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BIOLOGICAL CONTROL OF *Tetranychus urticae* KOCH (ACARI: TETRANYCHIDAE) WITH THE FUNGUS *Beauveria bassiana* (BALS.) VUILL., ON GRAPEYARD

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ABSTRACT

The spider mite (*Tetranychus urticae*), causes considerable damage in a large number of crops in Brazil. In the grape, the species causes serious damage to the leaves, with necrosis and premature fall, damaging the photosynthesis of the plant and consequently the production and quality of the fruits. The control of the spider mite is carried out predominantly in a chemical way, whose environmental impact can be significant. The incorporation of biological methods would reduce the legal, environmental and public risks of the use of chemical products, mainly for the *in natura* ones. In order to provide an alternative strategy to control *T. urticae* to grapeyard in the northwest region of São Paulo, the study aimed to evaluate the viability of biological control through the use of the pathogenic fungus *Beauveria bassiana* on the local climatic conditions under different dosages. They were sprayed in a vineyard area of the BRS-Vitória variety in Palmeira d'Oeste/SP, four combinations of products based on *B. bassiana*, with and without mineral oil, in addition to a treatment containing only mineral oil, which were compared with the control in relation to the effect on eggs and adults of the spider mite and also its effects on their mortality. The application of *B. bassiana* did not significantly reduce the population of eggs and adults of *T. urticae*, even with the addition of mineral oil. However, it had a significant influence on the mortality of mites. Longer evaluation periods could provide better efficiency results.

Keywords: Spider mite, entomopathogenic fungus, microbial control

CONTROLE BIOLÓGICO DE *Tetranychus urticae* KOCH (ACARI: TETRANYCHIDAE) COM O FUNGO *Beauveria bassiana* (BALS.) VUILL., EM VIDEIRA

RESUMO

O ácaro-rajado (*Tetranychus urticae*), considerado um dos ácaros de maior importância econômica em todo o mundo, causa consideráveis prejuízos em um grande número de culturas no Brasil. Na uva, a espécie ocasiona sérios danos às folhas, com necrose e queda prematura das mesmas, prejudicando a fotossíntese da planta e consequentemente a produção e qualidade dos frutos. O controle do ácaro-rajado é realizado predominantemente de maneira química, cujo impacto ambiental pode ser significativo. A incorporação de métodos biológicos reduziria os riscos legais, ambientais e públicos do uso de produtos químicos, principalmente para o consumo in natura da uva. De maneira a fornecer uma estratégia alternativa de controle do ácaro-rajado aos produtores de uva na região do Noroeste Paulista, o trabalho objetivou avaliar a viabilidade do controle biológico, através da utilização do fungo patogênico *B. bassiana* nas condições climáticas locais, sob diferentes dosagens. Foram pulverizadas em área de parreiral da variedade BRS-Vitória em Palmeira d'Oeste/SP, quatro combinações de produtos à base de *B. bassiana*, com e sem óleo mineral, além de um tratamento contendo apenas óleo mineral, que foram comparados com a testemunha em relação ao efeito sobre ovos e adultos do ácaro rajado e também seus efeitos sobre a mortalidade dos mesmos. The application of *B. bassiana* did not significantly reduce the population of eggs and adults of *T. urticae*, even with the addition of mineral oil. However, it had a significant influence on the mortality of mites. Maior período de aplicações e avaliações poderia evidenciar maior ação do fungo *B. bassiana* no manejo do ácaro-rajado na videira, nas condições climáticas do Noroeste Paulista.

Palavras-chave: Ácaro-rajado, fungo entomopatogênico, controle microbiano

INTRODUCTION

The grapeyard is of great importance worldwide due to the organoleptic characteristics of its fruits, which can be consumed *in natura*, or in the form of juices, jellies, raisins and wine (UNWIN, 2005).

Introduced in Brazil in 1532, the grape seed has grown in importance and is now produced in several states of the South, Southeast and Northeast (CAMARGO et al., 2011; UNWIN, 2005).

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In São Paulo state, which currently has the third national position in production, table grapes for fresh consumption are the most cultivated and have been increasing in importance due to the obtaining cultivars with characteristics aimed at the consumers taste (CAMARGO et al., 2011; MAIA, 2018). Within the state, the Northwest region has a significant increase in planted area year by year, thanks to the genetic improvement that enabled the development of several cultivars adapted to the climate of the region (TONDATO et al., 2010).

The climatic limitation of the Northwest region of the state of São Paulo, characterized by a dry winter and mild temperatures, and a hot, rainy summer, contribute significantly for the occurrence of numerous seasonal pests and diseases, where the diseases present particular importance in the rainy season and the spider mite (*Tetranychus urticae*) is important in the dry period of the year (COSTA et al., 2012).

Tetranychus urticae, considered one of the most economic importance worldwide, causes considerable damage in different cultures in Brazil, including cotton, papaya, strawberry, peach, grape, beans, soy, chrysanthemum, chili and tomato (ZHANG, 2003). In the grape, the species causes serious damage to the leaves, necrosis and premature fall, impairing the photosynthesis of the plant and consequently the production and quality of the fruits. Direct damage to fruits is also observed (BOTTON et al., 2004; VALADÃO et al., 2012).

The control of the spider mite is carried out predominantly in a chemical way, with the use of contact and ingestion acaricide based on abamectin, whose environmental impact can be significant. However, other problems have been occurring due to the recurrent use this type of control, such as the resurgence of secondary pests and, mainly, the development of mite populations resistant to the products used (STUMPF et al., 2001).

The incorporation of biological methods as part of an integrated program to *T. urticae* would reduce the legal, environmental and public risks of using chemicals, mainly for fresh consumption of grapes (JARONSKI, 2010). A promising way biological control systems that have been tested for countless pests, in different cultures, is the use of entomopathogenic fungi, with emphasis on *Beauveria bassiana*, which colonizes a wide variety of hosts and has a wide geographical distribution (KHERADMAND et al., 2021). However, it is necessary that there is a high potential for inoculums in the area, obtained through flooding applications in the field and favorable climatic conditions colonization (JARONSKI, 2010; FARGUES & LUZ, 2011).

In order to provide an alternative strategy for the control of *T. urticae* to the grapeyard producers in the Northwest region of São Paulo, the work aimed at evaluating the viability of biological control, through the use of the pathogenic fungus *B. bassiana* on the local climatic conditions, under different dosages. The idea of thinking only about increasing production is changing. Currently, the ecological cost of any agricultural technique is considered. Awareness of the adversities caused by the abusive use of pesticides, for example, is generating in consumers a search for organic food, which demands studies on alternative methods, including biological control.

MATERIAL AND METHODS

The experiment was carried out at Sitio São José, Córrego do Sucuri, Palmeira D'oeste, São Paulo State, Brazil (20º24'59"S and 50º45'43" W), which presents the AW climate according to Koppen classification (tropical with defined dry season, between the months of May and August). The region has an average annual rainfall of 1185 mm, an average temperature of 22.4 ° C and altitude of 429 meters. The studies were conducted in grape seeds (variety BRS-Vitória), aged five years, with a spacing of 2.5 meters between plants and 5.0 meters between lines, conducted in a "trellis" system.

The experimental design used was randomized blocks, with six treatments and four repetitions, each plot consisting of 2.4 m wide by 9.0 m long, making up 21.6 m^2. The treatments tested in this study were:

- Treatment 1: Witness (water only);
- Treatment 2: *B. bassiana** (1200 g / ha);
- Treatment 3: *B. bassiana* (2400 g / ha);
- Treatment 4: *B. bassiana* (1200 g / ha) + 3000 mL of mineral oil (*Assist*®) **;
- Treatment 5: *B. bassiana* (2400 g / ha) + 3000 mL of mineral oil (*Assist*®);
- Treatment 6: Mineral oil (*Assist*®).

* *Beauveria bassiana*: (*Ecobass*®): Concentration 1 x 109 conidia/ml of the IBCB 66 isolated strain of *B. bassiana* (11 g/kg). ** Mineral oil (*Assist*®): 756 g/L, chemical group Aliphatic hydrocarbons, adjuvant, insecticide and contact acaricide.

The installation of the blocks inside the vineyard was carried out respecting a border of 9 m towards the interior of the area. Each block was made up of 2.4 m wide by 54 m long, with 9 m for each plot (Table 1). The plots were delimited with stakes, and the treatments positioned at random within each block.

Table 1. Positioning of treatments in the experimental area. São José Farm, Palmeira d'Oeste, São Paulo State, 2018.

Treatments			
R1	R2	R3	R4
5A	4B	6C	3D
2A	1B	4C	5D
3A	2B	5C	6D
6A	5B	1C	2D
1A	6B	3C	4D
4A	3B	2C	1D

Considering the infestation of the spider mite in the experimental area, detected in previous sampling, the applications of the products, according to the respective treatments, were carried out in the afternoon, with a maximum interval of one hour between the first and last application. Three applications of treatments were carried out every three or four days, between the months of May and June 2018 (May/17, May/20, May/24, May/27, May/31 and June/03), which coincide with the time of greatest occurrence of the spider mite in the region.

The application was performed with a CO2 pressurized sprayer, with a 2.0 m spray bar, containing six empty cone spray tips (Magno®), 60 ° angle, spaced every 0.40 m, with equivalent flow 600 liters flow rate per hectare. The applications followed the sequence of treatments in the blocks (Table 1), starting with the control and the sequential increase in doses.

To evaluate the efficiency of each treatment on the population of *T. urticae,* five leaves were collected randomly per experimental unit, in the morning time. The collections were performed weekly, and occurred during the application and up to two weeks after the end of the application (Table 2).

The leaves collected from each plot were properly stored in a paper bag, identified and stored in a refrigerator for a maximum of three hours. Therefore, the spider mites (eggs, nymphs and adults - living and dead) were counted on the same day of each collection.

Table 2. Sequence of leaf collections and applications of treatments in the experimental area. São José Farm, Palmeira d'Oeste, São Paulo State, 2018.

Leaf collection number	Day and time of leaf collection		Day and time of application of treatments	
	Day	Time	Day	Time
1°	May/18	07:00-10:00	May/17	16:30-17:30
	-	-	May/20	16:30-17:30
2°	May/25	07:00-10:00	May/24	16:30-17:30
	-	-	May/27	16:30-17:30
3°	June/01	07:00-10:00	May/31	16:30-17:30
4°	June/08	07:00-10:00	-	-
5°	June/16	07:00-10:00	-	-

The counts were performed at the Laboratory of Zoology and Botany, University Center of Santa Fé do Sul (UNIFUNEC), Campus II in Santa Fé do Sul, State of São Paulo, Brazil with a stereoscope microscope, 20x magnification, in a quadrant of 4x4cm, fixed in the central region of the leaf. The data obtained were tabulated for further statistical analysis.

The data collected and tabulated were analyzed with the Sisvar® software, to verify differences between treatments and the control, through Analysis of Variance and later Tukey's Test, to separate the averages, at the level of 1% to 5%.

RESULTS AND DISCUSSION

In relation to the egg phase of *T. urticae*, no statistical differences were found between the treatments used in all evaluations. However, on June 15, the date of the last analysis, the treatments with *B. bassiana* at the lowest dose, with or without oil, had significantly positive results in reducing the number of eggs in the leaf, as well as pure mineral oil (Table 3). With that, it is evident that the application inoculation of the biological agent may be viable at the lowest dose tested, also aiming at economic viability of the biological management of the streaked mite on the grapevine.

Table 3. Average number of eggs of *Tetranychus urticae* in 16 cm^2 of vine leaves submitted to applications of *Beauveria bassiana*, without or with the addition of mineral oil. São José Farm, Palmeira d'Oeste, São Paulo State, 2018.

Treatments	Dose g i.a. ha^{-1}	Evaluation date					Total average
		May/17	May/24	May/31	June/07	June/15	
Control	----	158,50	130,25	35,75	66,50	89,00b	96,00
B. bassiana	1200	142,25	57,75	50,25	21,50	25,00a	59,35
B. bassiana	2400	124,37	91,75	36,00	43,00	50,00ab	69,03
B. bassiana + Mineral oil	1200 + 3L	125,37	73,00	47,75	56,75	35,00a	67,57
B. bassiana + Mineral oil	2400 + 3L	171,37	68,75	28,25	61,25	35,75ab	73,08
Mineral Oil	3L	161,37	111,50	23,75	29,00	31,25a	71,38
Total Average		147,21	88,83	36,95	46,33	44,33	72,73
CV (%)		35,55	63,81	91,98	83,31	52,59	24,13
F Calculated							$1,98^{ns}$

Means followed by the same letter in each column did not statistically differ between each other by Tukey Test at 5% of probability.

For live mites, no statistical differences were found in any of the treatments used on any of the evaluation dates (Table 4). That is, the treatments used did not significantly reduce the amount of live mites when compared with to control treatments.

However, when the same count was performed with dead mites, differences significant between treatments were observed, both in the evaluations of May/31/2018, of June/15/2018 and for the general average (Table 4). The leaves taken from the areas where there was application with *B. bassiana* (both at the dose of 1200 g c.p./ha and 2400 g c.p./ha), presented significantly greater amount of dead mites when compared to the other 12 treatments. The smallest amounts of dead mites were observed in the treatment with mineral oil and control, when compared to general averages.

When we compare the general averages of treatments, considering every day of evaluation, it was only for dead mites that significant differences were found (table 5). It is important to note that, the count of dead mites only started from the third evaluation date, indicating a probable cumulative effect of the biological product used.

Table 4. Average number of live mites of *Tetranychus urticae* in 16 cm2 of vine leaves submitted to applications of *Beauveria bassiana*, with or without the addition of mineral oil. São José Farm, Palmeira d'Oeste, São Paulo State, 2018.

Treatments	Dose g i.a. ha^{-1}	Evaluation date May/17	May/24	May/31	June/07	June/15	Total average
Testemunha	----	91,37	145,75	60,50	183,25	77,50	111,68
B. bassiana	1200	113,87	91,75	46,00	49,00	64,25	72,97
B. bassiana	2400	117,62	121,75	44,75	44,75	55,00	76,78
B. bassiana + Mineral oil	1200 + 3L	129,00	80,25	55,75	62,50	48,00	75,10
B. bassiana + Mineral oil	2400 + 3L	160,00	75,25	48,50	74,50	48,25	81,30
Mineral Oil	3L	129,37	110,50	27,75	36,50	39,00	68,62
Total Average		123,54	104,21	47,21	75,08	55,33	81,07
CV (%)		45,43	49,18	44,56	117,39	35,53	32,20
F Calculated							1,42 ns

Means followed by the same letter in each column did not statistically differ between each other by Tukey Test at 5% of probability.

Table 5. Average number of mites (eggs, adults and dead) of *Tetranychus urticae* found in 16 cm2 of vine leaves submitted to applications of *Beauveria bassiana*, with or without addition of mineral oil. São José Farm, Palmeira d'Oeste, São Paulo State, 2018.

Treatments	Dose g i.a. ha^{-1}	Evaluation date May/31/2018	June/07/2018	June/15/2018	Total average
Testemunha	----	16,75ab	7,50ns	3,25a	9,17b
B. bassiana	1200	19,50ab	18,75ns	22,75b	20,34a
B. bassiana	2400	24,25a	14,75ns	21,50ab	20,08a
B. bassiana + Mineral oil	1200 + 3L	9,75b	13,75ns	24,50b	16,00ab
B. bassiana Mineral oil	2400 + 3L	11,50ab	15,00ns	22,50b	16,34ab
Mineral Oil	3L	11,50ab	9,25ns	6,00ab	8,92b
Total average		15,54	13,16	16,71	15,14
CV (%)		38,68	59,47	48,23	28,80
F Calculated					5,39 **

Means followed by the same letter in each column did not statistically differ between each other by Tukey Test at 5% of probability.

For all fungal strains observed there was a low pathogenicity of the isolates of *B. bassiana*, both in the immobile and mobile phases of *T. urticae*, except in the mortality. However, the results obtained indicated that the need for a minimum period for the *B. bassiana* fungus presents significant results on the mortality of pest mites.

Despite fungal insecticides, such as the case of *B. bassiana*, present an action of contact, it takes about 15 days for target arthropod mortality to occur. CHETAN et al. (2013) comments that *B. bassiana* infection is established in about 24-48 hours, but that the process that occurs between the application of the product until the death of the arthropod occurs between 5-12 days.

According to Harith-Fadzilah (2021), *B. bassiana* conidia germinate on the host's surface, penetrating its integument and colonizing internally acari body. During the infection process, occurs the release of toxins that lead to death plague, about 6-12 days after application. These data correspond to the period of the experiment necessary for the mortality rates observed on May/31/2018, 14 days after the start of assessments.

The significant mortality of *T. urticae* found in some treatments with *B. bassiana*, correspond to the reduction in the amount of eggs found in the leaves. When mortalities started to be observed, then there was also a reduction in the amount of eggs present in the leaves. This matches the species biology, which takes about 14 days to complete its life cycle, according to SHIH et al. (1976). A reduction in the number of adults consequently leads to a reduction in egg laying.

Although positive results have been observed, it is clear that a longer period applications and evaluation could provide more consistent data. This factor needs to be carefully observed before considering the possibility of using *B.bassiana* for the control of *T. urticae* on grapevine, under the conditions where the experiment was conducted, since the population of pathogenic fungus are greatly affected by factors of climate change. (OLIVEIRA et al., 2016).

According to Jaronski (2010), *B. bassiana* shows maximum development in temperatures from 20 ° to 30 ° C and high relative humidity. However, these conditions do not correspond to the phases of highest occurrence of *T. urticae*, which has its largest populations in the driest and mildest times of the year.

Considering all these factors, more experiments are needed, but apparently there is a real possibility of using the *B. bassiana* fungus as an agent of control of *T. urticae*, in the Northwest region of São Paulo, which would be highly interesting from an ecological and social point of view.

CONCLUSIONS

The application of *B. bassiana*, alone or in association with mineral oil, does not cause a decrease in the population of eggs and adults of the spotted mite, however, it had an effect significant impact on mite mortality in the field.

A longer period of applications and evaluations could evidence greater action of the fungus *B. bassiana* in the management of the spider mite (*T. urticae*) on the vine, in the climatic conditions of the Northwest region of São Paulo.

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