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Potential of entomopathogenic fungi to control insect pests and disease at lettuce and arugula crops

Fernando Bellesini Vigna[1]*, Ana Paula Alves Silveira de Rensis[2], Luís Rodolfo Rodrigues[2], Matheus Barros Oliva[2], Marta Maria Rossi[2], Alexandre de Sene Pinto[2]

[1]Universidade Estadual Paulista, Jaboticabal-SP, Brasil
[2]Centro Universitário Moura Lacerda, Ribeirão Preto-SP, Brasil
*Corresponding author, e-mail: febelvi@gmail.com

Abstract

The lettuce (*Lactuca sativa* L.) (Asteraceae) and arugula (*Eruca sativa* Miller) (Brassicaceae) crops are attacked by various pests and diseases, but the control is usually done with agrochemicals. We evaluate the control of pests and diseases by systematic applications of the entomopathogenic fungi strains *Metarhizium anisopliae* IBCB425 and *Beauveria bassiana* IBCB66 both 300g c.p. in the planting groove and 200 g c.p. ha^{-1} on the foliage, in weekly applications at lettuce and arugula crops planted in association. For the evaluation of pests and diseases, 15 plants were evaluated at random in three beds per treatment. In the lettuce crop occurred the presence of aphids (*Myzus persicae* Hemiptera:Aphididae), whiteflies (*Bemisia tabaci* Hemiptera: Aleyrodidae), thrips (*Frankliniella schultzei* Thysanoptera:Thripidae), leafhoppers *Agallia albidula* (Hemiptera: Cicadellidae) and septoria disease (*Septoria lactucae)*. In the arugula crop the same pests occurred, but with any disease. The fungi *B. bassiana* and *M. anisopliae* controlled *F. schultzei* and *S. lactucae* at lettuce crop, *B. tabaci* in both crops, but did not control M. persicae and *A. albidula* in both crops. The fungus *B. bassiana* decreased the mean number of leaves per plant and the mean of dry mass weight of arugula plants in relation to the control treatment, but the sample size may have interfered with these results. The two fungi could be used as biological control agents of pests and diseases at lettuce and arugula crops, especially in organic farming.

Keywords: crop pest, leaf spot, microbial control, vegetables

Introduction

Lettuce (*Lactuta sativa* L.) (Asteraceae) and Arugula (*Eruca sativa* Mill.) (Brassicacea) are plants of Mediterranean origin. They are considered important vegetables in the world, with lettuce being the most consumed leafy vegetable in Brazil and in the world (Santos et al., 2001; Da Cunha et al., 2013). This preference occurs because of its taste, nutritious quality and low cost, as well as the ease of purchasing the product, available during all year round in supermarkets and fairs (Cometti et al., 2004).

The consumption of arugula in Brazil has been increasing in some regions of the country, because of its remarkable flavor in salads with softer leaves, pizza topping, pasta sauces and even soups (Sediyama et al., 2007). Vegetables are important in a balanced diet because they complement staple foods, as they are sources of vitamins, minerals and fiber, and often have medicinal value (Van Duyn & Pivonka 2000).

However, the olericulture can be responsible for considerable environmental impact, for the intense use of agrochemicals and natural resources, the intercropping of crops has been employed because it is a technique that contributes to the improvement of land efficiency , and maximizes the use of environmental resources, besides providing greater ecological balance (Koefender et al., 2016; Cecilio Filho & May, 2002). When choosing this cultivation system it is interest to use plants of different size, roots and cycles, facilitating their maintenance for the period that they are producing, such as lettuce and arugula (Silva et al., 2010).

There are several possibilities of intercropping vegetables, and among them, research has also shown that lettuce has been promising in intercropping (Caetano et al., 1999; Cecílio Filho & May 2002; Rezende et al., 2005; Tolentino Junior et al., 2008). Arugula has been

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used as an intermediate crop to other vegetables, mainly because it has a high price among some vegetables (Cecílio Filho et al., 2008; Rezende, 2004). Oliveira et al. (2010), demonstrated that all associations of lettuce and arugula, as well as their single crops, had better productive performance under organic fertilization, and the regrowth of arugula increases the agronomic efficiency of the intercropping system.

The advantages of this practice can offer is very well used in the cultivation of vegetables (Leite et al., 2011). In addition, to the control of pests and weeds in vegetable crops, due to existing ecological relationships (Vasconcelos et al. 2012). Entretanto, diversos problemas fitossanitários afetam a cultura da alface e da rúcula (Imenes et al., 2000; Gallo et al., 2002; Yuki, 2000; Kanashiro, 2017).

However, regardless of the production system adopted, problems with insects and pests are recurrent, since to implement an agroecosystem, it is necessary to disturb, to a greater or lesser extent, the natural ecosystem and, consequently, its balance. Although, agroecological management reduces problems with insect-pests, these organisms often limit the quantitative and qualitative production of vegetables. Quantitative losses, in general, are 10 to 30%, however, in certain situations these attacks can compromise 100% of production (Picanço et al. 2005). Thus, the need for alternative control methods to chemicals is highlighted, biological control is one of the most appropriate strategies for pest control in short growing crops, as they are efficient for pest control and have less impact on the agroecosystem (Alves, 1998; Tatchell, 2007). Few studies address the effects of entomopathogenic fungi on lettuce and rucula crops under field conditions.

In this context, this study aimed to evaluate the microbial control of pests and diseases in lettuce and arugula crops in intercropping, using the entomopathogenic fungi Beauveria bassiana IBCB-66 and Metarhizium anisopliae IBCB-425 in systematic applications.

Material and Methods

Study sites

The bioassays were carried out at Moura Lacerda University (Ribeirão Preto – São Paulo state). The field experiment is located at latitude 21°12'43''S and longitude 47°46'23''W , with an altitude of 620 m. The site has two rainy seasons: January–March and September–December, with an annual rainfall of 1508mm. In general the temperature ranges from 13 °C to 32 °C and is rarely below 9 °C or above 37 °C.

Fungus and production of inoculum

Two strains of entomopathogenic fungi were used in the experiments, the strains IBCB 425 *M. anisopliae* and IBCB-66 *B. bassiana* was gently provided by Instituto Biológico de São Paulo, Brazil.

The fungal isolates were cultured in Potato Dextrose Agar (PDA, KASVI) medium and 0.1 % stock antibiotics was added. The stock antibiotic solution consisted of 0.02 g each of tetracycline, streptomycin and penicillin, dissolved in10 ml sterile, distilled water, filter-sterilized through a 0.2 mm filter. The medium was added on Petri dishes and incubated in a B.O.D chamber at 26 ± 1 ° C with 12 hours of photophase for 7 to 10 days.

Subsequently, the solid fermentation was carried out to increase the amount of inoculum used for the bioassays. For the solid fermentation were used 250 mL capacity screw-cap borosilicate glass bottles (Schott Mark) containing 50 grams of parboiled rice type 1, purchased by local retailers. Initially, the substrate was hydrated with 100 mL of distilled water and kept at room temperature for 50 minutes. Thereafter, the rice was sieved to remove excess of water and returned to the Schott flasks to be autoclaved at 120° C for 20 minutes.

The inoculum of each fungal isolate was prepared by scraping the conidia present in the PDA culture medium and diluted in sterilized distilled water with 0.01% (v/v) surfactant (Tween 80®, Vetec Química Fina Ltda., Rio de Janeiro, RJ, Brazil). The inoculation of the flasks was done in an aseptic laminar flow chamber with the addition of 5 mL of the conidia suspension of each isolate adjusted to 1 x 10^8 conidia/mL,and thereafter all flasks were incubated at 20 °C, 90 ± 2% RH and a photoperiod of 16:8 (L:D) h for 10 days. Subsequently, the conidial suspension used in the bioassays was prepared from washing with distilled water the sporulated rice grains flasks with 0.01% (v/v) surfactant Tween 80 and the conidia concentration was adjusted to 1 x 10^{13} conidia/mL through counting in a Neubauer chamber with a light microscope. The viability of all fungus spores used in the bioassays exceeded 90%.

Field plots and experimental design

The soil preparation and raised beds were performed on August 31, 2017, using tanned manure and 200g of limestone per bed. A total of 180 lettuce seedlings and 180 arugula seedlings were used, 20 of each per bed. The seedlings were planted on September 18, 2017 in nine beds, each bed being 2.5 x 1.0 m, with plants spacing and rows of 0.20 m.

The syrup for each foliar spraying of the fungi was prepared at the time of application using a spreader-sticker (Agral - Syngenta, 200g / L Nonyl Phenoxy Poly

(Ethylenoxy) Ethanol). The entomopathogenic fungi used in the assays had conidia produced in rice as previously described. Three treatments were repeated seven times: (1) weekly leaf application of the fungus *Beauveria bassiana* IBCB66 at a dose of 200 g p.c. ha^{-1}; (2) weekly leaf application of the fungus *Metarhizium anisopliae* strain IBCB425, at a dose of 200 g p.c. ha^{-1}; (3) Control (no applications).

On the day of planting, the fungi that were used in the respective treatments were applied to the planting furrows at a dose of 300 g p.c. ha^{-1}. After the furrow treatments were applied, fertilization was made, based on Bulletin 200 described by Aguiar et al. (2014), and the N-P-K fertilizer used was 10-20-16 at a dose of 40 g every two meters. A fertilization coverage was performed on October 16th, using 46% of urea, with a dose of 21g for lettuce per ten plants and for 5.9 g arugula per ten plants. In the aerial part, the fungi were applied at a dose of 200g CFU ha^{-1} in a 300 L ha^{-1} syrup. The syrup was prepared whenever it was to be used and placed inside an electric backpack sprayer (one for each fungus), speed of 4 km h^{-1}, pressure of 60 lb and orange fan spray nozzle (0.1).

Four foliar applications of the fungi were made in consecutive weeks in the beds with lettuce and arugula crops, and a fifth application only in lettuce, since the arugula had already been harvested because it had already completed its cycle. The foliar applications were carried out on September 25th, October 3rd, 10th, 16th and 24th (the latter only in the lettuce crop), always at 18h00min. The evaluations were made weekly, being made in the next day after the foliar applications.

The evaluations were carried out in two distinct groups (lettuce and arugula) of five plants in a row. The number of plants with aphids (*Myzus persicae*,Hemiptera: Aphididae), Whiteflys (*Bemisia Tabaci* ,Hemiptera: Aleyrodidae), trips (*Frankliniella schultzei* Trybom ,Thysanoptera: Thripidae), leafhoppers (*Agallia albidula* Uhler ,Hemiptera:Cicadellidae) and the number of plants with Septoria disease *Septoria lactucae* were recorded.

At harvest time each plot was divided into two parts (lettuce and arugula) and from each part all consecutive plants of one linear meter were removed and evaluated for the number of leaves and leaves with disease or pest damage. Arugula was harvested on October 19 and lettuce was harvested on October 30.

After postharvest evaluations the plants were placed in separate paper bags and placed in a laboratory hot air oven at a temperature of 85 °C, after five days the plants were removed and weighed to calculate dry matter.

Data analysis

The data obtained from all evaluated parameters were transformed into means, compared to each other and submitted to the average comparison analysis by Tukey test, at 5% significance level. All statistical calculations were performed by Statistica for Windows (Statsoft, 1996).

Results and Discussion

In the lettuce crop occurred the presence of the insect pests *M. persicae*, *B. tabaci*, *F. schultzei*, *A. albidula* and *S. lactucae* disease. In the arugula crop the same pests occurred, but with no diseases.

The infestation of lettuce plants with *M. persicae* was very low and there were no significant differences between treatments. For arugula, the infestation was high, surpassing 70% of the plants with presence of *M. persicae*. In the arugula crop, at the second week of evaluation, the treatment with *M. anisopliae* IBCB 425 presented the highest value of percentage average of plants with *M. persicae*, differing statistically from the control treatment. In the third week, the fungal treatments showed the highest values and differed from the control, which presented few plants with *M. persicae* (**Figure 1**).

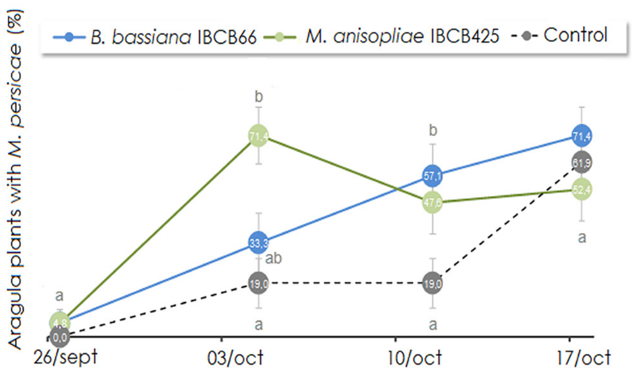

Figure 1. Average percentage of plants with *Myzus persicae* on different dates after fungus application in arugula crop. Ribeirão Preto, SP, 2017. Points (± standard error of the mean) followed by the same letter do not differ from each other by the Tukey's test ($p < 0.05$).

D Chandler (1997) tested the virulence of 25 *M. anisopliae* strains and just one strain of *M. anisopliae*-391.93 killed lettuce root aphids (*Pemphigus bursarius*) consistently, under laboratory conditions. Almeida et al. (2007) found that a 0.233 g L^{-1} of *B. bassiana* dose was the best to control *Brevicoryne brassicae*, that dose is more than double of that we have used in the current study (0.25 g L-1). Araújo et al. (2009) concluded that *B. bassiana* and *M. anisopliae* fungi were virulent to *L. erysimi*. Shrestha et al. (2015) reported that *B. bassiana* affected *Nasonovia* *ribisnigri* (Mosley) nymphs under laboratory conditions, with the greatest effect observed when 4th instar of *N. ribisnigri*'s was inoculated with *B. bassiana*. Loureiro and Moino Jr. (2006) also controlled *Aphis gossypii* and *M. persicae* with *B. bassiana* and *M. anisopliae* fungi in the laboratory.

The results obtained by the highlighted authors disagree with those obtained in the current study for *B. bassiana* IBCB 66 and *M. anisopliae* IBCB425 strains, which did not control aphids. In addition, the fungi increased the occurrence of *M. persicae* in arugula crop. Probably field trials may yield different results than in the laboratory, as most aphid studies were conducted under laboratory conditions, field conditions and other variables may have directly affected the expected results.

In the lettuce crop, the presence of *B. tabaci* was only on the second evaluation date, where fungi treatment showed values significantly lower than that observed in the control, which reached more than 50% (**Figure 2**).

The same occurred in arugula crop, where the control plant infestation exceeded 60% (**Figure 3**).

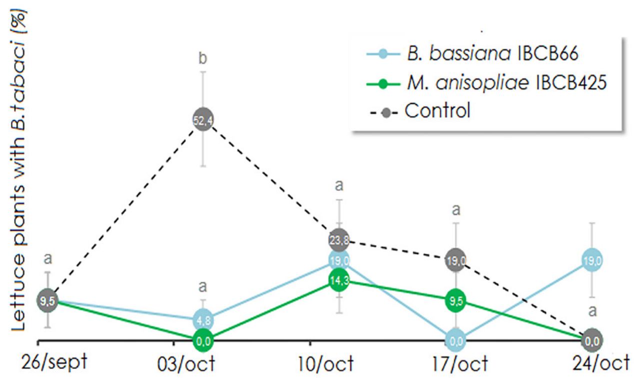

Figure 2. Average percentage of plants with *Bemisia tabaci* on different dates after fungus application in lettuce crop. Ribeirão Preto, SP, 2017. Points (± standard error of the mean) followed by the same letter do not differ from each other by the Tukey's test ($p < 0.05$).

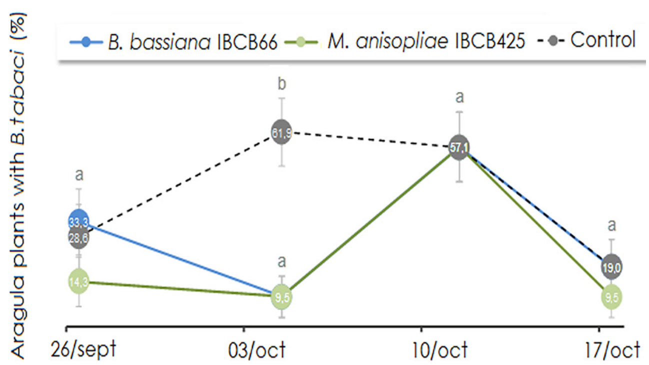

Figure 3. Average percentage of plants with *Bemisia tabaci* on different dates after fungus application in aragula crop. Ribeirão Preto, SP, 2017. Points (± standard error of the mean) followed by the same letter do not differ from each other by the Tukey's test ($p < 0.05$).

Against *B. Tabaci*, the two fungi were partially effective in the current study because they controlled the insect for a week. However, Rheinheimer et al. (2009) did not achieve good results of these fungi in the control of *B. tuberculata* in the laboratory. Islam et al. (2010) achieved good control of *B. tabaci* nymphs with *B. bassiana* fungus using a concentration of 10^7 conidia mL^{-1} plus neither oil (0.5%) in eggplant culture. Potrich et al. (2011) also achieved good results with *B. bassiana* and *Cordyceps* spp. on 3rd instar *B. tabaci* nymphs in kale leaves, but did not verify control exerted by *M. anisopliae*, as occurred in the current assay. Javed et al. (2019) reported that the fungus *B. bassiana* (Bb01) was found to be the most virulent against *B. tabaci* while *Verticillium lecanii* (V2) appeared to be the most virulent against aphids.

Espinosa et al. (2019) reported that the fungi *B. bassiana*, *Lecanicillium muscarium* and *M. riley* at bean were pathogenic to eggs and 3rd instar of *B. tabaci* Biotype B nymphs, *B. bassiana* JAB07 and *L. muscarium* LCMAP3790 strains presented the best results, with nymphs mortality of 96.68 ± 2.25% and 97.74 ± 1.56%, respectively.

For F. schultzei, the infestation was more severe in lettuce crop. The treatment with *B. bassiana* IBCB 66 showed significantly lower values of percentage average for thrips presence at plants than that observed in the control, on the second and fourth evaluation dates (Figure 4). *M. anisopliae* IBCB 425 treatment showed a statistically lower value than control only on the fourth assessment date (**Figure 4**).

The autors Jacobson et al. (2010) verified control of 65-87% of the *F. occidentalis* thrips with the *B. bassiana* fungus in cucurbit plant, laboratory and greenhouse crops. The authors recommend this fungus as a second option after the use of thrips predatory mites.

The results also agree with Al-Mazra'awi et al. (2006) who achieved effective control of *F. occidentalis* in

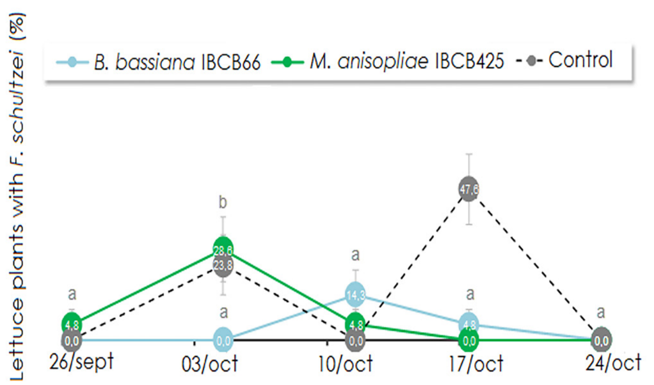

Figure 4. Average percentage of plants with *Frankliniella schultzei* on different dates after fungus application in lettuce crop. Ribeirão Preto, SP, 2017. Points (± standard error of the mean) followed by the same letter do not differ from each other by the Tukey's test ($p < 0.05$).

sweet pepper, using the *Bombus impatiens* pollinator as a *B. bassiana* disperser. Gao et al. (2012), verified effective control of *F. occidentalis* in laboratory (10^4 to 10^7 conidia mL^{-1}), 10 days after inoculation of 1st instar nymphs, and at greenhouse, for broccoli crop, they obtained significant reduction. of nymphs and adults on leaves. In lettuce, Dara (2017) found that *B. bassiana* has potential for the control of *F. occidentalis* thrips in lettuce, in agreement with the current study. The results of these authors agree with those obtained in the current study for thrips.

For *A. albidula*, the occurrence was not very high, but it reached almost 40% of presence at arugula plants. There were no significant differences between treatments at any evaluation date in the two crops, except for the arugula crop, where last treatment with *B. bassiana* IBCB 66 showed the highest percentage average value of plants with *A. albidula*, differing for control treatment, who did not present this insect pest (**Figure 5**).

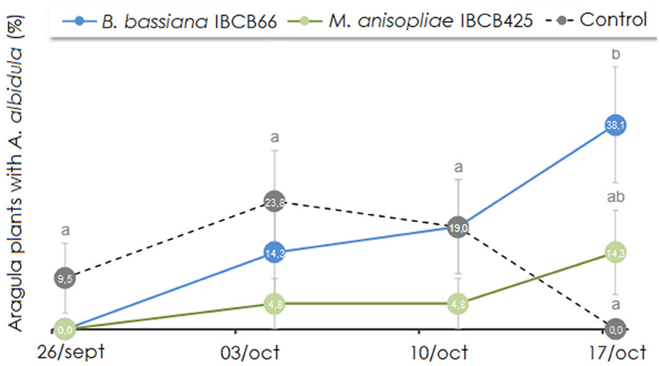

Figure 5. Average percentage of plants with *Agallia albidula* on different dates after fungus application in aragula crop. Ribeirão Preto, SP, 2017. Points (± standard error of the mean) followed by the same letter do not differ from each other by the Tukey's test ($p < 0.05$).

Despite this result that disadvantages the *B. bassiana* IBCB 66 fungus, including showing the increase of this pest at treated plants, as regards the control of *A. albidula*, this insect has wide dispersion, and it would be impossible to draw conclusions for such small and close plots.

The septoriosis disease caused by the fungus *S. lactucae* was very severe in lettuce crop, reaching 100% of infected plants in the control treatment. From the third evaluation date the fungi began to show interesting results. On the third date, *B. bassiana* IBCB 66 treatment showed the lowest percentage average value of diseased plants, differing from other treatments, which were around 70%. On the fourth evaluation date, the fungus *M. anisopliae* IBCB 425 showed the lowest value and differed statistically from the control. In the last evaluation there were no more sick plants in the fungal treatments, differing significantly from the control, which was completely compromised (**Figure 6**).

There are no studies showing the effect of *B. bassiana* or *M. anisopliae* fungi on controlo f *S. lactucae* in plants. The fungus *B. bassiana* IBCB 66 in the current study appeared to be the most effective in controlling the disease, but *M. anisopliae* IBCB 425 was not much lower, because both fungi have shown efficacy in controlling this disease.

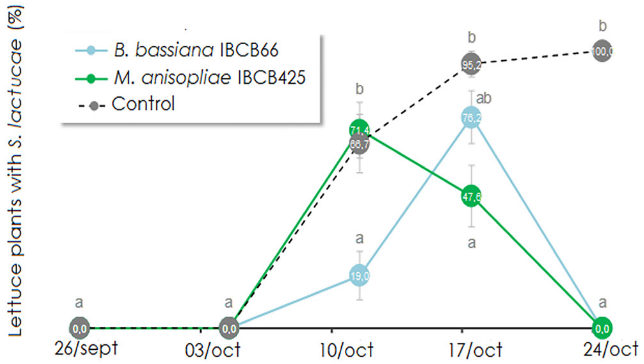

Figure 6. Average percentage of plants with *Septoria lactucae* on different dates after fungus application in lettuce crop. Ribeirão Preto, SP, 2017. Points (± standard error of the mean) followed by the same letter do not differ from each other by the Tukey's test ($p < 0.05$).

At harvest date, all plants of one linear meter per bed were harvested. In lettuce crop, there were no significant differences between treatments regarding the average number of leaves per plant, percentage average of leaves with diseases and leaves with pests (**Table 1**). In the arugula crop, there was a statistically higher number of leaves in the *M. anisopliae* IBCB 425 treatment, differing only from the *B. bassiana* IBCB 66 treatment (Table 1). For the other parameters, there were no significant differences between treatments (Table 1).

The results obtained for arugula crop indicate negative effect of *B. bassiana* on leaf production. Further trials should investigate this fact, as there are no reports of *B. bassiana* decreasing plant productivity.

In the evaluation of lettuce dry mass, there were no statistical differences between treatments (**Table 2**). In arugula crop, the control treatment showed the highest value, differing significantly only from the *B. bassiana* IBCB 66 treatment, which showed the lowest value (Table 2).

The results obtained for lettuce showed no improvement in yield with the use of fungi and showed negative results of *B. bassiana* IBCB 66 in arugula production. Perhaps the size of the samples evaluated has impaired the results obtained at harvest. In addition, the control was placed in beds a few meters further from those used for the treatments and this must have

Table 1. Average number of leaves per plant and average percentage of leaves with diseases and pests at harvest after systematic application of entomopathogenic fungi in lettuce and arugula crops. Ribeirão Preto, SP, 2017

Treatments	Leaves per plant	Sick leaves(%)	Pests on leaves(%)
	Lettuce		
B. bassiana IBCB-66	24.20 ± 1.34a[1]	0.91 ± 0.51a	1.53 ± 0.75a
M. anisopliae IBCB-425	23.40 ± 0.86a	2.32 ± 0.73a	3.78 ± 1.28a
Control	24.47 ± 0.70a	2.76 ± 0.88a	3.56 ± 1.04a
	Arugula		
B. bassiana IBCB-66	62.67 ± 5.57b	0.00 ± 0.00a	8.09 ± 3.79a
M. anisopliae IBCB-425	81.80 ± 6.08a	0.73 ± 0.35a	5.95 ± 0.99a
Control	73.13 ± 4.31ab	1.12 ± 0.56a	5.81 ± 1.22a

[1] Means (± standard error of the mean) followed by the same letters in the column do not differ from each other by the Tukey's test (p <0.05).

Table 2. Average mass dry weight of lettuce and arugula plants at harvest after systematic applications of entomopathogenic fungi. Ribeirão Preto, SP, 2017

Treatments	Lettuce	Arugula
B. bassiana IBCB-66	13.07 ± 1.34 a[1]	24.40 ± 1.07 b
M. anisopliae IBCB-425	11.93 ± 1.09 a	27.47 ± 1.16 ab
Control	13.40 ± 0.72 a	30.67 ± 1.43 a

[1] Means (± standard error of the mean) followed by the same letters in the column do not differ from each other by the Tukey's test (p <0.05).

compromised the homogeneity of the plots. New trials should determine this, this research creates expectations of new trials on the use of entomopathogenic fungi in the production of vegetable crops, especially in small crops and urban environments where insecticides cannot and should not be used.

Conclusion

The entomopathogenic fungi strains *B. bassiana* IBC66 and *M. anisopliae* IBCB425 (both at a concentration of 200 g pc ha^{-1}) controlled *B.tabaci* in lettuce and arugula crops and *F. schultzei* and *S. lactucae* in lettuce crop, however they did not control *M. persicae* and *A. albidula* in both crops. In addition, the tested fungi did not improve the vegetative production of the two studied crops.

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