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# Influence of Pesticides, Plant Oils and Antagonist on Entomopathogenic Fungus, *Metarhizium anisopliae* (Metsch.) Sorokin

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## ABSTRACT

Compatibility of the entomopathogenic fungus, *Metarhizium anisopliae* with representative pesticides, plant oils and a fungal antagonist were studied *in vitro*. The pesticides tested were: endosulfan, acephate, abamectin, ethion, chlorothalonil, iprodion + carbendazim, thiophanate methyl and dinocap, an antagonist, *Trichoderma harzianum* and the plant oils: coconut oil, groundnut oil, gingili oil, sunflower oil, neem oil, pongamia oil, and castor oil. Each product was tested in the laboratory at 27 ± 1°C and 65% RH, based on the recommended rates for application in the field. The products were added to PDA culture medium for growth of the entomofungal pathogen and the dual culture technique was followed in the case of *T. harzianum*. Reproductive and vegetative growth was evaluated for *M. anisopliae*. Results showed that the action of the pesticides on the vegetative growth and conidial spore formation of *M. anisopliae* varied as a function of the chemical nature of the products. Thiophanate methyl recorded maximum vegetative growth of 2.46 cm diameter, whereas the maximum conidial spore formation of $2.45 \times 10^7$ spores/ml was observed in chlorothalonil. Iprodion + carbendazim suppressed *M. anisopliae* completely. In the case of plant oils, sunflower oil yielded $5.77 \times 10^7$ spores/ml with a vegetative growth of 4.40 cm diameter indicating a synergistic effect when compared to all other treatments. However, 3.38 cm diameter and $3.4 \times 10^7$ spores/ml were recorded in the untreated control.

**Keywords:** biological control, colony diameter, compatibility, conidial spores, inhibition, mycelial growth, *Trichoderma harzianum*

## INTRODUCTION

Biological control agents in nature, especially within agricultural and horticultural ecosystems need to be protected from a wide range of lethal pesticides in order to preserve the eco-friendly biological microorganisms. Hence, it is necessary to know the action of these pesticides on the microorganism. An entomofungal pathogen, *Metarhizium anisopliae* (Metsch.) Sorokin is one such microorganism which naturally infects a wide range of insect species, pasture scarab, nuisance flies, cockroaches etc. Therefore, it is necessary to determine their compatibility and interaction with pesticides which forms an essential means for integrated pest management programs. So far several workers reported that different pesticides had selective action on the entomofungal pathogens *in vitro* (Alves 1986; Silva *et al.* 1993).

This fungi imperfecti, *M. anisopliae* had received much attention for its potential use in the control of a variety of lepidopterans (Robert and Marchal 1980) and insect pests (Vey *et al*. 1982). *M. anisopliae* is also employed worldwide to control spittle bug, *Mahanarva posticata* of sugar cane (Moscardi 1998). Although biopesticides represent approximately 1.3% of the phytosanitary products used in the world, those directed to insect control have 4.5% of the market share for insecticides (Menn and Hall 1999). Roberts and Campbell (1977), Osborne and Boucias (1985) published an extensive review on the influence of pesticides on entomofungal pathogen. In Brazil, Alves *et al.* (1998), based on the work of several authors, published several compatibility tables between chemical products and entomofungal pathogens of greater importance in the microbial control of insects, to be used for practical purposes.

During 1993, *M. anisopliae* was registered by the U.S. Environmental Protection Agency for control of nuisance flies and cockroaches. For this application, *M. anisopliae* is introduced in a patented bait-station, Bio-Path® (Agro Powder Development Inc., NJ, USA), where exposed insects can spread the fungus through direct contact.

In recent years, insect resistance to chemicals, high costs of research and development of new synthetic molecules, and technological breakthroughs on production, formulation and delivery of mycoinsecticides, have expanded the commercial success of these products (Wraight and Carruthers 1999).

Oil-based formulations are reported to increase the efficacy of entomopathogens probably by preventing conidial desiccation, increasing adhesion to the hydrophobic cuticle, spreading the inoculums over the host's body even into crevices, and possibly by interfering with the defensive nature of the cuticle (Prior *et al.* 1988). Also, an increase in conidial adhesion would be useful to bypass the first barrier to pathogenecity (Moor and Prior 1993). In addition, formulations based on oils (Thomas *et al.* 1996; Lomer *et al.* 1997; Milner *et al.* 1997) and adjuvant emulsifiable oils (Alves 1999; Bateman and Alves 2000) have proved suitable in many circumstances.

Hence, the objective of the present study was to investigate the toxic action of representative pesticides and to identify plant oils that could act as synergists for enhancing the effectiveness of *M. anisopliae*.

## MATERIALS AND METHODS

Eight commonly used pesticides in horticultural ecosystems, seven plant oils (Ganga Visalakshy *et al.* 2005, 2006a, 2006b), pesticides (Krishnamoorthy *et al.* 2007) and *Trichoderma harzianum*, an antagonist, were considered for the study to determine their influence on *M. anisopliae* growth *in vitro*. The effect of entomopathogenic

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**Table 1** Effect of different pesticides and antagonists on growth and sporulation of *Metarhizium anisopliae*.

| Chemical/control agent | Manufactures and Source | Dosage/l | Colony diameter (cumulative) (cm)* | | Spore yield/ml |
|---|---|---|---|---|---|
| | | | 7th day | 14th day | x0$^7$ |
| Endosulfan | Excel Crop Care Ltd., India | 2.00 ml | 1.63 bc | 1.73 ef | 0.58 (1.04) cd |
| Acephate | Crystal Phosphates Ltd., India | 0.75 g | 1.40 cd | 1.60 f | 1.00 (1.22) c |
| Abamectin | Crystal Phosphates Ltd., India | 0.60 ml | 2.00 a | 2.17 de | 0.35 (0.92) de |
| Ethion | Meerut Agro Chemical Industries Ltd., India | 1.00 ml | 1.83 ab | 2.73 c | 0.29 (0.89) de |
| Chlorothalonil | Syngenta Pvt. Ltd., India | 2.00 g | 1.27 d | 2.46 cd | 2.45 (1.72) b |
| Iprodion + Carbendazim | Indofil Pvt., Ltd., India | 2.00 g | 0.60 e | 0.60 g | 0.00 (0.71) e |
| Thiophanate methyl | Bayer Pvt. Ltd., India | 1.00 g | 1.83 ab | 3.97 a | 1.83 (1.53) b |
| Dinocap | Bayer Pvt. Ltd., India | 1.00 g | 1.40 cd | 1.80 ef | 0.11 (0.78) e |
| *T. harzianum* | I.I.H.R, Bangalore, India | - | 1.90 a | 1.90 ef | 0.15 (0.81) de |
| Control | - | - | 2.00 a | 3.37 b | 3.48 (1.99) a |
| CD (P=0.05%) | | | 0.26 | 0.46 | 0.25 |
| CV | | | 4.92 | 5.14 | 9.39 |
| SE ± | | | 0.03 | 0.08 | 0.06 |

* Mean of five replications
Means followed by the same letter within a column are non significant at 5%.
Figures in parentheses are square root transformed values.

fungus was determined in two ways: 1) Vigor of growth on mycelium; 2) Yield of conidial spores after the vegetative phase. The culture medium Potato Dextrose Agar (PDA) (HIMEDIA Laboratory Ltd., Mumbai, India) at 40 g/l was autoclaved at 1 atm for 20 min and the pesticides viz., endosulfan, acephate, abamectin, ethion, chlorothalonil, iprodion + carbendazim, thiophanate methyl and dinocap (**Table 1**) were added separately before solidification at approximately 45°C under aseptic condition at doses recommended by the manufacturer. Similarly, in another set of experiments, 0.2% (v/v) of plant oils viz. those of coconut (*Cocos nucifera* Linn. Fl. Zely), groundnut (*Arachis hypogaea* L.), gingili (*Sesamum indica* L.), sunflower (*Helianthus annuus*), neem (*Azadirachta indica* A. Juss), pongamia (*Pongamia glabra* Vent. Jard. Malm.) and castor (*Ricinus communis* L.) were amended separately to PDA before solidification at a temperature of approximately 45°C under aseptic conditions. The amended media was then poured into 9 cm in diameter Petri dishes, five dishes per pesticide/plant oil and were maintained in a BOD incubator (Newtronic Equipment Company, Mumbai, India) at 27±1°C, 65% RH and a 16:8 h photoperiod. Un-treated PDA medium served as the control. Thus, pesticides and plant oils were considered as separate treatments, and each was replicated five times. Moreover, the experiment was repeated once to ensure reproducibility.

## Vigor of mycelial growth

Isolate of *M. anisopliae* cultured on PDA for a period of 7 days. Actively growing mycelium was cut into 6 mm discs of *M. anisopliae* using a sterile cork borer. The cut blocks (mother culture) were inverted and transferred on to the center of the pesticide/plant oil + PDA amended Petri dishes using a sterilized inoculum loop and placed gently on the surface of the media and dishes were maintained in germination chambers (B.O.D, Newtronic Equipment Company, Mumbai, India) at 27±1°C, 65% RH and a 16:8 h photoperiod. A dual culture technique followed in the case of the *T. harzianum* to study the antagonistic effect on *M. anisopliae* (Morton and Straube 1995) in which a 6 mm diameter disc of *T. harzianum* was cut from a week-old stock culture grown on PDA and transferred on to the pesticide-amended PDA plate 2 cm away from the rim. A similar size disc of *M. anisopliae* was transferred on to the same plate and held 4 cm away from the *T. harzianum* disc. Further, the growth of *M. anisopliae* was determined by measuring the total growth of *M. anisopliae* (in diameter) from the transferred block at the 7th and 14th day after inoculation.

For evaluating the compatibility with *M. anisopliae*, the colony size of each microorganism (vegetative growth) and the number of conidia (reproductive growth or sporulation) were considered. The vegetative growth of the colonies was measured with a common ruler by measuring in two directions and calculating the mean for the two measurements.

## Yield of conidial spores

For counting the number of conidia grown 14 days after inoculation, the colonies were cut out together with the culture medium and transferred to wide rimmed test tubes (8.5 cm high × 2.5 cm in diameter) containing 10 ml sterile distilled water plus Triton X100, mixed well, centrifuged at 7000 rpm for 5 min and diluted with 1 ml of sterile water (with an adhesive spreader). Diluted microorganism suspensions were counted using an improved Neubauer double ruled haemocytometer and phase contrast microscope at 600X magnification. Compatibility was finally decided based on the production of conidial spores.

## Statistical procedures

The experiment was conducted in a completely randomized design (CRD). The data was expressed in centimeters of mycelial growth and spore yield expressed as the number of spores per milliliter. Mean colony sizes in each treatment were submitted to analysis of variance (one-way ANOVA) followed by mean separation by the Student Newman-Keul's test (p=0.05). All analyses were performed using the SAS (1996) package.

# RESULTS AND DISCUSSION

## Vigor of mycelial growth

The data showed that the pesticides and antagonist produced a varied level of inhibitory effect on mycelial growth of the fungus (**Table 1**). During the first week, mycelia of *M. anisopliae* grew to a maximum colony diameter of 2.0 cm in abamectin which is on par with the unamended control (2.0 cm). Ethion and thiophanate methyl were the next most effective pesticides which allowed *M. anisopliae* fungus to grow up to 1.83 cm diameter. Endosulfan recorded less growth (1.63 cm), while acephate and dinocap further reduced the mycelial growth to 1.40 cm. Chlorothalonil recorded the minimum mycelial growth (1.27 cm). However, iprodion + carbendazim (a combination of two fungicides - Quintol®) was observed to be the most toxic chemical recording no increase in mycelial growth.

However, in the second week, even though the maximum mycelial growth in terms of cumulative growth of colony diameter of 3.97 cm was observed with thiophanate methyl, it was significantly more than the 3.37 cm recorded in the control (**Table 1**). This was followed by 2.73, 2.46 and 2.17 cm mycelial radial growth in ethion, chlorothalonil and abamectin treatments, respectively, but all were on par with each other. Toxicity appeared to carry through to the second week with a colony diameter of 1.8, 1.73 and 1.6 cm for dinocap, endosulfan and acephate, respectively. Iprodion + carbendazim was the most toxic treatment and never allowed the mycelium to grow or develop.

Similarly, from the second set of experiments it is evident that most of the plant oils showed a significant interaction with the fungus, *M. anisopliae* over both weeks (**Table 2**). During the first week, sunflower and coconut oils

**Table 2** Effect of different plant oils on the mycelial growth of and sporulation of *Metarhizium anisopliae*.

| Treatment | Colony diameter (cumulative) (cm)* | | Spore yield/ml × $10^7$ |
|---|---|---|---|
| | 7th day | 14th day | |
| Sunflower oil | 2.40 a | 4.40 a | 5.77 a |
| Groundnut oil | 1.40 f | 2.50 e | 1.44 e |
| Coconut oil | 2.10 b | 3.80 b | 4.33 b |
| Gingili oil | 1.13 g | 1.40 f | 1.03 e |
| Castor oil | 1.87 cd | 2.90 d | 2.83 d |
| Neem oil | 1.70 e | 3.40 c | 4.00 b |
| Pongamia oil | 1.90 cd | 2.76 d | 1.33 e |
| Control | 2.00 bc | 3.38 c | 3.40 c |
| CD (P=0.05%) | 0.15 | 0.24 | 0.55 |
| CV | 4.64 | 4.20 | 10.62 |
| SE ± | 0.05 | 0.07 | 0.18 |

* Mean of five replications
Means followed by common letters within a column are non significant at 5%.

enhanced fungal mycelial growth (2.40 and 2.10 cm, respectively) compared to the control (2.00 cm). Castor and pongamia oils, however both inhibited mycelial growth, but not significantly (1.90 cm). Minimum mycelial growth of 1.40 and 1.13 cm was recorded in groundnut and gingili oil, respectively. A similar trend was observed during the second week where sunflower oil- and coconut oil-amended PDA enhanced mycelial growth (4.40 and 3.80 cm, respectively) compared to the unamended control (3.38 cm). Other plant oils, viz. those of groundnut, gingili, castor and pongamia caused a reduction in (i.e. inhibited) mycelial growth (2.50, 1.40, 2.90 and 2.76 cm, respectively). In the case of neem oil-amended PDA, the mycelial growth was inhibited over the first week, but in the second week it was insignificantly different to the control.

The above data show that there was a significant interaction among pesticides and antagonist with the entomopathogenic fungus, which resulted in the inhibition of growth of *M. anisopliae*. The action of the pesticides on the vegetative growth of the fungus varied as a function of the chemical nature of the products. Among fungicides, the iprodion + carbendazim interaction had a significant lethal effect on the entomopathogenic fungus since even after two weeks of association it produced 100% inhibition of mycelial growth. A similar observation was found by Loureiro *et al*. (2002) with iprodione when used alone and therefore, these fungicides are considered to be the most toxic and are incompatible. All the treatments except for thiophanate methyl, inhibited the fungus both in the first and second week.

In contrast, the interaction of the antagonist *T. harizianum* with the entamopathogenic fungus led to a highly significant level of suppression of mycelial growth as there was no further growth of mycelia after the first week. Such suppressive behavior of *T. harzianum* on entomopathogenic fungus, *M. anisopliae* in the present study is similar to the observations of Krauss *et al.* (2004) with *M. anisopliae*. A similar suppressing property was observd for *T. harizianum* and *T. viridae* towards *M. anisopliae* (Ganga Visalakshy *et al.* 2006b). Thus, it is appeared from present study, that the antagonist *T. harzianum* is more dominant than the *M. anisopliae* although both behave as mycoparasites.

## Yield of conidial spores

There was a significant difference observed in the yield of conidial spore caused by pesticides (**Table 1**) and plant oils (**Table 2**). The pesticides chlorothalonil and thiophanate methyl recorded maximum spore yield of 2.45 and 1.83 × $10^7$ conidial spores/ml respectively. Nevertheless a spore yield of 1.00, 0.58, 0.35, 0.29 and 0.11 × $10^7$ conidial spores/ml was recorded in acephate, endosulfan, abamectin, ethion and dinocap, respectively. All treatments were resulted in significantly less spore yield compared to untreated control with a spore yield of 3.4 × $10^7$ conidial spores/ml. However, conidial production was found to the totally inhibited in the presence of iprodion + carbendazim. Similar, Krishnamoorthy *et al*. (2007) observed the complete inhibition of mycelial growth and spore production in *Lecanicillium lecanii* in presence of iprodion + carbendazim *in vitro*. In case of antagonist, *T. harzianum* treated plates (dual cultured) recorded a spore yield of 0.15 × $10^7$ conidial spores/ml.

Of all the plant oils sunflower oil reported the maximum spore yield of 5.77 × $10^7$ conidial spores/ml followed by 4.33 × $10^7$ conidial spores/ml in coconut oil and neem oil 4.0 × $10^7$ conidial spores/ml as against 3.4 × $10^7$ conidial spores/ml in un-treated control. However, castor oil, groundnut oil, pongamia oil and gingili oil has yielded less conidial spores of 2.83 × $10^7$, 1.44 × $10^7$, 1.33 × $10^7$and 1.03 × $10^7$ /ml respectively. In a similar kind of study by Ganga Visalakshy *et al.* (2006b) reported *T. harzianum* and *T. viride* significantly inhibited the mycelial and as well the conidial spore yield compared *M. anisopliae* alone. Ibrahim *et al*. (1999) studied that, the conidia of *M. anisopliae* formulated in oils increasingly germinated over the surface of insect and plant cuticles compared with aqueous formulations. In another study, conidia of *M. anisopliae* var. *acridum*, strain CG 423 formulated in soybean oil and kerosene were sprayed under field conditions against the gregarious grasshopper. Significant reduction in population size i.e. 65.8% and 80.4% respectively was observed (de Faria *et al*. 2002).

## REFERENCES


**Alves RT** (1999) Development of mycoinsecticide formulations and application techniques appropriate for pest control. PhD thesis, University of London, UK, 225 pp

**Alves SB** (Ed) (1986) Fungos entomopatogênicos. In: *Controle Microbiano de Insetos*, Manole, São Paulo, Brazil, pp 73-126

**Alves SB, Moino A Jr., Almeida JEM** (1998) Produtos fitossanitários e entomopatógenos. In: Alves SB (Ed) *Controle Microbiano de Insetos*, FEALQ, Piracicaba, Brazil, pp 217-238

**Bateman RP, Alves RT** (2000) Delivery systems for mycoinsecticides using oil-based formulations. *Aspects of Applied Biology* **57**, 163-170

**de Faria MR, Magalhães BP, Alves RT, Schmidt FGV, da Silva JBP, Frazão H** (2002) Effect of two dosages of *Metarhizium anisopliae* var. *acridum* against *Rhammatocerus schistocercoides* Rehn. *Pesquisa Agropecuária Brasileira* **37 (11)**, 1531-1539

**Ganga Visalakshy PN, Krishnamoorthy A, Manoj Kumar A** (2005) Effects of plant oils and adhesive stickers on the mycelial growth and conidiation of *Verticillium lecanii*, a potential entomopathogen. *Phytoparasitica* **33 (4)**, 367-369

**Ganga Visalakshy PN, Krishnamoorthy A, Manoj Kumar A** (2006a) Compatability of plant oils and additives with *Paecilomyces farinosus*, a potential entomopathogenic fungus. *Journal of Food, Agriculture and Environment* **4 (1)**, 333-335

**Ganga Visalakshy PN, Manoj Kumar A, Krishnamoorthy A** (2006b) Compatability of plant disease antagonists *Trichoderma harzianum* Rifai, *Trichoderma viride* Pers. Fr. with entomopathogenic fungi of horticultural crop pests. *Entomon* **31 (2)**, 1-4

**Ibrahim L, Butt TM, Beckett A, Clark SJ** (1999) The germination of oil formulated conidia of the insect pathogen, *Metarhizium anisopliae. Mycological Research* **103**, 901-907

**Krauss U, Hidalgo E, Arroyo C, Piper SR** (2004) Interaction between the entomopathogens *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* and the Mycoparasites *Clonostachys* spp., *Trichoderma harzianum* and *Verticillium lecanii*. *Biocontrol Science and Technology* **14**, 331-346

**Krishnamoorthy A, Ganga Visalakshy PN, Manoj Kumar A, Mani M** (2007) Influence of some pesticides on entomopathogenic fungus, *Lecanicillium* (=*Verticillium*) *lecanii* (Zimm.) ZARE & GAMES. *Journal of Horticultural Science* **2 (1)**, 53-57

**Lomer CJ, Prior C, Kooyman C** (1997) Development of *Metarhizium* spp. for the control of grasshoppers and locusts. *Memoirs of the Entomological Society of Canada* **171**, 265-286

**Loureiro E de S, Moino Jr. A, Arnosti A, de Souza GC, Loureiro E de S, de Souza GC** (2002) Effect of chemical products used in lettuce and chrysanthemum on entomopathogenic fungi. *Neotropical Entomology* **31**, 263-269

**Menn JI, Hall FR** (Eds) (1999) Biopesticides: present status and future prospects. In: *Biopesticides: Use and Delivery*, Humana, Totowa, pp 1-10

**Milner RJ, Baker GL, Hooper GHS, Prior C** (1997) Development of a mycoinsecticide for the Australian plague locust. In: Krall S, Peveling R, Diallo

DB (Eds) *New Strategies in Locust Control*, Birkhäuser-Verlag, Basel, pp 177-183

**Moor D, Prior C** (1993) The potential of mycoinsecticides. *Biocontrol News Information* **14**, 31-40

**Morton DJ, Straube WH** (1995) Antagonistic and Stimulatory effect of microorganisms upon *Sclerotium rolfsii*. *Phytopathology* **45**, 417-420

**Moscardi F** (1998) Utilização de vírus entomopatogênicos em campo. In: Alves SB (Ed) *Controle Microbiano de Insetos*, FEALQ, Piracicaba, Brazil, pp 509-539

**Osborne LS, Boucias DG** (1985) A review of chemical antagonists to mycopathogens of citrus root weevils. *Florida Entomologist* **68**, 409-416

**Prior C, Jollands P, le Patourel G** (1988) Infectivity in oil and water formulations of *Beauveria bassiana* (Deuteromycotina:Hyphomycetea) to the cocoa weevil *Pantohytes plutus* (Coleopteran:Cucurculionidae). *Journal of Invertebrate Pathology* **52**, 66-72

**Robert P, Marchal M** (1980) Use of *Plutella maculipennis* larvae as insect pests for entomopathogenic hypomycetes (*Metarhizium* sp., *Beauveria* sp. *and Nomuraea* spp.). *Entomophaga* **25**, 83-89

**Roberts DW, Campbell AS** (1977) Stability of entomopathogenic fungi. *Miscellaneous Publications of the Entomological Society of America* **10**, 19-75

**SAS** (1996) SAS/STAT User's Guide, Version 6.12. SAS Institute Inc., Cary NC, USA

**Silva L, Silva RFP, Heineck MA** (1993) Avaliação "*in vitro*" do efeito de diferentes inseticidas sobre a esporulação do fungo *Nomuraea rileyi* (Farlow) Samson. *Anais da Sociedade Entomologica da Brasil* **22**, 99-103

**Thomas MB, Langewald J, Wood SN** (1996) Evaluating the effects of a biopesticide on populations of the variegated grasshopper, *Zonocerus variegatus*. *Journal of Applied Entomology* **33**, 1509-1516

**Vey A, Farques J, Robert P** (1982) Histological and ultra-structural studies of factors determining the specificity of pathotypes of the fungus *Metarhizium anisopliae* for scarabaeid larvae. *Entomophaga* **24**, 387-397

**Wraight SP, Carruthers RI** (1999) Production, delivery, and use of mycoinsecticides for control of insect pests on field crops. In: Menn JI, Hall FR (Eds) *Biopesticides: Use and Delivery*, Humana Press, Totowa, pp 233-269