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Research article

IN-VITRO EVALUATION OF THE ENTOMOPATHOGENIC FUNGAL ISOLATES OF *METARHIZIUM ANISOPLIAE* FOR COMPATIBILITY WITH PESTICIDES, FUNGICIDES AND BOTANICALS

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ABSTRACT: Isolates of the entomopathogenic fungus *Metarhizium anisopliae* were tested for their compatibility with insecticides, fungicides and botanical pesticides, which are being used in the field, as a prerequisite for developing as mycopesticides and their use in IPM programmes. Three concentrations (0.1X, 0.5X and 1X) of each chemical were evaluated in the laboratory based on the recommended dose for field application by food poison technique. Variation in vegetative growth and sporulation of *M. anisopliae* appeared to be related to the chemical nature of the formulations, its concentration and the fungal isolates in study. M19 and M48 isolates showed compatibility with imidacloprid at 0.5X and 0.1X and with fungicide sulphur at all the concentrations tested. All the four botanicals tested were found to be compatible to all the four fungal isolates and neem gold displayed maximum tolerance, at all the concentrations. M19 displayed an enhancement in the vegetative growth with imidacloprid (2%) and HIT (2-18%). 2% increase in the spore output was also recorded by M19 with chloropyrifos and sulphur. M19 and M48 isolates demonstrated compatibility with pesticides, fungicides and botanicals as well as with a cockroach management pesticide, HIT. The two isolates of *M. anisopliae* tested emerged as prospecting candidates for use as mycopesticide component in the combined application with pesticides like imidachloprid and fungicide, sulphur as well as botanicals in the IPM programmes.

Key words: Integrated pest management, biological control, *Metarhizium anisopliae*, cockroach control.

INTRODUCTION

Growing concern among people all over the world on accumulation of pesticides in the food chains and the consequent health hazards have prompted the scientific community to seek for non-toxic and eco friendly alternatives to chemical inputs. Knowledge about compatibility of the biocontrol agents with an array of chemicals in the form of pesticides, fungicides and botanicals in the agroecosystem is a prerequisite for their deployment in the IPM programmes. Combined utilization of selective insecticides in association with fungal pathogens can increase the efficiency of control by reduction of the amount of applied insecticides, minimizing environmental contamination hazards and pest resistance (Quintela and McCoy 1998). The use of incompatible pesticides with enthomopatogenic fungal propagules and products may inhibit the development and reproduction of biocontrol agents which adversely affect the efficacy of Integrated Pest Management programme. Neves et al. (2001) pointed out the importance of condial germination in compatibility studies and emphasized that the inhibition of this initial step affect plain development of the fungus in the field. Fungal biocontrol agents and selective insecticide may act synergistically increasing the efficacy of control, allowing the lower doses of insecticides, preservation of natural enemies, minimizing environmental pollution and decreasing the likelihood of development of resistance to either agent (Ambethgar, 2009). The increased virulence by combination of the entomopathogenic fungus *M. anisopliae* with the insecticide imidacloprid against the dengue vector *Aedes aegypti* has been reported by Paula et al. (2011). Gardner and Kinard (1998) propounded imidacloprid as not only safe to conidial germination but also to mycelial growth. Mancozeb and copper oxychloride were reported to be incompatible to *M. anisopliae* and caused complete inhibition of vegetative growth and spore germination (Shafa Khan et al., 2012).

In recent years there has been an attempt to replace the synthetic insecticides with less expensive, locally available, ecologically safe and socio-friendly options including botanicals (Is-man, 2007). Jayaraj (1988) hinted the possibility of combining botanicals with microbial for enhanced efficacy against insect pests. Neem oil was reported to be moderately toxic to *M. anisoplaie* (Hirose et al., 2001). The synergism between the botanical insecticide, azadirachtin, and destruxin, a mycotoxin from *M. anisopliae* against cotton aphid, *Aphis gossypii* was reported (Fei Yi et al., 2012).

Compatibility between *M. anisopliae* and insecticides can lead to reduced use of insecticides (Quintela and Mc Coy, 1997) for cockroach control thereby reducing human exposure in the urban structures (Sanyang and Van-Emden, 1996). Enhanced lethal effect of *M. anisopliae* on *P. americana* was observed when applied in combination with chlorpyrifos (Wakil et al., 2012). Among the pyrethroid compounds, deltamethrin and cypermethrin are often used in the form of miraculous *Chinese chalk* stick, (locally named as *Lakshman rekha*), powder and liquid to ward off the kitchen insects like cockroaches (Das and Sudip, 2006). In view of discrepancy in the results obtained by various workers in the studies on compatibility of entomopathogenic fungi with commercial pesticides, fungicides and botanicals, the present investigation is devised for testing selected isolates of *M. anisopliae* for compatibility against four each of the commonly used commercial pesticides, fungicides and botanicals as a prerequisite for their deployment in IPM programme.

MATERIALS AND METHODS

Fungal culture and maintenance

Metarhizium anisopliae isolates, M20 (*M. anisopliae sensu lato*, ARSEF-1823, isolated from *Nilaparvatha lugens*), M52 (*M. robertsii* (Bischoff et al. 2009), ARSEF-2575, isolated from *Curculio caryae*), M48 (*M. anisopliae sensu lato*, ARSEF-1882, isolated from *Tibraca limbativentres*), and M19 (*M. anisopliae sensu stricto* (Bischoff et al. 2009), ARSEF-1080, isolated from *Helicoverpa zea*), obtained from ARSEF (Agricultural Research Service Collection of Entomopathogenic Fungi), Ithaca-type culture collections. The microscopic cultures were grown on SDAY medium (Sabouraud dextrose Agar with yeast extract medium) - 4% dextrose, 1% peptone, 1% Yeast extract, 2% Agar, pH 7.0, incubation of slants at 25 $\pm 1^{o}$ C. The sporulated cultures seen with green colored powdery coating on the white mycelial mat were stored at 4^{o} C. Viability of the isolates was maintained through strain passage by infecting the natural insect hosts (*Spodoptera litura* and *Periplaneata americana*). All the solvents used as medium components of the culture media were from Merck (India) ltd.

Evaluating the compatibility with fungicides, pesticides and botanicals

Pesticides, fungicides and botanicals used in the experiment and their active ingredients were detailed in table 1. The culture medium SDAY was autoclaved at 15 lbs for 20 min. and the pesticides were added before solidification at a temperature of approximately 45°C. Requisite quantity of pesticides, fungicides and botanicals were added to the medium before solidification (medium temperature 48^{o}C) at 0.1X, 0.5X and 1X doses recommended by the manufacturer (Table 1) and mixed thoroughly before pouring into petri dishes measuring 9 cm in diameter. After medium solidification, a well of 0.5mm diameter was made using a sterile cork borer, into which 40µls of conidial suspension of *M. anisopliae* at $1x10^{8}$ ml^{-1} concentration was transferred using a micropipette. For each treatment three triplicates were maintained. Controls without the toxin (pesticide, fungicide and botanical) were kept for comparison under the same condition. The dishes were maintained in an incubator at 25°C for10 days.

For evaluating compatibility with the fungi, the colony size and spore output were taken in to consideration. Diameter of the colonies was measured on the 10^{th} day with the common ruler by measuring in two directions and the mean for the two values was tabulated. Inhibition of colony growth over untreated check was worked out for the respective chemicals. For estimating spore output, 5 ml of 75 per cent ethanol was added to 10 day old culture to arrest growth and washed 10 times with 9.5 ml of 0.02 per cent Tween 80 and aliquot was collected in vials. Number of conidia of each culture was determined using heamocytometer and the average number of conidia per colony in each plate was calculated to measure the spore output (Li and Holdom, 1994). Mean colony size and mean number of conidia in each treatment was submitted to analysis of variance. Compatibility assessment was done as per Alves *et al*. (1998) using the formula:

$$T = \frac{20[VG] + 80[SP]}{100}$$

Where *T* is the corrected value of vegetative and reproductive growth for product classification, *VG* is percent vegetative growth and *SP* is percent sporulation compared to control. The T values for classification of the effect of chemical products on the fungi are as: 0 to 30 (highly toxic), 31 to 45 (toxic), 46 to 60 (moderately toxic) and >60 (compatible).

Germination assay

For evaluating the germination rates of conidia of the fungal isolates, the method followed by Bugeme et al. (2008) was adopted with slight modifications. 500µl of conidial suspension at $1x10^{8}$ conidia/ml was spread on SDA plates amended with pesticides, fungicides, botanicals at 0.1X, 0.5X and 1X and pesticides against cockroach at 0.1%, 0.5% and 1% of the recommended doses.

A sterile cover slip was randomly placed before sealing the plates with Parafilm and incubated at 25°C in complete darkness. At 24 hr post-inoculation, 1 ml formaldehyde (0.5%) was added onto the plates to halt germination and the germination counts were made from 500 spores from each plate at 40X magnification. Triplicates were maintained for each treatment.

RESULTS

Compatibility of M. anisopliae isolates with commercial pesticides

Germination rates of *M. anisopliae* isolates observed at 24 hours post inoculation containing pesticides amended in the medium at the three concentrations 0.1X, 0.5X and 1X varied from 20 – 90%. A dose dependant decrease with increasing concentration of the pesticides was observed for chloropyrifos, imidacloprid, monocrotophos and quinolphos (Figure 1a).

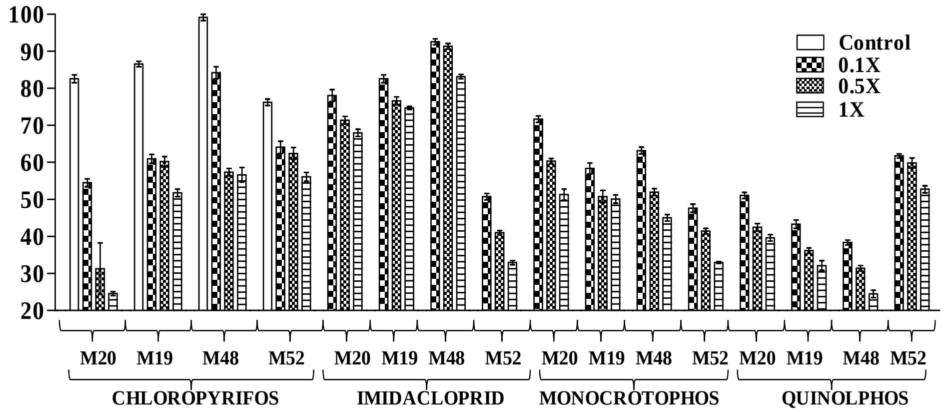

Fig-1: (a)

Compatibility and toxicity level assessment based on vegetative growth and spore output against the four pesticides revealed isolates M10, M48 and M52 to be incompatible at higher concentrations (1X). On the other hand isolate M19 displayed marginal increase (2%) in the vegetative growth and spore output at 0.1X of imidachloprid, and with chlorpyrifos at same concentration showed 2% increase in the spore output (Table 2). Isolate M20, at lower concentration (0.1X) was found to be compatible with quinolphos and imidacloprid and showed decrease in its vegetative growth up to 50% with 1X of quinolphos and graded as highly toxic. M52 has displayed compatibility with imidachloprid, monocrotophos and quinolphos only at a lower concentration of 0.1X while chlorpyrifos demonstrated high toxicity. Isolate M48 displayed compatibility with imidachloprid at at all the concentrations (Table 3). *Compatibility of M. anisopliae isolates with commercial fungicides.* With respect to commercial fungicides, the isolates M48 and M52 showed no germination at all the concentrations against bavistin and mancozeb. The germination rates ranged between 20% and 80% with 86% germination in M48 at 0.1X concentration of sulphur. On the other hand, isolates M20 and M19 recorded germination rates ranging between 60% and 75% with copper oxychloride and sulphur, while the corresponding values of these isolates revealed 24% - 68% of germination with bavistin and mancozeb at all the concentrations while for M52 they were found to be detrimental (Figure 1b).

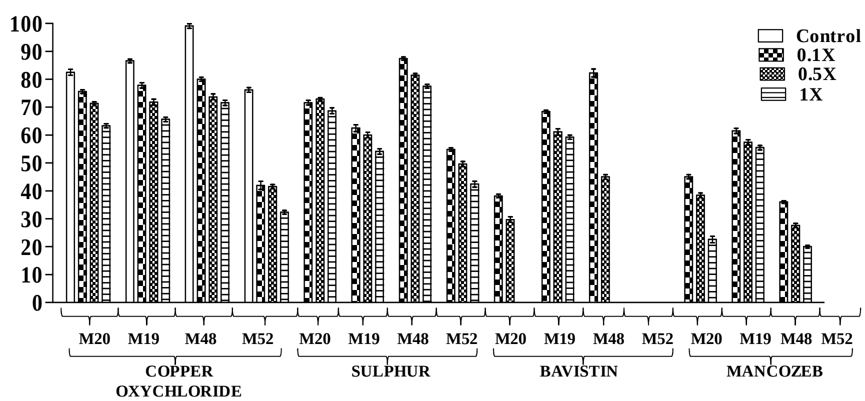

Fig-1: (b)

Among the fungicides, sulphur was found to be compatible to M19, M48 and M52 at 0.5X and 0.1X concentrations. Bavistin and mancozeb were found to be incompatible to all the fungal isolates in the study where complete inhibition in the vegetative growth and spore output (40% to 100%) was displayed reflecting their deleterious and toxic effect. Copper oxychloride, at lower concentrations, displayed compatibility to M19 and M48 where the vegetative growth ranged between 80-90%. There was an enhanced spore output of about 2% over the control as was displayed by M19 at lower concentration 0.1X of sulphur (Table 2). Bavistin and Mancozeb revealed to be deleterious to M52, in terms of vegetative as well as sporulation. Sulphur displayed compatibility for M48 at all the concentrations (Table 3).

Studies on compatibility of M. anisopliae isolates with commercial botanicals. The germination rates ranged between 28-86% among the isolates with the botanicals in the study. The maximum germination rate was revealed by M19 (86%) with neem gold at 0.1X concentration and the least by M52 (28%) with 1X of exodon. The rates of germination decreased with increase in the concentration of the botanicals (Figure 1c). Notably, M19 had displayed an increase in vegetative growth of about 4% in neem gold at 1X concentration. However, there was a decrease of 1-50% with respect to sporulation compared to that of the control. Herbastim was found to be moderately toxic to M19 with 15-20% decrease in vegetative growth and 50-60% of decrease in the sporulation (Table 2). All the botanicals tested were found to be compatible to the four isolates tested except herbastim, biospark at 1X (Table 3). *Studies on compatibility of M. anisopliae isolates with commercial pesticides used for cockroach management*

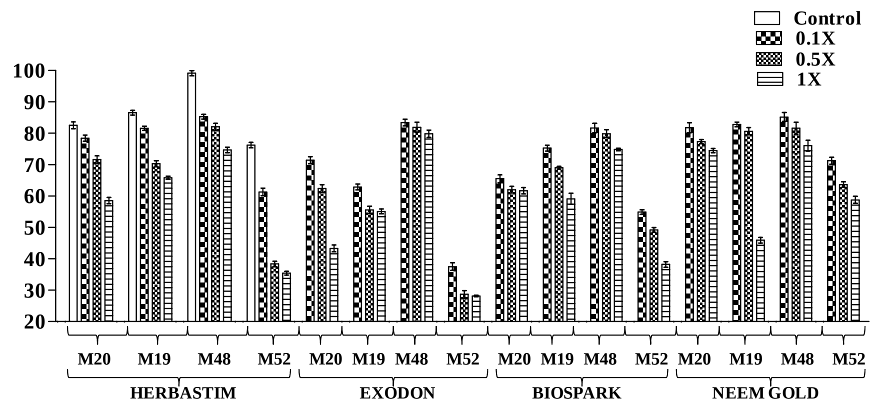

Fig-1: (c)

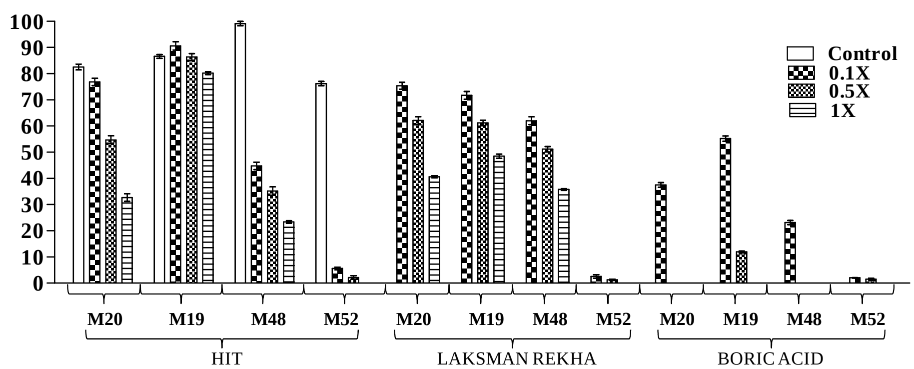

Fig-1: (d)

Figure 1. (a) Graph displaying percent (%) conidial germination of *M. anisopliae* isolates in combination with commercial pesticides, fungicides, botanicals and pesticides against cockroaches in three different concentrations at 24th hr post inoculation.

(b) Graph displaying percent (%) conidial germination of *M. anisopliae* isolates in combination with commercial fungicides in three different concentrations at 24th hr post inoculation.

(c) Graph displaying percent (%) conidial germination of *M. anisopliae* isolates in combination with commercial botanicals in three different concentrations at 24th hr post inoculation.

(d) Graph displaying percent (%) conidial germination of *M. anisopliae* isolates in combination with commercial pesticides against cockroaches in three different concentrations at 24th hr post inoculation.

The germination rates tested for the conidia of *M. anisopliae* isolates ranged from 0% to 90%. The product HIT demonstrated germination rates of 90% and 85% at 0.1% and 0.5% concentrations respectively and 71% and 60% of the germination with lakshman rekha at corresponding concentrations. Boric acid, on the other hand was found to be detrimental to all the isolates in the present study (Figure 1d). M19 displayed an increased vegetative growth of about 18%, 3.4% and 2% at 0.1X, 0.5% and 1% concentrations of HIT respectively compared to that of the control. M48 displayed no sporulation against all the pesticides tested (Table 2). HIT was found to be compatible with isolate M19 while lakshmanrekha and boric acid incompatible and detrimental to the remaining fungal isolates of the study (Table 3).

Table 1: Pesticides, fungicides, botanicals and pesticides against cockroaches with their active ingredients and chemical group.

	Active ingredient (Commercial name)	**IUPAC name**	**Chemical group (Formula)**	**RC* (per litre)**
Pesticides	Chlorpyrifos (Hilban ®)	*O*,*O*-diethyl *O*-3,5,6-trichloropyridin-2-yl phosphorothioate	Organophosphate ($C_9H_{11}C_{l3}NO_3PS$)	2 ml
	Imidacloprid (Media®)	*N*-[1-[(6-Chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl]nitramide	Chloronicotine ($C_9H_{10}ClN_5O_2$)	2 ml
	Monocrotophos (Monodhan 36)	Dimethyl *(E)*-1-methyl-2-(methylcarbamoyl)vinyl phosphate	Etylene ($C_7H_{14}N_3O_2$)	1.5 ml
	Quinalphos (Ekalux ®)	*O*,*O*-Diethyl *O*-2-quinoxalinyl phosphorothioate	Bisdithiocarbamate ($C_{12}H_{15}N_2O_3PS$)	3 ml
Fungicides	Mancozeb (Indofil® M- 45)	-	Carbon disulfide	3 gm
	Sulphur (Sulfex®)	-	Sulphur compounds	3 gm
	Copper oxychloride (Cuprogarb 500)	-	Copper compounds	2 gm
	Carbendazim (Bavistin)	Methyl *1H*-benzimidazol-2-ylcarbamate	Benzimidazole carbamate ($C_9H_9N_30_2$)	3 gm
Botanicals	- (Herbastim)	-	-	2 ml
	- (Exodos)	-	-	3 ml
	- (Biospark)	-	-	3 gm
	- (Neem gold)	-	-	2 ml
Pesticides used against cockroaches	- (HIT)	-	Cypermethrin ($C_{22}H_{19}C_{l2}N0_3$)	DA#
	Pyrethroid compund (Lakshman rekha)	Cyano-(3-phenoxyphenyl)methyl]3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate	Cypermethrin ($C_{22}H_{19}C_{l2}N0_3$)	DA#
	Boric acid (Borid)	Trihydrooxidoboron	- (H_3BO_3)	DA#

***Recommended dose, DA# Direct application**

Table 2: Effect of pesticides, fungicides, botanicals and pesticides against cockroaches in three different concentrations on vegetative growth and sporulation of the entomopathogenic fungal isolates of *M. anisopliae* in studies conducted on compatibility.

		Isolate	***CONCENTRATION***					
			0.1X		***0.5X***		***1X***	
			Vg	***So***	***Vg***	***So***	***Vg***	***So***
PESTICIDES	**Chlorpyrifos**	M20	-20.99 (±0.02)	-70.71 (±0.66)	-27.21 (±0.00)	-88.70 (±1.15)	-46.70 (±0.03)	-93.72 (±1.15)
		M19	-20.40 (± 0.02)	**+02.18 (± 2.60)**	-32.40 (±0.02)	-28.96 (± 2.40)	-29.10 (± 0.05)	-56.55 (± 3.05)
		M48	-10.96 (± 0.03)	-15.60 (± 1.15)	-41.12 (±0.04)	-48.71 (±1.76)	-46.86 (± 0.03)	-67.35 (± 1.45)
		M52	-04.93 (± 0.02)	-81.24 (± 1.20)	-27.10 (±0.09)	-100 (± 0.00)	-40.89 (± 0.04)	-100 (± 0.00)
	Imidacloprid	M20	-02.85 (± 0.03)	-45.60 (± 1.20)	-05.96 (±0.06)	-50.19 (± 0.66)	-27.47 (± 0.00)	-56.06 (± 1.76)
		M19	**+02.00 (± 0.00)**	**+56.30 (± 2.90)**	-01.10 (±0.02)	**+13.38 (± 2.60)**	-03.64 (± 0.05)	-01.09 (± 0.66)
		M48	**+07.00** (± 0.09)	50.00 (± 3.51)	-04.84 (±0.01)	-18.42 (± 2.40)	-14.55 (± 0.03)	-29.61 (± 2.90)
		M52	-13.80 (± 0.01)	-28.55 (± 1.45)	-27.34 (±0.06)	-85.79 (± 2.66)	-45.82 (± 0.05)	-91.12 (± 1.58)
	Monocrotophos	M20	-02.84 (± 0.01)	-76.15 (± 1.15)	-07.08 (±0.03)	-92.05 (± 1.85)	-34.46 (± 0.03)	-97.48 (± 0.00)
		M19	-28.40 (± 0.01)	-43.99 (± 1.20)	-40.55 (±0.03)	-74.86 (± 1.76)	-30.91 (± 0.05)	-80.60 (± 1.85)
		M48	-27.11 (± 0.03)	-55.60 (± 1.52)	-35.73 (±0.02)	-69.27 (± 1.33)	-38.96 (± 0.05)	76.50 (±1.76)
		M52	-10.60 (± 0.11)	-27.61 (± 2.30)	-28.58 (±0.09)	-33.22 (± 3.05)	-27.84 (± 0.03)	-41.68 (± 2.90)
	Quinolphos	M20	-06.47 (± 0.01)	-46.65 (± 1.64)	-36.52 (±0.02)	-58.36 (± 0.88)	-49.22 (± 0.02)	-92.26 (± 0.83)
		M19	-44.72 (± 0.04)	-59.56 (± 0.66)	-58.90 (±0.05)	-78.14 (± 1.20)	-65.45 (± 0.03)	-93.92 (± 1.76)
		M48	-47.40 (± 0.06)	-34.92 (± 3.05)	-63.02 (±0.02)	-77.80 (± 2.08)	-58.71 (± 0.10)	-81.53 (± 1.52)
		M52	-48.53 (± 0.00)	-29.21 (± 3.46)	-60.10 (±0.02)	-83.11 (± 1.73)	-50.00 (± 0.01)	-96.92 (± 0.88)
	Copper Oxychloride	M20	-09.56 (± 0.05)	-50.63 (± 0.88)	-09.81 (±0.05)	-66.74 (± 3.05)	-12.15 (± 0.01)	-88.07 (±0.57)
		M19	-09.37 (± 0.03)	-04.20 (± 0.88)	-13.33 (±0.05)	-28.39 (± 0.02)	-54.24 (± 0.02)	-61.56 (±0.00)
		M48	-05.22 (± 0.08)	-46.05 (± 1.20)	-05.76 (±0.12)	-78.59 (± 0.88)	-36.57 (± 0.04)	-90.63 (±0.33)
		M52	-17.47 (± 0.05)	-42.76 (± 0.45)	-19.90 (±0.11)	-85.93 (± 0.57)	-23.78 (± 0.02)	-95.32 (±0.09)

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		Isolate	*CONCENTRATION*					
			0.1X		***0.5X***		***1X***	
			Vg	***So***	***Vg***	***So***	***Vg***	***So***
FUNGICIDES	**Copper Oxychloride**	M20	-09.56 (± 0.05)	-50.63 (± 0.88)	-09.81 (±0.05)	-66.74 (± 3.05)	-12.15 (± 0.01)	-88.07 (±0.57)
		M19	-09.37 (± 0.03)	-04.20 (± 0.88)	-13.33 (±0.05)	-28.39 (± 0.02)	-54.24 (± 0.02)	-61.56 (±0.00)
		M48	-05.22 (± 0.08)	-46.05 (± 1.20)	-05.76 (±0.12)	-78.59 (± 0.88)	-36.57 (± 0.04)	-90.63 (±0.33)
		M52	-17.47 (± 0.05)	-42.76 (± 0.45)	-19.90 (±0.11)	-85.93 (± 0.57)	-23.78 (± 0.02)	-95.32 (±0.09)
	Sulphur	M20	-21.70 (± 0.03)	-57.32 (± 0.15)	-14.72 (±0.11)	-71.97 (± 0.40)	-15.76 (+ 0.08)	-88.28 (±0.66)
		M19	-14.24 (± 0.04)	+02.21 (± 0.76)	-20.55 (±0.04)	-11.47 (± 0.00)	-11.36 (± 0.08)	-31.69 (±0.76)
		M48	-14.77 (± 0.08)	-13.06 (± 0.40)	-17.83 (±0.10)	-42.83 (± 0.20)	-09.72 (± 0.05)	-30.85 (±0.52)
		M52	-12.62 (± 0.08)	-40.61 (± 0.33)	-18.44 (±0.06)	-73.19 (± 0.76)	-04.61 (± 0.00)	-86.20 (±0.20)
	Bavistin	M20	-46.78 (± 0.01)	-88.07 (± 0.57)	-64.35 (±0.01)	-94.96 (± 0.00)	-100 (± 0.00)	-100 (±0.00)
		M19	-11.72 (± 0.08)	-53.45 (± 0.05)	-15.86 (±0.05)	-73.03 (± 0.00)	-23.25 (± 0.05)	-100 (±0.00)
		M48	-44.00 (± 0.00)	-90.63 (± 0.33)	-71.64 (±0.00)	-94.09 (± 0.00)	-100 (± 0.00)	-100 (±0.00)
		M52	-100 (± 0.00)	-100 (± 0.00)	-100 (±0.00)	-100 (± 0.00)	-100 (± 0.00)	-100 (±0.00)
	Mancozeb	M20	-37.20 (± 0.09)	-93.12 (± 0.06)	-49.35 (±0.00)	-100 (± 0.00)	-78.29 (± 0.05)	-100 (±0.00)
		M19	-36.57 (± 0.00)	-63.04 (± 0.06)	-51.71 (±0.06)	-76.31 (± 0.00)	-73.87 (± 0.05)	-100 (±0.00)
		M48	-52.43 (± 0.03)	-83.57 (± 0.03)	-62.16 (±0.01)	-92.22 (± 0.06)	-75.49 (± 0.09)	-100 (±0.00)
		M52	-100 (± 0.00)	-100 (± 0.00)	-100 (±0.00)	-100 (± 0.00)	-100 (± 0.00)	-100 (±0.00)
	Herbastim	M20	-21.7 (± 0.00)	-40.99 (± 0.09)	-33.85 (±0.07)	-52.04 (± 0.05)	-45.73 (± 0.03)	-56.77 (±0.01)
		M19	-05.76 (± 0.01)	-29.51 (± 0.05)	-15.67 (±0.04)	-49.95 (± 0.01)	-22.16 (± 0.00)	-60.47 (±0.08)
		M48	-0.90 (± 0.08)	-18.65 (± 0.00)	-08.10 (±0.01)	-23.23 (± 0.03)	-11.71 (± 0.01)	-27.46 (±0.04)
		M52	-05.32 (± 0.09)	-24.83 (± 0.05)	-14.07 (±0.05)	-42.22 (± 0.04)	-22.33 (± 0.01)	-52.15 (±0.01)

***VG*: vegetative growth; *SO:* spore output**

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		Isolate	CONCENTRATION					
			0.1X		*0.5X*		*1X*	
			Vg	*So*	*Vg*	*So*	*Vg*	*So*
	Exodon	M20	-09.81 (± 0.05)	-30.92 (± 0.09)	-15.76 (±0.01)	-45.12 (± 0.01)	-21.25 (± 0.04)	-49.23 (±0.05)
		M19	-03.64 (± 0.05)	-12.25 (± 0.05)	-14.55 (0.55)	-21.46 (± 0.00)	-23.78 (± 0.09)	-34.50 (±0.03)
		M48	-10.27 (± 0.10)	-17.64 (± 0.07)	-16.57 (±0.02)	-25.47 (± 0.05)	-18.91 (± 0.01)	-30.40 (±0.00)
		M52	-24.75 (± 0.04)	-19.94 (± 0.04)	-30.82 (±0.09)	-32.05 (± 0.04)	-38.59 (± 0.03)	-42.15 (±0.01)
	Biospark	M20	-15.76 (± 0.01)	-24.63 (± 0.05)	-20.99 (±0.02)	-40.96 (± 0.06)	-27.21 (± 0.00)	-50.64 (±0.88)
		M19	-09.72 (± 0.05)	-10.13 (± 0.06)	-12.97 (±0.00)	-20.23 (± 0.05)	-16.39 (± 0.08)	-28.97 (±1.20)
		M48	-11.71 (± 0.01)	-14.47 (± 0.01)	-18.37 (±0.07)	-18.65 (± 0.00)	-26.12 (± 0.02)	-27.29 (±0.01)
		M52	-12.13 (± 0.03)	-25.02 (± 0.07)	-22.81 (±0.06)	-42.13 (± 0.05)	-33.25 (± 0.05)	-48.52 (±0.08)
	Neemgold	M20	-0.97 (± 0.03)	-24.68 (± 0.06)	-01.81 (±0.04)	-46.66 (± 0.09)	-01.81 (± 0.04)	-49.31 (±0.05)
		M19	**+04.86 (± 0.09)**	**+17.22 (± 0.06)**	+0.90 (±0.04)	**+14.75 (+ 0.06)**	-0.10 (± 0.08)	-01.63 (±0.01)
		M48	-07.38 (± 0.03)	-04.57 (± 0.09)	-08.28 (±0.01)	-08.47 (± 0.04)	-14.23 (± 0.08)	-12.54 (±0.03)
		M52	-02.91 (± 0.00)	-15.53 (± 0.09)	-11.89 (±0.07)	-27.60 (± 0.04)	-15.29 (± 0.03)	-37.25 (±0.03)
PESTICIDES AGAINST COCKROACH	**HIT**	M20	00.00 (±0.00)	-15.10 (±0.91)	-27.50 (± 0.35)	-52.83 (±0.15)	-36.75 (± 0.11)	-62.26 (± 0.32)
		M19	+18.00 (± 0.52)	-21.38 (± 0.75)	+03.40 (± 0.12)	-27.67 (±0.12)	+02.00 (± 0.56)	-43.39 (± 0.36)
		M48	-26.19 (± 0.32)	-100.0 (± 0.00)	-42.14 (± 0.44)	-100.0 (±0.00)	-65.00 (± 0.11)	-100.0 (± 0.00)
		M52	-	-	-	-	-	-
	Lakshman Rekha	M20	-11.75 (± 0.88)	-99.83 (± 0.86)	-20.75 (± 0.32)	-99.86 (±0.78)	-48.25 (± 0.72)	-99.88 (± 0.91)
		M19	-10.60 (± 0.01)	-9.83 (± 0.07)	-19.40 (± 0.32)	-22.13 (±0.31)	-30.60 (± 0.61)	-59.01 (± 0.76)
		M48	-11.19 (± 0.21)	-100.0 (± 0.00)	-38.80 (± 0.71)	-100.0 (±0.00)	-29.28 (± 0.39)	-100.0 (± 0.00)
		M52	-	-	-	-	-	-
	Boric acid	M20	-60.75 (± 0.41)	-99.93 (± 0.51)	-100.0 (± 0.00)	-100.0 (±0.00)	-100.0 (± 0.00)	-100.0 (± 0.00)
		M19	-38.60 (± 0.18)	-99.71 (± 0.74)	-89.40 (± 0.32)	-100.0 (±0.00)	-100.0 (± 0.00)	-100.0 (± 0.00)
		M48	-64.28 (± 0.11)	-100.0 (± 0.00)	-100.0 (± 0.00)	-100.0 (±0.00)	-100.0 (± 0.00)	-100.0 (± 0.00)
		M52	-	-	-	-	-	-

***VG*: vegetative growth; *SO*: spore output**

Table 3: The T values for classification of the effect of pesticides, fungicides, botanicals and pesticides against cockroaches on the entomopathogenic fungal isolates of *M. anisopliae*.

		ISOLATE	***CONCENTRATION***		
			0.1X	***0.5X***	***1X***
PESTICIDES	**Chlorpyrifos**	M20	T	HT	HT
		M19	MT	T	T
		M48	C	MT	T
		M52	T	HT	HT
	Imidacloprid	M20	C	MT	HT
		M19	C	C	C
		M48	C	C	C
		M52	C	HT	HT
	Monocrotophos	M20	T	HT	HT
		M19	C	MT	T
		M48	MT	T	T
		M52	C	C	MT
	Quinolphos	M20	C	MT	HT
		M19	T	HT	HT
		M48	MT	HT	HT
		M52	C	HT	HT
FUNGICIDES	**Copper Oxychloride**	M20	MT	MT	HT
		M19	C	C	T
		M48	C	T	HT
		M52	C	HT	HT
	Sulphur	M20	MT	T	HT
		M19	C	C	C
		M48	C	C	C
		M52	C	T	HT
	Bavistin	M20	HT	HT	HT
		M19	MT	T	HT
		M48	HT	HT	HT
		M52	HT	HT	HT
	Mancozeb	M20	HT	HT	HT
		M19	MT	T	HT
		M48	HT	HT	HT
		M52	HT	HT	HT
BOTANICALS	**Herbastim**	M20	C	MT	MT
		M19	C	MT	MT
		M48	C	C	C
		M52	C	C	MT
	Exodon	M20	C	C	MT
		M19	C	C	C
		M48	C	C	C
		M52	C	C	MT
	Biospark	M20	C	C	MT
		M19	C	C	C
		M48	C	C	C
		M52	C	C	MT

Contd..

	Neemgold	M20	C	C	C
		M19	C	C	C
		M48	C	C	C
		M52	C	C	C
		ISOLATE	***CONCENTRATION***		
			0.1%	***0.5%***	***1%***
PESTICIDES USED AGAINST COCKROACH	**HIT**	M20	C	MT	HT
		M19	C	C	C
		M48	HT	HT	HT
		M52	-	-	-
	Lakshman Rekha	M20	HT	HT	HT
		M19	C	C	MT
		M48	HT	HT	HT
		M52	-	-	-
	Boric acid	M20	HT	HT	HT
		M19	HT	HT	HT
		M48	HT	HT	HT
		M52	-	-	-

C: Compatible; T: Toxic; MT: Moderately toxic; HT: Highly toxic

DISCUSSION

Inconsistency prevailed in the compatibility relationship between the isolates of *M. anisopliae* and type of pesticides tested as reported by different workers. Therefore, the selected isolates of *M. anisopliae* for use as mycopesticides, require compatibility testing with insecticides, fungicides and botanical pesticides, for subsequent use in IPM programmes. Imidacloprid was found to be compatible to M19 and M48 at all the three concentrations teste and in some cases demonstrated synergistic effects. Batista Filho et al. (2001) also observed that pesticides can also act in a positive manner in combination with entomopathogens. At sub lethal doses, they interact with the latter causing or activating infectious disease by stress and making the insects more susceptible to the action of microbial infection. Shafa Khan et al. (2012) recommended imidachloprid to be highly safe and most compatible to *M. anisopliae.* On the other hand chlorpyrifos was compatible to M19 and monocrotophos against M52 isolate. Chlorpyrifos along with *M. anisopliae* at sub lethal doses was tested for mortality studies on German cockroach by Pachamuthu et al. (2000) and found a significant interaction between the entomopathogenic fungi and commercial pesticide. On the other hand, Muhammad Ramzan Asi et al. (2010**)** reported detrimental effects of chloropyrifos to *M. anisopliae*. High toxicity of Mancozeb towards all the isolates in the present study was in accordance with Duran et al. (2004) who mentioned that benomyl, dimethomorph-mancozeb, mancozeb, and mancozeb-cymoxanil mixture of fungicides significantly affect germination and growth of *B. bassiana* while fosetyl-Al, propamocarb, and copper oxychloride do not. It is interesting that one fungicide (fosetyl-aluminium) appeared to stimulate mycelial growth of *Lecanicillium longisporum*. Synergism has been identified between entomopathogenic fungi and insecticides (Shah et al., 2007). Background information about the different degrees of entomopathogenic fungi showing fungicide tolerance was reported by Maribel (2010).

With respect to botanicals, neem gold, biospark and exodon showed compatibility to all the isolates in the study and neemgold displayed synergism with M19 which was manifested by enhanced vegetative growth of the isolate when grown in combination. Sahayaraj et al. (2011) also observed that the commercial plant based pesticides were well tolerated by *B. bassiana.* Neemgold and biospark were relatively safe for combined use. Vyas et al. (1992) reported that, neemark, a biopesticide of neem was well tolerated by *M. anisopliae*. HIT and lakshmanrekha, among the pesticides used to control cockroaches, displayed compatibility with M19 while other isolates showed lack of tolerance to HIT, lakshman rekha as well as boric acid.

CONCLUSIONS

On the basis of results obtained, it was evident that the action of pesticides and fungicides on vegetative growth and sporulation of entomopathogenic fungal isolates varied as a function of the chemical nature of the products and their concentration, and isolates of *M. anisopliae* employed.

The present study demonstrated M19 and M48 isolates to be more promising for development as mycopesticides and for application along with the pesticides like imidachloprid and fungicide, sulphur in the IPM programmes. On the other hand, M19 can be used in combination with fungicides (bavistin and mancozeb), preferably in sequence with time lag either before or after the application of fungicide as the toxicity of fungicides will reduce as the day's progress after application. The same isolate, due to its compatible germination and enhanced vegetative growth along with HIT, may form effective biocontrol agent in the management of house hold pest cockroach.
However, laboratory results on artificial media may not be reproducible in the field as there will be degradation of the toxicants in the field environment and hence the effective dosage at field conditions should be studied.

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