JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2013. Vol. **7**(1), p. 721-726

Compatibility of Entomopathogenic Fungi *Metarhizium Anisopliae (*Ascomycota: Hypocreales) with Few Pyrethroid and Organophosphate Pesticides

S. Apoorva and K. Ramaswamy*

Food Protectants and Infestation Control Department,
CSIR-Central Food Technological Research Institute, Mysore - 570 020, India.

(Received: 21 July 2012; accepted: 11 September 2012)

There has been increasing number of reports indicating wide use of entomopathogenic fungi as biological control agents against broad range of insect pests, including stored product insects. However, the success of entomopathogenic fungi in controlling insect pests varies because of unfavourable and fluctuating enviraonmental conditions and other factors. One strategy to enhance entomopathogenic fungal efficacy is a combined use of entomopathogenic fungi with sub-lethal doses of pesticides. Therefore, in the present study compatability of *Metarhizium anisopliae* (with respect to mycelia growth, spore germination and biomass production) with different concentrations of pesticides was carried out. The results demonstrate that, of the five pesticides tested, only deltamethrin at 250ppm concentration has shown 100% spore count in Sabouraud Dextrose Agar (SDA) followed by 96.73% in Potato Dextrose Agar (PDA) medium. Almost similar results were obtained when 250ppm of chloropyriphos was amended to PDA. Also, in most of the treatments addition of pesticides to Czapek Dox Agar (CDA) yielded poor percentage of spore count than SDA and PDA. Furthermore, among the five pesticides studied for compatibility of *Metarhizium anisopliae*, only phorate was more toxic than other four pesticides. Therefore, it is possible to use the other four pesticides in low concentrations (sub-lethal levels) combining with *Metarhizium anisopliae* for Integrated Pest management (IPM).

Key words: Entomopathogenic fungi, Pesticide toxicity, Fungal biomass and Compatability.

Insecticides are needed to suppress the rapidly growing pest populations in agricultural crops and other stored product insect management. These synthetic chemicals provide number of benefits in protecting the agricultural crop; but they also cause certain health hazards on human beings. As an alternative to this, entomopathogenic fungal pesticides (bio-pesticides) are long been in use, although these cannot replace the synthetic chemical pesticides. Entomopathogenic fungi are found worldwide associated to insects and mites, which attribute to the biological control of these insect pests on number of economically important agricultural crops (Van Der Geest *et.al* 2000, Carruthers and Hural, 1990). Because of their potential use as biological control agents, these organisms have been commercially developed (Alves and Pereira 1998, Mc Coy 1990, Mc Coy and Couch 1982). The success of pest control programme using fungal pathogens mainly depends on conidial survival in the field environment (Benz 1987). However, conidial survival may be affected by number of environmental factors (Furlong and Pell 1997) or in combination of fungal pesticide or chemical products used in protecting the field crops

* To whom all correspondence should be addressed.
Tel.: +91-821-2513210; Fax: +91-821-2517233;
E-mail: rama60swamy@yahoo.co.in

(Anderson and Roberts 1983, Loria et al 1983, Alves and Lecuona 1998).

Numerous studies have been made to detect influence of pesticides on entomopathogenic fungi (Olmert and Kenneth 1974, Gardner and Storey 1985 and Neves et al 2001). These studies have revealed, only the effects of the products on vegetative growth and sporulation of the fungus, without giving much attention for spore germination studies. According to Neves et al., (2001) conidial germination is more important while studying compatibility of these organisms with the synthetic chemical pesticides. They emphasized that the fungal spore germination inhibition will affect the development of the fungus in the field because, the fungal spore germination and development is responsible for the onset of disease on susceptible insect pest populations. Anderson and Roberts (1983) reported, germination is an important step in evaluating compatability of pesticides with entomopathogenic fungi in vitro. An application of incompatible pesticides with entomopathogenic fungal organism and its products may inhibit the development and reproduction of entomopathogen, and may negatively affect the efficacy of integrated pest management (IPM) program (Anderson and Roberts 1983, Daurte et al 1992, Malo 1993). If an insect pathogen needs to incorporate in to the pest management programme, it is very much essential to determine the compatibility of the test fungus with various insecticides. Information on compatibility between entomopathogenic fungi and pesticides may facilitate the choice of these products in IPM programs, in which the fungus plays an important pest control agent (Neves et al 2001). Keeping this fact in view, the present investigations were, therefore, undertaken to evaluate the compatibility of *Metarhizium anisopliae* with certain pesticides such as phorate, malathion, chloropyriphos deltamethrin and permethrin, in-vitro.

MATERIAL AND METHODS

Media preparation, culture inoculation and quantification of spores

Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA) and Czapek Dox Agar (CDA) medium procured from Hi-media Ltd., Mumbai, was autoclaved at 120±1°C for 15 minutes, cooled to 40±5°C, and then amended with 0.3g/L of streptomycin sulphate, just before use. The required concentrations of pesticides viz., 250, 500 and 1000 ppm were prepared and added to respective media, while it was warm and mixed thoroughly to get a uniform distribution of pesticides in the culture media. For control petri plates, appropriate amount of streptomycin sulphate (0.3g/L) alone was added. After solidification, 7 day old culture of *Metarhizium anisopliae* was point inoculated at the centre of the plates. Triplicate sets were then incubated at 25±1^{0}C for one week. At the end of incubation, the colony area was measured and a central disk (1cm) was drawn from each treatment to quantify the conidial concentration. For this estimation, a standard sample colony (without pesticide) area against pesticide treated colony area was chosen from control plate. Each disk was placed in a sterile test tube and conidia were suspended in 10ml of sterile water containing 0.02% Tween-80, agitated with a vortex mixer for 2-3 minutes to dislodge and disperse the aggregated conidia in the suspension. The concentration of conidia was estimated under the compound microscope (Model CX-31, Olympus, Japan) using a neubauer haemocytometer.

Biomass production

Triplicate sets of 100ml sterile potato dextrose broth (PDB) supplimented with 0.3g/L of streptomycin sulphate was dispensed into 250 ml conical flasks. To this, desired concentration of pesticides and 1ml of fungal spore suspension containing 1x10^{6}spore/ml was added aseptically. Control flasks (without pesticides) were also run along with treated flasks. All inoculated flasks were incubated at 25±0.1^{0}C for 8days in BOD incubator (Ind Lab., Chennai). At the end of incubation, the mycelial mat was separated by Whatman ™ filter paper No.1 and dried at 80±1^{0}C for over-night to achieve constant dry weight.

Disc inoculation

One ml of required concentration (250, 500, 100ppm) of different pesticides was added to the sterile petri dishes. Following which, respective mycological media (PDA, SDA and CDA about 40^0 ±5°C) was added to the plates and the plates were agitated aseptically to get a uniform distribution of the pesticides. The plates were allowed to

solidify in the laminar air flow for 30 minutes. A young fungal colony (5 days old) of *Metarhizium anisopliae* was cut with sterile cork borer (8mm dia.) and placed aseptically in the centre of each petri plate containing poisoned medium. Control plates without pesticides were also maintained. All in triplicate sets were incubated at 25±1^{0}C for one week and, the colony diameter was measured and means were recorded.

Statistical analysis

The data was analysed by two way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) for radial growth rate of fungi in the different treatments performed (Gaucula and Singh, 1984). Mean and standard error (SE) were determined for all treatments and the results were expressed as mean ± SE. A value of P<0.05 was considered statistically significant.

RESULTS

Different pesticides and their chemical name along with concentrations used are shown in Table-1. The results on vegetative growth and colony development of *Metarhizium anisopliae* treated with different pesticides (three OP compounds and two pyrethroids) on three mycological media are presented in Table-2. Of the five pesticides studied, only phorate with all three concentrations has shown significant reduction in growth against the control PDA plates; where as deltamethrin and chloropyriphos have shown significant reduction (P<0.05) on colony diameter (in cm) against the control only at 500 and 1000ppm levels. However, it is interesting to note that no significant reduction was noticed when *M. anisopliae* was treated with higher concentrations of permethrin (500 and 1000ppm). Contrary to this, when the organism was grown on SDA and CDA medium, significant reduction in colony diameter was observed with all pesticides, irrespective of the concentrations tested (Table-2). None the less, phorate has shown least compatability than other four pesticides (melathion, permethrin, deltamethrin and chloropyrifos), in all three fungal media under study.

Table-3 shows the results on biomass production of *M. anisopliae* in the liquid medium treated with different pesticides. In general, the test organism was inhibited by all five pesticides than the control, except in CDB amended with lower concentrations (250 and 500 ppm) of chloropyriphos. However, this trend was not observed with 1000 ppm of chloropyriphos amended to CDB. The test organism however, could establish fairly a good growth in PDB compared to control than other two liquid media. As such in almost all pesticide amended liquid media of SDB and CDB there was significant reduction in biomass production compared to control (Table-3). On the other hand, the decrease in biomass in pesticide amended PDB was not much. Therefore, among

Table 1. Pesticides, their IUPAC name and the concentrations used

Pesticides	IUPAC Name	Concentration (ppm)
Phorate	O,O-Diethyl-s-(ethyl thio methyl) phosphorodithioate	250 500 1000
Malathion	Diethyl 2- (dimethoxyphosphorothioyl sulphanyl) butanedioate	250 500 1000
Chloropyrifos	O,O- diethyl o-3,5,6-trichloropyridin-2-ylphosphorothioate	250 500 1000
Deltamethrin	(S)-cyano-(3-phenoxyphynyl)-methyl (1R,3R)-(2,2-dibromoethenyl)-2,2-dimethyl-cyclopropane-1-carboxylate	250 500 1000
Permethrin	3-Phenoixybenzyl(1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	250 500 1000

Table 2. Compatibility of *Metarhizium anisopliae* with different pesticides: Vegetative growth and colony development (in cm) in three mycological media (Disc Inoculation)

Treatments (PPM)	Potato Dextrose Agar (PDA)	Sabouraud Dextrose Agar (SDA)	Czapek Dox Agar (CDA)
Phorate			
250	3.30bc	3.60ab	4.20bcd
500	3.10ab	3.60ab	3.30a
1000	2.90a	3.56a	3.00a
Malathion			
250	3.50cd	3.80bcd	5.10g
500	3.00a	3.70abc	4.80efg
1000	3.30bc	3.70abc	3.90b
Chloropyrifos			
250	3.70de	3.70aqb	4.30bcd
500	3.50cd	3.60ab	4.30bcd
1000	3.30bc	3.60ab	4.10bc
Deltamethrin			
250	3.70de	3.90cd	4.50cdef
500	3.30bc	3.80bcd	4.90fg
1000	3.50cd	3.60ab	4.90fg
Permethrin			
250	3.90a	4.00d	4.60def
500	3.70de	3.80bcd	4.60def
1000	3.70de	3.50a	4.40cdef
Control	3.70de	4.50e	5.80h

Mean scores in a column with different letters are significantly different at p<0.05 by Least Significant Difference (LSD)

Table 3. Synergistic activity of different pesticides on growth and biomass production of *Metarhizium anisopliae* in three liquid media

Treatments (PPM)	Potato Dextrose Agar (PDA)	Sabouraud Dextrose Agar (SDA)	Czapek Dox Agar (CDA)
Phorate			
250	0.32a	1.69h	0.70i
500	0.39b	1.26c	0.41g
1000	0.44cd	1.07ab	0.30f
Malathion			
250	0.61h	1.27c	0.51h
500	0.51fg	1.24c	0.31f
1000	0.30a	1.01a	0.14a
Chloropyrifos			
250	0.52g	1.42f	1.39j
500	0.42bc	1.34de	1.40j
1000	0.41bc	1.1b	0.22d
Deltamethrin			
250	0.46de	1.50g	0.39g
500	0.46de	1.28cd	0.26e
1000	0.44cd	1.26c	0.17bc
Permethrin			
250	0.48ef	1.39ef	0.19c
500	0.46de	1.30cd	0.17bc
1000	0.43cd	1.3cd	0.16ab
Control	0.53g	1.87i	0.72i

Mean scores in a column with different letters are significantly different at p<0.05 by Least Significant Difference (LSD)

the three broth cultures tested, PDB is relatively more compatible than other media (SDB and CDB), although phorate was more toxic to *M.anisopliae*, among the five pesticides studied.

Data on the compatibility of different pesticides on percentage of spore counts of *M.anisopliae* on three fungal media are presented in Table-4. It has been observed that the percentage of spore count was inversely proportional to the concentration of the pesticides used. Therefore, in almost all treatments the percentage of spore counts was higher at 250 and 500ppm concentration than 1000ppm. However, the recovery of spores was 100% (which is equivalent to control), when SDA was amended with 250ppm of deltamethrin, followed by 96.73% on PDA, with the same concentration. Similarly, when PDA was amended with 250ppm of chloropyriphos, the spore counts were as high as 95.86% followed by 86.2 and 83.9% at 500 and 1000ppm, respectively. In general, the addition of pesticides to CDA had shown relatively lesser spore counts, which was particularly observed with chloropyriphos; although permethrin at 1000ppm concentration on SDA has recorded the least spore count (9.89%).

Table 4. Influence of pesticides on Percentage of spore count on different mycological media

Treatments (PPM)	Potato Dextrose Agar (PDA)	Sabouraud Dextrose Agar (SDA)	Czapek Dox Agar (CDA)
Phorate			
250	58.65e	77.47k	83.09i
500	39.06bc	51.09f	22.53cd
1000	25.07a	14.28b	16.90b
Malathion			
250	79.31gh	56.59j	73.23h
500	67.77f	45.05e	39.43f
1000	59.31e	21.97c	19.79bc
Chloropyrifos			
250	95.86i	69.23j	26.78de
500	86.20i	64.83i	19.79bc
1000	83.90hi	38.51d	11.26a
Deltamethrin			
250	96.73j	100.00l	47.88g
500	48.27d	69.78j	25.35de
1000	35.65b	59.01f	23.94cd
Permethrin			
250	75.86g	43.67cd	62.63h
500	14.83b	92.78j	80.25i
1000	35.63b	9.89a	28.57e

Mean scores in a column with different letters are significantly different at $p<0.05$ by Least Significant Difference (LSD)

DISCUSSION

Multiplication of bio-pesticidal fungus and its spore germination, is vital factors of pesticide compatibility evaluation with respect to dual interactions during integrated pest management (IPM) program. Hence evaluation studies were performed on different parameters such as vegetative growth and colony diameter, biomass production and spore counts, in determining the compatibility of *M.anisopliae* with varied concentrations of pesticides. The results of carried experiments indicated significant reduction ($P<0.05$) in colony development with most of the pesticides tested. Among the five pesticides under investigation, phorate was more toxic to the test organism than other four pesticides. Similar results were also obtained with other experiments such as fungal disc inoculation and biomass production. Other investigators have also reported significantly reduced vegetative growth and sporulation of *M.anisopliae* combined with hexaflumoron; while very less fungal inhibition was observed wih fipronil and pyriproxyfen at the concentrations of 50 and 100ppm (Marzieh et al 2010). The observed reduction in fungal growth is in agreement with the earlier report. This suggests that *M.anisopliae* was not compatible with most of the pesticides studied (both OP compounds and synthetic pyrethroids).

Viability of entomopathogenic fungal conidia and sporulation may be affected by number of environmental factors, including the strains of biopesticides and chemical products applied on the agricultural crops. In any given bio-pesticide or bio-pesticidal formulations, conidial germination is a prime factor because, unless the conidia were multiplied in the insect host tissue, the biological pesticides or their formulations (products) are ineffective. Anderson and Roberts, 1983 stated that fungal germination is important factor of pesticide compatibility evaluation with entomopathogenic fungi in IPM management. These observations were also confirmed by Alizadeh et al., 2007. If the chemical insecticide under test is compatible in vitro, there are higher chances of its selectivity under field conditions; similarly, if the fungal strain is highly toxic in vitro, it does not mean that the same phenomenon will occur in the field (Alves, et. al., 1998).

Recently, Schumacher et. al., (2012), studied in vitro effect of different concentration of pesticides viz., fipronil, imidacloprid, neemazal and amitraz against two strains of *M. anisopliae*. These authors reported that only fipronil at 200ppm concentration was moderately toxic to *M.anisopliae*. Further, they also noticed only higher concentration of pesticides, caused little inhibition on size of colony and fungal spore germination. In our findings, except phorate the other four pesticides have not shown much detrimental/toxic effects on *M.anisopliae*. The remaining four pesticides (two organo chlorine and two pyrethroid pesticides) although there was a slight inhibitory effect on the test fungus, the combined use of the fungus and insecticide cannot be completely ruled out. Therefore, it is imperative that certain insecticides have been combined at sub-lethal doses with strains of entomopathogens for achieving better control of the target insect species.

ACKNOWLEDGEMENTS

The authors wish to thank Dr.R.Ravi, Senior Technical Officer, Department of Sensory Science, CFTRI, Mysore, for analysing the statistical data.

REFERENCES

1. Alizadeh, A., Amim samih, M., Khezri, M. and Saberi Riseh, R. Compatibility of Beauveria bassiana (Bals.) Vuill. with several pesticides. *International Journal of Agriculture and Biology.,* 2007; **9**: 31-34.
2. Alves, S.B. and Lecuona R.E. Epizootiologia aplicada ao controle microbiano de insectos., p.97-170. In: *Controle microbiano de insetos.* S.B. Alves (ed.), 1898; Sao Paulo, Fealq, 1163.
3. Alves, S.B., Monino Jr. A and Almeida J.E.M. Produtos fitossanitarios e entomopatogenicos., P.289-370. In: "*Controle Microbiano de Insectos"* S.B.Alves (ed.),1998. Sao Paulo, Fealq,1163.
4. Alves, S.B and Pereira, R.M. Producao de fungos entomopatogenicos. p.845-870. In: *Controle microbiano de insetos.* 1998. S.B Alves (ed.), Sao Paulo, Fealq, 1163.
5. Anderson,T.E and Roberts D.W. Compatibility of *Beauveria bassiana* isolate with insecticide formulations used in Colarado Potato Beetle (coleopteran: *Chrysomelidae*) Control. *Journal of Economic Entomology.,* 1983; **76:** 1437-1441.
6. Benz, G. Environment, p.177-214. In:R. Epizootiology of insect diseases. Fuxa and Y.Tanada (eds.), New York, Wiley, 1987; 960.
7. Carruthers, R.I and Hural, K. Fungi as naturally occurring entomopathogens. UCLA Symposium on Molecular Cell Biology (USA)., 1990; **112:** 115-138.
8. Duarte, A., Menendez, J. M and Trigueiro, N. Estudio preliminar sobre la compatibilidad de Metarhizium anisopliae com algunos plaguicidas quimicos. *Revista Baracoa.,* 1992; **22:** 31-39.
9. Furlong, M.J and Pell, J.K. The influence of environmental factors on the persistence of *Zoophthora radicans* conidia. *J. Invertebrate Pathology*., 1997; **69**: 223-233.
10. Gacula, M.C and Singh, J. Statistical methods in food and consumers research. 1984. Academic Press, Orlando, Florida.
11. Gardner, W and Storey, G.W. Sensitivity of *Beauveria bassiana* to selected herbicides. J Economic Entomol., 1985; **78:** 1275-1279.
12. Loria. R., Galaini. S and Roberts D.W. Survival of inoculum of the entomopathogenic fungus Beauveria bassiana as influenced by fungicides. *Environ. Entomol.,* 1983; **12:**1724-1726.
13. Malo, A.R. Estudio sobre la compatibilidad del hongo *Beauveria bassaina* (Bals.) Vuill. Con formulaciones comerciales de fungicidas e insecticidas. *Revista Colombiana de Entomologia.,* 1993; **19:** 151-158.
14. Marzieh, R., Ahmad, B., Aziz, S, Hamid-Reza, P and Mehran, G. Compatibility of Metarhizium anisopliae (Ascomycota: Hypocreales) with several insecticides. *Journal of Plant Protection Research.,* 2010; **50** (1): 22-27.
15. McCoy, C.W. and Couch, T.I. Microbial control of the citrus rust mite with the mycoacaricide, Mycar ^{R}. *Fla.Entomol*., 1982; **65:** 116-126.
16. McCoy, C.W. Entomogenous fungi as microbial pesticides, p.139-159. In R.R. Baker and P.E.Dunn (eds.), *New Directions in Biological Control*. New York, Liss, 1990; 860.
17. Neves,P.M.O.J., Hirose, E., Tchujo, P.T and Moino Jr.A. Compatibility of entomopathogenic fungi with neonicotinoid insecticides. *Neotrop. Entomol*., 2001; **30**: 263-268.
18. Olmert, I. and Kenneth, R.G. Sensitivity of entomopathogenic fungi, Beauveria bassiana, Verticillium lecani, and Verticillium sp. to fungicides and insecticides. *Environ. Entomol*., 1974; **3**:33-38.
19. Schumacher, V. and Poehling, H.M. In vitro effect of pesticides on the germination, vegetative growth, and conidial production of two strains of Metarhizium anisopliae. Fungal Biology., 2012; **116**: 121-132.
20. Van Der Geest, L.P.S., Elliot, S.L., Breeuwer, J.A.J and Beerling, E.A.M., Diseases of mites. *Exp. Appl. Acarol*. 2000; **24**: 497-560.