Research, Society and Development, v. 12, n. 3, e3612336541, 2023
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i3.36541

# Effects of commercial entomopathogen-based formulations on *Neoseiulus* sp. in pine trees

**Efeitos das formulações comerciais à base de entomopatógenos sobre *Neoseiulus* sp. em pinheira**

**Efectos de formulaciones comerciales basadas en entomopatógenos sobre *Neoseiulus* sp. en pinos**

Received: 10/15/2022 | Revised: 02/10/2023 | Accepted: 02/14/2023 | Published: 03/08/2023

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**Abstract**
Biological control is a growing branch in Brazil, and with it comes the need to obtain efficient enemies in the control of pests. The use of entomopathogens has expanded and with it comes the need to investigate their selectivity to non-target organisms. The present work was conducted in a pinecone (*Annona squamosa* L.) orchard with the objective of evaluating in which locations of the plant (lower, middle or upper third) and the effects of the entomopathogens: *Beauveria bassiana*, *Metharhizium anisoplie* and *Bacillus thuringiensis* on the predatory mite. Sixteen weekly applications were made between March and August 2017 with a constant pressure knapsack sprayer, nozzle type JA-2 with the jet directed at the entire plant. The treatments consisted of the use of three entomopathogenic biological products: Dipel, *Beuaveria bassiana* JCO and *Metarhizium anisopliae* JCO applied alone and in combination. Eight treatments were performed, as follows: T1-Spraying with *M. anisopliae* + phfos (Quimifol) + adhesive spreader (Agral), T2-Spraying with *B. bassiana* + phfos (Quimifol) + adhesive spreader (Agral), T3-Spraying with *B. thruringiensis* + phfos (Quimifol) + adhesive spreader (Agral), T4-Spraying with *M. anisopliae* + *B. basisiana* + phfos (Quimifol) + adhesive spreader (Agral), T5- Spraying with *M. anisopliae* + *B*. thruringiensis + phfos (Quimifol) + adhesive spreader (Agral), T6- Spraying with *B. bassiana* + *B. thruringiensis* + phfos (Quimifol) + adhesive spreader (Agral); T7- Spraying with *M. anisopliae* + *B. bassiana* + *B. thruringiensis* + phfos (Quimifol) + adhesive spreader (Agral) e T8-Testify, spraying with phfos (Quimifol) + adhesive spreader (Agral). The results indicate a preference of the *Neoseiulus* sp. mite for the middle third of the plant canopy. The results obtained indicated that the entomopathogens influenced the population of the *Neoseiulus* sp. mite until the sixth week after treatment, where the combination of *Metarhizium anisopliae* + *Beauveria bassiana* + *Bacillus thuringiensis* was the one that most negatively affected the population of the predatory mite *Neoseiulus* sp.
**Keywords:** *Annona squamosa*; *Bacillus thuringiensis; Beauveria bassiana; Metarhizium anisopliae*; Biological control; Selectivity.

**Resumo**
O controle biológico é um ramo crescente no Brasil, e com isso surge a necessidade de obter inimigos eficientes no controle das pragas. A utilização de entomopatógenos tem se expandido e com ela surge a necessidade de investigar a

sua seletividade aos organismos não-alvo. O presente trabalho foi conduzido em pomar de pinha (*Annona squamosa* L.) com o objetivo de avaliar em quais locais da planta (terço inferior, médio ou superior) e os efeitos dos entomopatógenos: *Beauveria bassiana, Metharhizium anisoplie* e *Bacillus thuringiensis* sobre o ácaro predador. Foram realizadas dezesseis aplicações semanais entre março e agosto de 2017 com pulverizador costal de pressão constante, bico tipo JA-2 com jato dirigido a toda planta. Os tratamentos consistiram no uso de três produtos biológicos entomopatogênicos: Dipel, *Beuaveria bassiana* JCO e *Metarhizium anisopliae* JCO aplicados isolados e combinados. Foram realizados oito tratamentos, sendo eles: T1-Pulverização com *M. anisopliae* + phfos (Quimifol) + espalhante adesivo (Agral), T2-Pulverização com *B. bassiana* + phfos (Quimifol) + espalhante adesivo (Agral), T3-Pulverização com *B. thruringiensis* + phfos (Quimifol) + espalhante adesivo (Agral), T4-Pulverização com *M. anisopliae* + *B. basisiana* + phfos (Quimifol) + espalhante adesivo (Agral), T5-Pulverização com *M. anisopliae* + *B*. thruringiensis + phfos (Quimifol) + espalhante adesivo (Agral), T6-Pulverização *com B. bassiana* + *B. thruringiensis* + phfos (Quimifol) + espalhante adesivo (Agral); T7-Pulverização com *M. anisopliae* + *B. bassiana* + *B. thruringiensis* + phfos (Quimifol) + espalhante adesivo (Agral) e T8-Testemunha, pulverização com phfos (Quimifol) + espalhante adesivo (Agral). Os resultados apontam uma preferência do ácaro *Neoseiulus* sp. pelo terço médio do dossel da planta. Os resultados obtidos indicaram que os entomopatógenos influenciaram a população do ácaro *Neoseiulus* sp. até a sexta semana após o tratamento, onde a combinação de *Metarhizium anisopliae* + *Beauveria bassiana* + *Bacillus thuringiensis* foi aquela que mais afetou negativamente a população do ácaro predador *Neoseiulus* sp.
**Palavras-chave:** *Annona squamosa; Bacillus thuringiensis*; *Beauveria bassiana; Metarhizium anisopliae;* Controle biológico; Seletividade.

**Resumen**
El control biológico es una rama en auge en Brasil, y con él viene la necesidad de obtener enemigos eficaces para controlar las plagas. El uso de entomopatógenos se ha ampliado y con él llega la necesidad de investigar su selectividad para los organismos no objetivo. El presente trabajo se realizó en huertos de piñas (*Annona squamosa* L.) con el objetivo de evaluar en qué lugares de la planta (tercio inferior, medio o superior) y los efectos de los entomopatógenos: *Beauveria bassiana, Metharhizium anisoplie* y *Bacillus thuringiensis* sobre el ácaro depredador. Se realizaron 16 aplicaciones semanales entre marzo y agosto de 2017 utilizando un pulverizador de mochila a presión constante, boquilla tipo JA-2 con chorro dirigido a toda la planta. Los tratamientos consistieron en el uso de tres productos biológicos entomopatógenos: Dipel, *Beuaveria bassiana* JCO y *Metarhizium anisopliae* JCO aplicados de forma aislada y combinada. Se llevaron a cabo ocho tratamientos, como se indica a continuación: T1-Pulverización con *M. anisopliae* + phfos (Chemifol) + esparcidor adhesivo (Agral), T2-Pulverización con *B. bassiana* + phfos (Chemifol) + esparcidor adhesivo (Agral), T3-Pulverización con *B. thruringiensis* + phfos (Chemifol) + esparcidor adhesivo (Agral), T4-Pulverización con *M. anisopliae* + *B. basisiana* + phfos (Chemifol) + esparcidor adhesivo (Agral), T5-Pulverización con *M. anisopliae* + *B. thruringiensis* + phfos (Chemifol) + esparcidor adhesivo (Agral), T6-Pulverización con *B. bassiana* + *B. thruringiensis* + phfos (Chemifol) + esparcidor adhesivo (Agral), T7-Pulverización con *M. anisopliae* + *B. bassiana* + *B. thruringiensis* + phfos (Chemifol) + esparcidor adhesivo (Agral) y T8-Testigo, pulverización con phfos (Chemifol) + esparcidor adhesivo (Agral). Los resultados indicaron una preferencia del ácaro *Neoseiulus* sp. por el tercio medio de la cubierta vegetal. Los resultados obtenidos indicaron que los entomopatógenos influyeron en la población del ácaro *Neoseiulus* sp. hasta la sexta semana después del tratamiento, donde la combinación de *Metarhizium anisopliae* + *Beauveria bassiana* + *Bacillus thuringiensis* fue la que más afectó negativamente a la población del ácaro depredador *Neoseiulus* sp.
**Palabras clave:** *Annona squamosa; Bacillus thuringiensis; Beauveria bassiana; Metarhizium anisopliae;* Control biológico; Selectividad.

## 1. Introduction

According to Embrapa, in 2005, Brazil exported US$440 million in fresh fruits, against US$73 million in 1995, showing the growth capacity of these crops. In the world ranking, Brazil is the third largest producer, representing 5.4% of all fruits produced worldwide. In the field of fruit trees, the Anonaceae are fruits of the genus Annona, which include the pinecones, condessas, soursop and atemoias, occupying about 10,500ha (PEREIRA, 2011) of the Brazilian territory. Rich in vitamin C and with medicinal effects (EMBRAPA, 2000), the pinecone, also known as custard fruit (*Annona squamosa L.*), in terms of production by state, Bahia is the largest producer. According to CGEA/IBGE 2013, the municipality of Presidente Dutra - BA, has around 2,500 ha of pinecones, with an annual production of 21,250 tons in 2013.

However, one of the greatest risks to the productivity of pinecones is represented by the attack of pests, some of which are the twig borer or sawfly (*Oncideres dejeani, Oncideres saga*), the fruit borer (*Cerconota anonella*), the stem borer

(*Heilipus velamen*), the white mealybug or white louse (*Planococcus citri*) and the red mite (*Tenuipalpus granati).* Due to the losses caused by the attack of these pests, it is necessary to observe the population fluctuation in the field, in order to recognize at what level it will be necessary to control them in the orchard. It is also necessary to use efficient control methods that are economically, socially and environmentally sustainable.

When it comes to pest control, for many years the use of chemical molecules (chemical control) was seen as the only viable way to avoid losses from these attacks. However, in addition to damaging the environment, some species have developed resistance to these products and thus saw the need to seek new alternatives. Biological control is based on the use of natural enemies to control pests, avoiding the use of synthetic products and generating greater environmental balance. This control method is expanding, where the use of agricultural biodefensives has a potential annual growth of 20%, according to the Brazilian Association of Biological Control Companies (ABCBio).

One of the biological control agents are entomopathogens, usually fungi, bacteria and nematodes with effects on reducing the population of pest insects. Some entomopathogenic organisms are *Metarhizium anisopliae, Beauveria bassiana*, and *Bacillus thuringiensis,* species that have already been used successfully to suppress various pests in different crops. *Bacillus thuringiensis* is a bacterium with toxicity to insects of the orders Hemiptera and Orthoptera and mites, while *Metarhizium anisopliae* is associated with the control of more than three hundred pests such as *Cleonus punctiventris* and *Anisoplia astríaca*.

The use of these organisms is directly linked to its selectivity to target organisms in the field, research on the selectivity of entomopathogens to beneficial entomofauna is essential for the correct use of entomopathogenic organisms in the control of pest mites (Alves et al., 2002; Oliveira et al., 2002; Shi; Feng, 2004). However, there are few studies on the effects of the use of these species on non-target organisms, as in the case of natural enemies of other pests or pollinating insects.

Mites are not insects and are characterized by being cell content sucking organisms, belonging to the phylum Arthropoda and class Arachnida. They are diverse organisms with species that are predators, phytophages, mycophages, scavengers, forbivores, saprophytes, coprophages and parasites (Hickman et al., 2003; Moraes, Flectman, 2008; Evans, 1992; Damasceno, 2008). Mites of the family Phytoseidae have been widely used in Integrated Pest Management Programs for the control of phytophagous mites in the aerial part of several plants.

The genus Neoseiulus, mites belonging to the family Phytoseidae, is composed of predatory mites of phytophagous mites that are pests in several fruit crops, including the pinecone. One characteristic that makes mites of the genus mentioned is their ability to survive even with scarcity or extinction of phytophagous mites, since they can also feed on fungi, pollen, sweetish secretions and some insects. *Neoseiulus californicus, Phytoseiulus persimilis* and *Phytoseiulus macropilis* are among the phytoseiid species most used in biological control in some fruit crops and also ornamentals (Sato et al., 2006; Monteiro, 2002).

Thus, the objective of this work was to evaluate the effects of using products based on the bacteria *Bacillus thuringiensis* and the fungi *Beauveria bassiana* and *Metarhizium anisopliae*, alone and in mixtures, on the non-target organism, mites of the genus Neoseiulus in pine trees (*Annona squamosa L.)*.

## 2. Methodology

The experimental trial was implanted and conducted in the village of Palmeiras, Baixa das Mangueiras I farm, municipality of Central, State of Bahia. Located at 11º 09' 21.9" South latitude; 42º 02' 05.6" West longitude, at an altitude of 682m.

The average monthly temperatures between March and August 2017 according to the station of (INMET, 2022) in Irecê, twenty-four kilometers from the experimental trial site were: March 26.6ºC, April 24.6ºC, May 23.8ºC, June 22.5ºC, July

20.2ºC and August 22.0ºC. In the selected orchard high technological level applies, the spacing used was 4 m x 3 m, and the irrigation system is by drip with six year old plants with good vegetative aspect.

The experimental design was in randomized blocks with eight treatments and four repetitions, with nine plants, considering the central plant useful for evaluation purposes, totaling 32 plots, including the controls, in a total of 288 plants. The treatments consisted in the use of three entomopathogenic biological products: *Bacillus thuringiensis*, *Beuaveria bassiana e Metarhizium anisopliae* applied alone and in combination. The respective doses (g of i.a/20L of water) were: *Metarhizium anisopliae* (*Metarhizium anisopliae* JCO): 0,11(T1); *Beauveria bassiana* (*Beauveria bassiana* JCO) 0,24 (T2); *Bacillus thuringiensis* (Dipel SC) 0,23 (T3); *M.anisopliae* + *B.bassiana* (JCO) 0,11+0,24(T4); *M.anisopliae* + *B.thuringiensis* (JCO e Dipel SC) 0,11+0,23 (T5); *B.bassiana* + *B. thuringiensis* (JCO e Dipel SC) 0,24+0,23 (T6); *M.anisopliae* + *B.bassiana* + *B.thuringiensis* (JCO, JCO, DipelSC) 0,11+0,24+0,23 (T7); Testify (T8). An application water pH corrector (phfos) and an adhesive spreader were applied to all treatments.

The first application was performed on 04/19/2017 and the first count one week later. The morning period was determined for the applications, but due to the large number of treatments, there were significant variations in temperature and humidity during the applications. From the period between the first count on 04/26/2017 until 05/31/2017, the final collection for sampling, mites were collected, and stored in alcohol, in all treatments, which formed the sample sent to the Agronomy laboratory for identification and confirmation.

Mite counts were performed on the upper, middle and lower thirds of the plant. The mite population of the genus Neoseiulus was counted on the entire leaf lamina sampled. Twenty-four leaves were removed and counted from the useful plant, one at a time, and carefully analyzed on site, with a magnifying glass of ten magnifications being 6 leaves to the north, 6 to the south, 6 to the east and 6 to the west of the useful pine tree, and of each six leaves evaluated, 2 were in the upper third of the plant, 2 in the middle third and 2 in the lower third, after this count the leaves were discarded (Oliveira et al., 1982; Cruz et al., 2012). Evaluations were weekly and totaled sixteen counts between April and August 2017. Fertilization was done with 200 grams per plant of a 150-kilogram mixture of the NPK formula (04-30-10). Pollination was performed artificially with firecracker and brush.

The mite population count data were subjected to the square root transformation of (X+1) and the normality and homogeneity of variance tests. Afterwards, variance analysis was performed using the "F" test, at 5% probability level. If significant, the means of the treatments were compared using the Tukey test, at a 5% probability level. For the statistical procedures, the Sisvar program (Ferreira, 2011) was used.

## 3. Results and Discussion

The effect of applying the bioinsecticides was most prominent in the first six weeks after spraying (SAP). From the seventh week, with the exception of the tenth week, no effect of the treatment with entomopathogenic agents was observed. The mite population on pine cone plants varied between the thirds of the plant in the period between weeks three and 13 SAP, with the exception of the fourth and eighth SAP. No interaction was observed between the factors spraying and third of the plant, in this variable (Table 1). The comparison of the averages of the number of mites in the pine trees sprayed with different bioinsecticides and combinations, in each of the evaluation periods, were presented in (Table 2).

In the first, third and fourth evaluation periods, the treatment with the combination of three entomopathogenic agents (*Metarhizium anisopliae* + *Beuaveria bassiana* + *Bacillus thuringiensis*) resulted in fewer mites when compared to the control treatment. However, the other combinations or bioinsecticides applied alone did not alter the mite population. The exception was *Metarhizium anisopliae* in the fourth period.

**Table 1 -** Summary of the analysis of variance and coefficients of variation (CV) of the count of the number of predatory mites in *Annona squamosa L.* plants submitted to the application of different entomopathogenic biological agents (P), in the different thirds of the plant (T), evaluated from the first to the sixteenth week (1 - 16) after the application. Central - BA, 2017.

| | | **MEAN SQUARES** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **FV** | **GL** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| P | 7 | 0,15$^{**}$ | 0,08$^{ns}$ | 0,24$^{**}$ | 3,00$^{**}$ | 2,03$^{**}$ | 1,48$^{**}$ | 0,35$^{ns}$ | 0,13$^{ns}$ |
| T | 2 | 0,01$^{ns}$ | 0,26$^{ns}$ | 0,60$^{**}$ | 0,24$^{ns}$ | 3,62$^{**}$ | 2,82$^{**}$ | 2,17$^{**}$ | 0,14$^{ns}$ |
| P*T | 14 | 0,07$^{ns}$ | 0,08$^{ns}$ | 0,05$^{ns}$ | 0,19$^{ns}$ | 0,30$^{ns}$ | 0,19$^{ns}$ | 0,20$^{ns}$ | 0,37$^{ns}$ |
| BL | 3 | 0,06$^{ns}$ | 0,24$^{*}$ | 0,05$^{ns}$ | 0,11$^{ns}$ | 0,41$^{ns}$ | 0,98$^{*}$ | 0,41$^{ns}$ | 0,45$^{ns}$ |
| Res | 69 | 0,05 | 0,09 | 0,08 | 0,49 | 0,36 | 0,27 | 0,21 | 0,30 |
| CV (%) | 19,37 | | 22,19 | 21,59 | 32,47 | 18,08 | 18,92 | 18,11 | 25,27 |
| | MEAN SQUARES | | | | | | | | |
| FV | GL | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| P | 7 | 0,03$^{ns}$ | 0,27$^{*}$ | 0,18$^{ns}$ | 0,07$^{ns}$ | 0,15$^{ns}$ | 0,07$^{ns}$ | 0,05$^{ns}$ | 0,09$^{ns}$ |
| T | 2 | 2,20$^{**}$ | 1,18$^{**}$ | 1,80$^{**}$ | 0,98$^{**}$ | 0,52$^{*}$ | 0,45$^{ns}$ | 0,06$^{ns}$ | 0,02$^{ns}$ |
| P*T | 14 | 0,18$^{ns}$ | 0,10$^{ns}$ | 0,09$^{ns}$ | 0,07$^{ns}$ | 0,12$^{ns}$ | 0,06$^{ns}$ | 0,06$^{ns}$ | 0,05$^{ns}$ |
| BL | 3 | 0,68$^{*}$ | 0,14$^{ns}$ | 0,11$^{ns}$ | 0,08$^{ns}$ | 0,18$^{ns}$ | 0,08$^{ns}$ | 0,06$^{ns}$ | 0,04$^{ns}$ |
| Res | 69 | 0,19 | 0,12 | 0,16 | 0,15 | 0,13 | 0,14 | 0,12 | 0,09 |
| CV (%) | 22,33 | | 20,30 | 22,37 | 24,32 | 22,32 | 26,54 | 26,55 | 23,87 |

$^{ns}$, $^{*}$ e $^{**}$: not significant, significant by the "F" test at 5% and 1% probability, respectively. Source: Authors.

This result is in agreement with Parra (2006) for whom entomopathogenic fungi play important roles in the natural regulation of insects and many species of mites. And he states that they can develop severe epizootics that lead to a rapid decline in host populations. The result agrees with Gravina et al. (2014) who found that the mixture of the fungi Beauveria bassiana and Metarhizium anisopliae reached nineteen percent mortality of the natural enemy, Neoseiulus californicus.

In the fifth week after spraying, the isolated treatment with Metarhizium anisopliae and the combination of the three insecticides showed lower mean numbers of mites compared to the control. However, the control did not differ from the other treatments evaluated. This result is in agreement with Tamai et al. (2002), in a study using *Metarhizium anisopliae* to control the mite *Tetranychus urticae*, found that 8 isolates (80%) presented values greater than 80% of corrected mortality on the fifth day, and 4 isolates presented mortalities greater than 90%.

**Table 2 -** Number of predatory mites on *Annona squamosa L.* plants submitted to the application of different entomopathogenic biological agents and combinations, evaluated in the period between the first and the sixteenth week (1 - 16) after application. Central - BA, 2017.

| | SPRAYING | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Trial period | *M. anisopliae* (*Ma*) | *B. bassiana* (*Bb*) | *B. thuringiensis* (*Bt*) | *Ma + Bb* | *Bb + Bt* | *Ma + Bt* | *Ma + Bb + Bt* | Testify |
| 1 | 1,17ab | 1,17ab | 1,07ab | 1,30a | 1,20ab | 1,07ab | 1,00b | 1,32a |
| 2 | 1,23a | 1,27a | 1,40a | 1,44a | 1,34a | 1,35a | 1,23a | 1,23a |
| 3 | 1,21ab | 1,24ab | 1,36ab | 1,46ab | 1,23ab | 1,19ab | 1,13b | 1,54a |
| 4 | 1,60b | 2,32ab | 2,74a | 1,57b | 2,63a | 1,70b | 2,00ab | 2,67a |
| 5 | 2,63b | 3,28ab | 3,36ab | 3,86a | 3,20ab | 3,35ab | 2,99b | 3,85a |
| 6 | 2,93ab | 2,61b | 2,67b | 2,81b | 2,50b | 2,56b | 2,35b | 3,49a |
| 7 | 2,39a | 2,52a | 2,63a | 2,43a | 2,62a | 2,43a | 2,41a | 2,89a |
| 8 | 2,06a | 2,08a | 2,26a | 2,17a | 2,32a | 2,08a | 2,15a | 2,31a |
| 9 | 1,97a | 1,95a | 2,04a | 1,94a | 1,92a | 1,88a | 2,00a | 1,94a |
| 10 | 1,55b | 1,52b | 1,71ab | 1,69ab | 1,69ab | 1,70ab | 1,72ab | 2,02a |
| 11 | 1,58a | 1,71a | 1,88a | 1,76a | 1,90a | 1,68a | 1,78a | 1,93a |
| 12 | 1,54a | 1,54a | 1,55a | 1,63a | 1,72a | 1,60a | 1,53a | 1,70a |
| 13 | 1,49a | 1,54a | 1,50a | 1,72a | 1,50a | 1,70a | 1,59a | 1,77a |
| 14 | 1,44a | 1,52a | 1,46a | 1,49a | 1,35a | 1,30a | 1,40a | 1,46a |
| 15 | 1,30a | 1,31a | 1,25a | 1,37a | 1,29a | 1,33a | 1,27a | 1,44a |
| 16 | 1,23a | 1,30a | 1,30a | 1,26a | 1,23a | 1,20a | 1,35a | 1,47a |

Means followed by the same letter, in each evaluation period, do not differ by the Tukey test, at 5% probability. Source: Authors.

All entomopathogenic agents and combinations between them, with the exception of spraying with *Metarhizium anisopliae*, reduced the non-target mite population on pine cone plants evaluated at the sixth week after treatment. The effect of several chemical and biological products on the predatory mite *Neoseiulus californicus* was analyzed by Castagnoli et al. (2005). The authors found that *Beauveria bassiana* caused mortality of 60% of the predator's eggs, which can be defined as non-selective.

In the tenth evaluation period, it was observed that the isolated treatments with *Metarhizium anisopliae* and *Beauveria bassiana* reduced the number of non-target mites on pinecone plants, compared to untreated pine trees. The other treatments did not differ. After the tenth evaluation period mentioned above, there was no significant effect of spraying with entomopathogenic agents on the predatory mite population. These results corroborate those of Sato et al. (n. t. s.) that verified in several strains (strains) of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, which also proved selective to predatory mites.

The results of the comparison of the number of mites in the different thirds of the pine trees in each of the evaluation periods are presented in Table 3. In the period between the third and thirteenth week after spraying with bioinsecticides, the population of predatory mites was higher in the middle third of the pine trees when compared to the upper and lower thirds. Except for the fourth and eighth evaluation periods, in which the number of mites did not vary between the thirds of the plant.

**Table 3 -** Number of predatory mites in different thirds of *Annona squamosa L.* plants, evaluated in the period between the first and sixteenth week (1 - 16) after the application of entomopathogenic biological agents. Central - BA, 2017.

| Trial period | THIRD OF THE PLANT | | |
|---|---|---|---|
| | UPPER | MIDDLE | LOWER |
| 1 | 1,18a | 1,15a | 1,15a |
| 2 | 1,33a | 1,39a | 1,21a |
| 3 | 1,25b | 1,45a | 1,19b |
| 4 | 2,05a | 2,20a | 2,21a |
| 5 | 3,21b | 3,69a | 3,04b |
| 6 | 2,56b | 3,08a | 2,58b |
| 7 | 2,43b | 2,84a | 2,36b |
| 8 | 2,12a | 2,25a | 2,16a |
| 9 | 1,83b | 2,26a | 1,78b |
| 10 | 1,60b | 1,92a | 1,58b |
| 11 | 1,61b | 2,05a | 1,66b |
| 12 | 1,51b | 1,80a | 1,49b |
| 13 | 1,70a | 1,65ab | 1,46b |
| 14 | 1,43a | 1,56a | 1,31a |
| 15 | 1,37a | 1,30a | 1,29a |
| 16 | 1,28a | 1,32a | 1,28a |

Means followed by the same letter, in each evaluation period, do not differ by the Tukey test, at 5% probability. Source: Authors.

After the 13 weeks of treatment with bioinsecticides, the predatory mite population was higher in the upper third of the pine cone plants compared to the lower third, but did not differ from the middle third.

## 4. Conclusion

The entomopathogens affected the population of the *Neoseiulus* sp. mite until the sixth week after application. From the seventh week on, the applications with entomopathogens did not affect the population of *Neoseiulus* sp. The treatment that combined *Metarhizium anisopliae* + *Beauveria bassiana* + *Bacillus thuringiensis* was the one that most affected the mite population. The mite population is concentrated in the middle third of the pine tree.

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