Original Research Article

Compatibility of Entomopathogenic Fungi, *Metarhizium anisopliae* with Pesticides

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ABSTRACT

The present investigation was conducted to test the compatibility of pesticides on entomopathogenic fungi, *Metarhizium anisopliae*. The *in vitro* toxicity of five insecticides(Fipronil 5% SC, Chlorpyriphos 20% EC, Imidacloprid + Fipronil 80% WG, Imidacloprid 17.8% SL, Chlorantraniliprole 0.4% G), two fungicides (Hexaconazole 5% SC and Propiconazole 25% EC) at different concentration for their effect on colony diameter, growth inhibition (%) and spore yield of *M. anisopliae* by standard poison food technique. Compatibility of *M. anisopliae* revealed that among the various insecticides tested with *M. anisopliae* Fipronil 5% SC and Chlorantraniliprole 0.4% G showed highly compatible with least percent growth inhibition with highest spore yield. Chlorpyriphos 20% EC, Hexaconazole 5% SC and Propiconazole 25% EC showed cent percent inhibition and were most detrimental to the entomopathogen.

Keywords
Compatibility, *Metarhizium anisopliae*, Pesticides etc.

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Introduction

Entomopathogenic fungi are important natural biological control agents of many insects, including several pests (Carruthers and Hural, 1990). In Integrated Pest Management (IPM) programs accomplishment of entomopathogens, is one of a technique that should be considered as an important pest reduction factor. The use of incompatible insecticides may affect the growth, development and germination of these pathogens, affecting IPM (Anderson and Roberts, 1983). One of the adverse effects of insecticides is killing the non-target organisms which also feed on the pests, such as entomopathogenic fungi. However, it is very likely that interaction might have occurred between pesticides and entomopathogenic fungi (Akbar et al., 2012).
On the other hand, the utilization of selective pesticides in association with pathogens can increase the efficiency of control, allowing the reduction of the amount of applied pesticides, minimizing environmental contamination hazards and the expression of pest resistance. The objective of this study was to evaluate the in vitro fungi toxic effect (selectivity /compatibility) of some newer insecticides and pesticides in relation to the entomopathogenic fungus *M. anisopliae*, an important natural control agent. Thus, the present study will be useful to generate some information regarding compatibility of fungal bio-pesticide with some newer pesticides and increasing the effectiveness of bio-pesticides for managing the pest.

**Materials and Methods**

An experiment was carried out in the post graduate laboratory of Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad. The statistical design used was factorial completely randomised design with control (FCRD) with seven treatments and three replications. Three different concentrations of insecticides and fungicides (Table.1) commonly used in the field for the control of white grubs and diseases were used at their recommended field doses to check their compatibility with the entomopathogenic fungi.

Five insecticides and two fungicides were evaluated by standard poison food technique (Moorhouse *et al.*, 1992) in Potato Dextrose Agar (PDA) medium for the colony growth and germination of *M. anisopliae*. Requisite quantity of insecticides was incorporated into the melted sterile PDA media (60 ml) in flask before solidification to get desired concentration aseptically, thoroughly mixed, poured into the 3 Petri plates of 9 cm diameter sterile petri dishes and allowed to solidify under laminar flow cabinet. Small disk (5 mm dia.) cut from a 15 days old actively growing mycelium mat of *M. anisopliae* fungal be cut with sterile cork borer and placed aseptically into the centre of Petri plate containing the poison medium.

Growth medium (PDA) without insecticide but inoculated with mycelia disc served as untreated check. Each treatments were replicated thrice and incubated under room condition. The plates was sealed with Parafilm and incubated at at 27±2 °C temperature.

**Colony diameter and per cent inhibition**

Colony diameter of the fungus was measured at 7 and 10 days after inoculation and compared either standard check to measure the degree of toxicity (Nene and Thapliyal, 1997) of different pesticides used in the study. The per cent inhibition in radial growth over control was calculated by using the formula of Vincent (1947).

\[
I = \frac{C - T}{C} \times 100
\]

Where;

I = Per cent inhibition  
C = Radial growth in control  
T = Radial growth in treatment (pesticide).

After measuring the colony diameter on 10th day, 5 ml of 75 % ethanol was applied to each plate to kill the fungus and wet the conidia. The plate was then washed 10 times with 9.5 ml of 0.02 % Tween 80 and aliquot was collected in separate vials. Number of conidia in each vial was determined using standard counting methods and the average number of conidia per colony in each plate was counted to measure the rate of sporulation (Li and Holdom, 1994).
Results and Discussion

Results of the studies indicated that at 7th days after inoculation (DAI) among the different pesticides tested against *M. anisopliae*, fipronil 5% SC at ½RD, RD and 2RD resulted the least per cent inhibition of 37.57, 44.44 and 50.26 which is statistically on par with chlorantraniliprole 0.4% G with per cent inhibition of 38.62, 43.39 and 50.69, followed by imidacloprid 40%+ fipronil 40% WG with per cent inhibition of 43.92, 51.32 and 53.44. Imidacloprid 17.8% SL at 2RD recorded the highest per cent of inhibition 82.12 whereas at ½ RD and RD recorded the per cent inhibition of 56.61 and 65.61. However all the three concentrations of chlorpyriphos 20% EC, hexaconazole 5% SC and propiconazole 25% EC recorded 100 per cent growth inhibition compared to control. (Table.2 and Fig.1).

On 10th DAI fipronil 5% SC at ½RD, RD and 2RD resulted the least per cent inhibition of 30.89, 32.11 and 39.84 which is statistically on par with chlorantraniliprole 0.4% G with per cent inhibition of 32.60, 39.84 and 41.63, followed by imidacloprid 40%+ fipronil 40% WG with per cent inhibition of 35.77, 39.84 and 45.12. Imidacloprid 17.8% SL at 2RD recorded the highest per cent of inhibition 81.30 whereas at ½ RD and RD recorded the per cent inhibition of 44.72 and 67.48. Irrespective of pesticidal concentrations chlorpyriphos 20% EC, hexaconazole 5% SC and propiconazole 25% EC recorded 100 per cent growth inhibition compared to control. (Table.3 and Fig.1).

The spore yield of *M. anisopliae* when mixed with pesticides depends upon its inhibitory action on colony growth. Result varied from 0.94x10^8 to 1.41x10^8 conidia per plate. Among the different pesticides fipronil 5% SC and chlorantraniliprole 0.4% G recorded significantly higher spore yield of 1.41x10^8 and 1.23x10^8, while the lower spore yield was observed in imidacloprid 40%+ fipronil 40% WG of 1.15x10^8 followed by imidacloprid 17.8% SL of 0.94x10^8, whereas chlorpyriphos 20% EC, hexaconazole 5% SC and propiconazole 25% EC yielded no spores.

Table.1 The details of the pesticides evaluated for compatibility with *Meta rhizium anisopliae*

| no | Active ingredient | Formulation | Trade name | Dose/100 ml media in µL or g |
|----|------------------|-------------|------------|-----------------------------|
| 1  | Fipronil         | 5% SC       | Regent     | 100 200 400                 |
| 2  | Chlorpyriphos    | 20% EC      | Hyban      | 500 1000 2000               |
| 3  | Imidacloprid +  | 80 % WG     | Lesenta    | 0.0375 0.075 0.1125         |
|    | Fipronil         |             |            |                             |
| 4  | Imidacloprid     | 17.8% SL    | Confidor   | 50 100 150                  |
| 5  | Chlorantraniliprole | 0.4% G   | Ferterra   | 3 6 12                      |
| 6  | Hexaconazole     | 5% SC       | Contaf     | 50 100 200                  |
| 7  | Propiconazole    | 25% EC      | Tilt       | 50 100 200                  |
| 8  | Control          | -           | -          | -                           |

*RD = Recommended dose.
Table 2 Effect of pesticides on colony diameter and growth inhibition of *Metarhizium anisopliae* on 7th days after inoculation

| Sl. No. | Pesticides                              | Colony diameter (mm) | Growth inhibition (%) |  
|---------|-----------------------------------------|----------------------|-----------------------|
|         |                                         | 7th DAI*             |                       |
|         |                                         | 1/2RD**   | RD | 2RD | 1/2RD | RD | 2RD |
| 1       | Fipronil 5% SC                          | 19.7      | 17.5| 15.7| 37.57 (37.75) | 44.44 ab (41.87) | 50.26 a (45.15) |
| 2       | Chlorpyrifos 20% EC                     | 0.00      | 0.00| 0.00| 100 (90) d | 100 (90) d | 100 (90) c |
| 3       | Imidacloprid 40%+ Fipronil 40% WG       | 17.7      | 15.3| 14.7| 43.92 (41.50) b | 51.32 b (45.76) b | 53.44 a (46.97) a |
| 4       | Imidacloprid 17.8% SL                   | 13.7      | 10.8| 5.6 | 56.61 c (48.80) | 65.61 c (54.11) c | 82.12 b (65.00) b |
| 5       | Chlorantraniliprole 0.4% G              | 19.3      | 17.8| 15.5| 38.62 (38.41) ab | 43.39 a (41.20) a | 50.69 a (45.39) a |
| 6       | Hexaconazole 5% SC                      | 0.00      | 0.00| 0.00| 100 (90) d | 100 (90) d | 100 (90) c |
| 7       | Propiconazole 25 % EC                   | 0.00      | 0.00| 0.00| 100 (90) d | 100 (90) d | 100 (90) c |
| 8       | Control                                 | 31.5      | 31.5| 31.5| - | - | - |
| Factors |                                        | S.Em ±     | C.D. at 1%               |
| Insecticides (I) |                          | 0.42      | 1.62 |
| Concentration (C) |                                | 0.27      | 1.06 |
| IxC     |                                        | 0.74      | 2.81 |
| CV (%)  |                                        |           | 1.97 |

DAI = Days after inoculation, **RD = Recommended dose, Figures in parentheses are arc sine transformed values, means followed by the same alphabets in columns do not differ significantly (p = 0.01) by DMRT
Table 3: Effect of pesticides on colony diameter, growth inhibition and spore yield of *Metarhizium anisopliae* on 10th days after inoculation

| Sl. No. | Pesticides                      | Colony diameter (mm) | Growth inhibition (%) | Spore yield (×10^8) |
|--------|---------------------------------|----------------------|-----------------------|--------------------|
|        |                                 | 10th DAI*            | 10th DAI              |                    |
|        |                                 | ½RD | RD | 2RD | ½RD | RD | 2RD |                  |
| 1      | Fipronil 5% SC                  | 28.33 | 27.83 | 24.66 | 30.89 (33.75)^a | 32.11 (34.51)^a | 39.84 (39.13)^a | 1.41 |
| 2      | Chlorpyriphos 20% EC            | 0.00 | 0.00 | 0.00 | 100 (90)^d | 100 (90)^d | 100 (90)^d | 0.00 |
| 3      | Imidacloprid 40% + Fipronil 40% WG | 26.33 | 24.66 | 22.50 | 35.77 (36.72)^b | 39.84 (39.13)^b | 45.12 (42.20)^b | 1.15 |
| 4      | Imidacloprid 17.8% SL           | 22.66 | 13.33 | 7.66 | 44.72 (41.96)^c | 67.48 (55.23)^c | 81.30 (64.39)^c | 0.94 |
| 5      | Chelantraniliprole 0.4% G       | 27.63 | 25.33 | 23.93 | 32.60 (34.81)^ab | 38.21 (38.18)^b | 41.63 (40.18)^ab | 1.23 |
| 6      | Hexaconazole 5% SC              | 0.00 | 0.00 | 0.00 | 100 (90)^d | 100 (90)^d | 100 (90)^d | 0.00 |
| 7      | Propiconazole 25 % EC           | 0.00 | 0.00 | 0.00 | 100 (90)^d | 100 (90)^d | 100 (90)^d | 0.00 |
| 8      | Control                         | 41.00 | 41.00 | 41.00 | - | - | - | 2.01 |

**Factors**

| Insecticides (I) | S.Em. + | C.D. at 1% |
|------------------|---------|------------|
| Concentration (C)| 0.21    | 0.81       |
| I×C              | 0.56    | 2.15       |
| CV               |         | 1.57       |

*DAI = Days after inoculation, **RD = Recommended dose, Figures in parentheses are arc sine transformed values, means followed by the same alphabets in columns do not differ significantly (p = 0.01) by DMRT*
Fig 1. Effect of pesticides on colony diameter, growth inhibition and spore yield of \textit{M. anisopliaeon} 10th days after inoculation

The findings of present study is in line with the findings of Joshi \textit{et al.}, (2018) reported that at lowest concentration of chlorantraniliprole 0.4% G produced 76 per
cent of spore germination. Faraji et al., (2016) reported that imidacloprid at lower concentration significantly allowed the germination, mycelial growth and sporulation of *M. anisopliae*. Abdul et al., (1987), Asiet al., (2010) and Akbar et al., (2012) their findings reveals that chlorpyriphos was most toxic organophosphate insecticide which inhibited *Metarhizium* growth completely (Plate.1). The findings of Samson et al., (2005), Joshi et al., (2018) results are in line with outcomes of present studies, they reported that propiconazole and hexaconazole found to be highly incompatible with *M. anisopliae*.

In conclusion, fipronil 5% SC and Chlorantraniliprole 0.4% G found most compatible with *M. anisopliae*. Chlorpyriphos 20% EC, Hexaconazole 5% SC and Propiconazole 25% EC found highly incompatible.

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