BIOLOGICAL CONTROL

Compatibility of Entomopathogenic Fungi with Neonicotinoid Insecticides

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Compatibilidade de Fungos Entomopatogênicos com Inseticidas Nicotinóides

RESUMO - O efeito fungitóxico, *in vitro,* de três inseticidas neonicotinóides, acetamiprid (Saurus 200 PS), imidacloprid (Confidor 700 GrDA) e thiamethoxam (Actara 250 WG) em três concentrações (RM = recomendação média para campo, 0,7 RM e 1,3 RM), foi avaliado sobre os fungos entomopatogênicos *Beauveria bassiana*, *Metarhizium anisopliae* e *Paecilomyces* sp. O efeito dos inseticidas sobre a germinação dos conídios, o crescimento vegetativo e a produção de conídios também foi avaliado. Os resultados mostraram que os produtos não afetaram a germinação dos conídios, com exceção do acetamiprid na maior concentração (1,3 RM) onde ocorreu uma inibição ($P \leq 0,05$) de *M. anisopliae*. O crescimento vegetativo foi inibido nos tratamentos acetamiprid, nas três concentrações, para *B. bassiana* e *M. anisopliae*. Também no tratamento thiamethoxam, na concentração RM e 1,3 RM o crescimento vegetativo foi inferior à testemunha para *B. bassiana*. Para *Paecilomyces* sp. o crescimento vegetativo foi superior ao da testemunha para os tratamentos 0,7 RM e RM de acetamiprid, todas as concentrações de imidacloprid e as duas menores de thiamethoxam. A produção de conídios foi menor para o tratamento de acetamiprid na maior concentração (1,3 RM) para os fungos *Paecilomyces* sp. e *M. anisopliae* e para imidacloprid na maior concentração (1,3 RM) para *Paecilomyces* sp. Já thiamethoxam inibiu a produção de conídios somente na menor concentração (0,7 RM) para *M. anisopliae*. Aumento de produção de conídios foi observado somente nos tratamentos acetamiprid nas duas menores concentrações (0,7 RM) e RM e thiamethoxam nas duas maiores (RM e 1,3 RM) para o fungo *Paecilomyces* sp. Assim, os inseticidas testados nas concentrações e formulações utilizadas mostraram, na maioria dos casos, compatibilidade com os entomopatógenos *B. bassiana*, *M. anisopliae* e *Paecilomyces* sp. Portanto, estes produtos poderão ser recomendados em programas de MIP, nas formulações e concentrações testadas, para o controle de pragas que tenham como inimigos naturais os entomopatógenos testados.

PALAVRAS-CHAVE: Insecta, *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces* sp.

ABSTRACT - The *in vitro* fungitoxic effect of the neonicotinoid insecticides acetamiprid (Saurus 200 SP), imidacloprid (Confidor 700 WDGr) and thiamethoxam (Actara 250 WG) in three concentrations (AR= average field recommendation; 0.7 AR and 1.3 AR) to the entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces* sp. was studied. The effect of the insecticides on conidia germination, vegetative growth and conidiogenesis was compared. The insecticides had no effect on conidia germination, except under the highest concentration (1.3 AR) of acetamiprid, in which significant inhibition of *M. anisopliae* occurred. Vegetative growth of *B. bassiana* and *M. anisopliae* was significantly inhibited only by the three concentrations of acetamiprid treatment. Thiamethoxam treatment, in the AR and 1.3 AR concentrations, was significantly lower for *B. bassiana* vegetative growth. *Paecilomyces* sp. vegetative growth was higher than the control in the following treatments: 0.7 AR and AR of acetamiprid; all concentrations of imidacloprid; and in the two smallest concentrations, 0.7 AR and AR, of thiamethoxam. Conidia production was significantly smaller for *Paecilomyces* sp. and *M. anisopliae*, in the highest concentration (1.3 AR) of acetamiprid treatment and, for *Paecilomyces* sp., in the highest concentration (1.3 AR) of imidacloprid. Thiamethoxam significantly inhibited *M. anisopliae* conidia production, only in the smallest concentration (0.7 AR).

Significant increase in the conidia production of *Paecilomyces* sp. fungus was observed in the following treatments: two concentrations, 0.7 AR and AR of acetamiprid; and in the two highest concentrations, AR and 1.3 AR, of thiamethoxam treatments. The use of insecticides, in the recommended formulations and other concentrations tested, in most cases, had no negative effect on conidia germination, conidia production and vegetative growth of *B. bassiana*, *M. anisopliae* and *Paecilomyces* sp. Consequently, these products, in the formulations and concentrations tested, may be used in IPM programs in which the entomopathogens are important pest regulators.

KEY WORDS: Insecta, *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces* sp.

Entomopathogenic fungi are important as natural control agents of many insects, including several pests (Carruthers & Hural, 1990). In Integrated Pest Management (IPM), biological control with entomopathogens should be considered as an important reduction factor in pest population density. So, the conservation of entomopathogens is needed if they occur naturally, are applied or introduced with the objective of controling pests. However, the use of incompatible pesticides 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 use of selective pesticides is an important strategy in IPM programs. In some cases, compatible products may be associated with entomopathogenic fungi, increasing the control efficiency, decreasing the amount of insecticides required and minimizing the risks of environmental contamination and pests resistance expression (Moino & Alves 1998, Quintela & McCoy 1998).

Heterocyclic nitromethylenes also called neonicotinoids, belong to a new insecticides group, in which acetamiprid, imidacloprid and thiamethoxam are the most used. Since they have the same mode of nicotine action, linking in the sinapses of the nervous connections to the acetylcholin receptors (Abbink, 1991), they are classified as neonicotinoids.

Due to the recent development of those insecticides, the compatibility of commercial formulations of acetamiprid, imidacloprid and thiamethoxam with the entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill, *Metarhizium anisopliae* (Metsch.) Sorok. and *Paecilomyces* sp. Bainier is reported. These compatibility data are essential before such associations (chemical insecticides + entomopathogens) are employed in the field.

Material and Methods

The following fungi strains were used: *B. bassiana* - strain 447 (ESALQ/USP), *M. anisopliae* - strain E9 (ESALQ/USP), and *Paecilomyces* sp. strain CNPSo P77 (EMBRAPA - Soja). Conidia were produced in potato-dextrose-agar (P.D.A.) medium.

The insecticide concentrations were: an average recommendation for field application in 100 l/ha (AR), 0.7AR (AR -30%) and 1.3AR (AR +30%). These values were chosen because they include all field recommended concentrations: for acetamiprid (Saurus 200 SP) (AR = 25 g c.f. /100 l); for imidacloprid (Confidor 700 WDGr) (AR = 30 g c.f. /100 l); and for thiamethoxam (Actara 250 WG) (AR = 20 g c.f. /100 l).

Germination Assessments. The formulations were dissolved in water containing 0.02% of Tween 20. Conidia of *B. bassiana*, *M. anisopliae* and *Paecilomyces* sp. were suspended in the respective aqueous solution. After one hour, 0.5 ml aliquots of each suspension were spread in four Petri dishes containing water-agar medium. The dishes were then incubated at 25±1°C and 12h photophase and 24h later the percentage of germinated conidia was quantified.

Vegetative Growth and Conidia Production Assessments. Formulations were incorporated in P.D.A. medium amended with streptomycin (0.5g/l), at 45±5°C, and poured into Petri dishes. After medium solidification, the fungi were inoculated in three points per plate, (five dishes/treatment). The dishes were then incubated at 25±1°C and 12h photophase. Seven days after inoculation, for *B. bassiana* and *Paecilomyces* sp., and nine days for *M. anisopliae*, the colony areas were measured on a vegetable paper with a planimeter. After the area of the colonies were drawn 10 central colony disks (2,27cm^2) from each treatment were collected for conidia production quantification. A standard sample colony area in relation to all colony area was chosen for conidia production quantification. Each disk was placed in a glass tube and the conidia were suspended in 10 ml of water containing 0.02% Tween 20 and quantified using a Neubauer chamber.

The assessments had a complete randomised design. Data were submitted to ANOVA and means compared by the Tukey's test ($P \leq 0.05$).

Compatibility Calculation. The formula proposed by Alves *et al*. (1998) was used for toxicity classification of chemical products regarding tests with entomopathogenic fungi *in vitro*. In this model values calculation of the vegetative growth (VG) and sporulation (SP) is made in relation to the control (100%): T= 20 (VG) + 80 (SP) / 100 (0 to 30 = very toxic; 31 to 45 = toxic; 46 to 60 = moderately toxic; > 60 = compatible).

Results and Discussion

B. bassiana germination and conidia production were not affected by the treatments (Table 1). Significant reduction was observed in colony area for all acetamiprid (0.7 AR, AR and 1.3AR) and thiamethoxam (AR and 1.3 AR) treatments. For the imidacloprid treatments however, there were no

Table 1. Percent conidia germination (means ± SE), colony area (means ± SE), at seven days after inoculation and number of conidia (means ± SE), produced by the entomopathogenic fungus *B. bassiana* (strain 447), grown at 25±1ºC and 12h photophase, on potato-dextrose-agar medium + 0.5 g/l streptomycin, amended with neonicotinoid insecticides.

Treatments		Germination (n=4)		Colony area (n=15)		Conidia number (n =10)	
		(%)	(%) reduction	(cm^2)	(%) reduction	(X x 10^7)	(%) reduction
Control		88.1±3.8 a[(2)]	0.00	6.5±0.90 a	0.00	69.5±13.75 ab	0.00
Acetamiprid	0.7AR[1]	82.6±4.07 a	-6.24	4.6±0.88 b	-29.89	65.7±13.82 ab	-5.47
	AR	83.0±5.61 a	-5.79	4.9±0.56 b	-24.35	61.2±10.59 b	-11.94
	1.3AR	79.7±6.49 a	-9.53	3.1±0.51 c	-52.83	59.6±10.59 b	-14.24
Imidacloprid	0.7AR	81.5±4.55 a	-7.49	6.2±0.73 a	-5.08	67.1±15.67 ab	-3.45
	AR	86.2±3.28 a	-2.16	6.4±0.68 a	-1.69	64.4±15.53 ab	-7.34
	1.3AR	84.8±3.16 a	-3.75	6.0±0.60 a	-6.78	57.0±13.02 b	-17.99
Thiamethoxam	0.7AR	83.5±5.80 a	-5.22	6.0±0.97 a	-7.55	78.8±14.54 a	+13.38
	AR	86.6±1.73 a	-1.70	5.1±0.70 b	-20.96	60.9±11.86 ab	-12.37
	1.3AR	85.2±3.85 a	-3.29	4.9±0.57 b	-24.96	67.1±15.67 ab	-3.45

Means followed by different letters within each column are significantly different (P≤0.05) from control treatment; Tukey's studentized range test.
[1]AR = field average recommendation.

significant differences in any of the variables ($P \leq 0.05$).

For *M. anisopliae*, acetamiprid (1.3 AR) treatment showed a significant reduction in germination, vegetative growth and conidia production (Table 2). In the other two acetamiprid concentrations (0.7 AR and AR), the vegetative growth was also significantly reduced. Conidia production was significantly reduced ($p \leq 0.05$) by the thiamethoxam treatment (0.7 AR). However, this negative effect was not

Table 2. Percent conidia germination (means ± SE), colony area (means ± SE), at seven days after inoculation, and number of conidia (means ± SE) produced by the entomopathogenic fungus *M. anisopliae* (strain E-9) grown at 25±1ºC and 12h photophase, on potato-dextrose-agar medium + 0.5 g/l streptomycin, amended with neonicotinoid insecticides.

Treatments		Germination (n=4)		Colony area (n=15)		Conidia number (n =10)	
		(%)	(%) reduction	(cm^2)	(%) reduction	(X x 10^7)	(%) reduction
Control		97.6±1.33 a	0.00	7.2±0.37 ab	0.00	78.4±5.07 abcd	0.00
Acetamiprid	0.7AR[1]	96.4±2.35 a	-1.23	6.2±0.61 cd	-14.64	65.4±8.52 cde	-16.58
	AR	92.7±2.95 ab	-5.02	5.6±0.57 d	-22.10	69.8±4.13 bcde	-10.97
	1.3AR	83.9±7.41 b	-14.04	4.6±1.17 e	-36.46	23.0±7.65 f	-70.66
Imidacloprid	0.7AR	96.9±1.42 a	-0.72	7.1±0.77 abc	-2.35	70.9±2.95 bcde	-9.57
	AR	97.4±1.48 a	-0.20	7.5±0.74 a	+3.45	87.2±9.08 a	+11.22
	1.3AR	95.3±2.92 a	-2.36	6.4±0.76 bcd	-11.19	81.0±7.61 ab	+3.32
Thiamethoxam	0.7AR	92.8±6.26 ab	-4.92	6.7±0.87 abc	-7.46	59.6±20.67 e	-23.98
	AR	94.6±1.30 a	-3.07	6.9±0.70 abc	-5.11	60.9±6.83 de	-22.32
	1.3AR	96.5±2.17 a	-1.13	6.5±1.11 bcd	-10.36	72.6±6.90 abcd	-7.40

Means followed by different letters within each column are significantly different (P≤0.05) from control treatment; Tukey's studentized range test.
[1]AR = field average recommendation.

observed in the highest concentrations (1.3 AR). Imidacloprid treatments had no negative effect on germination, vegetative growth and conidia prodution (see Table 2).

production apparently conflicting data were obtained since treatments with acetamiprid (0.7 AR and AR) and thiamethoxam (1.3 AR and AR) provided higher figures than those of the control. Similar results were frequent in *in vitro* experiments (Alves *et al*. 1998). Moino Jr. & Alves (1998) suggested two possible explanations for these results: 1 - in fungi, as a physiologic mechanism of resistance, insecticides can be metabolized and liberate compounds that can be used by the fungus as secondary nutrients; and 2 - in a toxic medium, the fungus could be making a reproductive effort, increasing conidia production. Another possible explanation is that substances present in the insecticide formulations can be used directly as nutrients increasing the vegetative growth and conidia production of the pathogen.

Table 3. Percent conidia germination (means ± SE), colony area (means ± SE) at seven days after inoculation, and number of conidia (means ± SE) produced by the entomopathogenic fungus *Paecilomyces* sp., (strain CNPSo P77) grown at 25±1°C and 12h photophase, on potato-dextrose-agar medium + 0.5 g/l streptomycin, amended with neonicotinoid insecticides.

Treatments		Germination (n=4)		Colony area (n=15)		Conidia number (n =10)	
		(%)	(%) reduction	(cm^2)	(%) reduction	(X x 10^7)	(%) reduction
Control		97.7±0.60 ab	0.00	3.6±0.30 de	0.00	57.1±3.64 bc	0.00
	0.7AR[1]	95.0±0.21 b	-2.76	4.7±0.48 b	+30.81	91.4±9.21 a	+60.07
Acetamiprid	AR	98.7±1.15 ab	+1.02	4.3±0.30 bc	+21.29	80.5±7.93 a	+58.49
	1.3AR	99.2±0.62 a	+1.54	3.5±0.33 e	-1.40	33.0±4.31 d	-42.21
	0.7AR	99.1±0.72 ab	+1.43	5.5±0.33 a	+53.22	50.5±16.5 c	-11.56
Imidacloprid	AR	97.5±0.64 ab	-0.20	5.3±0.39 a	+48.18	73.3±8.92 ab	+28.37
	1.3.AR	97.9±3.32 ab	+0.20	5.3±0.42 a	+47.62	32.1±17.27 d	-43.78
	0.7.AR	97.8±1.27 ab	+0.10	4.7±0.40 b	+31.09	73.4±3.78 ab	+28.55
Thiamethoxam	AR	94.7±2.16 b	-3.07	4.6±0.30 b	+30.25	81.9±6.78 a	+43.43
	1.3.AR	98.2±1.97 ab	+0.51	4.0±0.59 cd	+12.32	89.8±15.76 a	+57.27

Means followed by different letters within each column are significantly different (P≤0.05) from control treatment; Tukey's studentized range test.
[1]AR = field average recommendation.

No significant differences were observed between control and the formulations in *Paecilomyces* sp. germination (Table 3). However, vegetative growth was significantly higher in the treatments in relation to control, except for acetamiprid (1.3 AR) and thiamethoxam (1.3 AR) treatments. Conidia production, for *Paecilomyces* sp., was also higher for acetamiprid (0.7 AR and AR) and thiamethoxam (AR and 1.3 AR). High reduction in the conidia production was observed with the acetamiprid (1.3 AR) and imidacloprid (1.3 AR) treatments (Table 3).

In general, the insecticides tested did not affect the entomopathogenic fungi. Formulations were, probably, partially responsible for this result. Anderson & Roberts (1983) pointed out that an insecticide formulation is more important than the active ingredient, and wettable powder formulations generally do not cause inhibition and may increase *B. bassiana* colonies number. Duarte *et al*. (1992) observed a synergic effect between wettable powders insecticides and *M. anisopliae*. However, the hypothesis that active ingredient have a fungitoxic effect is not discarded. This was confirmed by Moino Jr. & Alves (1998) that observed a high compatibility of imidacloprid with fipronil for *B. bassiana* and *M. anisopliae*, although both formulations contained water dispersible granules.

For *Paecilomyces* sp., vegetative growth and conidia

In agreement with the proposed model, all products were compatible with *B. bassiana* (Table 4). For this entomopathogen, the average " T " values, for the three tested concentrations, were 95.8; 91.4 and 84.5 respectively for thiamethoxam, imidacloprid and acetamiprid. Only acetamiprid (Saurus) in the highest concentration (1.3 AR) was toxic to *M. anisopliae* with " T " values of 36.2. The average " T " values, considering the three concentrations were 100.8; 84.3 and 69.0 for imidacloprid, thiamethoxam and acetamiprid, respectively. For *Paecilomyces* sp., all products in all concentrations were compatible. The average "T" values, considering the three concentrations, were 139.1, 118.84 and 102.62 respectively for thiamethoxam, acetamiprid and imidacloprid.

Table 4. "T" values and compatibility classification of neonicotionid insecticides, in relation of fungitoxic effect on *B. bassiana* (strain 447), *M. anisopliae* (strain E9), and *Paecilomyces* sp. (strain CNPSo P77). photophase.

Treatments		*B. bassiana*		*M. anisopliae*		*Paecilomyces* sp.	
		"T" values[1]	Classification [2]	"T" values	Classification	"T" values	Classification
Acetamiprid	0.7AR[3]	89.8	C	84,0	C	154,17	C
	AR	85.5	C	86.8	C	136,67	C
	1.3AR	78.1	C	36.2	T	65,68	C
Imidacloprid	0.7AR	96.3	C	92.1	C	101,31	C
	AR	93.8	C	109.8	C	132,14	C
	1.3.AR	84.1	C	100.4	C	74,42	C
Thiamethoxam	0.7.AR	109.2	C	79.4	C	128,95	C
	AR	85.8	C	81.3	C	140,30	C
	1.3.AR	92.3	C	92.1	C	148,04	C

[1]Formula proposed by Alves *et al*. (1998).
[2] T = toxic; MT = moderately toxic; C = compatible.
[3]AR = field average recommendation.

The formula proposed by Alves *et al*. (1998) represents, in an appropriate way, the effect of products on the pathogens *in vitro*. These studies have the advantage of exposing the pathogen to the maximum action of the chemicals, a fact that could not occur under field conditions. In these conditions, growth inhibition may not be a good indication of other fungicidal effects, such as those of spore viability (Loria *et al.* 1983). Thus, when the insecticide is compatible *in vitro*, there are strong evidences about its selectivity under field conditions (Alves *et al*. 1998). Likewise, a high toxicity *in vitro* does not always mean that the same will occur in the field, but it shows a possibility for this to occur (Alves *et al*. 1998). On the other hand, for compatibility in the field we should consider the effect of products on conidia germination as one of the most important factors (Anderson & Roberts 1983, Malo 1993). This is due to the fact that pathogens infect insects through the conidium germination, by ingestion or contact. In addition, the inoculum survival of the entomopathogenic fungus in the field is made by conidia. In the beginning of an epizootic, the conidia are responsible for the occurrence of the first disease focuses (Alves & Lecuona, 1998). Thus, if germination inhibition occur, the pathogen control efficiency will be committed if the pathogen is been applied in an inundative form, together or separately with the product, or if the pathogen is naturally present in the plants and contacts the product.

Pathogen vegetative growth will occur, or will be inhibited only inside the host. Chemical concentration inside the insect is probably smaller than that used in *in vitro* tests. Thus, *in vitro* results represent, in a more drastic way, what happens with the vegetative growth of the pathogen inside the insect body. In addition, for the same reasons discussed for the vegetative growth, data obtained *in vitro* for conidia production are not of fundamental importance. In the case of insecticides, the insects that die by contact with the products will probably be quickly colonized by saprophytic bacteria, eliminating the chances for fungal development and consequently conidia formation.

The *in vitro* compatibility tests should consider inhibition or not of conidia germination when mixed with the formulations since this is an important factor at field conditions as mentioned before. A consequence of this is that the formula proposed by Alves *et al*. (1998) should be reevaluated considering conidia germination as an important factor for " T " values calculation. Considering germination as a compatibility parameter, in general all products/ formulations were compatible with the entomopathogens with no significant differences from the control presenting only a very small inhibitory effect.

The use of the insecticides acetamiprid, imidacloprid and thiamethoxam, in the formulations and concentrations tested, has no negative effect on the germination, vegetative growth and conidia production of *B. bassiana*, *M. anisopliae* and *Paecilomyces* sp. Therefore, in an IPM program they can be recommended for pest control where this entomopathogens are important natural enemies. Field studies with these products applied in mixture or not with the pathogens can give additional information and help in the strategies using IPM production systems including these insecticides.

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