diameter and polar diameter of the fruits by 17.5% and 8.1% respectively, in addition to increasing the total average weight of a sample of 20 fruits by 23.8% compared to fruits in the control. In the second harvest, with the applications of the two biostimulants, Serrano pepper fruits were obtained on average significantly larger ($p=0.0061$) and heavier ($p=0.0001$) compared to the fruits of plants in the control. With the T22® biostimulant, the average fruit weight increased by 12.7% and with Mix® by 9.6%. In addition, the two products increased the equatorial diameter of the fruits by 5.6%, however for polar diameter, the application of T22® increased it by 5.3%, and with Mix® by 4.3%. Regarding the average weight of the sample of 20 fruits, the applications of T22® increased it by 18.5% and with Mix® by 15.8%, compared to fruits of the plants in the control (Table 1).

The interactions of biostimulants with insecticides improved the weight and size of Serrano pepper fruits. In the first harvest of fruits, significant effects were obtained on the weight ($p=0.0001$), equatorial diameter ($p=0.0027$) and polar diameter ($p=0.0109$) of fruits. With the applications of the T22® biostimulant, to improve plant nutrition and the chemical insecticide Movento® or the biological one *Isaria javanica* for the control of aphids, wider and heavier Serrano pepper fruits were obtained compared to the fruits obtained from control plants or with applications of the biostimulant Mix® (Figure 1A and Figure 1B). Regarding fruit length, only the fruits of plants with applications of the T22® and Movento® products were significantly different from the fruits of the control plants (Figure 1C).

In the second harvest, differences were also recorded among the interactions of the treatments in weight ($p=0.0001$), equatorial diameter ($p=0.0001$) and polar diameter ($p=0.0461$) of the fruits. As in the first fruit harvest, the plants treated with T22® and the insecticides *I. javanica* and Movento® produced wider and heavier Serrano pepper fruits than those in the control plants (Figure 1D and Figure1E). The length of the fruit was improved by the application of the bio-stimulant Mix® and insecticide Movento®

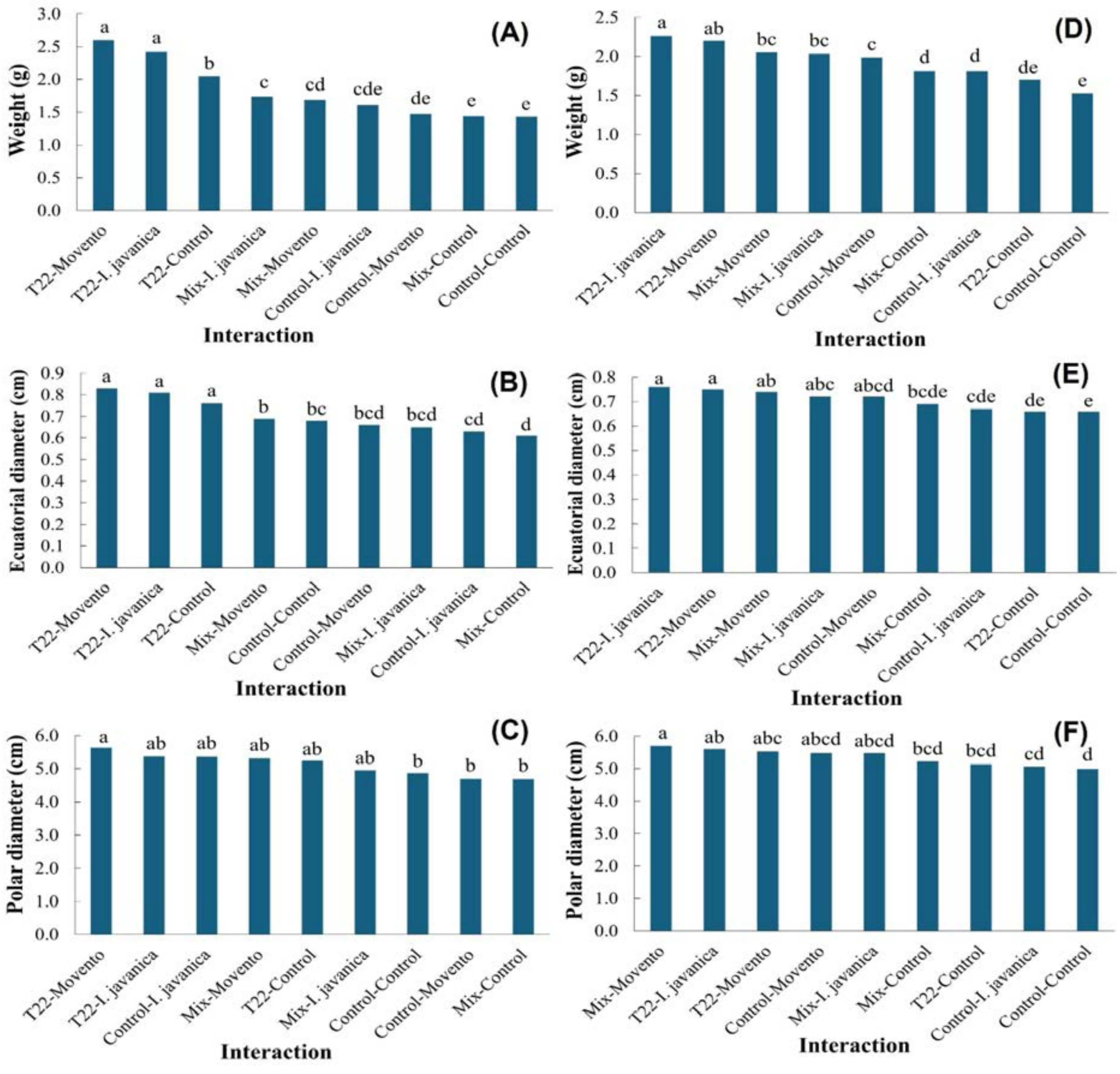


Figure 1. Effect of biostimulant × insecticide interactions on the mean weight (g), equatorial diameter (cm) and polar diameter (cm) in two harvest cuts of Serrano pepper fruits produced under macrotunnel protected conditions. A, B, C: harvest 1 and D, E, F: harvest 2. Means with different letters indicate statistical difference ($p \leq 0.05$).

compared to those interactions of treatments with the control, except control × Movento® (Figure 1F).

Regarding the average weight of samples of 20 fruits, in the first harvest, the interaction of the applications, for aphid control, of T22® plus Movento® or *I. javanica* produced on average fruits of greater weight ($p=0.0221$) compared to all other interactions (Figure 2A). In the second harvest, with the interactions of the applications of T22® plus *I. javanica*, and Mix® plus Movento® or *I. javanica*, it was possible to obtain samples of 20 fruits with significant and greater average weight ($p=0.0103$) than the fruit samples obtained with those interactions from control plants (Figure 2B).

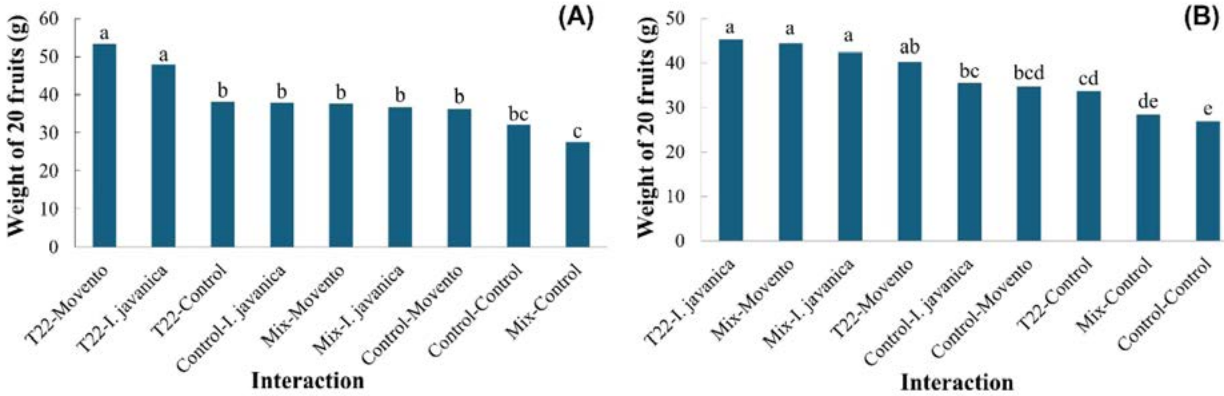


Figure 2. Effect of the biostimulant × insecticide interaction on the mean weight (g) of samples of 20 Serrano pepper fruits in two harvests produced in protected macrotunnel conditions. A: harvest 1 and B: harvest 2. Means with different letters indicate statistical difference ($p \leq 0.05$).

The biostimulants T22® and Mix® showed significant effects in promoting the growth of Serrano pepper fruits compared to the fruits of plants in the control. Similar results are reported in other horticultural crops such as habanero peppers, bell peppers, tomatoes and eggplants, although with variations in efficiency depending on the product and the crop evaluated. For example, the biostimulant T22® showed a greater increase in the weight of habanero pepper and eggplant fruits, while the Mix® product was more effective in bell peppers and tomatoes. In percentage terms, the largest increases were recorded in eggplant (T22®=27.4% and Mix®=17.8%) and habanero pepper (T22®=18.8% and Mix®=17.9%), followed by bell pepper (T22®=10.5% and Mix®=11.4%) and tomato (T22®=2.7% y Mix®=6.9%) (Adame-García *et al.*, 2023; Murillo-Cuevas *et al.*, 2021; 2023).

Compared to the crops reported in other studies, the biostimulant T22® showed a higher increase in the weight of Serrano pepper fruits in the first harvest, and also exceeded the results obtained in tomatoes and bell peppers during the second harvest. These differences could be attributed to the different levels of compatibility between *Trichoderma* spp. strains and host plant species. The efficiency of microbial biostimulants depends largely on the physiological and biochemical compatibility of plant-microorganism interactions, which implies genetic recognition between the two (Cano, 2011).

On the other hand, significant differences were also observed between the effects of the two biostimulants evaluated, particularly in the first harvest. This may be due to the specific formulation of each product: T22® contains a unique strain of *Trichoderma harzianum* (T22 strain), while Mix® is composed of a mixture of several species (*T. harzianum*, *T. viride*, *T. asperellum* and *T. koningii*), which implies a possible variability in the production of bioactive compounds such as auxins, organic acids, or in the solubilization mechanisms of inorganic phosphate, which influence the promotion of growth. This variability in effects between strains was confirmed by Larios *et al.* (2019), who evaluated the efficiency of the co-application of two native strains of *Trichoderma* sp. (SP6 and Clombta), and a commercial product (Tri-HB® with *T. harzianum* and *Bacillus subtilis*) on *Capsicum chinense* var. *Chichen Itza*. Those authors reported that plants treated with the Clombta strain presented better results in height (11 cm), stem diameter (2.6 mm), aboveground biomass (0.8 g fresh and 0.13 g dry), and root volume (0.13 g fresh and 0.4 g dry) than other treatments at 28 days after germination.

The benefits of fungi of the *Trichoderma* genus are related to their ability to control fungal pathogens, as well as a plant growth-promoting effect, by improving nutrition and the development of plants and fruits (Ruiz-Cisneros *et al.*, 2018; Elshahawy & El-Mohamedy, 2019). These effects are due, in part, to the production of phytohormones, peptides, volatile compounds, and secondary metabolites that stimulate root growth and increase nutrient absorption capacity, resulting in increased plant vigor and improvements in plant productivity (Shahnaz *et al.*, 2022).

The results obtained confirm that the T22® and Mix® biostimulants formulated with *Trichoderma* spp. strains have a significant effect on the development of Serrano pepper fruits. The incorporation of these products into biorational management programs can contribute to reduce the excessive use of chemical fertilizers or to make a reduced application more efficient, thus favoring more sustainable production. Espinoza *et al.* (2019) reported positive interactions between *Trichoderma* spp. and Serrano pepper varieties, attributed to the complex chemical activity of volatile secondary metabolites, phytohormones, and antibiotics released into the rhizosphere, which favored plant development, by improving nutrient absorption and increasing phytopathogen control.

Before applying the insecticides (day 0) the average number of live aphids per plant ranged from 21.77 to 23.29, and were statistically equal ($p>0.05$) among the established treatments. At 3 daa, significant differences ($p=0.0009$) were obtained in the average number of aphids per plant. Plants treated with the insecticide Movento® only had an average of 3.69 aphids, which is equivalent to an average reduction of 21.8 aphids (85.5%). Unlike the plants treated with the bioinsecticide *I. javanica*, which had an average reduction of 6.2 aphids (24.4%), which was non-significant compared to the control plants (Table 2).

At 5 daa, plants treated with the insecticides, both chemical and biological, had a significant lower average in the number of aphids ($p=0.0001$) than the control plants (Table 2). With the insecticide Movento® an average reduction of 26.4 aphids (94.3%) was obtained, and with *I. javanica* of 10.7 aphids (38.4%) less than control. At 7 and 14 daa, the reduction of aphids also was significant ($p=0.0001$) compared to control plants (Table 2). The insecticide Movento® maintained the average reductions at 23.5 (95.7%) and 13.6

Table 2. Effect of insecticides on the average number of aphids per plant present in the Serrano pepper crop at 3, 5, 7 and 14 daa under macrotunnel protected conditions.

Treatments	Days after application (DAA)			
	3	5	7	14
Movento®	3.69 ± 1.15^{a}	1.58 ± 0.97^{a}	1.06 ± 0.67^{a}	1.04 ± 0.67^{a}
Isaria javanica	19.25 ± 1.16^{b}	17.21 ± 0.97^{b}	10.69 ± 0.67^{b}	6.15 ± 0.64^{b}
Control	25.46 ± 1.15^{b}	27.94 ± 0.97^{c}	24.52 ± 0.68^{c}	14.68 ± 0.64^{c}
C.V. (%)	15.44	14.98	15.37	15.46

Means with different letters indicate statistical difference ($p\leq0.05$). Values show mean plus standard error, $\bar{X}\pm$S.E.

(92.9%) less aphids than control at 7 and 14 daa. Whereas the bioinsecticide *I. javanica* decreased the average number of aphids by 13.8 (56.4%) and 8.5 (58.1%) at 7 and 14 daa.

In the interaction of insecticides with biostimulants, there were significant differences ($p \leq 0.05$) at 3, 5, and 7 daa (Figure 3). In addition, some interactions of biostimulants without insecticides were statistically different from the zero control in relation to a lower number of aphids per plant (Figure 3). At 3 daa, interactions with Movento® were those that registered on average a lower number of live aphids; these interactions were statistically equal to each other, with or without the addition of biostimulants (Figure 3A). At 5 daa, the Movento® interactions with or without biostimulants further reduced the average number of live aphids per plant, remaining non-significant with each other.

The interaction of *I. javanica* plus the biostimulant T22® significantly decreased the average number of live aphids compared to the other interactions with I. javanica and the zero control (Figure 3B). At 7 and 14 daa, interactions with Movento® continued to be those that presented on average the lowest number of live aphids. However, the interaction of I. javanica plus the biostimulant T22® turned out to be statistically equal to the interactions of Movento® insecticide, with an average of live aphids lower than those obtained from plants in the control (Figure 3C and Figure 3D).

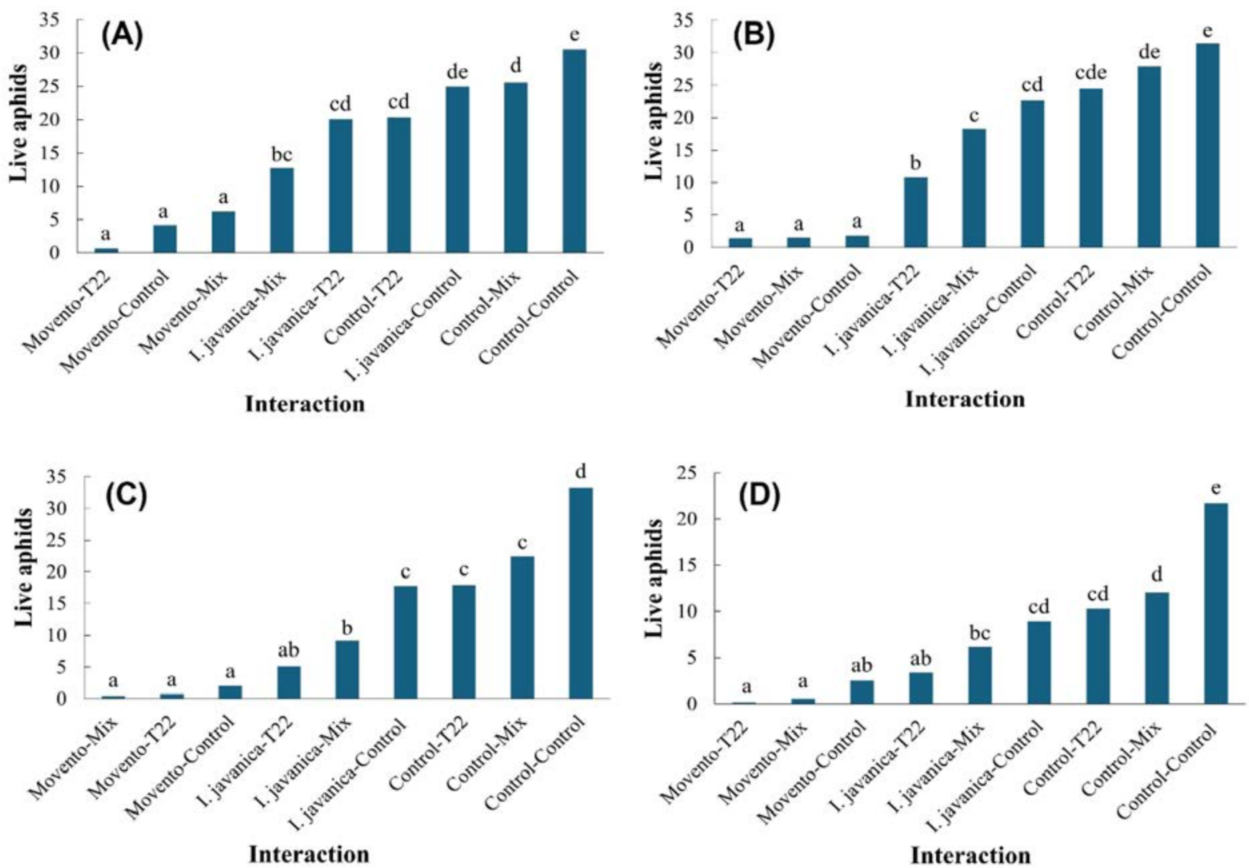


Figure 3. Effect of insecticide × biostimulant interactions on the average number of live aphids present in the Serrano pepper crop under protected macrotunnel conditions. A: 3 daa, B: 5 daa, C: 7 daa, and D: 14 daa. Means with different letters indicate statistical difference ($p \leq 0.05$).

The results obtained corroborate the high efficiency of the insecticide Movento® in the control of green aphids, which is attributed to its active ingredient, spirotetramat, an inhibitor of lipid biosynthesis that mainly affects the juvenile stages of the insect and reduces adult fecundity (Gong *et al.*, 2016). After foliar application, spirotetramatenol, with bimodal allocation through xylem and phloem, reaching both growing shoots and roots. This bidirectional systemic activity ensures the control of hidden sucking pests and protects new shoots (Gong *et al.*, 2016).

The high efficiency of spirotetramat has already been previously documented. Cortez *et al.* (2018) reported 91% and 100% mortality of the yellow sorghum aphid (*Melanaphis sacchari*) at 72 hours, and 120 hours after application. Similarly, Díaz *et al.* (2019) indicated a biological effectiveness of 89.33% in the control of *Aphis gossypii*, the aphid in pumpkin crops, when using the dose recommended by the manufacturer. Recently Murillo-Cuevas *et al.* (2023) recorded a 93.3% reduction of whitefly populations in eggplant crops when spirotetramat was used. These data coincide with our results in this study; we recorded mortalities of 85.5%, 94.3%, 95.7% and 92.9% at 3, 5, 7 and 14 daa respectively, confirming the high efficiency of spiroteramat against *Myzus persicae* in Serrano peppers.

On the other hand, the entomopathogenic fungus *I. javanica* strain 304 showed an important effect on the control of the aphid *M. persicae* in the Serrano pepper crop under macrotunnel protected conditions, since mortalities of 56.4% and 58.1% were reached at 7 and 14 daa. These results are comparable to those reported by Spinel *et al.* (2009) for *Isaria fumosorosea* in the control of whitefly nymphs *Bemisia tabaci* under laboratory conditions, as well as by Mweke *et al.* (2018), who reported, by an Isaria species, a mortality of 64.2% of *Aphis craccivora* in the legume *Vigna unguiculata*. However, in this latter study, it was also observed that *Isaria* caused lower mortality than the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae*, with respective efficiencies of 74.7% and 90%.

Under field conditions, it has also been reported that the fungus *I. fumosorosea* presented a lower efficiency compared to *B. bassiana* and *M. anisopliae* in the control of whiteflies in tomato and zucchini crops grown on lands in the open (Murillo-Cuevas *et al.*, 2020). Similarly, in the cultivation of eggplant, *I. javanica* reduced the whitefly population by 75.6%, in contrast to 83.8% achieved with *B. bassiana* (Murillo-Cuevas *et al.*, 2023). These results suggest that, although *I. javanica* has biocontrol capacity, its efficiency may be lower than that of other entomopathogenic fungi, such as *B. bassiana* and *M. anisopliae*.

CONCLUSIONS

The biostimulant formulated with *Trichoderma harzianum* strain T22 presented the greatest potential to a significant improvement of the size and weight of Serrano pepper fruits in a protected agricultural production. The insecticide based on spirotetramat was the one that best controlled the aphid *Myzus persicae* with mortalities greater than 85% and a persistence of up to 14 daa in the Serrano pepper crop in protected production system. These products based on *T. harzianum* strain T22 and spirotetramat can be integrated into a Serrano pepper production scheme with biorational management in protected macrotunnel conditions, since they offer relevant agronomic benefits with a low environmental impact.

The entomopathogenic fungus *Isaria javanica* strain 340 only showed a medium efficiency, reaching mortality percentages close to 60% at 7 and 14 daa. However, its inclusion in integrated pest management strategies in Serrano peppers can contribute to reduce dependence on synthetic insecticides in production systems with biorational management. The interactions between biostimulants and insecticides had positive effects on both fruit development and the evaluated pest control. This emphasizes the potential of their combined use in Serrano pepper production systems with biorational management in protected agriculture conditions.

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