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Effect of *Pleurotus ostreatus* (Jacq.) and *Trichoderma harzianum* (Rifai) on *Meloidogyne incognita* (Kofoid & White) in tomato (*Solanum lycopersicum* Mill.)

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ABSTRACT. Two isolations of fungi from a bank of microorganisms in the Biological Sciences Laboratory at Escuela Superior Politécnica de Chimborazo were tested on the galling caused by *Meloidogyne incognita* in tomato seedlings grown in pots with substrate infested with a suspension of nematodes, with approximately 2000 juvenile stages (J2) from root galls of plants infested with *M. incognita*, taken from the Nematology laboratory of the Ecuadorian Agricultural Quality Assurance Agency (AGROCALIDAD). *Pleurotus ostreatus* was a fungus with nematicidal characteristics through production of toxins; while *Trichoderma harzianum* is a widely known fungus, although it is a plant growth promoter rather than a nematicide. The two fungi were formulated in wheat straw and rabbit manure. A complete randomized design (CRD) with three replications was used, with a chemical control (Fenamiphos) and an absolute control. Five grams of each formulation was applied per plant before the transplant. The number of galls in the roots, plant height, stem diameter, number of leaves and fresh and dry weight of the aerial part and roots of 180 tomato plants grown in greenhouse were evaluated at 60 days after transplant. The results showed that the two fungi reduced the number of galls and made it possible to obtain dry weights of the aerial and radicular part very close to the chemical control (10.09 and 3.39 g) with 8.68; 8.04; 2.96 and 3.25 g respectively. Besides *Trichoderma harzianum* proved to be a good promoter of root growth, therefore, the use of these bioformulates is a promising measure for the control of this phytonematode.

Keywords: nematicide; nematophagous fungus; bioformulates; juvenile stages.

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Introduction

The nematode *Meloidogyne* sp., is one of the most harmful pests of tomato worldwide, because it severely affects the roots of this crop, due to its rapid expansion and high frequency of infestation, reducing the yield by up to 68% (Salazar-Antón & Guzmán-Hernández, 2013). Current agriculture demands the reduction of chemical pesticides and the introduction of sustainable systems with the use of biological management agents. The use of fungi as an alternative for the management of phytoparasitic nematodes can be a very important biological option (Piedra-Naranjo, 2008).

Nematophagous fungi are microorganisms with the ability to attack, kill and digest nematodes (adults, juvenile stages and eggs). Aside from its nematophagous ability, many of these fungi can also live saprophytically on dead organic matter, attack other fungi (mycoparasites) and colonize plant roots as endophytes (Piedra-Naranjo, 2008). Some species of *Pleurotus* are a source of nematicides and *P. ostreatus* proved to be more effective in larval mortality (Okorie, Ononuju, & Okwujiako, 2011). *Pleurotus ostreatus* is worldwide a common basidiomycete, whose hyphae can produce an effective nematotoxin. It has been active in many nematodes, such as *Hirschmanniella oryzae*, *Bursaphelenchus xylophilus*, *Pratylenchus* sp., *Meloidogyne incognita*, *M. arenaria,* among others (Xiang & Feng, 2002).

When purifying a nematicidal toxin of *Pleurotus ostreatus* NRRL 3526 grown in wheat straw treated in an autoclave and moistened for 30 days at room temperature (21-33°C), trans-2-decenedioic acid was identified as the active compound. A 300-ppm concentration of this acid, immobilized 95% of the nematodes (*Panagrellus redivivus*) in 1 hour. The immobilized nematodes did not recover, even after rinsing with deionized water (Kwok, Plattner, Weisleder, & Wicklow, 1992).

When evaluating the predatory activity of the fungus *P. ostreatus* (PLO 06) and of its proteases PLO 06 in the larvae of *Panagrellus* sp., the percentage reduction of *P. ostreatus* was 95%, while the action of its proteases were 65.6% demonstrating the great potential of use in integrated biological control of both the fungus and its proteases, which have a very effective predatory activity against this nematode (Genier et al., 2015).

The antagonistic effect of five species of *Pleurotus* on the second juvenile stage (J2) of *Meloidogyne javanica* was studied *in vitro*. In water agar, all tested species produced small droplets of toxin. The nematodes that touch such drops are shown after 24-48 hours colonized by the fungi. The filtrate of *P. ostreatus* tested in malt extract broth showed the highest nematicidal activity towards *M. javanica* J2 (Heydari, Pourjam, & Goltapeh, 2006).

Additionally, *Trichoderma harzianum* BI was evaluated for its ability to reduce the incidence and pathogenicity of the root-knot nematode *Meloidogyne javanica* in tomato, by studying the culture filtrations of *T. harzianum* BI at different concentrations (standard, 1:1, 1:10 and 1:100). *In vitro* studies revealed that incubation of *M. javanica* eggs was inhibited by culture filtrates and this inhibition correlated positively with the increase in the concentration of culture filtrates. The parasitism of eggs of *M. javanica* by *T. harzianum* BI varied from 21 to 84% in the control and the damage of the nematode to the tomato was reduced (Naserinasab, Sahebani, & Etebarian, 2011).

The strains of *Trichoderma* with biostimulant plant activities represent one of the solutions for the disadvantages of the systems of cultivation with high content of residues, being able to compensate the disadvantages of the system of conventional production. The ability to colonize plant and soil residues and generate a suppressive environment reduces the risks of pathogens in the soil, improves resistance to biotic and abiotic stress resulting from the activation of the plant's defense system and increases efficiency in the use of nutrients, which is reflected in the stimulation of growth and development of cultivated plants (Oancea et al., 2017).

Finally, because of serious affectations that environment suffers by the excessive use of chemical synthesis nematicides, the use of the nematophagous fungi constitutes a sustainable option for phytonematode management. For this reason, the objective of this research was to evaluate the effect of *Pleurotus ostreatus* (Jacq.) NE1 and *Trichoderma harzianum* (Rifai) NE2 on the galling caused by *Meloidogyne incognita* (Kofoid & White) in tomato (*Solanum lycopersicum* Mill.).

Material and methods

Laboratory phase

Juvenile stages 2 (J2) of phytonematode *Meloidogyne incognita* were obtained from egg masses of females present in intentionally infested tomato roots, from AGROCALIDAD (Tumbaco - Ecuador), the eggs were incubated until hatching of the juveniles in the second stage. The J2 were captured by means of a 100 µL micropipette (micro-fishing) until a sufficient population was completed for laboratory mortality tests.

For the extraction of eggs the methodology of McClure et al. (1973) and Taylor and Sasser (1978) was followed, for which 20 g of infested tomato roots were weighed, cut into pieces of approximately one centimeter, then disinfected with 4% sodium hypochlorite, rinsed with sterile distilled water and liquefied for 20 seconds at 3000 rpm. Finally, the liquid was filtered and sieved in two 60 and 500 mesh sieves; the samples of this last sieve were diluted to 20 mL. The eggs were disinfected with sodium hypochlorite, rinsed and incubated at average laboratory temperature (20°C).

Greenhouse phase

For this essay, the species *Pleurotus ostreatus* NE1 and *Trichoderma harzianum* NE2 grown in the substrates (wheat straw and rabbit manure) were considered. The criteria for the selection of these two species of fungi and substrates were as follows: a) *Pleurotus ostreatus* NE1 was the best fungus from the laboratory mortality test since it was able to immobilize and kill nematodes in the shortest time (24 hours), b) *Trichoderma harzianum* NE2 was taken into account even though it was not the best in the laboratory test, however, this genus is referred to in many investigations as a plant growth promoter, c) the rabbit manure substrate being organic matter, is assumed to exert double action when carrying the fungus and fertilizing the plants, d) the wheat substrate, because it was one of the best substrates that allowed the development of these two species of fungi in the laboratory.

Due to the temperature requirements of tomato plants, the test was established in a greenhouse, where the average recorded temperature was ± 24°C, the tomato variety used was Daniela, sown in one-litre polystyrene pots (Flex foam), which were filled with soil from the place that had the following characteristics: pH: 8,4 (slightly alkaline), MO: 0.6% (low); NH_4: 15.6 (low); P: 31.8 (high); K: 0.95 (high). Approximately 2000 *Meloidogyne incognita* J2 phytonematodes were inoculated into each of the pots, the same ones that were obtained from root galls of 4-month-old tomato plants purposely infested. They were washed with plenty of water to remove the soil, then they were disinfected with 4% sodium hypochlorite for 5 minutes and with the help of a scalpel and dissecting needle, the nodules and egg masses were removed and deposited in Petri dishes with sterile distilled water plus chloramphenicol (0.01 g 100 mL^{-1}) for 2 minutes and finally they were placed in a container with sterile distilled water at room temperature until the eggs hatched (6-10 days).

Once the pots were irrigated to field capacity, 5 g $plant^{-1}$ of each of the selected bioformulates (Nematofagous fungi plus solid substrates) selected from the previous stage (*Pleurotus ostreatus* NE1 developed in wheat straw and rabbit manure, *Trichoderma harzianum* NE2 developed in wheat and rabbit manure) were applied. Finally, in the pots with sterile Canadian peat, tomato seedlings with 4 leaves were transplanted. In 180 tomato plants were evaluated the following parameters: plant height, stem diameter, number of leaves, number of galls in the roots, fresh and dry weight of the aerial and roots at 60 days after transplant.

Statistical analysis

A complete random design (CRD) with three replications was used, the coefficient of variation and the Tukey's Studentized Range (HSD) Test were determined at 5% using the INFOSTAT program, trough ANOVAS. The correlation analysis was determined with the Pearson test and the Z-test was applied for the hypothesis test.

Results and discussion

Effect of selected bionematicides on the galling and development of tomato seedlings grown in the greenhouse 60 days after transplant

When analyzing the effect of evaluated treatments on the galling and development of tomato in greenhouse, we can observe mainly a very close tendency between the Chemical control (Fenamiphos) with the selected bionematicides, reflected in a summary of all results obtained ± SD (Standard deviation) and the level of significance of each one (Table 1).

Data are average value of three replications, mean values of different superscript letters are significantly different and same letters are not statistically different by Tukey's Studentized Range (HSD) Test ($p < 0.05$).

Table 1. Average results obtained in tomato seedlings grown in the greenhouse 60 days after transplant (media ± SD)

Treatments	Parameters evaluated at 60 days after transplant					
	Height (cm)	Number of galls	Aerial fresh weight (g)	Fresh root weight (g)	Aerial dry weight (g)	Dry root weight (g)
C.CH	34.90 ± 3.60^{a}	0.27 ± 0.52^{a}	59.61 ± 5.39^{a}	9.96 ± 1.43^{bc}	10.09 ± 1.20^{a}	3.39 ± 0.67^{a}
P.RM.	33.97 ± 4.70^{bc}	1.70 ± 1.12^{a}	52.56 ± 9.42^{ab}	10.12 ± 2.70^{ab}	8.68 ± 1.79^{ab}	2.96 ± 0.82^{b}
P.WS.	33.67 ± 3.81^{bc}	2.47 ± 0.97^{a}	51.97 ± 8.71^{bc}	9.29 ± 2.65^{bc}	8.38 ± 1.44^{ab}	2.94 ± 0.83^{b}
T.RM.	34.07 ± 2.99^{ab}	1.63 ± 1.07^{a}	51.55 ± 13.54^{bc}	11.15 ± 3.64^{a}	8.04 ± 2.23^{abc}	3.25 ± 1.25^{ab}
T.WS.	31.00 ± 3.99^{bc}	2.07 ± 0.83^{a}	41.60 ± 8.70^{c}	8.07 ± 2.37^{bc}	6.93 ± 1.80^{bc}	2.56 ± 0.78^{b}
C.ABS.	27.60 ± 2.72^{c}	18.27 ± 8.31^{b}	38.58 ± 5.12^{c}	6.66 ± 1.37^{c}	6.25 ± 0.67^{c}	1.75 ± 0.45^{b}

CH: Chemical, C: Control, P: *Pleurotus ostreatus* NE1, RM: rabbit manure, WS: wheat straw, T: *Trichoderma harzianum* NE2, ABS: Absolute

Height of plants, diameter of the stem and number of leaves at 60 days after transplant

The analysis of variance for plant height at 60 days after transplant showed a significant difference for treatments, with a coefficient of variation of 4.32%. According to the Tukey test at 5%, it was observed that the chemical control (Fenamiphos) occupies the level "a" with 34.90 cm, while the level "c" was occupied by the absolute control with 27.60 cm. In addition, according to the analysis of variance for stem diameter and number of leaves, there was no significant difference.

Number of galls at 60 days after transplant

According to the analysis of variance for number of galls at 60 days after transplant, there was a highly significant difference between treatments, with a coefficient of variation of 20.76%. Using the Tukey test at 5% in the treatments: Chemical control (Fenamiphos), *Trichoderma*-rabbit manure, *Pleurotus*-rabbit manure, *Trichoderma*-wheat straw and *Pleurotus*-wheat straw, a lower amount of galls per plant was observed, occupying level “a” (since 0.27 to 2,47); while the absolute control occupies level “b” with the highest number of galls per plant (18.27). These results showed that these antagonistic fungi are an effective option for the protection of this crop against the phytonematode *Meloidogyne incognita*.

Fresh weight of the aerial part of the plant at 60 days of transplant

In the same way, the analysis of variance for fresh weight of the aerial part of the plant at 60 days after transplant showed a highly significant difference for treatments, with a coefficient of variation of 8.08%. According to the Tukey test at 5%, the chemical control (Fenamiphos) occupied level “a”, with 59.61 g, while the treatment *Pleurotus*-rabbit manure occupied level “ab” with 52.56g and finally level “c” was the absolute control with 38.58 g.

Fresh root weight at 60 days after transplant

The analysis of variance for fresh weight of the root at 60 days after transplant showed a highly significant difference for treatments, with a coefficient of variation of 8.10%. According to the Tukey test at 5% *Trichoderma*-rabbit manure treatment occupied level “a” with 11.15 g, followed by *Pleurotus*-rabbit manure (level “ab”) and *Pleurotus*-wheat straw (level “bc”) with 10.12 and 9.29g respectively and the last level “c” was occupied by absolute control with 6.66 g.

Dry weight of plant’s aerial part at 60 days after transplant

The analysis of variance for dry weight of the aerial part of the plants at 60 days after transplant showed a highly significant difference for treatments, with a coefficient of variation of 9.34%. The Tukey test at 5% showed that the chemical control (Fenamiphos) treatment occupied level “a” with 10.09 g, followed by *Pleurotus*-rabbit manure and *Pleurotus*-wheat straw (level “ab”) with 8.68 and 8.38 g respectively. *Trichoderma*-rabbit manure occupied level “abc” with 8.04 g and at the level “c” the absolute control was found with 6.25 g.

Dry weight of plant’s root after 60 days of transplant

The analysis of variance for root dry weight at 60 days after transplant was highly significant for treatments, with a coefficient of variation of 13.40%. The Tukey test at 5% indicated that with the chemical control (Fenamiphos), greater root weight was achieved, represented by level “a” with 3.39 g, while the treatment *Trichoderma*-rabbit manure occupied level “ab” with 3.25 g and the absolute control occupied level “b” with 1.75 g.

In addition, it is appreciated that the treatments formed by the bioformulates constituted by *Pleurotus ostreatus*-rabbit manure and *Trichoderma harzianum*-rabbit manure contributed with fresh and dry weights of the aerial part and roots similar to chemical control shows that biological measures can be implemented with the purpose of diminishing the use of pesticides of chemical synthesis, which produce vegetables with traces of dangerous substances, risking the health of producers, consumers and the environment.

Correlation between number of galls and dry weight of the aerial and radicular part of the plant

According to Figures 1 and 2, a correlation between number of galls and dry weight of the aerial and root parts of the plant was determined, reflected in the values of r with 0.640 and 0.792 respectively.

According to the hypotheses raised in this research, consisting of:

Ho (null): Bionematicides formulated with native fungal strains are not able to reduce the number of galls in tomato plants grown in the greenhouse by at least 60% compared to a synthetic nematicide.

Hi (alternating): Bionematicides formulated with strains of native fungi are able to reduce the number of galls in tomato plants grown in the greenhouse by at least 60% compared to a synthetic nematicide.

According to the results obtained, the value -1.65 indicates that the alternating hypothesis is accepted, that is to say that the bionematicides formulated with strains of local nematophagous fungi, controls the galling of the roots of tomato plants grown in greenhouses in at least 60% of a synthetic nematicide (Table 2) (Figure 3).

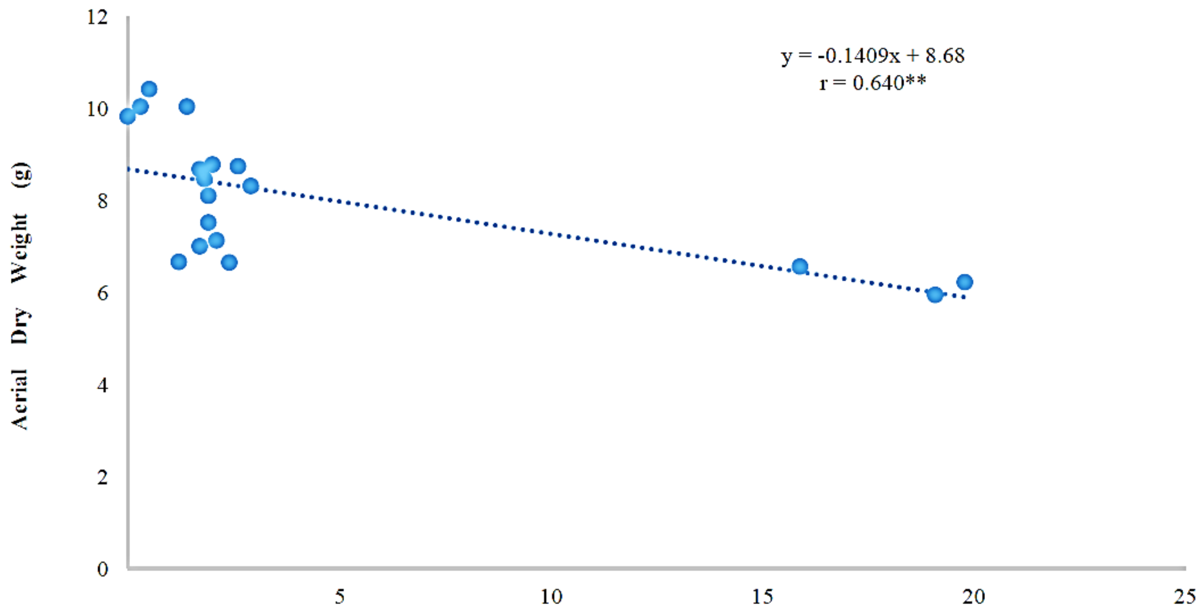

Figure 1. Correlation between number of galls and dry weight of the aerial part of the plant. ** Highly significant correlation

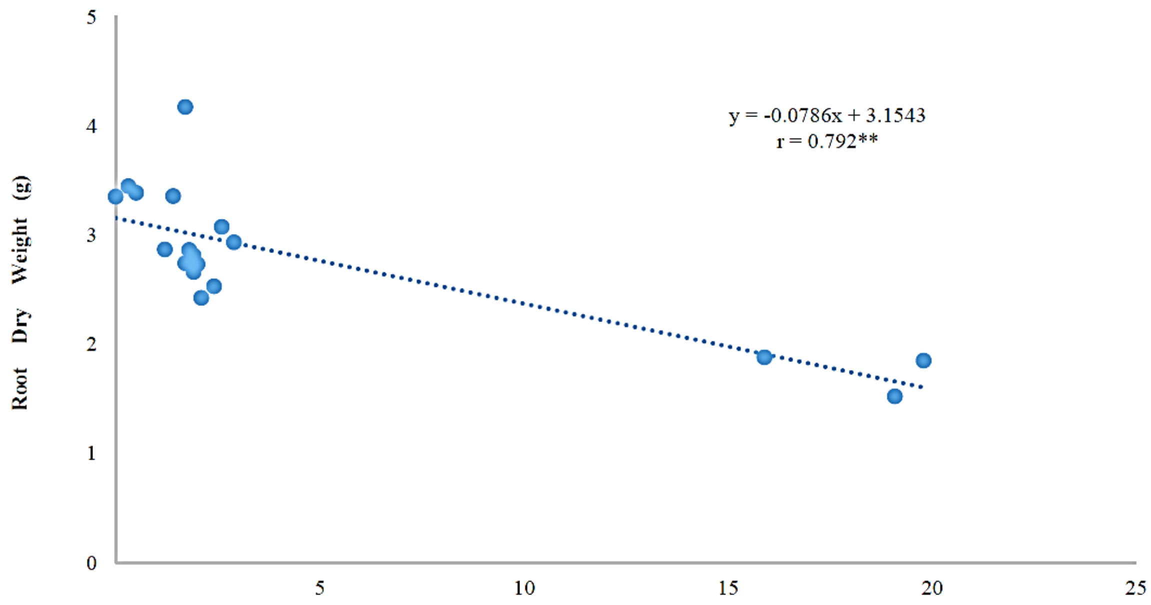

Figure 2. Correlation between number of galls and dry weight of the plant root. ** Highly significant correlation

Table 2. Z test to determine the effect of bionematicides and the number of galls on the roots of plants

Statistical	Value
Effectiveness	60%
Number of observations	12
µ	3.39
Alpha	5%
Z tabulated	-1.65
Np	7.2
Standard deviation typified	1.70
Calculated z	-2.25

Figure 3. Tomato seedlings in greenhouse phase (A), Root infested with *Meloidogyne incognita* (absolute control) (B), Roots inoculated with bionematicides (C and D).

According to the results obtained, the potential damage caused by *Meloidogyne incognita* was significantly reduced, whose effect was probably increased by the addition of organic matter (rabbit manure), and so, the development of the seedlings was evidenced in a higher radicular and aerial dry weight and lesser number of galls. *Trichoderma harzianum*-rabbit manure, *Pleurotus ostreatus*-rabbit manure and *P. ostreatus*-wheat straw allowed obtaining higher fresh and dry weight values of the plant at 60 days after the greenhouse transplant. The benefit of the addition of organic matter to the soil has been widely recognized, which is able to reduce some diseases and pests such as phytonematodes (Singht & Sitaramaiah, 1970).

The results obtained in this research can be related to those achieved in the study on the potential role of bioinoculants and organic matter for the management of the root knot nematode in infested chickpea, where it was demonstrated that the combined application of biological agents and organic matter is very useful for the producers of this legume to reduce root knot disease, in addition to minimizing environmental disturbances, caused by pesticides of synthetic origin and preserve the flora, fauna and microbial diversity in soil (Taylor & Sasser, 1978).

Other studies show that organic matter is beneficial not only when combined with biological controllers, but also with plants with nematicidal action such as neem. As evidenced by the work on sustainable management of root knot disease in tomato with neem cake and *Glomus fasciculatum* in Aligarh, India, the nematicidal potential of the organic matter and the neem cake and the biological agent, *G. fasciculatum* and its positive influence on tomato growth, was determined when inoculated individually, as well as in combination, while providing sources of nutrients for the proper growth of plants, as the intensity of the disease caused by the knot nematode decreased (Akram, Rizvi, Sumbul, Ansari, & Mahmood, 2016).

Likewise, the positive effect of *Trichoderma harzianum* and *Nerium indicum* has been proven either alone or in combination, that it reduced the severity of the disease complex caused by the nematode *Meloidogyne javanica* and *Rizoctonia solani*. It is stated that, the incorporation of biofertilizers significantly reduced the multiplication of the nematode, root-galls, egg mass/root system, and also reduced the root rot of *R. solani* to the hundred. It was concluded that the combined application of *N. indicum* and *T. harzianum* showed maximum improvement in growth variables compared to their individual application, as well as untreated control. In addition, the maximum inhibition in the incidence and multiplication of the nematode of the root knot was observed (Vásquez & Álvarez, 2011).

Probably, another explanation is the resistance induced in plants, caused by some microorganisms, as shown by the study carried out with the genus *Arthrobotrys oligospora*, the same one that grew inter and intracellularly in roots of barley and tomato, but did not penetrate the vascular tissues of plant roots. In addition, it also induced plant defense reactions without impairing their development, making them more resistant to phytoparasitic nematodes and/or other pathogens (Askary & Martinelli, 2015).

Conclusion

In the greenhouse phase on the effect of two bionematicides on root galling and development of tomato seedlings, it is concluded that both: *Trichoderma harzianum*-rabbit manure and *Pleurotus ostreatus*-rabbit manure exerted a positive effect on the development of the plants, as well as the decrease of galls. The two bionematicides exerted a positive effect on the dry weight of both aerial part and roots, being in average just 20.64 and 13.64% minor to the obtained with the chemical control (Fenamiphos), respectively, showing that it is possible to replace or reduce the application of chemical pesticides by biological products.

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