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BIOASSAY OF CHEMICAL INSECTICIDE AND ENTOMOPATHOGENIC FUNGI (*METARHIZIUM ANISOPLIAE*) HYBRID AGAINST COTTON APHIDS

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Abstract: *The studies were undertaken in present investigations with aim to manipulate the effects of insecticides on M. anisopliae, as well as to check the compatibility of these chemicals with M. anisopliae. The selectivity of agrochemicals to entomopathogenic fungi under laboratory conditions was determined by using Biological Index (BI) formula. The compatible insecticides and their compatible doses were evaluated against cotton aphids. The hybrid insecticides were prepared by mixing compatible chemical insecticides at their compatible doses with M. anisopliae. From the results it was observed that treatments with hybrid insecticide and alone insecticide were comparatively more effective than alone treatment of M. anisopliae (1x10^6 conidia/ml). The combination treatments of Fipronil and Thiamethoxam with M. anisopliae were numerically more effective than alone treatments of insecticides. The insecticide Fipronil was most compatible insecticide with M. anisopliae.*

Key words: *M. anisopliae, entomopathogenic fungi, insecticide.*

Introduction

Entomopathogenic fungi are important as natural control agents of many insects, including several pests. A number of examples exist where application of different selective chemical insecticides and fungi when used in combination, provided satisfactory control against many agricultural insect pests. On the other hand, the use of nonselective or incompatible chemical pesticides may possibly have the potential to hinder the vegetative growth and development of entomopathogenic fungi adversely affecting the IPM. In some cases, compatible products may be associated with entomopathogenic fungi, increasing the control efficiency, decreasing the amount of insecticides required and minimizing the risks of environmental contamination and pest resistance expression.For this reasons, an understanding about the adverse effects of different insecticides on entomopathogenic fungi is essential.

Keeping this in mind, to avoid the possibility of developing resistance in insects against chemical insecticides in near future and resurgence of non-target pests, the studies were undertaken in present investigations with aim to manipulate the inhibitory effects of different insecticides on *M. anisopliae,* as well as to check the compatibility of these chemicals with *M. anisopliae.*

Materials and Methods

The present investigation was conducted at Plant Pathology Section, College of Agriculture, Dhule during *Kharif*, 2016. The pure cultures of *M. anisopliae* were collected from Entomology Section, College of Agriculture, Dhule and maintained on PDA slants. The standard poison food technique was followed to assay the effect of insecticide on entomopathogenic fungi using CRD with 4 replications and 8 treatments. The insecticide doses were used 1xRD, 0.75xRD, 0.50xRD and 0.25xRD (RD: Recommended dose). The insecticides were *viz.,* Imidaclopride 17.80% SL (RD@20gm a.i./ha), Fipronil 5% SC (RD@50gm a.i./ha), Thiamethoxam 25% WG (RD@50gm a.i./ha), Trizophos 40 EC (RD@400gm a.i./ha), Acetamiprid 20% SP (RD@20gm a.i./ha), Lamdacyhalothrin 5% EC (RD @12.4gma.i./ha) and Dimethoate 30% EC (RD@262.5 gma.i./ha). Separate experiment was conducted for each insecticide dose. Suitable check without poison was kept for comparison under the same condition.

The radial mycelial growth was measured on 5, 7, 9, 12 and 14 days after incubation. The conidial production rate(colony sporulation) andviability/germination were measured at 9, 12 and 14 days after incubation by placing 1.0 cm diameter central parts of poison food colonies in a test tube containing 10 ml of sterile distilled water and Tween 20 (0.02%)and conidia were quantified with haemocytometer. For the measurement of conidia viability CFU count was taken at 10^{12} dilution.

To determine the selectivity of agrochemicals to entomopathogenic fungi under laboratory conditions, the Biological Index *(BI)* formula proposed by Rossi-Zalaf*et al.* (2008) was used,

BI = [47*VG+43*SP+10*GER]/100

Where:

VG : the percentage of vegetative growth of fungal colony in relation to control

SP : the percentage of colony sporulation in relation tocontrol

GER: the percentage of conidial germination in relation to control

The values of *BI* were clustered into three categories of toxicological classification of insecticidesto the fungus *viz.,* Toxic (*BI*: 0 to 41), Moderately toxic (BI: 42 to 66) and Compatible (BI: > 66).

Bioassay of chemical insecticide and entomopathogenic fungi (*M. anisopliae*) hybrid against cotton aphids

The compatible insecticides and their compatible doses were selected for further studies against cotton aphids.The mature conidia of *M. anisopliae* were transferred in to a conical flask containing the compatible insecticide at compatible concentration. The spore concentration in this suspension was adjusted at $1x10^6$ conidia/ml and the bioassay of chemical insecticide and entomopathogenic fungi (*M. anisopliae*) was followed against cotton aphids using CRD with 3 replications and 16 treatments. The experimental details were as fallows.

Tr. No.	Treatments
T1	0.25X Imidacloprid + *MA*
T2	0.25X Fipronil + *MA*
T3	0.50X Fipronil + *MA*
T4	0.75X Fipronil + *MA*
T5	1X Fipronil + *MA*
T6	0.25X Thiamethoxam + *MA*
T7	0.50X Thiamethoxam + *MA*
T8	0.25X Imidacloprid

Tr. No.	Treatments
T9	0.25X Fipronil
T10	0.50X Fipronil
T11	0.75X Fipronil
T12	1X Fipronil
T13	0.25X Thiamethoxam
T14	0.50X Thiamethoxam
T15	*MA*
T16	Control

MA: M. anisopliae ($1x10^6$ conidia/ml) X: Recommended dose

To evaluate the hybrid pesticide against cotton aphids, the cotton plants were selected in the field of Entomology Section, College of Agriculture, Dhule. For each treatment one leaves per plants was selected and tagged. Twenty aphids population per leaves was maintained. Each treatment was replicated thrice on same plant. The insecticides and hybrid pesticides were sprayed and the observations of mortality/natality were recorded on the 3rd, 6th, 9th and 12th days after spraying with the help of hand lens.

Statistical analysis was carried out as per the procedure given by Panse and Sukhatme (1995). To compare different numerical observations, the data was statistically analyzed by using CRD.

Results and Discussion

On the basis of effect of insecticides on growth and reproduction characteristics of *M. anisoplia*e by poison food technique on PDA the compatibility of insecticides with *M. anisopliae* were tested by using Biological Index formula *(BI)* proposed by Rossi-Zalaf *et al*. (2008). From the observations presented in Table 1, the *BI* values were clustered into three categories. Among the seven insecticides tested Fipronil (0.25X, 0.50X, 0.75X and 1X) wasmost compatible insecticide to *M. anisopliae* followed by Thiamethoxam (0.25X and 0.50X) and Imidacloprid (0.25X). At recommended dose (@50gm a.i./ha)Fipronil was compatible with *M. anisopliae*. The insecticide Thiamethoxam was compatible with *M. anisopliae*up to 50% of recommended dose while, it was moderately toxic at 75% and full recommended dose (@50gm a.i./ha). But insecticide Imidacloprid was only compatible at 25% of recommended dose, it was moderately toxic at 50% and 75% of recommended dose and toxic at full recommended dose.

The insecticides *viz.,* Imidaclopride 17.80% SL (RD@20gm a.i./ha), Trizophos 40 EC (RD@400gm a.i./ha), Acetamiprid 20% SP (RD@20gm a.i./ha), Lamdacyhalothrin 5% EC (RD @12.4gma.i./ha) and Dimethoate 30% EC (RD@262.5 gma.i./ha) were toxic to *M. anisopliae* at recommended dose. The insecticides Lamdacyhalothrin, Acetamipride and Dimethoate were toxic at 75% and 50% of recommended dose. Among the seven insecticides Lamdacyhalothrin was highly toxic to *M. anisopliae* followed by Dimethoate and Acetamipride at recommended dose.

Bioassay of compatible hybrid insecticide against cotton aphids

Only compatible insecticides and their compatible doses were selected for further bioassay against cotton aphids. The data presented in Table 2 showed that after the application of all treatments the aphid population was decreased significantly over the untreated control. Also it was observed that with increased in time intervals the aphid population was decreased significantly but in some treatments it was remained constant.

At all-time intervals, it was observed that treatments with compatible hybrid insecticide and alone insecticide were comparatively more effective than alone treatment of *M. anisopliae* ($1x10^6$ conidia/ml). At all the time intervals all the treatments were at par with each other except T9 [Fipronil (0.25X)], T15 [*M. anisopliae* ($1x10^6$ conidia/ml)] and T16 [Control] at 3 days after spraying, T10 [Fipronil (0.50X)], T9 [Fipronil (0.25X)], T15 [*M. anisopliae* ($1x10^6$ conidia/ml)] and T16 [Control] at 6 days after spraying, T2 [Fipronil (0.25X) + *M. anisopliae* ($1x10^6$ conidia/ml)], T15 [*M. anisopliae* ($1x10^6$ conidia/ml)] and T16 [Control] at 9 days after spraying and T15 [*M. anisopliae* ($1x10^6$ conidia/ml)] and T16 [Control] at 12 days after spraying. While, the highest aphid population was recorded in untreated control followed by treatment T15 [*M. anisopliae* ($1x10^6$ conidia/ml)].

At 12 days after spraying, no aphid population was recorded in treatment T2 [Fipronil (0.25X) + *M. anisopliae* ($1x10^6$ conidia/ml)], T4 [Fipronil (0.75X)+ *M. anisopliae* ($1x10^6$ conidia/ml)], T6 [Thiamethoxam (0.25X) + *M. anisopliae* ($1x10^6$ conidia/ml)], T7 [Thiamethoxam (0.50X) + *M. anisopliae* ($1x10^6$ conidia/ml)] and T12 [Fipronil (1X)] but these treatments were at par with rest of all treatments except T15 [*M. anisopliae* ($1x10^6$ conidia/ml)] and T16 [Control]. These results clearly indicated that combination treatment of Fipronil (0.25X and 75X) + *M. anisopliae* ($1x10^6$ conidia/ml) and Thiamethoxam (0.25X and 0.50X) + *M. anisopliae* ($1x10^6$ conidia/ml) were numerically more effective than alone treatments of Fipronil (0.25X and 75X) and Thiamethoxam (0.25X and 0.50X). The combination treatment of Fipronil (0.25X and 75X) + *M. anisopliae* ($1x10^6$ conidia/ml) were as effective as alone treatment of Fipronil with full recommended dose @50gm a.i./ha. These results clearly indicated that the insecticide Fipronil was the most compatible insecticide with *M. anisopliae* and can be used in reduced doses upto 25% of recommended dose which was as effective as full recommended dose.

Anderson *et al*. (1989) assessed the combinations of *Beauveriabassiana* (Balsamo) Vuillemin and five insecticide with Colorado potato beetle (CPB), *Leptinotarsadecemlineata* (Say). *In vitro* tests with abamectin 0.15 emulsifiable concentrate, triflumuron 4 flowable, thuringiensin ABG-6162A (1.5% AI), and carbaryl 50 wettable powder demonstrated non significant inhibition of *B. bassiana* colony growth. Combinations of *B.bassiana* with a given insecticides were consistently more toxic than *B. bassiana* alone. This results indicate that the two treatments (*B. bassiana* and insecticide) were additive.

Pachamuthu and Kamble (2000) studied the effect of *Metarhizium anisopliae* strain ESC1 alone and in combination with sublethal doses of commercial formulations of chlorpyrifos, propetamphos and cyfluthrin on mortality of CSMA strain of German cockroach, *Blattellagermanica*(L.). Percentage of cockroach mortality resulting from insecticide + *M.anisopliae* combinations was significantly higher than insecticide alone. Purwar and Sachan (2006) were investigated the toxicity of imidacloprid and dimethoate on *Beauveriabassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin against 10-11 days old larvae of *Spilarctiaobliqua* (Walker). The combination treatments showed higher mortality response than the sole treatment of fungal conidia or the insecticide. Vijila*et al.* (2011) evaluatedmortality of rice weevil, *Sitophilusoryzae* (L.) caused by *Metarhizium anisopliae* (Metschnikoff) Sorokin alone and in combination with a neonicotinoid insecticide, acetamiprid in laboratory bioassays. Spray application of *M. anisopliae* alone at a spore concentration of 6 x 10^7 conidia/ml required 6 days to cause 10%mortality whereas 4 x 10^7conidia/ml required 8 days to cause 10% mortality.Acetamiprid alone at a concentration of 30.00 µg/dl required 96h to cause 10% mortalityand at 20.00 µg/dl required 120 h to cause 20% mortality. In combination with acetamiprid, *M. anisopliae* killed *S. oryzae*significantly faster than without acetamiprid. The *M. anisopliae* – acetomiprid combination resulted higher mortality of *S. oryzae*.

Farooq and Freed (2016)assessed the effectiveness of the entomopathogenic fungi *Metarhizium anisopliae* var. *anisopliae* (Metschnikof) Sorokin and *Isariafumosorosea*(Wize) Brown and Smith applied in combination with some synthetic insecticides against the house fly, *Muscadomestica* L. (Diptera: Muscidae). The insecticides acetamiprid and imidacloprid in combination with insect pathogenic fungi showed higher mortality. The results highlight the potential of combined use of entomopathogenic fungi and synthetic insecticides for the control of *M. domestica*.

Table 1: Toxicological classification of insecticides based on the Biological Index formula

Tr. no	Insecticide & Recommended Dose (X)	Chemical group	Toxicological classification of insecticides based on the BI formula							
			0.25X		0.50X		0.75X		1X	
			BI	Categories	BI	Categories	BI	Categories	BI	Categories
T1	Imidaclopride 17.80% SL (RD@20gm a.i./ha)	Neonecotinoid (Systemic)	67.43	C	60.20	MT	45.92	MT	41.32	T
T2	Fipronil 5% SC (RD@50gm a.i./ha)	Phenyl pyrazole (Systemic)	97.54	C	95.47	C	87.58	C	83.24	C
T3	Thiamethoxam 25% WG (RD@50gm a.i./ha)	Neonecotinoid (Systemic)	77.97	C	70.25	C	58.60	MT	51.93	MT
T4	Trizophos 40 EC (RD@400gm a.i./ha)	Organophosphate (Non-systemic)	51.16	MT	46.88	MT	44.75	MT	39.97	T
T5	Acetamiprid 20% SP (RD@20gm a.i./ha)	Neonecotinoid (Systemic)	48.16	MT	41.69	T	39.58	T	37.66	T
T6	Lamdacyhalothrin 5% EC (RD@12.4gma.i./ha)	Pyrethroid (Non-systemic)	42.67	MT	40.24	T	38.32	T	35.60	T
T7	Dimethoate 30% EC (RD@262.5 gma.i./ha)	Organophosphate (Systemic)	44.09	MT	41.23	T	39.59	T	36.24	T

Table 2: Effect of compatible insecticides and hybrid insecticides on cotton aphid

Tr. No.	Treatment	Aphid population			
		3DAS	6DAS	9DAS	12DAS
T1	Imidacloprid (0.25X) + *MA*	2.33 (1.64)	0.67 (1.05)	0.33 (0.88)	0.33 (0.88)
T2	Fipronil (0.25X) + *MA*	3.00 (1.82)	1.33 (1.29)	1.00 (1.17)	0.00 (0.71)
T3	Fipronil (0.50X) + *MA*	2.67 (1.74)	1.00 (1.17)	0.67 (1.05)	0.33 (0.88)
T4	Fipronil (0.75X) + *MA*	1.67 (1.39)	1.33 (1.29)	0.00 (0.71)	0.00 (0.71)
T5	Fipronil (1X) + *MA*	2.00 (1.56)	1.00 (1.17)	0.67 (1.05)	0.33 (0.88)
T6	Thiamethoxam (0.25X) + *MA*	1.33 (1.34)	0.67 (1.05)	0.33 (0.88)	0.00 (0.71)
T7	Thiamethoxam (0.50X) + *MA*	1.67 (1.46)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T8	Imidaclopird (0.25X)	2.67 (1.76)	1.00 (1.17)	0.67 (1.05)	0.33 (0.88)
T9	Fipronil (0.25X)	3.67 (2.04)	1.67 (1.46)	0.67 (1.05)	0.33 (0.88)
T10	Fipronil (0.50X)	3.33 (1.90)	1.33 (1.34)	0.33 (0.88)	0.33 (0.88)
T11	Fipronil (0.75X)	2.33 (1.64)	1.00 (1.17)	0.33 (0.88)	0.33 (0.88)
T12	Fipronil (1X)	2.33 (1.57)	1.33 (1.29)	0.00 (0.71)	0.00 (0.71)
T13	Thiamethoxam (0.25X)	2.00 (1.56)	1.00 (1.17)	0.33 (0.88)	0.33 (0.88)
T14	Thiamethoxam (0.50X)	2.00 (1.47)	1.00 (1.17)	0.67 (1.05)	0.67 (1.05)
T15	*M. anisopliae* ($1x10^6$ conidia/ml)	13.00 (3.67)	11.33 (3.44)	9.33 (3.13)	6.67 (2.68)
T16	Control	20.00 (4.53)	20.00 (4.53)	19.67 (4.49)	19.67 (4.49)
	S.Em.±	0.24	0.21	0.15	0.13
	C.D. at 5 %	0.68	0.59	0.44	0.37

Note: Figures in parenthesis are square root transformation ($\sqrt{n+1}$) values

X: Recommended dose DAS : Days after spraying MA: *M. anisopliae* ($1x10^6$ conidia/ml)

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