BIOLOGICAL CONTROL

Compatibility of *Beauveria bassiana* with Acaricides

Renato C. de Oliveira[1] and Pedro M.O.J. Neves[2]

[1]*Depto. Ciências Biológicas, Faculdade Assis Guagacz, 85806-095, Cascavel, PR*
[2] *Depto. Agronomia, Centro de Ciências Agrárias, UEL, C. postal 6001, 86051-990, Londrina, PR*

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RESUMO - Visando avaliar a compatibilidade *in vitro*, entre o fungo entomopatogênico *Beauveria bassiana* (Bals.) Vuil. e acaricidas, 12 formulações com os ingredientes ativos clorfenapir, fenpiroximate, amitraz, acrinatrim, hexitiazox, abamectina, piridine, dimetoato, piridaphetion, óxido de fenbutatim, azociclotim e cihexatim, foram testadas em três concentrações (concentração Média - CM, metade da CM e duas vezes a CM). Para os parâmetros germinação de conídios, crescimento vegetativo e esporulação, apresentaram diferenças significativas na compatibilidade entre o fungo e as formulações utilizadas. As formulações mais compatíveis com o fungo, para os parâmetros avaliados, foram as pertencentes ao grupo químico da avermectina e piretróide. As formulações pertencentes ao grupo químico do organofosforado e organostânico afetaram drasticamente a germinação de conídios, bem como o crescimento vegetativo e a esporulação.

PALAVRAS-CHAVE: Seletividade, fungitoxicidade, fungo entomopatogênico, MIP

ABSTRACT - The *in vitro* compatibility of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuil. with 12 acaricide formulations was evaluated. The active ingredients Clorfenapyr, Fenpyroximate, Amitraz, Acrinathrin, Hexythiazox, Abamectin, Pyridine, Dimethoate, Pyridaphethion, Fenbutatin Oxide, Azocyclotin and Cyhexatin were tested in three different concentrations (mean concentration - MC, half MC and twice the MC). The formulations tested affected conidial germination, vegetative growth and sporulation of the fungus in different levels. The formulations more compatible with *B. bassiana* were Avermectin and the pyrethroids. The acaricides belonging to the organophosphate and organostanic chemical groups, however, drastically affected conidial germination as well as vegetative growth and sporulation.

KEY WORDS: Selectivity, fungitoxicity, entomopathogenic fungus, IPM

Entomopathogenic fungi are found worldwide associated to insects and phytophagous mite populations, contributing to the biological control of these arthropods on several economically important crops (Carruthers & Hural 1990, Van Der Geest *et al.* 2000). Seeking to use the potential of these organisms for pest control, commercial products have been developed with entomopathogenic fungi (McCoy & Couch 1982, McCoy 1990, Alves & Pereira 1998).

The success of a pest control program using entomopathogenic fungi, however, depends on conidia survival in the field environment (Benz 1987). Conidia survival may be affected either by environmental factors (Furlong & Pell 1997) or by bio-pesticides and/or chemical products used to protect crop plants (Anderson & Roberts 1983, Loria *et al.* 1983, Alves & Lecuona 1998).

Many experiments have been carried out aiming to detect pesticides side effects on entomopathogenic fungi (Olmert & Kenneth 1974, Gardner & Storey 1985, Neves *et al*. 2001). Most of them evaluated only the effects of the products on vegetative growth and sporulation, disregarding conidial germination. However Neves *et al*. (2001) pointed out the importance of conidial germination in compatibility studies. They emphasized that the inhibition of this initial step will affect the plain development of the fungus in the field because this fungal structure is responsible for initiating the disease on insect pest populations.

According to Anderson & Roberts (1983), germination is also an important criterion to evaluate compatibility of pesticides with entomopathogenic fungi *in vitro*. Todorova *et al*. (1998) reinforced the importance of pesticides influence on conidial germination, since those fungal structures are responsible for the occurrence of the first disease foci in the field. This author also emphasized that germination inhibition compromises the efficiency of the pathogen, either for those already present in the agro-ecosystem or for those applied by overflow, associated or not to pesticide products. Duarte

et al. (1992) pointed out the importance in considering the antagonistic effect of the pesticides on all developmental phases of entomopathogenic fungi since these products may affect the bio-insecticide potential as well as may reduce the occurrence of epizootics. The knowledge of the compatibility between entomopathogenic fungi and pesticides may facilitate the choice of these products in Integrated Pest Management (IPM) programs where the fungus is an important pest control agent (Neves *et al*. 2001).

This study aimed to evaluate the *in vitro* effect of acaricides on the entomopathogenic fungus *B*. *bassiana* at different stages of development.

Material and Methods

Entomopathogenic Fungus. Embrapa – Recursos Genéticos located in Brasília, Federal District, provided the strain CG424 of *B*. *bassiana*. Conidium of this strain were stored at -4°C (in Eppendorf tubes) and subsequently grown and multiplied on PDA (Potato-Dextrose-Agar) medium (25°±1°C; 12h photophase). Conidia produced on PDA were used for germination, vegetative growth and sporulation studies.

Pesticides. Information on active ingredient, trade name, formulation, chemical group and recommended doses of the acaricides as well as the doses used (ppm) are shown on Table 1. For compatibility tests, the acaricides were used in three different concentrations: mean concentration (MC), half MC and twice the MC. For MC determination, the arithmetic mean was computed using the various concentrations of the same acaricide recommended by the manufacturers for field application on different crops, diluted in 100 liters of water per ha.

Conidial Germination. For conidial germination tests, the acaricides, in the pre-established concentration, were diluted in sterile distilled water amended with 0.02% Tween 20 and 1 ml of *B*. *bassiana* conidia suspension, standardized to $1x10^6$ conidia/ml. Sixty minutes after components mixture, 0.5 ml aliquots of each suspension was transferred to four 9-cm culture plates (petri dishes) containing solidified water-agar medium and smeared with the aid of a Drigalsky loop. The same aliquot of sterile distilled water standard spore suspension and 0.02% Tween 20, without the acaricides, was used as control. There were four replications (plates) for each treatment. Culture plates were then randomly transferred to an incubator (25 ± 1°C; 12h photophase) for 24h. After incubation, lines were drawn on the external bottom surface of each plate, dividing them into four quadrants, in which approximately 100 germinated or non-germinated conidia/quadrant were counted under light microscope. Data were used to compute percent germinated or non-germinated conidia.

Vegetative Growth and Conidia Production. Standard PDA medium was autoclaved at 120°C and 1.5 atm for 20 min, cooled to 40 ± 5°C and amended with 0.5 g/L streptomycin. The acaricides, in the pre-established concentrations, were then added. Approximately 20 ml of each one of these amended media was poured into eight 9 cm culture plates. The same amount of streptomycin-amended medium without the acaricides was used as control. After media solidification, each plate was inoculated with *B*. *bassiana* conidia using the three points method (Alves *et al*. 1998b). The plates were then randomly transferred to an incubator (25 ± 1°C; 12h photophase) for eight days. After incubation, the diameter of colonies in each culture plate was determined with the aid of a sliding caliper, measuring three times each colony in different directions. The means of the values obtained were used to compute the area of each colony, using the formula pR^2.

For conidia production evaluations, one 15 mm disk was cut with a cork borer from the center of 10 (replications) fully sporulated *B*. *bassiana* colonies per treatment. Disks were then separately transferred to flat bottom glass vials (8.40 cm tall x 2.30 cm diameter), suspended in 10 ml of a 0.02% Tween

Table 1. Formulations (acaricides) used *in vitro* studies of compatibility with the entomopathogenic fungus *B. bassiana* (strain CG424).

Trade name	Active ingredient	Formulation	Chemical group	MC[1]	ppm
Citrex	Clorfenapyr	CS[2]	Pyrrole	47 ml	470
Dimetoato	Dimethoate	CE	Organophosphate	280 ml	2800
Kendo	Fenpyroximate	CS	Pyrazole	100 ml	1000
Ofunack 400	Pyridaphenthion	CE	Organophosphate	140 ml	1400
Parsec	Amitraz	CE	Formamidine	175 ml	1750
Partner	Fenbutatin oxide	CS	Organostanic	60 ml	600
Peropal	Azocyclotin	WP	Organostanic	100 ml	1000
Rufast 50	Acrinathrin	CS	Pyrethroid	10 ml	100
Sanmite	Pyridine	CE	Pyridazinone	62,5 ml	625
Savey	Hexythiazox	WP	Carboxamide	3 g	30
Sipcatin 500	Cyhexatin	CS	Organostanic	50 ml	500
Vertimec 18	Abamectin	CE	Avermectin	65 ml	650

[1]Mean concentration of commercial product for application in 100 liters of water per ha
[2]CS - concentrate solution; CE - concentrate emulsion; WP - wetable powder

20-amended sterile distilled water and agitated until complete conidia release. Conidia suspensions were subsequently diluted and quantified using a Neubauer chamber.

A completely randomized experimental design was used in all experiments. Data were submitted to ANOVA and means were compared by Tukey's multiple range test ($P \leq 0.05$) using SAS (Statistical Analytical System, inc. 1998). The statistical correlation was estimated by means of Pearson analysis.

Results

All acaricides tested caused different levels of inhibition on germination, vegetative growth and sporulation of *B. bassiana* (Table 2). These levels were mainly dependent on the chemical nature of the compounds as well as on the concentrations used.

Conidial germination was significantly affected by all acaricides used, independently of concentration (Table 2). The formulation with Febutatim Oxide, Azocyclotin, Cyhexatin and Dimethoate drastically reduced (>91%) *B. bassiana* conidial germination at the three concentrations (MC, half MC and twice the MC). The formulations with Pyridaphethion and Clorfenapyr, similarly, induced high reduction (>88%) at MC and twice the MC concentrations. These two formulations, together with Pyridaben caused, respectively, 83.9%, 78.6% and 70.6% reduction on conidial germination at half MC concentration. The formulations with Amitraz, Fenpyroximate and Hexythiazox also caused significant reduction on conidial germination at the three concentrations used, with percent reductions ranging from 47% to 85%. Likewise, the reductions caused by the formulations with pyretroid Acrinathrin and avermectin Abamectin. In addition, Abamectin also significantly differed from the control at the three concentrations. Nevertheless, the reductions on percent germination for these two formulations were lower than 45%.

As far as the effect of the acaricides on *B. bassiana* vegetative growth is concerned, results have shown that almost all formulations studied significantly inhibited fungal development (Table 2). However, the formulation with the pyrethroid Acrinathrin at half MC induced a fungal growth 10% higher than the control treatment. Moreover, the vegetative growth inhibition induced by this formulation at twice the MC was not significantly different from the control treatment. On the other hand, the formulations with Pyridaphethion, Azocyclotin and Cyhexatin induced fungal growth inhibition higher than 89%. Dimethoate, although inducing percent growth inhibition higher than 98% at MC and twice the MC, caused only 40.3% reduction at half MC. The remaining formulations induced percent growth inhibition ranging from 21.1% (Clorfenapyr, at half MC) to 80.0% (Pyridaben, at twice the MC).

The formulations with Clorfenapyr, Fenpyroximate, Acrinathrin, Hexithioazox and Abamectin induced intermediate levels of inhibition of sporulation at all concentrations used, ranging from 69.2% (Fenpyroximate at half MC) to 29.2% (Abamectin at MC). However, only data on Clorfenapyr and Acrinathrin at half MC and Abamectin at the three concentrations, were not statistically different from each other although significantly different from the control treatment. The formulations with Dimethoate, Amitraz, Fenbutatin Oxide, and Pyridaben induced levels of sporulation inhibition higher than 75%. Whereas formulations with Pyridaphenthion, Azocyclotin and Cyhexatin totally prevented sporulation (100% reduction) at all concentrations used (Table 2).

A positive correlation ($r = 0.80$) between vegetative growth and sporulation was observed, indicating that the amount of conidia produced was directly dependent on colony size (Table 2). When germination and vegetative growth data were compared, a positive correlation ($r = 0.70$) could also be observed. Nevertheless, when a product presents compatibility with vegetative growth of a given fungus it almost never will show the same compatibility level with conidia germination. The formulatons with active ingredient Clorfenapyr, Fenpyroximate and Hexythiazox that induced high levels of inhibition on germination but a relatively low level of inhibition on vegetative growth confirmed this statement.

Discussion

Conidial germination is a very important step in pest control programs with fungi because the beginning of epizootics is conditioned to the capacity of these structures to germinate on the host. The entomopathogenic fungus success, however, depends on conidial viability (Feng *et al*. 1994, Batista Filho *et al*. 1998).

In the germination tests carried out in this study a simulation of procedures used in the field for simultaneous application of pesticides and entomopathogenic fungi, was attempted. These procedures allow to evaluate more completely the effect of the chemicals on the fungus since conidia are fully exposed to the products. Boucias *et al*. (1988) used similar methodology to demonstrate that the treatment of entomopathogenic fungi conidia with chemical products, either ionic or molecular ones, may neutralize the electrostatic charge of the surface and/or remove the mucous layer covering conidia, thus affecting the substrate recognition process and the transduction of the signal that initiates germination. St. Leger & Cooper (1987) and St. Leger *et al*. (1991) have demonstrated the role of this coat removal on substrate recognition and on transduction of the signal that triggers germ tube formation in *M. anisopliae*.

The drastic inhibition induced on germination of *B. bassiana* conidia by the acaricides may be related to evidences already reported by Moore-Landecker (1982). This author has found that the presence of chemical products may block conidia metabolic functions and thus drastically affect germination. Metabolic blockage in phytopathogenic fungi conidia due to ions accumulation on the surface of the cellular membrane has also been described by Ghini & Kimati (2000). The authors reported that molecules, analogous to prosthetic groups, diffuse to the cytoplasm where they bind to specific receivers affecting membrane permeability and enzymatic synthesis, consequently affecting metabolic processes. The same authors also emphasize that organophosphate compounds

Table 2. Effect of acaricide formulations in three different concentrations, on conidia germination, vegetative growth and sporulation of the entomopathogenic fungus *B. bassiana* (strain CG424) in studies conducted on formulation-amended PDA media at 25 ± 1°C and 12h photophase.

Treatments		Germination (%) (n = 4)		Vegetative growth (mm) (n = 10)		Conidia number (x10^6 conidia per ml) (n = 8)	
		Means ± SE	% reduction	Means ± SE	% reduction	Means ± SE	% reduction
Abamectin	0,5x	62.2 ± 3.35 B	23.8	20.3 ± 0.96 D	22.7	81.3 ± 11.39 BCD	40.8
Abamectin	1x	53.9 ± 2.58 C	18.0	18.0 ± 1.00 EF	31.2	97.4 ± 10.97 B	29.1
Abamectin	2x	45.7 ± 1.99 D	44.0	16.6 ± 0.98 FGH	36.5	78.3 ± 19.00 BCD	43.0
Acrinathrin	0,5x	54.5 ± 1.07 C	33.3	28.9 ± 1.86 A	10.0	83.6 ± 8.51 BC	39.2
Acrinathrin	1x	52.6 ± 2.33 C	35.6	24.0 ± 1.56C	8.7	71.9 ± 7.70 CDE	47.7
Acrinathrin	2x	46.6 ± 0.74 D	42.9	25.4 ± 1.45 BC	3.0	65.2 ± 16.88EFG	52.6
Amitraz	0,5x	42.8 ± 1.83 D	47.6	0.2 ± 0.10 JK	49.3	34.2 ± 8.62 IJ	75.1
Amitraz	1x	12.3 ± 0.64 JKL	84.9	0.1 ± 0.06 L	58.9	24.3 ± 5.01 JK	82.3
Amitraz	2x	12.6 ± 0.54IJK	84.5	0.1 ± 0.06 L	60.4	20.9 ± 3.35 K	84.8
Azocyclotin	0,5x	2.5 ± 0.56 OPQR	96.9	11.7 ± 0.94 Q	99.2	0.0 ± 0.00 P	100.0
Azocyclotin	1x	1.9 ± 0.08 PQR	97.6	8.2 ± 1.63 Q	99.3	0.0 ± 0.00 P	100.0
Azocyclotin	2x	0.9 ± 0.10 R	98.8	5.2 ± 0.72 Q	99.5	0.0 ± 0.00 P	100.0
Clorfenapyr	0,5x	17.5 ± 1.17 HI	78.6	20.7 ± 2.43 D	21.1	82.5 ± 11.77 BCD	39.9
Clorfenapyr	1x	9.7 ± 0.31 JKLM	88.0	15.0 ± 1.85 HIJ	42.7	54.5 ± 9.25 FGH	60.3
Clorfenapyr	2x	7.9 ± 0.27 KLMN	90.2	14.6 ± 1.45 IJ	44.3	49.5 ± 5.86 GH	63.9
Cyhexatin	0,5x	2.9 ± 1.22 NOPQR	96.3	1.0 ± 0.00 OPQ	96.2	0.0 ± 0.00 P	100.0
Cyhexatin	1x	1.5 ± 0.62 QR	98.1	1.0 ± 0.00 OPQ	96.2	0.0 ± 0.00 P	100.0
Cyhexatin	2x	0.9 ± 0.10 R	98.9	1.0 ± 0.00 OPQ	96.2	0.0 ± 0.00 P	100.0
Dimethoate	0,5x	5.9 ± 0.38 MNOPQR	92.7	15.7 ± 1.01 GHI	40.3	16.8 ± 7.29 KL	87.7
Dimethoate	1x	2.0 ± 0.06 PQR	97.5	0.4 ± 0.09 PQ	98.5	0.1 ± 0.05 O	99.9
Dimethoate	2x	1.1 ± 0.23 R	98.5	0.2 ± 0.06 Q	99.2	0.1 ± 0.05 O	99.9
Fenbutatin oxide	0,5x	7.1 ± 0.43 MNO	91.3	13.3 ± 0.47 KL	55.3	9.5 ± 2.43 LM	93.0
Fenbutatin oxide	1x	3.4 ± 0.53 NOPQR	95.7	10.8 ± 0.75 M	68.8	6.7 ± 2.87 MN	95.1
Fenbutatin oxide	2x	1.5 ± 0.60 QR	98.1	10.4 ± 0.58 N	79.8	5.6 ± 1.25 MN	95.9
Fenpyroximate	0,5x	24.2 ± 0.30 FG	70.3	19.4 ± 0.73 DE	26.1	42.4 ± 10.74 HI	69.1
Fenpyroximate	1x	25.1 ± 1.12 FG	69.2	19.7 ± 0.81 DE	24.9	57.6 ± 11.40 EFG	58.1
Fenpyroximate	2x	22.6 ± 1.15 FG	72.3	17.3 ± 1.44 FG	34.3	52.8 ± 10.15 FGH	61.6
Hexythiazox	0,5x	33.7 ± 0.94 E	58.7	17.3 ± 1.38 FG	34.2	66.6 ± 18.26 CDEFG	51.5
Hexythiazox	1x	26.1 ± 0.62 F	68.0	15.4 ± 0.83 GHI	41.1	67.1 ± 8.61 CDEF	51.2
Hexythiazox	2x	20.3 ± 0.47 GH	75.1	13.6 ± 1.62 IJK	48.0	66.0 ± 12.01 CDEFG	52.0
Pyridaphenthion	0,5x	13.1 ± 0.91 IJ	83.9	2.7 ± 0.00 O	89.8	0.0 ± 0.00 P	100.0
Pyridaphenthion	1x	6.8 ± 0.82 MNOP	91.6	2.3 ± 0.00 OP	91.1	0.0 ± 0.00 P	100.0
Pyridaphenthion	2x	3.4 ± 0.52 NOPQR	95.7	2.0 ± 0.00 OPQ	92.3	0.0 ± 0.00 P	100.0
Pyridine	0,5x	24.0 ± 1.36 FG	70.6	11.7 ± 1.16 KL	55.2	6.7 ± 1.87 MN	95.1
Pyridine	1x	6.3 ± 1.47 MNOPQ	92.2	7.7 ± 1.24 M	70.6	2.7 ± 0.97 NO	98.0
Pyridine	2x	7.5 ± 0.80 LMN	90.7	5.2 ± 046 N	80.0	4.3 ± 1.48 MN	96.8
Water with Tween	--[1]	81,7 ± 0,95 A	0.0	26.2 ± 0.70 B	0.0	137.6 ± 5.66 A	0.0

[1]X= field mean dose recommendation

Means followed by different letters within each column are significantly different (P ≤ 0.05) from control treatment; Tukey's studentized range test. SE = standard error

directly interfere on cell wall formation due to inhibition of the enzyme that converts phosphatydilethanalamine into chitin. The same mechanism of inhibition was probably responsible for the drastic reduction on *B. bassiana* conidia germination, vegetative growth and sporulation observed in this study. The organophosphates as well as the organostanic formulations used in the experiments comprise metallic and semi-metallic elements in their composition, which confers to them high ionic activity.

Information on the effect of the pyrethroid Acrinathrin and the avermectin Abamectin on fungi are scarce in the literature. According to Halley *et al*. (1993) Abamectin has no significant anti-fungal activity. Results of tests conducted by AgroEvo (1998) reported selectivity of Acrinathrin to beneficial insects and natural enemies. However, no reference is made to its effect on fungi.

Another possible explanation for inhibition of conidia germination is the accumulation of chemical compounds against a concentration gradient, starting from diluted solutions, as described by Kimati (1995) for phytopathogenic fungi. In this process the compound molecules get in contact with conidia and slowly diffuse to the inner cytoplasm allowing fungal germination before achieving a lethal dose. According to the same author carboxamide compounds have their primary site of action on succinate oxidation that variably affects cellular respiration and, consequently, the metabolic activity of the fungus.

Concerning the effect of the products presently used on vegetative growth and sporulation, a significant reduction was found in relation to the control treatment. Alves *et al*. (1998), however, pointed out that results obtained *in vitro* may be more drastic then what happens under field conditions where several factors may impair the fungal exposure to the chemical compounds. Thus, when the innocuousness of a given product is determined in the laboratory, no doubts that its selectivity under field conditions will stand. On the other hand, the high toxicity *in vitro* of a given formulation may suggest similar toxicity under field conditions. However for field studies the inhibition of conidial germination should be the key factor to be considered, as discussed by Neves *et al*. (2001).

Regarding the effect of the chemical formulations studied on different developmental stages of *B. bassiana*, Abamectin and Acrinathrin presented lower toxicity than the other formulations, mainly when conidial germination was considered. Data obtained in this study may guide future recommendations of these active ingredients in IPM programs where *B. bassiana* is intended to be used as a control agent of a given mite through inundative strategy. In these cases and when the pathogen and acaricide are used in combinations, compatible products should be prefered for recommendation.

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