SIDE EFFECTS OF INSECTICIDES ON THE ENTOMOPATHOGENIC FUNGUS *METARHIZIUM ANISOPLIAE* (MERCH.) SOROKIN

Raul P. de Almeida[1]

National Center for Research on Cotton , Embrapa, P.O. Box 174
Campina Grande, Brazil, email:raul@cnpa.embrapa.br

Keywords: pesticides inhibitory effect, toxicity, entomopathogenic fungus, *Metarhizium anisopliae*

Summary

The green muscardine fungus, *Metarhizium anisopliae* (Merch.) Sorokin is used worldwide in biological control programs. In Brazil many researchers have done basic and applied studies on this entomopathogenic fungus and this has contributed to the success of the insect pests control in different crops. Here I evaluate the inhibitory effects of insecticides recommended to cotton boll weevil control on the growth and reproduction of *M. anisopliae*. Results show that deltametrhin did not affect the fungus in a negative way. In while the other insecticides influenced these characters in to some extend.

INTRODUCTION

Entomopathogenic fungi play an important role in the biological control programs in the world. The utilisation of Metarhizium anisopliae has gained attention principally due to its efficacy as a biological control agent. On the other hand the strong negative effect of chemicals insecticides have been considered a limiting factor on this fungal biocontrol agent's efficiency. Therefore, ecological measures allowing the combination of both fungal and chemical applications have been implemented in IPM programs. In addition, studies on large scale production of Metarhizium spp. have been initiated (Patel et al., 1990, Nelson et al., 1996, Alvarez et al., 1997, Singh et al., 1997, Jenkins et al., 1998). However, many factors can limit its use under field conditions. The side effects of insecticides on entomopathogenic fungi have been studied. (Ramaraje et al., 1967, Olmert & Kenneth, 1974, Ignoffo et al., 1975, Bajan et al., 1977, Alves et al., 1980).

Attempts to use fungi in integrated pest management programs against insect pests in different crops have failed because of side effects of the chemical pesticides. Gonzalez *et al.*(1996) studied the viability of the fungus *M. anisopliae* in combination with several agrochemicals. The inhibitory effect on the pathogenic fungi on pests of rice and potato was evalutated by Cadatal & Gabriel (1970) and Mietkiewski & Sapieha (1995), respectively. The effect of insecticides recommended for the control of the brown planthopper (*Nilaparvata lugens*) on fungal spores was studied by Aguda *et al.* (1984). In Brazil, *M. anisopliae* is widely used in conjunction with chemical insecticides to control cercopids on pasture grasses (Barbosa & Moreira, 1982).

In this study I determine the insecticides with the least side effects on *M. anisopliae* for incorporation in integrated pest management programs.

[1] Current address: Laboratory for Entomology, Wageningen Agricultural University, P.O.Box 8031, 6770 EH, Wageningen, THE NETHERLANDS, email: Raul.Almeida@USERS.ENTO.WAU.NL

MATERIAL AND METHODS

This study was carried out at Entomology Laboratory of Embrapa Cotton, in Paraíba State, Brazil in 1997. Climate chamber type BOD was used at 25 ± 1°C and 14:10 (L:D). The data were analysed using a completely random design with six treatments and four replications. The data were submitted to analysis of variance and the means compared by Tukey test (P≤0,01).

Five insecticides (g.a.i./ha): deltamethrin CS 50 (10,0), lambdacyhalothrin EC 50 (15,0), betacyfluthrin SC 125 (10,0), metamidophos SC (300,0) e endosulfan EC 350 (700,0) were tested in vitro for their inhibitory effects on the development of *M. anisopliae* strain PL-43. The chemical products were used at concentrations equivalent to those recommended for field application considering the cotton boll weevil as target pest.

The fungus was grown in potato dextrose agar medium, transferred (disks of 0.8mm diameter) to petri dishes with insecticide plus culture media. The medium diameter of the colonies, the mycelial growth rate (mm/day) and the number of *M. anisopliae* conidia/ml of the suspension were evaluated.

RESULTS AND DISCUSSION

The inhibitory effect on the *M. anisopliae* mycelial growth is shown in Table 1. The first day after the fungus disks were placed on potato dextrose agar medium, no mycelial growth was found in any treatment. According to Gonzalez *et al.*(1996) fungistatic effect of the pesticides on the *M. anisopliae* fungus decreased 48 h after germination and the inhibition on the spore germination by endosulfan, 24 h after germination was of 96.94 and 69.75%, respectively for the commercial and half commercial doses.

From the second day the fungus growth started and ensodulfan was the insecticide most harmful to the enthomopathogenic fungus in all of the days evaluated, except for the 3rd and 6th days. The mycelial growth was highest in the control (31.50mm diameter) and lowest in endosulfan (24.00mm diameter) treatment the last day of the experiment. Alves (1978) verified that endosulfan exhibited inhibition of fungus growth. Cadatal & Gabriel (1970) and Aguda *et al.* (1984) also found inhibitory effect on sporulation.

Betacyfluthrin and metamidophos only in thirty percent of the days evaluated had a negative effect on the mycelial growth and metamidophos was the most harmful after endosulfan. No difference was found to the mean diameter of the fungus between the control, and deltamethrin and lambdacyhalothrin in all days studied, showing that these chemical products were harmless to *M. anisopliae*. Mietkiewski & Sapieha (1995) and Barbosa & Moreira, (1982) determined the same effect of deltamethrin on *M. anisopliae* in recommended and increased dose and in a dose dependent manner even at concentrations below the recommended application rates, respectively.

The use of endosulfan caused the lowest mycelial growth rate of *M. anisopliae* (Table 2). For all treatments, except endosulfan, the fungi growth peak fell between the second and third days and it was not smaller than 2.83 mm/day and from the third day on it began reduce gradually (Data no shown). For endosulfan the maximum fungal growth peak was 1.88mm on the third day. The effect of the products on the number of *M. anisopliae* conidia/ml of suspension produced was shown in Table 2. The number of conidia/ml of suspension was the smallest for endosulfan (0.25×10^7), although no differences were found when compared with lambdacyfluthrin and metamidophos. Deltamethrin and betacyfluthrin had no negative effect on the conidia yield compared with the control.

From this study the conclusion can be drawn that the insecticide deltamethrin was innocuous to *M. anisopliae*. Endosulfan was harmful and methamidophos, betacyfluthrin and lambdacyfluthrin were less harmful in decreasing order.

Table 1. *Means[1] of the medium diameter of the colony (mm/day) of M. anisopliae, strain PL-43. Paraíba State, Brazil 1997*

Treatment	Day of the evaluation								
	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Testemunha	11.75 a	15.50 a	19.00 a	21.87 a	23.13 a	25.38 a	27.63 a	30.13 a	31.50 a
deltamethrin	12.00 a	15.63 a	18.63 a	21.50 ab	23.13 a	25.38 a	26.63 a	28.13 ab	29.25 ab
lambdacyhalothrin	11.63 a	15.25 a	17.00 a	17.88 bc	19.63 a	22.13 ab	24.63 ab	26.13 abc	27.63abc
betacyfluthrin	12.00 a	15.48 a	17.25 a	19.00 ab	20.13 a	21.63 b	23.88 ab	25.00 bc	26.00 bc
methamidophos	10.75 ab	14.25 a	17.38 a	19.00 ab	21.00 a	21.88 ab	22.25 b	23.38 c	24.00 cd
endosulfan	9.38 b	11.25 a	13.00 b	14.25 c	15.25 a	16.38 c	17.75 c	18.75 d	19.63 d
F-test	10.31**	22.42**	9.78**	11.67**	12.86**	16.14**	15.95**	15.40**	14.62**
CV (%)	5.69	4.90	8.02	8.56	7.97	7.42	7.43	8.00	8.31

[1] Means followed with the same letter are not significantly different by Tukey test (P≤ 0.01)
** Significantly different by F-test (P≤ 0.01)
CV- Coefficient of variability

Table 2. *Means[1] of the mycelial growth rate (mm/day) and the conidia number/ml of suspension of M. anisopliae, strain PL-43. Paraíba State, Brazil 1997*

Treatment	Mycelial growth rate[1] (mm/day)	*M. anisopliae* conidia numbers/ml of suspension[2]
Control	2.35 a	1.74×10^7 a
Deltamethrin	2.14 ab	2.02×10^7 a
lambdacyhalothrin	1.98 abc	0.73×10^7 bc
betacyfluthrin	1.80 bc	1.44×10^7 ab
methamidophos	1.66 c	0.52×10^7 c
endosulfan	1.16 d	0.25×10^7 c
F-test	15.41**	15.62*
CV (%)	11.44	32.14

[1] Means followed with the same letter are not significantly different by Tukey test (P≤ 0.01)
[2] Means followed with the same letter are not significantly different by Tukey test (P≤ 0.05)
** Significantly different by F-test (P≤ 0.01)
* Significantly different by F-test (P≤ 0.05)
CV- Coefficient of variability

ACKNOWLEDGEMENTS

I am thankful to Richard Stouthamer for his valuable comments on the manuscript.

REFERENCES

AGUDA, R.M., SAXENA, R.C., LITSINGER, J.A. & ROBERTS, D.W. (1984). Inhibitory effects of insecticides on entomogenous fungi *Metarrhizium anisopliae* and *Beauveria bassiana*. *International Rice Research Newsletter* **9**: 6-17.

ALVAREZ, M.I.G., RIVERO, L.F.V. & COTES, A.M. (1997). Produccion masiva y preformulacion de Metarhizium spp. para el control biologico de la langosta llanera. *Revista Colombiana de Entomologia* **23**: 119-124.

ALVES, S.B. (1978). Efeito tóxico de defensivos "in vitro" sobre patógenos de insetos. Piracicaba, ESALQ. 66p. (Tese de Doutorado).

ALVES, S.B.; CARVALHO, S.M.; CARVARLHO, A.O.R. (1980). Avalição da toxicidade de alguns inseticidas sobre *Metarhizium anisopliae* (Metsch.) Sorokin. *Revista de Agricultura* **55**:133-138.

BAJAN, C.; KMITOWA, K.; WOJCIECHOWSKA, M. (1977). The effect of enolos 50 and its active substance chlorfenvinphos on growth and pathogenicity of entomopathogenic fungi. *Polish Ecological studies*.**3**: 67-77.

BARBOSA, F. R. & MOREIRA, W.A. (1982). Efeito 'in vitro' de seis inseticidas sobre Metarhizium anisopliae (Metsch.) Sorokin, 1883. *Anais-da-Sociedade-Entomologica-do-Brasil* **11**: 245-250.

CADATAL, T.D.; GABRIEL, B.P. (1970). Effect of chemical pesticides in the development of fungi pathogenic to some rice insects. *Phlippine Entomologist* **1**:379-395.

GONZALEZ, D.M.E., VALBUENA, P.B.F., RIVERA, M.A., BUSTILLO,P.A.E. & CHAVES, B. (1996). Viabilidad del hongo Metarhizium anisopliae en mezcla con agroquimicos. *Revista Colombiana de Entomologia*, **22**: 31-36.

IGNOFFO, C.M.; HOSTETTER, D.L.; GARCIA, C.; PINNELL, R.R. (1975). Sensitivity of the entomopathogenic fungus Nomurea rileyi to chemical pecticides used on soybeans. *Environmental Entomology* **4**:765-768.

JENKINS-NE; HEVIEFO-G; LANGEWALD-J; CHERRY-AJ; LOMER-CJ. (1997). Development of mass production technology for aerial conidia for use as mycopesticides. *Biocontrol News and Information* **19**: 3p.

MIETKIEWSKI-R; SAPIEHA-A. (1995). Wplyw pestycydow stosowanych w ochronie ziemniaka na wzrost grzybow owadobojczych izolowanych z gleb pol ziemmniaczanych. *Acta-Agrobotanica* **48**: 65-73.

NELSON,T.L., LOW, A., GLARE, T.R. & O'CALLAGHAN, M. (1996). Large scale production of New Zealand strains of Beauveria and Metarhizium. *Proceedings of the Forty Ninth New Zealand Plant Protection Conference*: 257-261.

OLMERT, I.; KENNETH, R.G. (1974). Sensitivity of the entomopathogenic fungus *Beauveria bassiana*, *Verticillium lecanii* and *Verticillium* sp. to fungicides and insecticides. *Environmental Entomology* **3**:33-38.

PATEL, K.C., YADAV, D.N., DUBE, H.C. & PATEL, R.J. (1990). Laboratory and mass production studies with Metarhizium anisopliae. *Annals of Biology Ludhiana* **6**:135-140.

RAMARAJE, N.V.U., GOVINDU,M.C.; SHASTRY, K.S.S. (1967). The effect of certain insecticides on the entomogenous fungi *Beauveira bassiana* and *Metarhizium anisopliae*. *Journal of the Invertebrate Pathology* **9**:398-403.

SINGH, B.S.A. (1997). Development of Metarhizium anisopliae (Metschnikoff) Sorokin (Deuteromycete: Hyphomycete) as a commercial bioinsecticide at Caroni (1975) Limited in Trinidad (1991-1996). *Proceedings of the West Indies Sugar Technologists 26th Conference:*180-186.