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Annals of Biological Research, 2012, 3 (11):5028-5033
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Toxicity of the pyrethroid deltamethrin on the entomopathogenic fungus *Metarhizium anisopliae* var. *anisopliae* (Metsch) Sorokin assessed by germination speed parameter

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ABSTRACT

The objective of this study was to evaluate the toxicity of the pyrethroid deltamethrin on the entomopathogenic fungus Metarhizium anisopliae var. anisopliae, considering the conidia germination speed as parameter. Suspended conidia in a concentration of 5.42 x10^7 conidia/ml were treated with deltamethrin at concentrations of 50 µg/ml, 250 µg/ml, 500 µg/ml, 750 µg/ml (diluted treatments), 31.25 ρg/ml, 31.25 ηg/ml and 31.25 µg/ml (ultra diluted treatments) and incubated for 24 h. Samples were collected among 0 and 24 h of incubation, then germinated conidia were counted, assessing the percentage of germination and germination speed. The results showed that 50 µg/ml of deltamethrin reduced and delayed conidia germination and it was 100% inhibited by the concentrations of 250 µg/ml to 750 µg/ml. Ultra diluted treatments with deltamethrin were not inhibitory and treatments with 31.25 ρg/ml and 31.25 ηg/ml of deltamethrin showed a significant increase of germinated conidia, indicating a possibly hormesis, that is, the biological effects of low level exposures of these concentrations of deltamethrin on conidia germination of M. anisopliae.

Key words: Biological-chemical combination, Entomopathogen, Hormesis, Insecticide, Vegetative development.

INTRODUCTION

The modern mass agriculture prioritizes the safe use of pesticides, assuring the food quality and sustainable productivity. Although often their use is beneficial, the misuse of these compounds can affect the environment and consumers health [12], since at a sufficient dose they can cause genetic toxicity, cancer, birth defects, kidney and liver disease [27].

Several chemical products are applied for the implantation and maintenance of a high agricultural production. Pyrethroid insecticides, synthetic derivatives of pyrethrins, are used as wide-spectrum insecticides [25] due to their high insecticidal potency, slow development of insect resistance, relatively low acute toxicity in mammals, not being persistent in the environment [41]. However, effects of exposures to pyrethroids have been documented in potentially sensitive subpopulations, such as pregnant women, infants and children [11, 23, 44, 45].

Pyrethroids insecticides are classified in two classes, based on their structure and toxic effects: type I, compounds that not contain a cyano group, whereas type II compounds that contain it [25]. Deltamethrin ((*S*) alpha-cyano-3-phenoxy-benzyl-(1R,*cis*)-2,2-dimethyl-3-(2,2-dibromvinyl)-cyclopro-panecarboxylate), a type II pyrethroid, is effective by contact, ingestion and repellency. It has a wide action spectrum against insects and mites, including *Spodoptera frugiperda*, and in general it shows selective toxicity, favoring natural enemies [20, 28, 43].

It is considered a potent neurotoxic pyrethroid and among the major signs of acute poisoning are salivation, hyperexcitability, choreoathetosis, and seizures. Interaction with neuronal voltage-sensitive sodium channels is the primary mode of action of this insecticide [5, 6, 35, 39, 41].

The asexual filamentous fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin (order Moniliales, family Moniliaceae) was used for the first time as a microbial control agent of insects by Elie Metchnikoff, in 1879, for controlling of wheat grain beetle (*Anisoplia austriaca*). This entomopathogenic fungus is capable of infect several species of insect-pests, such as beetles [26], flies [13, 21, 22, 31], spittlebugs [24, 33], whitegrubs [2], locusts and grasshoppers [32].

Studies using the conidia germination speed parameter can be conducted *in vitro* by adding products to the synthetic culture media used for fungal growth, to evaluate whether physical and/or chemical variables in the substrate on conidia are produced influence fungal development and conidiogenesis [30, 37]. To determine the rate of germination, conidia can be inoculated into a liquid medium and sampled periodically for analysis with a microscope, counting the number of germinated conidia [29].

Germination speed of conidia has been used as parameter to evaluate the effects of different factors on *M. anisopliae*, such as employed by Rangel et al. [37], that verified the influence of natural or artificial substrates on the conidial UV-B tolerance and germination speed of two isolates *M. anisopliae*. Also, Rangel et al. [38], which demonstrated that the conidia germination speed of *M. anisopliae* can be strongly influenced by culture conditions.
Considering the importance of *M. anisopliae* as a microbial agent of a wide variety of insect-pests, it is of critical importance to evaluate the effect of chemical products on this fungus, assessing the conidia germination speed parameter, which is directly associated with virulence, and also, conidia represent the infective unit and the inoculum source in the field after application [4]. Therefore, this present study aimed to evaluate the toxicity of different concentrations of deltamethrin on *M. anisopliae*, in order to verify the possibility of a combined use of this entomopathogen and the pyrethroid.

MATERIAL AND METHODS

Fungal strain and culture media

The strain of *M. anisopliae* var. *anisopliae* was isolated from the insect host *Deois* sp. and belongs to the fungal culture collection of Laboratório de Biotecnologia Microbiana from Universidade Estadual de Maringá, Paraná, Brazil. Complete Medium (CM) and Liquid Complete Medium (LCM) [36] were employed.

Conidia germination speed in the presence of deltamethrin

M. anisopliae was incubated in Petri dishes containing CM (20 ml) in biological oxygen demand (BOD) at 28ºC. Conidia were obtained directly from seven days-old sporulating cultures by scraping and then suspending in aqueous solution of 0.01% Tween 80. Into nine Erlenmeyer flasks were inoculated the suspended conidia in a concentration of $5.42x10^6$ conidia/ml.

For treatments, seven Erlenmeyer flasks also received (in a final volume of 5 ml) LCM and deltamethrin (Decis 25 EC, Bayer®) at different concentrations: 50 µg/ml (T1), 250 µg/ml (T2), 500 µg/ml (T3), 750 µg/ml (T4) (diluted treatments), 31.25 ρg/ml (T5), 31.25 ηg/ml (T6) and 31.25 µg/ml (T7) (ultra diluted treatments). Two Erlenmeyer flasks also received only 5ml of LCM and were used as negative controls, one for diluted treatments (C1) and another for ultra diluted treatments (C2).

All Erlenmeyer flasks were incubated in BOD at 28ºC for 24 h. Samples from C1, T1, T2, T3 and T4 were collected at 0, 6, 8, 10, 12 and 24 h of incubation. Samples from C2, T5, T6 and T7 were collected at 8 and 24 h of incubation. Germinated conidia were counted using Neubauer hemocytometer. Samples were analyzed in triplicate. The percentage of germination and germination speed were assessed by randomly observing 100 conidia. A conidium was considered germinated when a germ-tube projected from it [29].

RESULTS AND DISCUSSION

The biological control using entomopathogens represents a promising alternative with low environmental impact that may contribute to reduce or eliminate the use of chemical products in agriculture and studies evaluating the effects of the combination of entomopathogens and chemical pesticides are being intensified [3, 7, 15, 30]. Among entomopathogenic microorganisms, fungi are the most wide spread group closely associated with agriculture [1].
In this present analysis of the toxicity of deltamethrin on *M. anisopliae* var. *anisopliae*, assessed by germination speed parameter, was possible to observe that T1 (50 µg/ml) delayed the speed of *M. anisopliae* conidia germination

(Figure 1) in comparison to C1, since the conidia germination started before 6 h of incubation for C1 whereas it started after 12 h for T1 (Figures 1 and 2). At the end of incubation time (24 h), a decrease in the number of germinated conidia was observed for T1 (20%) compared to C (90%) (Figures 1 and 2). A strong inhibition of germination was caused by treatments 2, 3 and 4, where conidia germination was 100% inhibited (Figure 1), indicating a possible toxic effect of deltamethrin on *M. anisopliae* when this pyrethroid is used in concentrations of 250 µg/ml, 500 µg/ml and 750 µg/ml. When deltamethrin was employed as ultra diluted treatments (Figure 3), there was no germination inhibition, moreover, at the end of incubation period (24 h), T5 and T6 showed a significant increase of germinated conidia compared to C2, where 81% and 96% of conidia were germinated in T5 (31.25 ρg/ml) and T6 (31.25 ηg/ml), respectively. In special, the results obtained for these two reduced concentrations of deltamethrin indicate a possibly hormesis effect of this pyrethroid on conidia germination of *M. anisopliae*, favoring it.

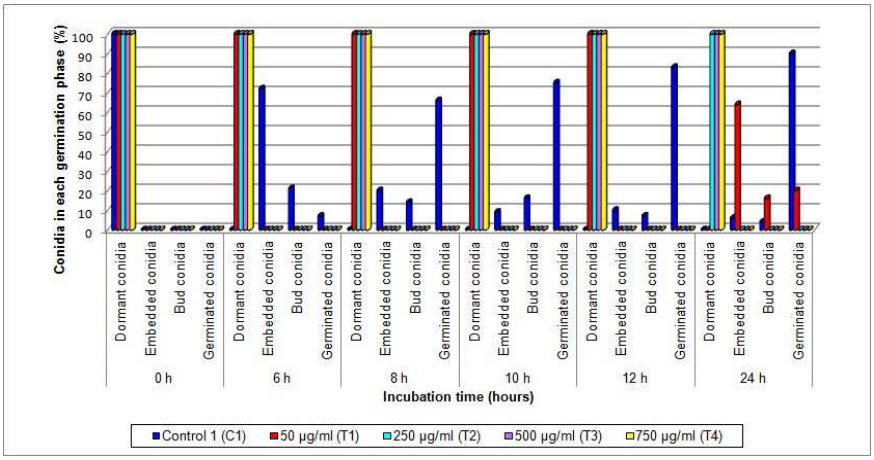

Figure 1. Percentage of *M. anisopliae* conidia at different phases of germination in the presence of diluted treatments of deltamethrin.

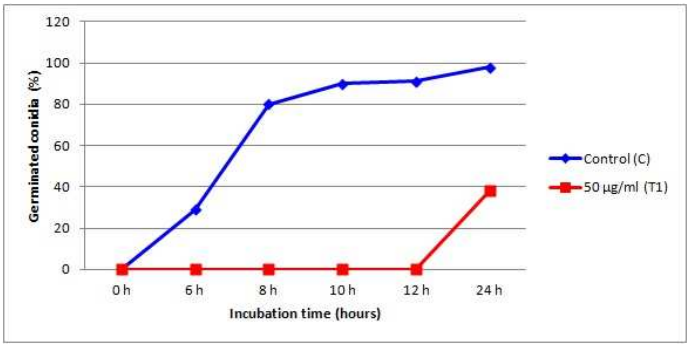

Figure 2. Comparison of the germination speed of *M. anisopliae* conidia in the control 1 and treatment 1 (50 µg/ml of deltamethrin).

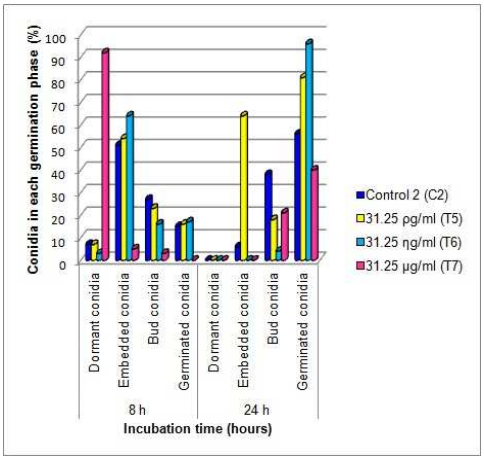

Figure 3. Percentage of *M. anisopliae* conidia at the beginning and end of germination in the presence of ultra diluted treatments of deltamethrin.

Hormesis or biological effects of low level exposures (BELLE) in the field of toxicology, is characterized by non-monotonic dose response which is biphasic, displaying opposite effects at low and high doses [42]. Similarly to the present study, the occurrence of hormesis has been documented across several biological models that received different types of exposure [4, 14, 16, 17, 18, 34, 42].

The growth of *M. anisopliae* colonies was the parameter used by Camargo [19] to check the effects of deltamethrin at different concentrations, observing that high inhibition occurred in concentrations from 30 up to 480 ppm. Batista Filho et al. [10] evaluated the effects of deltamethrin (in concentrations recommended for field, 30 to 400 ml a.i./ha) on the reproductive and vegetative growth of *M. anispoliae*, obtaining a compatibility when deltamethrin was used at the maximal dose and moderate toxicity when it was used at the minimum dose. The toxic effects of deltamethrin (50 ml/100 l^{-1}) and other pesticides on respiratory activity of *M. anisopliae* were checked by Mochi et al. [30], showing that no significant difference in fungal respiratory activity was observed between treatment with deltamethrin and the control.

Bahiense et al. [8] evaluated the compatibility of a strain of *M. anisopliae* (obtained from *Boophilus microplus*) and commercial product composed by deltamethrin to control larvae of *B. microplus* tick. The chemical product was used at concentrations of 0.39, 0.78, 1.56, 3.12 and 6.25 ppm and *M. anisopliae* was used at concentrations of 10^5, 10^6, 10^7 and 10^8 conidia/ml^{-1}. As results the authors observed that in all associations of *M. anisopliae* and deltamethrin (except of the combination of 10^8 conidia/ml^{-1} and 1.56 ppm deltamethrin) the mortality rates were higher than those treatments using only fungus or pyrethroid. Regarding the concentration of $2.2x10^5$ conidia/ml^{-1}, the best result was obtained for its combination with 6.25 ppm of deltamethrin, with 68% of larvae mortality. The concentration of $2.2x10^6$ conidia/ml^{-1} had better compatibility with 3.12 and 6.25 ppm of deltamethrin, with 54% of larvae mortality. For the combinations of $2.2x10^7$ conidia/ml^{-1} and either 1.56 or 6.25 ppm of deltamethrin, 99.1% and 100% of larvae mortality occurred, respectively. And the combination of $2.2x10^8$ conidia/ml^{-1} and 6.25 ppm of deltamethrin resulted in 100% of larvae mortality.

In a subsequent study, Bahiense et al. [9] tested the compatibility of deltamethrin and *M. anisopliae* to control a *B. microplus* strain resistant to the pyrethroid. Engorged females that naturally dropped off from calves were treated with deltamethrin (25 ppm) and the concentration of 10^8 conidia/ml^{-1} of *M. anisopliae*, used in combination or alone. As results, 32.57% mortality was observed for treatment with *M. anisopliae*, 38.58% for treatment with deltamethrin and 30.92% the combination of fungus and chemical.

Alves et al. [4] assessed the toxicity of the insect growth regulator lufenuron on MT strain of *M. anisopliae* using the conidia germination speed as parameter. The results indicated an increase of conidia germination when lufenuron was used at lower concentration (700 µg/ml) and the opposite effect was observed for the highest concentration (2 mg/ml), when inhibitory conidia germination occurred. This compatibility between 1.0 mg/ml and 700 µg/ml of lufenuron and *M. anisopliae* indicates that it is not toxic to the entomopathogenic fungus and suggests that they can be mixed and used to combat insect-pests, maintaining the inoculum source (conidia) in the field after application. Their results are in agreement with hormesis effect presented herein, where the lowest concentrations of deltamethrin (31.25 ρg/ml and 31.25 ηg/ml) increased the conidia germination, whereas high concentrations (250 to 750 µg/ml) had negative action.

Recently, Schumacher and Poehling [40] assessed the effects of five potential candidates for combined applications (fipronil, permethrin, imidacloprid, NeemAzal, and amitraz) on two strains of *M. anisopliae*, using germination, vegetative growth and sporulation as parameters. All pesticides were tested in concentrations of 0.32, 1.6, 8.0, 40, and 200 ppm, Also, permethrin and imidacloprid were combined in a ratio of 5:1 and tested in four combinations (1.6 ppm permethrin and 0.32 ppm imidacloprid; 8 ppm permethrin and 1.6 ppm imidacloprid; 40 ppm permethrin and 8 ppm imidacloprid; 200 ppm permethrin and 40 ppm imidacloprid). As results, the maximum inhibition of germination caused by these pesticides was ≤ 15% and most of the pesticides had no negative influence on the germination. It was concluded that the low dosages of the five pesticides (dissolved in 1% dimethyl sulfoxide) were compatible with *M. anisopliae* for an integrated pest management.

CONCLUSION

The results presented herein showed that high concentrations of deltamethrin delayed or inhibited the germination of *M. anisopliae*. The lower concentrations tested (31.25 ρg/ml and 31.25 ηg/ml) significantly increased the conidia germination of *M. anisopliae*, what points to the hormesis effect of them. Considering the foregoing, the integrated use of low doses of deltamethrin and *M. anisopliae* in pest management could be proposed to the future, with the objective of evaluating the action of this biological-chemical combination on the control of insect-pests.

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