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Compatibility of Entomopathogenic Fungi, *Metarhizium anisopliae* with Pesticides

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A B S T R A C T

The present investigation was conducted to test the compatibility of pesticides on entomopathogenic fungi, *Metarhizium anisopliae.* The *in vitro* toxicity of five insecticides(Fipronil5% SC, Chlorpyriphos20% EC, Imidacloprid + Fipronil80 % 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* Fipronil5% SCandChlorantraniliprole0.4% G showed highly compatible with least per cent growth inhibition with highest spore yield. Chlorpyriphos 20% EC, Hexaconazole 5% SC and Propiconazole 25 % ECshowed cent per cent 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 Para film 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 0.94×10^8 to 1.41×10^8 conidia per plate. Among the different pesticides fipronil 5% SC and chlorantraniliprole 0.4% G recorded significantly higher spore yield of 1.41×10^8 and 1.23×10^8. while the lower spore yield was observed in imidacloprid 40% + fipronil 40% WG of 1.15×10^8 followed by imidacloprid 17.8% SL of 0.94×10^8, 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 *Metarhiziumanisopliae*are here under

no	Active ingredient	Formulation	Trade name	Dose/100 ml media in µL or g		
				½ RD*	RD	2RD
1	Fipronil	5% SC	Regent	100	200	400
2	Chlorpyriphos	20% EC	Hyban	500	1000	2000
3	Imidacloprid + Fipronil	80 % WG	Lesenta	0.0375	0.075	0.1125
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*			7th DAI		
		½RD**	RD	2RD	½RD	RD	2RD
1	Fipronil 5% SC	19.7	17.5	15.7	37.57 (37.75)[a]	44.44 (41.87)[ab]	50.26 (45.15)[a]
2	Chlorpyriphos 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 (45.76)[b]	53.44 (46.97)[a]
4	Imidacloprid 17.8% SL	13.7	10.8	5.6	56.61 (48.80)[c]	65.61 (54.11)[c]	82.12 (65.00)[b]
5	Chlorantraniliprole 0.4% G	19.3	17.8	15.5	38.62 (38.41)[ab]	43.39 (41.20)[a]	50.69 (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		
I×C		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 ($\times 10^8$)
		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	Chlorantraniliprole 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		**S.Em. +**			**C.D. at 1%**			
Insecticides (I)		0.32			1.24			
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 *M. anisopliaeon* 10th days after inoculation

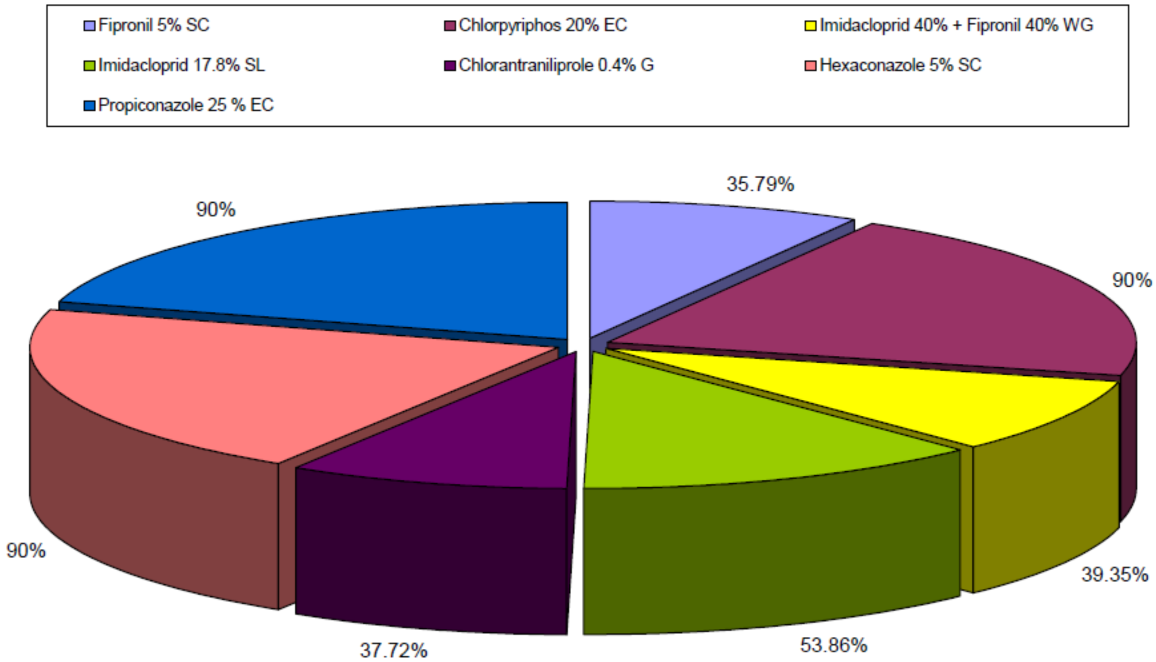
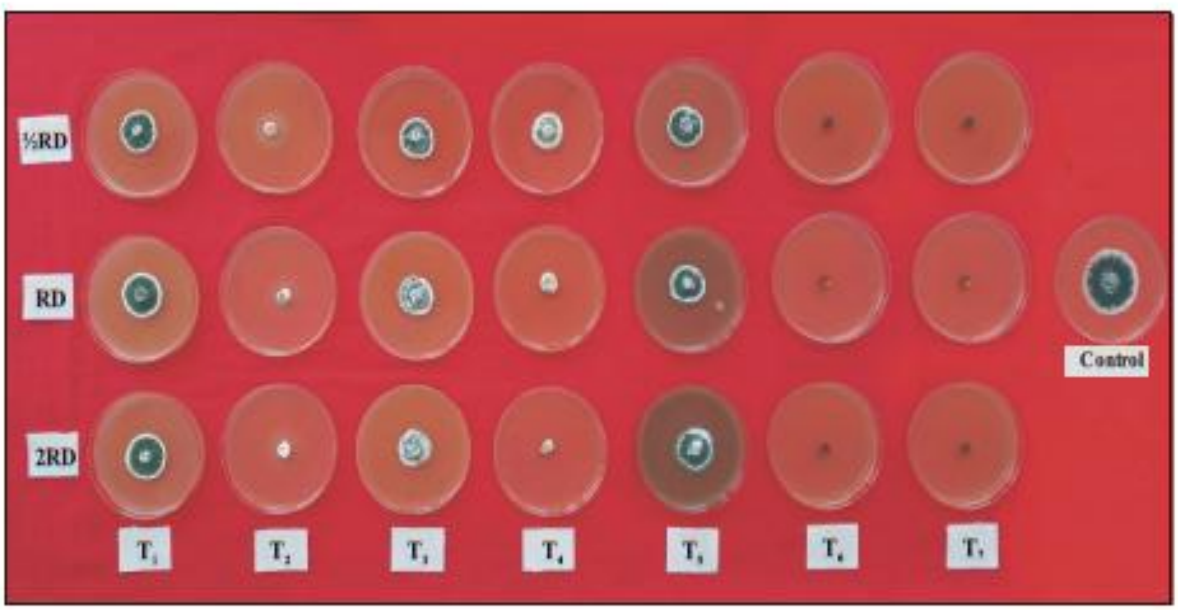


Plate.1 Compatibility of *Metarhizium anisopliae* with pesticides



The findings of present study is in line with the findings of Joshi *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), Asi*et 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*. Chlopyriphos 20% EC, Hexaconazole 5% SC and Propiconazole 25% EC found highly incompatible.

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