Cultivos Tropicales, 2019, vol. 40, no. 3, e01
ISSN impreso: 0258-5936
ISSN digital: 1819-4087

julio-septiembre
Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas

http://ediciones.inca.edu.cu

Original article

Effect of three beneficial fungi and Sulfur on harmful insects in the common bean crop (*Phaseolus vulgaris* L)

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ABSTRACT

The present work was carried out in the "La Berta" farm located in the municipality of Alquízar, Artemisa province, with the objective of evaluating the effectiveness of the fungi *Lecanicillium lecanii* (Zimm) Zare & Gams, *Trichoderma harzianum* Rifai and *Metarhizium anisopliae sensu lato* (Metsch) Sorokin at a dose of 10^{12} conidia ha-^{1} and Sulfur at a dose of 3 kg ha^{-1} (ai), applied weekly in the leaf area of the plant, in the control of thrips (*Thrips palmi* Karny), leaves jumps (*Empoasca kraemeri* Ross & Moore) and whitefly (*Bemisia tabaci* Gennadius), in the cultivation of common bean (*Phaseolus vulgaris* L.), variety CC-25-9N. A randomized block design with five treatments and four replications was used. The population of the insects was evaluated weekly, after the start of the applications, and a total of six evaluations were made. In addition, performance and some of its components were evaluated. All three fungi reduced insect populations compared to control, although the greatest effect was achieved with *Metarhizium* and L*ecanicillium*. The applications with fungi and sulfur also caused a positive effect on crop yield and its components.

Key words: Beans, entomopathogenic fungi, sulfur, yield

INTRODUCTION

The common bean is the most important species in the legumes of food grains, due to the high nutrient content it has. This grain provides an essential source of protein, vitamins and minerals to the diet of America populations, especially in developing countries [(1)].

The world production of this crop exceeded 26 000 000 t in 2016 with a yield of 0.91 (t ha^{-1}), while in Cuba, in the same year there was a production volume of more than 130 000 t yield of 1.11 (t ha^{-1}) [(2)]. In Cuba, the production that is reached does not satisfy the population's demand, due to the incidence of different factors that limit the expression of the genetic potential of the varieties. Among the factors that have contributed to this situation is the high incidence of phytophagous insects, among which *Thrips palmi* Karny, *Empoasca kraemeri* Göethe, *Bemisia tabaci* Gennadius, capable of causing severe damage due to their direct feeding or as disease vectors [(3)].

Traditionally chemical pesticides have been used to counteract the incidence of phytophagous insects and thus obtain higher yields to meet and meet the food demands of the population [(4)]. The control of harmful organisms in this crop has been based on chemical control, which has a set of side effects.

In recent years, alternatives have been sought, where biological control has been most studied for contributing to agroecological control of pests, which have a lower environmental impact, less harmful to human and animal health, among other advantages.

Microbial agents constitute a phytosanitary alternative, which can decrease chemical inputs in integrated agricultural systems [(5)]. Some authors have investigated the use of entomopathogenic fungi for the control of harmful organisms, which constitute the most versatile biological control agents, due to the wide range of hosts [(6)].

Lecanicillium lecanii (Zimm) Zare & Gams, is a natural bioregulator of pests of economic importance [(7)]. For its part, *Trichoderma harzianum* Rifai is known as mycoparasite, but it has been proven that it can act on insects, demonstrating its entomopathogenic effect [(8)]. *Metarhizium anisopliae* sensu lato (Metsch) Sorokin, is considered the second of the most fungal entomopathogenic species with the most work in the world, in relation to its mass production and commercialization as biopesticides [(9)].

Based on the above, the objective of this work is to evaluate the effectiveness of foliar applications with the fungi *Lecanicillium lecanii* (Zimm) Zare & Gams, *Thrichoderma* *harzianum* Rifai, *Metarhizium anisopliae* sensu lato and sulfur in the control of *Thrips palmi* Karny, *Empoasca kraemeri* Göethe and *Bemisia tabaci* Gennadius in the cultivation of common beans (*Phaseolus vulgaris* L.)

MATERIALS AND METHODS

This work was carried out at the farm "La Berta", located in Alquízar municipality, in Artemisa province. The Cuba Cueto 25-9N bean variety was used, planted in a typical red Ferralitic soil [(10)].

Entomopathogenic fungi *L. lecanii*, strain Y-57, *T. harzianum*, strain T-29 and *M. anisopliae*, isolated Ma-005 were used for the assembly of the experiments. From the isolates of fungi that are preserved in the Plant Health Laboratory of the Faculty of Agronomy, they were reactivated on malt extract agar in test tubes. To obtain the fungal material necessary for the test, these fungi were reproduced using conventional solid state fermentation methodologies (FES).

In the case of the treatment where sulfur is used, the population incidence of insects was evaluated, using a dose of 3 kg ha^{-1} (a.i.), as directed in the Phytosanitary Strategy 2013-2014, for bean cultivation [(11)]. During the course of the experiment, no chemicals were used. Bean sowing in the field was done manually on November 11th, 2015, at a distance between rows of 0.70 and a distance between plants of 0.05 m. For planting a soil preparation was conceived according to the technical standards of the crop.

The design of the experiment was a randomized block with four replicas. Each plot (experimental unit) had four rows and five meters in length for an area of 14 m^2.

From the moment of planting a constant observation was maintained in the experiment and in the fields near it, to detect the presence of harmful organisms. At 17 days after sowing (DAS) it was decided to start with a frequency every seven days, as the first harmful organisms began to appear in the surrounding fields. In total, nine applications were made from November 28th, 2015 to January 23th, 2016, using the treatments shown in Table 1. After each application a gravity irrigation was performed in the experiment.

Table 1. Treatments used in the test for the control of harmful insects in the cultivation of common beans (*Phaseolus vulgaris* L)

Treatments	Doses	Applications
T. harzianum	1,26 x 10^{12} conidia ha^{-1}	9
L. lecanii	1,14 x 10^{12} conidia ha^{-1}	9
M.anisopliae	1,18 x 10^{12} conidia ha^{-1}	9
Sulfur	3 kg ha^{-1} (i.a.)	9
Control	-	No application

After checking the theoretical assumptions of normality and homogeneity of variance, the statistical analysis was carried out applying a double classification analysis of Variance and the means were compared through the Tukey multiple range test with a significance level of 5 % [(12)].

Effect of three beneficial fungi and sulfur on populations of harmful insects in beans

For the application of the products, a backpack, manual, with a capacity of 16 L and a discharge of 300 L ha^{-1} was used, making the necessary adjustments to achieve the required dose.

The evaluations began on December 19th, 2015, three weeks after the first application (when the first organisms appeared in the field trial) and concluded on January 23, 2016, being carried out as follows: ten were selected random plants of the two central furrows of the plots, a leaflet of the central level of the plant was observed. In each leaflet with the help of a magnifying glass, 10X magnification and manually counted the number of thrips nymphs and adults and jumps leaves, the number of whitefly nymphs and the average of the 10 leaflets. It was expressed as the number of insects per leaflet (insert $leaflet^{-1}$).

With the data of the six evaluations, a line graph was made to analyze the behavior of the populations of each of the harmful organisms evaluated, using Microsoft Excel 2010. With the data of the first, second and sixth evaluation and with the average from the data of the six evaluations, double classification variance analyzes were performed and the treatment means were compared using the Tukey Test at 95 % probability [(12)]. Before the analysis, the population data were transformed, according to the expression $(X+1)^{½}$.

Effect of three beneficial fungi and sulfur on yield and its components

The harvest was carried out when each of the plots reached the level of harvest maturity (90 % defoliation of the plant and 20 % moisture content of the grains). The first two plots with treatments of *L. lecanii* and Sulfur were harvested at 87 days, after germinated plots, treatments with *M. anisopliae* and *T. harzianum* at 94 days, after sowing and control plots they were collected 100 days after planting. In the harvest process the following indicators were evaluated:

- Number of legumes per plant
- Number of grains per legume
- Mass of 100 seeds (g)
- Yield (t ha^{-1})

The number of legumes per plants was evaluated after harvesting and for this, ten plants were selected per random plot and from the central rows, the number of legumes was counted and averaged. The number of grains per leg was determined by taking the legumes of the ten previously selected plants, the grains were extracted, counted and the average obtained for each replica. For the yield, a linear meter (0.70 m^2) of each plot was harvested, the grains were dried, their mass was determined and it was estimated in t ha^{-1} at 12 % humidity. With the data obtained for each of the variables evaluated, double classification variance analyzes were performed and the means were compared using the 95 % Tukey Test [12].

RESULTS AND DISCUSSION

Effect of three beneficial fungi and sulfur on populations of harmful insects in common beans

The results of the population fluctuations of *T. palmi* during the period evaluated are shown in Figure 1. In fungal treatments, a lower number of insects per leaflet can be seen than in the control, highlighting the effectiveness of *M. anisopliae* and *L. lecanii* fungi, with results close to sulfur. The treatments with *T. harzianum* and the control presented a greater population of insects.$leaflet^{-1}$, during the experiment a period of heavy rains developed.

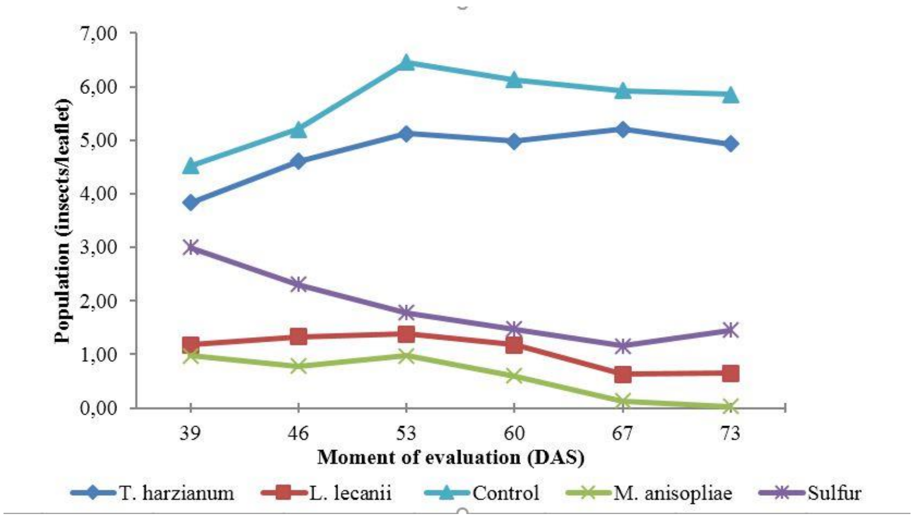

Figure 1. Effect of the applications of three beneficial fungi and sulfur in the population of nymphs and adults of *T. palmi* in beans, under field conditions

Some authors [5,13] state that *L. lecanii* and *M. anisopliae* are efficient as biological control agents and compatible with each other. Treatments with *M. anisopliae* can protect the roots of crops of various insects and some pathogens.

The statistical analyzes performed showed highly significant differences; the treatments with *Metarhizium* and *Lecanicillium* maintained a better behavior with respect to the other fungi, with significant differences between them when evaluating the population of nymphs and adults of *T. palmi*, as can be seen in Table 2.

Table 2. Effect of applications with beneficial fungi and sulfur on the population of *T. palmi* in beans, under field conditions

Treatments	Population of nymphs and adults (foliolo^{-1} insects)							
	1st evaluation (39 DAS)		4 th evaluation (60 DAS)		6th evaluation (73 DAS)		Average of 6 evaluations	
	X. orig.	X. transf.	X. orig.	X. transf.	X orig.	X. transf.	X. orig.	X. transf.
Trichoderma	3,83	2,19a	4,98	2,44a	4,93	2,43a	4,88	2,41a
Lecanicillium	1,18	1,47b	1,18	1.47b	0,65	1,28c	1,01	1,43c
Metarhizium	0,98	1,40b	0,60	1.26b	0,03	1,01d	0,50	1,26c
Sulfur	3,00	1,98a	1,48	1.57b	1,45	1,56b	1,86	1,69b
Control	4,53	2,35a	6,13	2,65a	5,85	2,62a	5,80	2,58a
C.V. (%)		9,82		10,96		5,96		5,66
ESx		0,092**		0,103**		0,053**		0,053**

Means with equal letters, within each column, do not differ significantly, according to Tukey (p≤0, .05)

In the first evaluation it was observed that the treatments of *Lecanicillium* and *Metarhizium* were the ones with the greatest effect on *T. palmi* populations, without significant differences between them.

On the other hand, in the fourth evaluation the treatments of *Lecanicillium*, *Metarhizium* and Sulfur showed the least number of insects per leaflets with significant differences with respect to the control.

In the sixth evaluation, *Metarhizium* stands out as the best treatment, with significant differences, with respect to the rest; On the other hand, *Lecanicillium* maintained a positive effect on insect populations, with significant differences in relation to sulfur, while *Trichoderma* and the control did not show significant differences between them.

The applications of *Metarhizium* and *L. lecanii* in beans have been directed to harmful organisms, such as *T. palmi*, with good results, both alone, and in combination with chemical pesticides, with which they can be compatible [14,15].

Different authors have emphasized the importance of this species as a biological control agent for grasshoppers in various regions of the world. In the state of Chihuahua, Mexico, it has been reported to obtain native isolates of *M. anisopliae*, with potential as biological control agents of Brachystola magna Girard (*Orthoptera: Romaleidae*), an important bean pest (*Phaseolus vulgaris* L.) and other crops [16,17].

Figure 2 shows the effect of the applications of the three beneficial fungi on the population of nymphs and adults of *E. kraemeri* in beans, under field conditions. It is shown that the application of the treatments positively influenced the inhibition of nymphs and adults, with respect to the control, highlighting the treatments of *M. anisopliae* and *L. lecanii*, which at 73 days managed to inhibit insect populations, while that sulfur and control kept populations of this species high.

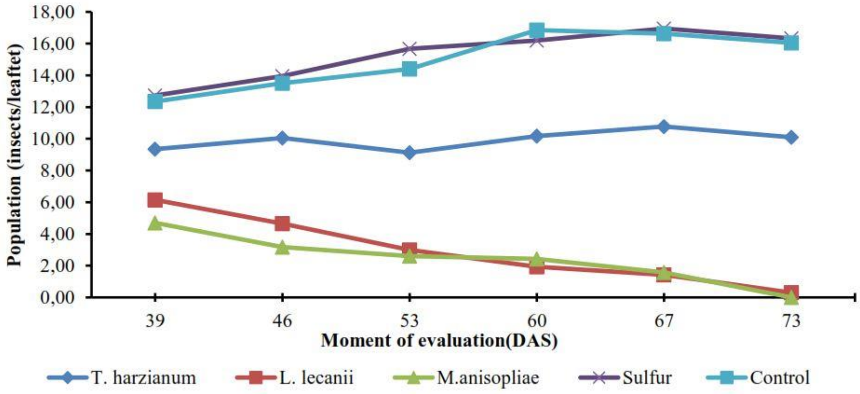

Figure 2. Effect of the applications of three beneficial fungi and sulfur on the population of nymphs and adults of *E. kraemeri* in beans, under field conditions

The foregoing coincides with that raised by different authors, who consider *M. anisopliae* and *L. lecanii* as fungal pathogens that are found in many ecosystems and are used in biological control against insects (18).

Authors report the use of *Trichoderma* species successfully for insect control in bean, cucumber, tomato crops. Also suggesting that this genus helps plants to absorb nutrients, increase roots and triggers growth (19).

The statistical analysis performed with the data of 1st, 4th and 6th, independent, and the average of the six evaluations, showed that there are highly significant differences between treatments. As can be seen in Table 3, in the first evaluation, carried out at 39 days, the treatments with Sulfur and *Trichoderma* turned out to be the smallest inhibitors with a behavior similar to the control, while *Metarhizium* proved to be the best inhibitor of nymphs and the adults of *E. kraemeri*.

Table 3. Effect of applications with beneficial fungi and sulfur on the population of *E. kraemeri* in beans, under field conditions

Treatments	Population of nymphs and adults (foliolo^{-1} insects)							
	1 rst evaluation (39 D DS)		4 th evaluation (60 DDS)		6 th evaluation (73 DDS)		Average 6 evaluations	
	X. orig.	X. transf.	X. orig.	X. transf.	X orig.	X. transf.	X. orig.	X. transf.
Trichoderma	9,35	3,19ab	10,18	3,35b	10,10	3,33b	9,93	3,31b
Lecanicillium	6,15	2,65bc	1,93	1,71c	0,30	1,13c	2,52	1,87c
Metarhizium	4,70	2,38c	2,43	1,84c	0,00	1,00c	2,07	1,75c
Sulfur	12,73	3,71a	16,20	4,15a	16,33	4,16a	15,41	4,05a
Control	12,35	3,65a	16,85	4,20a	16,05	4,12a	14,96	4,00a
C.V. (%)		8,10		8,36		6,08		4,25
ESx		0,126**		0,127**		0,083**		0,064**

Means with equal letters, within each column, do not differ significantly, according to Tukey ($p \leq 0.05$)

On the other hand, the 4th. and 6th, independent and the average of the six evaluations, showed a similar treatment behavior, highlighting *Lecanicillium* and *Metarhizium* with positive effects on the reduction of insect populations without significant differences between them; while control and treatment with Sulfur, even when they did not show significant differences between them, inhibited the growth of nymphs and adults of *E. kraemeri* to a lesser extent in the cultivation of common beans.

M. anisopliae has been recommended against a great diversity of phytophagous insects, of different orders and families, as well as of mites, in many crops. Research carried out ensures that working with these fungi leads to minimal risks for humans, vertebrates and the environment (16).

Figure 3 shows the effect of the applications of the three beneficial fungi on the population of nymphs of *B. tabaci* in beans, under field conditions, in which it can be seen that their application positively influenced the inhibition of nymphs with respect to control, highlighting *L. lecanii* as the best treatment, achieving, after 73 days, the total inhibition of nymphs of *B. tabaci* in beans. However, the treatment where sulfur was used, decreased populations to a lesser extent than the rest of the treatments where fungi were used.

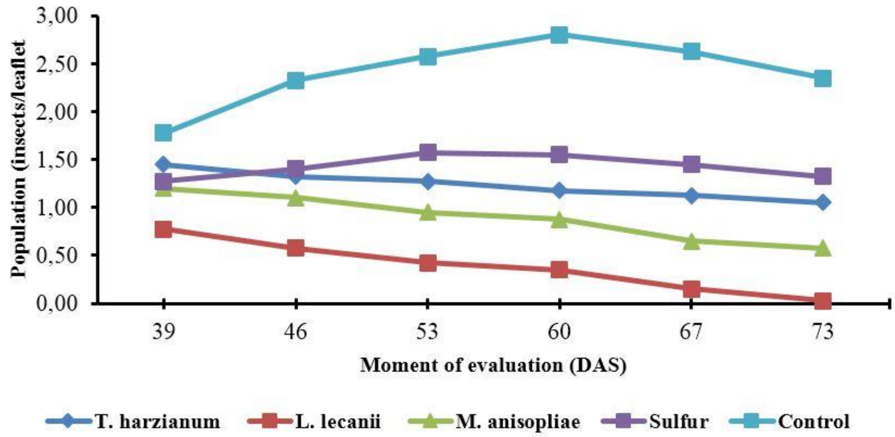

Figure 3. Effect of the applications of three beneficial fungi and sulfur on the population of nymphs of *B. tabaci* in beans, under field conditions

The statistical analysis performed with the data of 1st, 4th and 6th, independent and the average of the six evaluations, showed that there are highly significant differences between treatments. As can be seen in Table 4 from the first evaluation, at 39 days *Lecanicillium* reduced the population of nymphs of *B. tabaci*; while the rest of the treatments did not differ from the control.

Table 4. Effect of applications with beneficial fungi and sulfur on the population of *B. tabaci* in beans, under field conditions

Treatments	**Population of nymphs (foliolo^{-1} insects)**							
	1th evaluation (39 DAS)		**4th evaluation (60 DAS)**		**6th evaluation (73 DAS)**		**Average 6 evaluations**	
	X. orig.	**X. transf.**	**X. orig.**	**X. transf.**	**X orig.**	**X. transf.**	**X. orig.**	**X. transf.**
Trichoderma	1,45	1,57a	1,18	1,47c	1,05	1,43b	1,23	1,50b
Lecanicillium	0,78	1,33b	0,35	1,16e	0,03	1,01d	0,38	1,18d
Metarhizium	1,20	1,48ab	0,88	1,37d	0,58	1,25c	0,89	1,38c
Sulfur	1,28	1,51ab	1,55	1,59b	1,33	1,53b	1,43	1,56b
Control	1,78	1,66a	2,80	1,95a	2,35	1,83a	2,41	1,85a
C.V. (%)		5,75		2,49		5,09		2,57
ESx		0,043**		0,019**		0,036**		0,019**

Means with equal letters, within each column, do not differ significantly, according to Tukey ($p \leq 0.05$)

On the other hand, from the fourth evaluation, the best inhibitor of the insect population was Lecanicillium, with significant differences with respect to the rest of the treatments and control; while the treatment with *Trichoderma* and Sulfur, inhibited, to a lesser extent, the growth of nymphs and adults of *B. tobacco*, in the cultivation of common beans, even when they presented better results than the control.

This demonstrates that, although the three fungal species exert a certain degree of control over the populations of nymphs of *B. tabaci*, there are differences between them, standing out as the most effective entomophagus *L. lecanii*, which was able to inhibit in greater proportion of these populations.

Different authors have indicated that *L. lecanii* is a biological control agent widely used as a natural enemy of important insect and pathogen pests in crops. On the other hand, the entomophage effect of species of the genus *Trichoderma* on different insects has been demonstrated, such as the cabbage root fly, *Delia radicum* L. (Diptera: Anthomyiidae). While other authors evaluated the pathogenicity of *T. harzianum* on nymphs and adults of *B. tabaci*, its control being effective [20-22].

On the other hand, sulfur plays an important role in the defense mechanisms of plants against pests and diseases, applied in a foliar way is absorbed and metabolized, acting against mites, thrips and whitefly [23].

Effect of three beneficial fungi and sulfur on bean yield and components

The results obtained for the components evaluated and the estimated yield are presented in Table 5. The statistical analysis performed showed highly significant differences between treatments.

As for the legumes component per plant, it was obtained that all variants differ statistically between them, with the greatest value in the case of sulfur treatments, followed by *Lecanicillium*. In the case of grains/legumes it was not the same, because, although it remained in the same order between these last treatments, the values corresponding to the two remaining fungi were statistically similar. The arrangement in the mass component of 100 grains was similar to the number of legumes per plant. A similar trend was also obtained in the different variables in the case of the estimated yield.

Table 5. Effect of beneficial fungi and sulfur applications on bean yield and some of its components

Treatments	legumes/plant	Grains/legume	100 seed mass (g)	Yield (t ha^{-1})
Trichoderma	11,48 c	5,10 c	16,28 c	1,28c
Lecanicillium	15,68 b	6,15 b	20,13 b	1,47 b
Metarhizium	10,95 d	5,10 c	13,30 d	1,21d
Azufre	17,03 a	7,08 a	21,43 a	1,56 a
Control	7,13 e	4,33 d	10,20 e	1,10e
C.V. (%)	1,70	4,20	2,02	1,96
ESx	0,106***	0,116***	0,164***	0,012***

Means with equal letters, within each column, do not differ significantly, according to Tukey ($p \leq 0.05$)

Regarding this indicator, it is noted that in all variants, including the control, values greater than 1 t ha^{-1} were obtained. The treatments behaved in descending order as follows: Sulfur, *L. lecanii*, *T. harzianum*, *M. anisopliae* and control, with values of 1.56, 1.47, 1.28, 1.21 and 1.10 t ha^{-1}, respectively.

Despite the climatic conditions not favorable to the crop, with unusual rains, as well as temperatures and relative humidity somewhat higher than normal for the period, the treatments reached good yields, since this variety, in optimal conditions, should have average yields from 2.5 to 3 t ha^{-1} [(24)].

On the other hand, it should be noted that the results obtained here exceed the average yield reported for Cuba in 2016, of 1.11 t ha^{-1} and for the world, in that same year, of 0.91 t ha^{-1} [(2)].

The results achieved allow us to affirm that the weekly applications of these fungi and sulfur are very beneficial for the productive development of this crop, which corroborates what was stated by authors who assured that the weekly applications of *T. harzianum* produce positive effects on the agroproductive behavior in the cultivation of beans, having results of mass of one hundred seeds, number of legumes per plant, number of grains per legume and yields above the national average [(25)].

Recent research shows that *M. anisopliae* stimulates plant growth and has endophytic activity. They also indicated that the effect of *M. anisopliae* on bean cultivation stimulates the growth of both the root and the aerial part of the plant and that this fungus produces good effect in vitro and *in vivo* on *Fusarium* in bean cultivation; both cell-free filtration and conidia of this cause stabilization in the soil, so its use is suggested against some plant pathogens in the rhizosphere [(16,26)].

Among the main benefits of *Trichoderma* spp. It can be mentioned that it offers effective control in the control of insects and plant diseases and stimulate their growth. In addition, it preserves the environment by decreasing the use of fungicides. Also, by replacing synthetic agrochemicals with these beneficial organisms, producers save production costs (27).

Different authors have evaluated the benefits of sulfur on the yield in bean cultivation, obtaining numerous results, since it is part of the processes of plant development because it is a nutrient considered within the macroelements required by crops for their production (28).

CONCLUSIONS

- Applications with entomopathogenic fungi *L. lecanii*, and *M. anisopliae* had a positive effect on the regulation of *T. palmi* and *E. kraemeri*, highlighting *L. lecanii* in the case of *B. tabaci*.
- The applications of the selected fungi had a favorable impact on crop yields and some of its components, highlighting *L. lecanii* with values very close to those of Sulfur.

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