COMPATIBILITY BETWEEN THE ENTOMOPATHOGENIC FUNGUS *Beauveria bassiana* AND INSECTICIDES USED IN COFFEE PLANTATIONS

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ABSTRACT: Microbial control in integrated pest management (IPM) programs of coffee plantations is an important factor for the reduction of pest population densities. The use of selective pesticides can be associated with entomopathogens, increasing the efficiency of the control and reducing the use of required insecticides. The *in vitro* fungitoxic effect of insecticide formulations of Thiamethoxam, Cyfluthrin, Deltamethrin, Alpha-Cypermethrin, Triazophos, Chlorpyrifos, Fenpropathrin and Endosulfan and *Beauveria bassiana* (CG 425 strain) was evaluated at three concentrations (FR = average field recommendation; 0.5 × FR and 2 × FR). Effects of these products on conidia germination, vegetative growth and sporulation were compared. Only five insecticides, at the FR concentration, promoted conidia viability higher than 60%. Viability should be considered the most important factor to be evaluated since it is the first step of the infection process. The insecticide formulations of Alpha-Cypermethrin, Thiamethoxam and Cyfluthrin caused the lower inhibition level on conidia germination at the two lower concentrations, with no difference in relation to the control. With respect to vegetative growth analysis, Thiamethoxam at the two lower concentrations was not found to cause radial growth inhibition. Thiamethoxam caused the smallest inhibition level with regard to conidia production. The use of Alpha-Cypermethrin and Thiamethoxam formulations in coffee IPM programs for a *B. bassiana* inoculum conservation strategy are recommended, since these products were compatible with the entomopathogenic fungus *Beauveria bassiana* (CG 425), an important natural control agent of the coffee berry borer, *Hypothenemus hampei*.
Key words: microbial control, toxic effect, pesticides

COMPATIBILIDADE ENTRE O FUNGO ENTOMOPATOGÊNICO *Beauveria bassiana* E INSETICIDAS USADOS NA CULTURA DO CAFEEIRO

RESUMO: Em programas de Manejo integrado de pragas (MIP) deve-se considerar o controle microbiano como um importante fator de redução da densidade populacional de pragas. A utilização de produtos seletivos quando associados a patógenos, pode aumentar a eficiência de controle, reduzindo assim a quantidade de inseticidas. O efeito fungitóxico *in vitro* das formulações inseticidas de Tiametoxan, Ciflutrin, Deltametrin, Alfacipermetrina, Triazofos, Clorpirifós, Fenpropatrin e Endosulfan em três concentrações (RC= recomendação média para campo, 0,5 × RC e 2 × RC), foi avaliado sobre a germinação dos conídios, crescimento vegetativo e produção de conídios no fungo *Beauveria bassiana.* Apenas cinco formulações inseticidas, na concentração RC, proporcionaram viabilidade dos conídios acima de 60%. A viabilidade deve ser considerada o parâmetro mais importante a ser avaliado por ser o passo inicial no processo de infecção. As formulações de Alfacipermetrina, Tiametoxan e Ciflutrin, nas menores concentrações, causaram a menor inibição da germinação, sem diferença em relação à testemunha. Analisando-se o crescimento vegetativo, observou-se que a formulação de Tiametoxan nas menores concentrações, não inibiu o crescimento radial, sendo o crescimento vegetativo nos demais tratamentos inferior à testemunha. A formulação de Tiametoxan também proporcionou a menor inibição na produção de conídios. Os inseticidas com formulações de Alfacipermetrina e Tiametoxan mostraram-se compatíveis com o fungo *Beauveria bassiana* (CG 425), importante agente natural de controle da broca do café *Hypothenemus hampei* e podem ser recomendados para MIP no cafeeiro
Palavras-chave: controle microbiano, efeito tóxico, agrotóxico

INTRODUCTION

Entomopathogenic fungi are important natural control agents of many insects, including several pests (Carruthers & Hural, 1990). Biological control, in particular when accomplished by entomopathogens, is a technique that should be considered as an important pest population density reduction factor in Integrated Pest Management (IPM) programs. Therefore, the conservation of such entomopathogens, whether they occur natu-

rally or when they are applied or introduced to control insects, is an interesting practice. However, the use of incompatible insecticides may inhibit the development and reproduction of these pathogens, affecting IPM (Malo, 1993; Duarte et al., 1992; Anderson & Roberts, 1983). On the other hand, the utilization of selective insecticides in association with pathogens can increase the efficiency of control, allowing the reduction of the amount of applied insecticides, minimizing environmental contamination hazards and the expression of pest resistance (Moino & Alves, 1998; Quintela & McCoy, 1998).

The coffee berry borer, *Hypothenemus hampei*, is naturally controlled by the entomopathogenic fungus *Beauveria bassiana*. Therefore, the selectivity/compatibility of products/formulations utilized as sprays, such as insecticides, leaf fertilizers, herbicides and fungicides, with the natural control agent, namely the fungus *B. bassiana* in the case of the coffee berry borer, is a factor of great importance for the development of an IPM strategy for the crop.

Alves et al. (1998) proposed a formula to classify chemical products according to their toxicity over entomopathogenic fungi in *in vitro* tests, based on the calculation of a factor T, relating values of vegetative growth (VG) and conidiogenesis (SPR) to a control (%): T= {20 (VG) + 80 (SPR)}/100. The values for T are classified according to the following limits: 0 to 30 = very toxic, 31 to 45 = toxic, 46 to 60 = moderately toxic and >60 = compatible. However, this formula does not take viability into account as an important compatibility factor but, according to a discussion presented by Neves et al. (2001) that should be done. These authors have shown the importance of the formula and have suggested that the viability should be considered to calculate compatibility values, especially considering that the pathogen infects the insect through conidial germination and also that the survival of the entomopathogenic fungus inoculum in the field depends primarily on the conidia, which are responsible for the first foci of the disease.

The objective of this study was to evaluate the *in vitro* fungitoxic effect (selectivity/compatibility) of the most important insecticides used in the coffee crop in relation to the entomopathogenic fungus *Beauveria bassiana,* an important natural control agent of the coffee berry borer, *Hypothenemus hampei.*

MATERIAL AND METHODS

The fungus *Beauveria bassiana*, strain CG 425, stored in the form of pure conidia, in Eppendorf vials at -4ºC, and selected among 60 strains as the most virulent to the coffee berry borer, was utilized in the experiments. The fungus was multiplied in a complete medium previously autoclaved and poured onto sterilized Petri dishes (Alves et al., 1998). Plating was performed according to the full dish method, the conidia transferred from the Eppendorf vial to the dish containing medium by platinum loop and then streaked with a Drigalsky loop. Plates were incubated in a B.O.D. incubator at 25 ± 1ºC with, 12-hour photophase, for fungus growth and sporulation. After eight days, conidia were scraped with a spatula and transferred to flat-bottom vials, containing 10 mL sterilized distilled water with 0.02% spreader sticker (Tween 20). The conidia concentrations in the suspensions were quantified directly under the optical microscope, with a Neubauer chamber. Then the suspensions were standardized until the desired concentration (1×10^6 conidia mL^{-1}) was obtained.

The insecticide tests consisted of the following formulations*: (1) Thiamethoxam, (2) Cyfluthrin, (3)Deltamethrin, (4) Alpha-Cypermethrin, (5) Triazophos, (6) Chlorpyrifos, (7) Fenpropathrin and (8) Endosulfan. Insecticides were tested at three concentrations: average recommendation utilized for aplication in the field with 100 L water (FR), 0.5 × FR (FR–50%) and 2 × FR (FR+100%). These values were chosen since they cover all concentrations recommended for the tested products, providing information that could be used in field compatibility tests. The recommended or mean commercial product dosages contemplate the different pests that attack the coffee plant.

In the viability (VIA) test, the formulations, at the pre-established concentrations, were mixed in sterilized distilled water containing Tween 20 spreader (0.02%) and *B. bassiana* conidia suspended in their respective mixtures, and left to rest for one hour. Later, 0.5 mL were distributed to each plate containing agar-water. Two replicates (plates) were prepared for each treatment (concentration). The viability quantification (germination %) was made after 24-h incubation period (T=25 ± 1ºC, 12 hour photophase), using an optical microscope. The vegetative growth (VG) and sporulation (SPR) tests were performed only for formulations that presented viability equal to or higher than 60% at the FR.

For the vegetative growth and sporulation tests, the formulations were mixed at the different concentrations, with autoclaved P.D.A. culture medium when still liquid, at a temperature of 40 ± 5ºC. After mixing (medium + formulation), approximately 20 mL of the mixture were poured onto Petri dishes (9 cm in diameter). After solidification of the medium, the fungal conidia were inoculated at three points per dish with a platinum wire loop (five dishes/treatment). After eight-day incubation period (T= 25 ± 1ºC; 12-hour photophase), radial growth measurements were randomly taken on 10 colonies, per treatment, with a caliper rule. A central disc was

*Commercial names of products: (1) Actara, (2) Baytroid, (3) Decis, (4) Fastac 100, (5) Hostathion 400, (6) Lorsban 480, (7) Meothrin 300 and (8) Thiodan.

removed with a hole-puncher from 10 colonies chosen at random, for each treatment, to quantify the production of conidia. Each disc was individually placed inside a flat-bottomed vial, containing 10 mL of sterilized distilled water with Tween 20 spreader (0.02%), and agitated until conidia were totally released from the medium surface. The suspension thus obtained was submitted to dilutions, as necessary, for later quantification of the conidia in Neubauer chamber. Two readings (16 fields) were taken per colony/replicate and their mean was utilized for statistical analysis. The same procedures adopted for the other treatments were utilized for the control in all evaluations, but without mixing the products/formulations.

Trials were set up in a completely randomized, design with two replicates for the viability test and 10 replicates for the VG and SPR tests. Data were submitted to analysis of variance and means were compared by the Tukey test (P = 0.05). The formula proposed by Alves et al. (1998) for classifying chemical products according to their toxicity was utilized to calculate compatibility.

RESULTS AND DISCUSSION

The insecticides based on Triazophos, Chlorpyrifos and Endosulfan formulations inhibited 100% of the germination, i.e., viability (VIA) was null at the three concentrations. The Alpha-Cypermethrin formulation at the lowest concentrations (0.5 × FR and FR) caused the least inhibition of conidium viability, as well as the Thiamethoxam formulation at the concentrations 0.5 x FR and 2 x FR (Table 1). Neither of these differed from the control. Treatments containing formulations of

Table 1 - Mean percentage of conidia germination after 24 hours, colony area, and number of conidia produced by the fungus *B. bassiana*, strain CG 425 (25±1ºC and 12D:12L photophase) mixed with different insecticide formulations.

Treatment		Germination[1] (n=2)		Colony area[1] (n=10)		Number of conidia[1] (n=10)	
		%	(%) reduction/ increase	mm²	(%) reduction/ increase	X × 10^6	(%) reduction/ increase
Control		92.8 ± 2.88 a[2] b	0.00	27.2 ± 1.72 a[2]	0.00	132.3 ± 28.66 a	0.00
Alpha-Cypermethrin	0.5 × FR	94.4 ± 4.62 a	+ 1.83	22.0 ± 1.04 bc	- 19.12	64.0 ± 22.25 c	- 51.63
	FR[3]	92.9 ± 6.14 ab	+ 0.22	19.8 ± 1.05 cd	- 27.21	63.3 ± 23.80 c	- 52.15
	2 × FR	72.1 ± 2.57 cde	- 22.22	15.2 ± 2.63 e	- 44.12	58.9 ± 9.64 c	-55.48
Thiamethoxam	0.5 × FR	93.5 ± 0.04 a	+ 0.86	28.6 ± 1.64 a	+ 5.15	156.9 ± 30.23 a	+ 18.59
	FR	62.3 ± 3.18 de	- 32.80	25.2 ± 3.19 ab	- 7.35	104.0 ± 29.36 b	- 21.39
	2 × FR	75.4 ± 4.25 abcd	- 18.66	22.1 ± 3.73 bc	- 18.75	143.0 ± 32.79 a	+ 8.09
Cyfluthrin	0.5 × FR	91.5 ± 1.29 abc	- 1.29	17.5 ± 1.64 de	- 35.66	146.2 ± 31.11 a	+ 10.51
	FR	79.9 ± 8.54 abcd	- 13.81	14.1 ± 1.36 e	- 48.16	132.6 ± 14.86 ab	+ 0.23
	2 × FR	15.2 ± 2.20 fg	- 83.60	9.6 ± 1.61 f	- 64.71	59.6 ± 24.03 c	- 54.95
Fenpropathrin	0.5 × FR	92.2 ± 3.14 ab	- 0.54	---	---	---	---
	FR	54.0 ± 3.27 e	- 41.75	---	---	---	---
	2 × FR	5.0 ± 0.92 g	- 94.61	---	---	---	---
Deltamethrin	0.5 × FR	73.4 ± 1.60 bcde	- 20.91	---	---	---	---
	FR	33.3 ± 10.7 f	- 64.08	---	---	---	---
	2 × FR	17.9 ± 11.0 fg	- 80.70	---	---	---	---
Endosulfan	0.5 × FR	0	- 100.00	---	---	---	---
	FR	0	- 100.00	---	---	---	---
	2 × FR	0	- 100.00	---	---	---	---
Chlorpyrifos	0.5 × FR	0	- 100.00	---	---	---	---
	FR	0	- 100.00	---	---	---	---
	2 × FR	0	- 100.00	---	---	---	---
Triazophos	0.5 × FR	0	- 100.00	---	---	---	---
	FR	0	- 100.00	---	---	---	---
	2 × FR	0	- 100.00	---	---	---	---

[1]original data.
[2]means followed by a common letter in the columns do not differ by Tukey test (P = 0.05).
[3]FR= field recommendation, 0.5 × FR=(-50%) and 2 × FR=(+100%).

Cyfluthrin, at the two smallest concentrations, Fenpropathrin, Deltamethrin at the smallest, and Thiamethoxam at the highest concentration, also did not show differences relative to the control. The other treatments, however, presented significant reductions relative to the control (Table 1). Based on the results obtained for *B. bassiana* conidia viability, VG and SPR tests were performed with the Alpha-Cypermethrin**,** Thiamethoxam and Cyfluthrin formulations**.**

Thiamethoxam formulation at the smallest concentrations did not inhibit radial growth, without differences in relation to the control. In the other two formulations, Alpha-Cypermethrin and Cyfluthrin, when the same concentrations were compared, the colony area was smaller than the areas in the control and in the product containing a Thiamethoxam formulation. In addition, the Cyfluthrin treatment at the highest concentration was different from all other treatments, with the greatest percentage of reduction relative to the control (64.7%).

With respect to conidia production, the Thiamethoxam formulation also presented the best results at the highest and lowest concentrations, with increases of 8.1% and 18.6%, respectively, relative to the control, but without statistical differences. The treatment containing a Cyfluthrin formulation at the smallest concentrations also had increases of 10.5% (0.5 × FR) and 0.2% (FR) in the number of conidia, but without significant differences as compared to the control and to the Thiamethoxam formulation. The product containing the Alpha-Cypermethrin formulation at the three concentrations was poorer than the other treatments with regard to conidia production, except from the Cyfluthrin formulation at the highest concentration, with reductions relative to the control ranging between 51.6% and 55.5%, respectively, for 0.5 × FR and 2 × FR.

Table 2 - T factor and compatibility classification of different insecticide formulations, with respect to their fungitoxic effect against *B. bassiana* (strain 425).

Treatment		*B. bassiana*	
		T values[1]	Classification[2]
Alpha-Cypermethrin	0.5 × FR	54.87	MoT
	FR[3]	52.84	MoT
	2 × FR	46.79	MoT
Thiamethoxam	0.5 × FR	115.00	C
	FR	81.42	C
	2 × FR	102.6	C
Cyfluthrin	0.5 × FR	101.27	C
	FR	90.55	C
	2 × FR	43.09	T

[1]Formula proposed by Alves et al. (1998).
[2]MoT= moderately toxic, C= compatible, T= toxic.
[3]FR= field recommendation.

Considering the formula proposed by Alves et al. (1998), Thiamethoxam formulations at the three concentrations and Cyfluthrin at the two smallest concentrations were compatible to the fungus (Table 2). This formula adequately represents the effect of the tested products/formulations over the *in vitro* pathogens. *In vitro* studies have the advantage of exposing the microorganism to the products/formulations to the maximum extent possible, which does not occur under field conditions, where several factors act as barriers against such exposure, protecting the entomopathogen. For example, imperfect plant coverage by the product provides a spatial refuge for the entomopathogen. Therefore, once an agrochemical has been verified to be innocuous in the laboratory, it will, undoubtedly be selective under field conditions. On the other hand, the high toxicity of a product *in vitro* not always means that the same will occur in the field, but is an evidence that it is possible to occur (Alves et al., 1998).

With regard to product compatibility in the field, the effect of insecticides on conidia germination/viability as one of the most important aspects (Anderson & Roberts, 1983; Malo, 1993; Neves et al., 2001) must be considered. This happens because the entomopathogenic fungi infect insects through the germination of conidia, either by ingestion or by contact, the latter form being more frequent. The entomopathogenic fungus preservation in the field (inoculum) also occurs mainly in the form of conidia. At the onset of epizootics, the surviving conidia are responsible for the occurrence of primary foci of infection (Alves & Leucona, 1998). The importance of VG and SPR is relative as well, as discussed by Neves et al. (2001) and Hirose et al. (2001). These authors commented that VG will only occur or become inhibitted inside the body of the host and rarely in the environment in the saprophytic form. In the case of insecticides, insects that die from their contact with products will probably be quickly colonized by saphophytic bacteria, eliminating the chances for the development of diseases; as a consequence, no conidia formation will occur. The authors also suggest that the formula proposed by Alves et al. (1998) should regard viability as an utterly important factor.

When germination/viability is considered as a compatibility factor, it can be observed that, in addition to the Thiamethoxam and Cyfluthrin formulations, the Alpha-Cypermethrin formulation can also be deemed as compatible, as well as the Fenpropathrin and Deltamethrin formulations at the lowest concentrations. Therefore, when VG and SPR are taken into account, the values for the factor T do not attribute actual toxicity values, because germination is only the initial step in the infection process. It is also important to point out that effects observed in the tests result not only from the formulations, but also from the active ingredients (Table 3).

Table 3 - Chemical names and formulas of active ingredients utilized.

Active ingredient	Chemical name	Formula
Thiamethoxam	(EZ)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4 -ylidene(nitro)amine	$C_8H_{10}ClN_5O_3S$
Cyfluthrin	(RS)-α-cyano-4-fluoro-3-phenoxybenzyl (1RS)-cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	C22H18Cl2FNO3
Deltamethrin	(S)-α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate	C22 H19 BR2 N O3
Alpha-cypermethrin	(R) and (S) Enantiomorph Isomeres of α-cyano-3-phenoxybenzyl (2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	C22 H19 CL2 NO3
Triazophos	O,O-diethyl O-1-phenyl-1H-1,2,4-triazol-3-yl phosphorothioate	C12 H16 N3 O3 PS
Chlorpyrifos	O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate	C9H11CL3 NO3PS
Fenpropathrin	(RS)-α-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate	C22 H23 NO3
Endosulfan	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide	C9H6O3CL6S

Thus, when an IPM strategy is devised, it is important to take into account the compatibility of products sprayed on the crop, avoiding the use of the most toxic, or using them during seasons when the effect over a natural control agent is minimized. Therefore, the toxic effect impact on the control agent will be smaller, contributing indirectly to control the host pest-insect and, consequently, to reduce damage in the cultivated field. In the case of coffee fields, it is important to utilize other control alternatives, obeying an ecological selectivity perspective. This type of selectivity takes the application site into consideration, so as to avoid the contact of the toxic agent with the natural enemy, in this case *B. bassiana*, which can be achieved through the application of systemic fungicide products, such as soil granular fungicides.

The Alpha-Cypermethrin, Cyfluthrin and Thiametoxan formulations can be utilized in coffee IPM programs, since they are compatible with the entomopathogenic fungus *Beauveria bassiana* (strain CG 425). Because of their compatibility, the use of these products will conserve the *B. bassiana* conidia in the environment, contributing to biological control of the coffee berry borer. As natural control is implemented, less chemical insecticides will have to be used, resulting in benefits for the farmer and the environment.

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