



Assessment of Insecticide Compatibility with *Metarhizium anisopliae* for Fall Armyworm (*Spodoptera frugiperda* J.E. Smith) Management in Maize

**G Sugeetha ^a, M V Adwaitha ^a, K S Nikhil Reddy ^{a*},
P Mahadevu ^b and J Mahadeva ^c**

^a Department of Entomology, College of Agriculture, V. C. Farm, Mandya – 571405, India.

^b Department of Genetics and Plant Breeding, College of Agriculture, V. C. Farm, Mandya – 571405, India.

^c Department of Forestry and Environmental Sciences, College of Agriculture, V. C. Farm, Mandya – 571405, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The fall armyworm (*Spodoptera frugiperda*) is an invasive pest of maize, which are kept under control by mostly chemical insecticides. The environmental impact and potential for resistance development due to continuous use of insecticides has resulted in the search for alternatives for the

*Corresponding author: E-mail: vikkyvirat4@gmail.com;

management of FAW. *Metarhizium anisopliae*, an entomopathogenic fungus (EPF), is a promising biological control agent that has been recorded effective against several lepidopteran pests including *S. frugiperda*. An array of insecticides have been recommended for the management of FAW, while simultaneous studies pertaining to evaluation of EPF are made to target the pest through non chemical approaches. However, the compatibility between insecticides and the entomopathogenic fungus *M. anisopliae* has not been studied at large. The present study focuses on the compatibility of insecticides with EPF in the management of FAW. Six insecticides, representing different chemical classes, were tested at their field recommended doses for their inhibitory effects on *M. anisopliae* fungal colony growth, at various concentrations (50%, 75%, and recommended concentrations). Mycelial growth inhibition was measured at 7, 14, and 21 days after inoculation (DAI). Results showed that most insecticides exhibited varying degrees of compatibility with *M. anisopliae*, with growth inhibition ranging from 6.25% to 51.88% at 7 DAI. The lowest growth inhibition occurred with Emamectin benzoate 5 SG and Novaluron 5.25 + Emamectin benzoate 0.9 SC, showing minimal suppression of fungal growth. In contrast, Spinetoram 11.7 SC and Thiodicarb 75 WP caused significant inhibition at higher concentrations, with the latter showing up to 51.88% inhibition at 7 DAI. At 14 and 21 DAI, the growth inhibition generally decreased across treatments, with Chlorantraniliprole 9.3 + Lambda-cyhalothrin 4.6 ZC and Emamectin benzoate 5 SG demonstrating the best compatibility. Overall, the results suggest that most insecticides tested were compatible with *M. anisopliae*, with Spinetoram and Thiodicarb being exceptions, particularly at higher concentrations. This study provides valuable insights into selecting insecticide-fungus combinations for integrated pest management.

Keywords: Compatibility; fall armyworm; inhibition; *Metarhizium anisopliae*.

1. INTRODUCTION

The battle against agricultural pests in maize crop production is a perpetual struggle for farmers worldwide. Among these pests, the fall armyworm (*Spodoptera frugiperda*) stands out as a notorious pest due to its rapid spread and devastating impact on maize production. In recent years, the fall armyworm has emerged as a significant threat to global food security, causing substantial yield losses and economic hardship for farmers, particularly in regions where maize is a staple crop. It has been observed that over the initial 9 months of infestation across 10 Indian states, the Fall Armyworm (FAW) has inflicted significant damage on maize, resulting in a yield loss of 30 percent (Anon, 2024). Chemical insecticides, have been widely employed to mitigate the damage caused by the fall armyworm. However, the indiscriminate use of chemical pesticides poses environmental risks, disrupts ecological balances, and may lead to the development of resistance in pest populations (Sparks & Nauen, 2015). In this context, the integration of biological control agents, such as entomopathogenic fungi, has garnered increasing attention as a sustainable and environmentally friendly alternative for pest management. Entomopathogenic fungi are capable of infecting and killing a wide range of insect species, including the fall armyworm. Unlike chemical pesticides, which often target specific

biochemical pathways in insects, entomopathogenic fungi utilize multiple modes of action, making them less susceptible to resistance development (Alizadeh et al., 2007; Rachappa et al., 2007). Moreover, these fungi are biodegradable and pose minimal risk to non-target organisms and the environment. Despite the potential of entomopathogenic fungi in controlling the fall armyworm, their effectiveness can be influenced by various factors, including environmental conditions and interactions with other pest management strategies, such as chemical insecticides (Kachhadiya et al., 2014; Rajeshwari et al., 2020). Understanding the compatibility between insecticides and entomopathogenic fungi is crucial for optimizing integrated pest management (IPM) programs and maximizing their efficacy in controlling fall armyworm infestations. Therefore, this study aims to investigate the compatibility of different insecticides commonly used against fall armyworm with entomopathogenic fungi strains. By assessing the impact of insecticide-fungus interactions on the viability and effectiveness of entomopathogenic fungi, this research seeks to provide valuable insights into the development of more sustainable and integrated approaches for fall armyworm management in maize crops.

2. MATERIALS AND METHODS

Compatibility studies between entomopathogenic fungi and *Ad-hoc*

recommended insecticides were undertaken (Table 1). under laboratory condition by employing poisoned food technique (Moorhouse et al., 1992). The effect of insecticides on the radial growth and germination of entomopathogenic fungi was evaluated (Table 2). The insecticide concentrations were calculated based on active ingredient (ai) recommended per hectare. The different concentration of insecticides viz., recommended, 75 percent of the recommended concentration (3/4th) and 50 percent of the recommended concentration (1/2) was tested for compatibility with the entomopathogenic fungi.

2.1 Inoculum and Maintenance of Pure Culture of Entomopathogenic Fungi

Inoculum for pure culture of *Metarhizium anisopliae* was obtained by spraying commercial fungal spore products on different larval instars of fall armyworm, which were then incubated for one week. After incubation, the infected larvae, which exhibited mycelial growth, were used to maintain the pure culture. To maintain the culture, PDA medium was sterilized at 15 psi and 121°C for 30 minutes in an autoclave, then poured into sterilized Petri plates and cooled. A loopful of inoculum from the infected larvae was transferred under aseptic conditions to the Petri plates. The plates were incubated at room temperature (26 ± 2°C) for 10 days. The pure culture was sub-cultured for use in subsequent experiments.

2.2 Preparation of Test Chemical Insecticide Concentrations

Six insecticides were evaluated by poisoned food technique (Moorhouse et al., 1992) in Potato Dextrose Agar (PDA) medium mentioned in Table 2. Five hundred ml of PDA medium was sterilized in individual boiling tubes and the insecticide emulsions of required concentration were incorporated into the melted sterile PDA aseptically, thoroughly mixed, poured into sterile

Petri plates and allowed to solidify under laminar air flow cabinet.

2.3 Inoculation of the Entomopathogenic Fungi to the Poisoned PDA Media

An agar disc along with mycelium mat of fungi will cored from the periphery of 10 days old colony of fungi by needle and transferred into the centre of the PDA plates which are poisoned by test insecticides. The growth medium (PDA) without insecticide but inoculated with mycelia disc served as untreated check. The plates were incubated at room temperature for 14 days to allow maximum growth. Each treatment was replicated three times.

2.4 Calculation of Growth, Diameter and Growth Inhibition by the Test Chemicals

The diameter of growing culture in excess of the plugs in each Petri dish was measured at 7 days after inoculation (DAI) (when radial growth in the control plate fully covered the medium) and also on 14 and 21 days after inoculation. The data was expressed as diameter of colony growth and percentage growth inhibition of entomopathogenic fungi (Hokkanen & Kotiluoto, 1992). The percent growth inhibition is calculated by using the formula,

$$X = \frac{Y - Z}{Z} \times 100$$

Where, X, Y, Z stand for percentage of growth inhibition, radial growth of fungus in untreated check and radial growth of fungus in poisoned medium, respectively. The insecticides were classified into evaluation categories of 1- 4 scoring index in *in vitro* toxicity tests (Table 3) according to Hassan's classification scheme (Hassan, 1989). Also test insecticides were classified into evaluation categories of 1 - 4 scoring index of Compatibility (Table 4) according to Jayasing's classification (Jayasing, 2011).

Table 1. Ad-hoc recommended insecticides for fall armyworm management

Sl. No.	Name of insecticides	Dosage/ ha (ml or gm a.i.)
1)	Chlorantraniliprole 9.3+Lambda-cyhalothrin 4.6 ZC	35
2)	Spinetoram 11.7 SC	30
3)	Chlorantraniliprole 18.5 SC	40
4)	Thiodicarb 75 WP	750
5)	Emamectin benzoate 5 SG	20
6)	Novaluron 5.25 + Emamectin benzoate 0.9 SC	92.25

Table 2. Treatment details of compatibility studies between test insecticides and entomopathogenic fungi

Sl. No.	Treatment details	Concentration (%)
1	Chlorantraniliprole 9.3 + Lambda-cyhalothrin 4.6 ZC (RC)	0.050
2	Chlorantraniliprole 9.3 + Lambda-cyhalothrin 4.6 ZC (75%RC)	0.037
3	Chlorantraniliprole 9.3+ Lambda-cyhalothrin 4.6 ZC (50%RC)	0.025
4	Spinetoram 11.7 SC (RC)	0.050
5	Spinetoram 11.7 SC (75% RC)	0.037
6	Spinetoram 11.7 SC (50% RC)	0.025
7	Chlorantraniliprole 18.5 SC (RC)	0.043
8	Chlorantraniliprole1 8.5 SC (75%RC)	0.032
9	Chlorantraniliprole 18.5 SC (50%RC)	0.021
10	Thiodicarb 75 WP (RC)	0.200
11	Thiodicarb 75 WP (75% RC)	0.150
12	Thiodicarb 75 WP (50% RC)	0.100
13	Emamectin benzoate 5 SG (RC)	0.080
14	Emamectin benzoate 5 SG (75% RC)	0.060
15	Emamectin benzoate 5 SG (50% RC)	0.040
16	Novaluron 5.25 + Emamectin benzoate 0.9 SC (RC)	0.300
17	Novaluron 5.25 + Emamectin benzoate 0.9 SC (75% RC)	0.224
18	Novaluron 5.25 + Emamectin benzoate 0.9 SC (50% RC)	0.150

Table 3. Categories of 1-4 scoring index in in vitro toxicity tests according to Hassan's classification scheme (Hassan, 1989)

Score	Definition	Reduction in beneficial capacity
1	Harmless	<50%
2	Slightly harmful	50-79%
3	Moderately harmful	80-90%
4	Harmful	>90%

Table 4. Compatibility ratings for test insecticides were classified in evaluation categories of 1 -4 scoring index

Sl. No.	Compatibility status	Average reduction in growth
1	Highly compatible	< 20%
2	Compatible	20-50%
3	Partially compatible	50-80%
4	Incompatible	> 80%

3. RESULTS AND DISCUSSION

To understand extent of compatibility between insecticides and entomopathogenic fungi, beneficial fungal growth inhibition was calculated by measuring diameter of fungal colony growth. Six insecticides belonging to different groups or classes of insecticides were tested for compatibility with common entomopathogenic fungi that affects fall armyworm.

3.1 Mycelial Growth of *Metarhizium Anisopliae* on Insecticide-treated Media and Inhibitory Growth Effect of Various Test Insecticides

Observations at 7 days after inoculation. Compatibility between *Metarhizium anisopliae* and insecticides showed significant reduction in mycelial growth when compared with control. Mycelial growth inhibition by tested insecticides varied from 6.25 to 51.88 per cent and were significant with one other.

All insecticides combined with *Metarhizium anisopliae* were highly compatible and harmless except Chlorantraniliprole 18.5 SC, Thiodicarb 75 WP and Spinetoram 11.7 SC (Table 5). Lowest colony growth inhibition (6.25%) and highest colony growth (75 mm) were recorded in case of half RC of Emamectin benzoate 5 SG, followed by 15.38 per cent reduction in growth capacity and 67.5 mm colony growth in case of 3/4th RC.

Emamectin benzoate 5% SG showed high compatibility and harmless effect to *Metarhizium anisopliae*. Chlorantraniliprole 9.3 + Lambda-cyhalothrin 4.6 ZC showed high compatibility and harmless effect to *Metarhizium anisopliae*. Colony growth of fungus was 73.2, 72.2 and 67.5 mm of mean mycelial growth with 8.5, 9.75 and 15.63 percentage of reduction in growth capacity of fungal colony at different concentrations.

Novaluron 5.25 + Emamectin benzoate 0.9 SC showed high compatibility and harmless effect to

Metarhizium anisopliae irrespective of concentrations. Better colony growth was observed (70, 72.5 and 67.5 mm) with lesser growth inhibition (12.5, 9.38 and 15.63%) at various concentrations.

At low concentrations (1/2 of RC and 3/4th of RC) of Chlorantraniliprole 18.5 SC, high compatibility and harmless effect were observed, with 9.38 and 16.75 per cent suppression of fungal colony over control with colony growth of 72.5 mm and 66.6 mm respectively.

Thiodicarb 75 WP at low concentrations (1/2 of RC and 3/4th of RC), fungus was able to grow to mean growth of 68.8 and 70 mm and showed highly compatible and harmless effect towards fungus, with growth inhibitory effect of 14 and 12.5 per cent respectively.

Spinetoram 11.7 SC had inhibitory effect on growth about 51.88 per cent, which found to be partially compatible and slightly harmful to fungus. At 3/4th RC of same molecule, fungal growth was inhibited up to 40.63 per cent with colony growth of 47.5 mm was found to be compatible and harmless (Table 5)."

3.2 Observations at 14 Days after Inoculation

All insecticides combined with entomopathogenic fungus *Metarhizium anisopliae* showed significant difference with respect to control. Among tested insecticides at various concentrations showed significant difference with one another. Fungal mycelial growth inhibition ranged from 2.94 to 32.12 per cent.

Lowest growth inhibition (2.94%) was recorded in case of half RC of Novaluron 5.25 + Emamectin benzoate 0.9 SC, with mean mycelial colony diameter of 82.5 mm. Molecule showed 5.88 and 11.76 per cent growth inhibition with 80- and 75-mm colony growth at respective concentrations of 3/4th RC and full RC of Novaluron 5.25 + Emamectin benzoate 0.9 SC (Table 6).

Lowest growth inhibition of 3.53 per cent, with 82 mm colony growth were recorded at half RC of Chlorantraniliprole 9.3 + Lambda-cyhalothrin 4.6 ZC, whereas in other two concentrations, (3/4th RC and full RC) mean mycelial colony growth recorded were 80 and 77.7 mm respectively, with 5.88 and 8.59 per cent growth inhibition towards fungus. Insecticide at all concentrations showed high compatibility and harmless effect towards *Metarhizium anisopliae*.

Emamectin benzoate 5% SG at two lower concentrations i.e., at 1/2 RC and 3/4th RC inhibited *Metarhizium anisopliae* fungal colony growth with only 4.71 and 5.88 percentage, followed by 11.76 per cent inhibition in growth at full RC. Mean fungal colony diameter recorded at 1/2 and 3/4th RC was 81 and 80 mm respectively and in case RC mean colony diameter was 75 mm. Emamectin benzoate 5 SG was found to be highly compatible and harmless towards *M. anisopliae*.

Chlorantraniliprole 18.5 SC showed high compatibility and harmless effect at two concentrations i.e., at 1/2 RC and 3/4th RC. Insecticide concentration inhibited beneficial capacity of fungus in negligible percentage i.e., 7.29 and 17.65 per cent respectively. Insecticide was found to be compatible at RC by inhibiting fungal colony growth up to 20.35 per cent. Mean colony growth was 78.8, 70 and 67.7 at 1/2 of RC, 3/4th RC and RC respectively (Table 6).

Thiodicarb 75 WP showed 9.65, 14.71 and 23.53 per cent growth inhibitory effect towards *M. anisopliae* at concentrations 1/2 RC, 3/4th RC and RC respectively. Colony growth recorded were 76.8, 72.5 and 65 mm respectively. Insecticide concentrations showed high compatibility and harmless effect at 1/2 RC and 3/4th RC, at RC insecticide was found to be compatible and harmless.

At 14 days after inoculation, highest growth inhibition was recorded in case of Spinetoram 11.7 SC. At RC of insecticide, there was 32.12 percent reduction in growth capacity of *M. anisopliae* and contributed growth of 57.7 mm. At 3/4th RC, growth inhibition was reduced to 20.59 percentage.

Spinetoram 11.7 SC showed compatible and harmless effect against *M. anisopliae* while 1/2 RC showed high compatibility and harmless effect with only 8.82 percent reduction in growth capacity of fungus. Colony growth recorded were 77.5, 67.5 and 57.7 mm at concentrations 1/2 RC, 3/4th RC and full RC respectively (Table 6).

Observations at 21 days after inoculation showed all insecticide concentrations combined with *M. anisopliae* were significant in relation to control. Growth inhibition by insecticides varied from 0.71 to 20 percent, with all treatments significant among one another.

Table 5. Effect of insecticides on the growth of entomopathogenic fungus, *Metarhizium anisopliae* at 7 days after inoculation

Sl. No.	Treatments	Growth inhibition (%)			Colony growth (mm)		
		RC	75% of RC	50% of RC	RC	75% of RC	50% of RC
1	Chlorantraniliprole 9.3 + Lambda-cyhalothrin 4.6 ZC	15.63 (23.29)*	9.75 (18.20)	8.50 (16.95)	67.50	72.20	73.20
2	Spinetoram 11.7 SC	51.88 (46.08)	40.63 (39.60)	11.25 (19.60)	38.50	47.50	71.00
3	Chlorantraniliprole 18.5 SC	32.50 (34.76)	16.75 (24.16)	9.38 (17.83)	54.00	66.60	72.50
4	Thiodicarb 75 WP	41.75 (40.26)	12.50 (20.71)	14.00 (21.98)	46.60	70.00	68.80
5	Emamectin benzoate 5 SG	18.75 (25.66)	15.38 (23.09)	6.25 (14.48)	65.00	67.70	75.00
6	Novaluron 5.25 +Emamectin benzoate 0.9 SC	16.00 (23.58)	9.75 (18.20)	12.50 (20.71)	67.20	72.20	70.00
7	Control	0.00			80.00		
	Particulars	S.E m \pm			CD@1%		
	Insecticides(I)	0.69			2.67		
	Concentration I	0.49			1.89		
	I*C	1.20			4.63		

RC: Recommended Concentration, *Figures in parenthesis are arcsine transformed values

Table 6. Effect of insecticides on the growth of entomopathogenic fungus, *Metarhizium anisopliae* at 14 days after inoculation

Sl. No.	Treatments	Growth inhibition (%)			Colony growth (mm)		
		RC	75% of RC	50% of RC	RC	75% of RC	50% of RC
1	Chlorantraniliprole 9.3+ Lambda-cyhalothrin 4.6 ZC	8.59 (17.04)*	5.88 (14.04)	3.53 (10.83)	77.70	80.00	82.00
2	Spinetoram 11.7 SC	32.12 (34.53)	20.59 (26.99)	8.82 (17.28)	57.70	67.50	77.50
3	Chlorantraniliprole 18.5 SC	20.35 (26.82)	17.65 (24.84)	7.29 (15.67)	67.70	70.00	78.80
4	Thiodicarb 75 WP	23.53 (29.02)	14.71 (22.55)	9.65 (18.1)	65.00	72.50	76.80
5	Emamectin benzoate 5 SG	11.76 (20.06)	5.88 (14.04)	4.71 (12.53)	75.00	80.00	81.00
6	Novaluron 5.25 +Emamectin benzoate 0.9 SC	11.76 (20.06)	5.88 (14.04)	2.94 (9.88)	75.00	80.00	82.50
7	Control	0.00			85.00		
	Particulars	S.E m \pm			CD@1%		
	Insecticides(I)	0.55			2.13		
	Concentration I	0.39			1.50		
	I*C	0.96			3.68		

RC: Recommended Concentration, *Figures in parenthesis are arcsine transformed values

Lowest growth inhibition (0.71%) with highest colony growth (84.4 mm) was observed at 1/2 RC of Chlorantraniliprole 9.3 + Lambda-cyhalothrin 4.6 ZC. Maximum growth inhibition (20%) with smallest colony growth (68 mm) was observed in RC of Thiodicarb 75 WP (Table 7).

At 21 days after inoculation, all test insecticides showed highly compatible and harmless effect towards fungus *M. anisopliae*. Insecticide Chlorantraniliprole 9.3 + Lambda-cyhalothrin 4.6 ZC recorded least growth inhibition of 0.71 percent and promoted colony growth of 84.4 mm at 1/2 of RC.

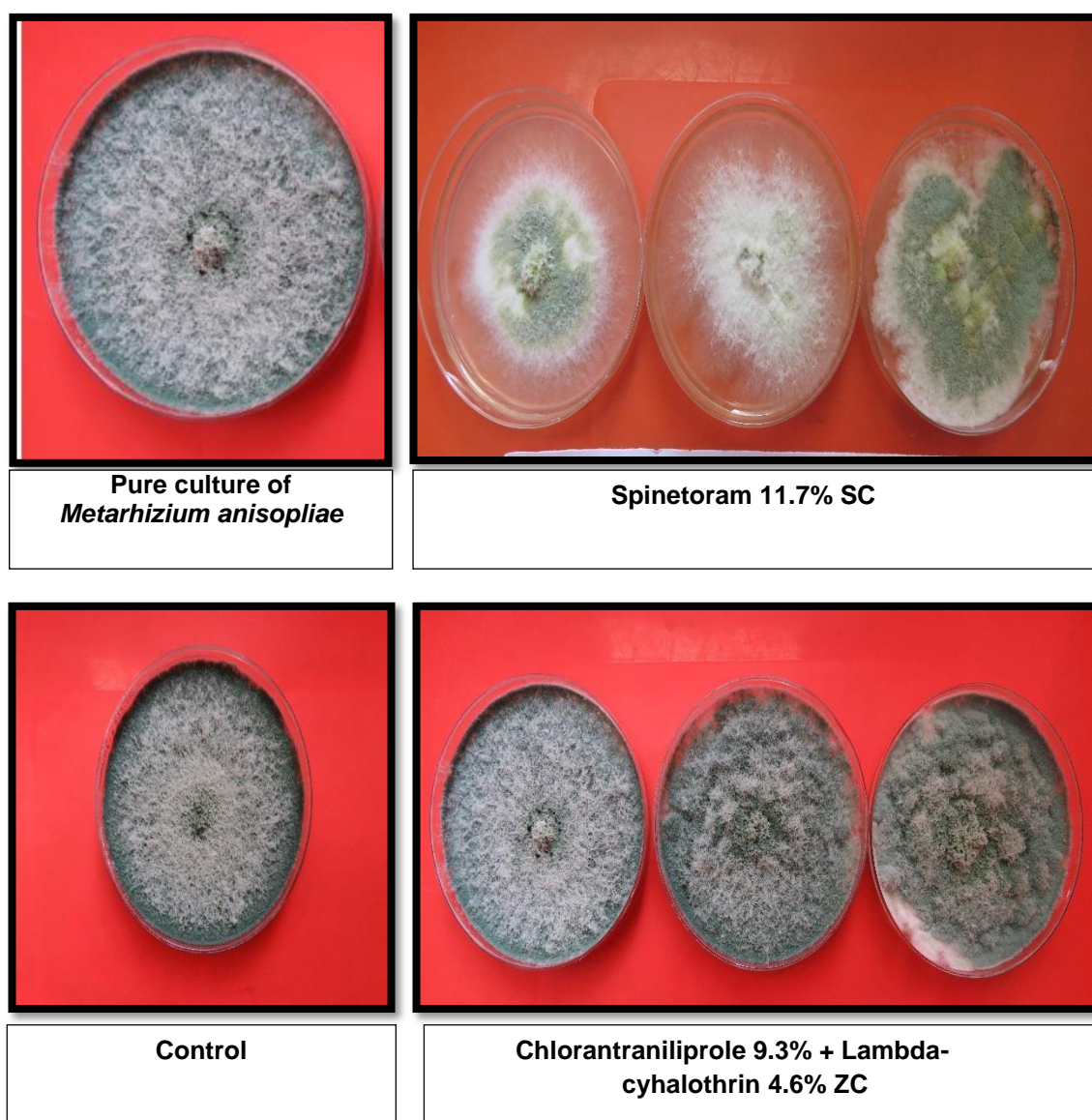


Plate 1. Compatibility of *Metarhizium anisopliae* with different test insecticides at various concentrations

Table 7. Effect of insecticides on the growth of entomopathogenic fungus, *Metarhizium anisopliae* at 21 days after inoculation

Sl. No.	Treatments	Growth inhibition (%)			Colony growth (mm)		
		RC	75% of RC	50% of RC	RC	75% of RC	50% of RC
1	Chlorantraniliprole 9.3 + Lambda-cyhalothrin 4.6 ZC	5.88 (14.04)*	3.29 (10.46)	0.71 (4.82)	80.00	82.20	84.40
2	Spinetoram 11.7 SC	17.65 (24.84)	11.06 (19.43)	7.29 (15.67)	70.00	75.60	78.80
3	Chlorantraniliprole 18.5 SC	17.65 (24.84)	11.76 (20.06)	5.88 (14.04)	70.00	75.00	80.00
4	Thiodicarb 75 WP	20.00 (26.57)	16.47 (23.95)	11.18 (19.53)	68.00	71.00	75.50
5	Emamectin benzoate 5SG	8.82 (17.28)	3.53 (10.83)	4.71 (12.53)	77.50	82.00	81.00

Sl. No.	Treatments	Growth inhibition (%)			Colony growth (mm)		
		RC	75% of RC	50% of RC	RC	75% of RC	50% of RC
6	Novaluron 5.25 + Emeactin benzoate 0.9 SC	8.24 (16.68)	3.29 (10.46)	1.41 (6.82)	78.00	82.20	83.80
7	Control	0.00			85.00		
	Particulars	S.E m \pm			CD@1%		
	Insecticides (I)	0.49			1.88		
	Concentration I	0.34			1.33		
	I*C	0.84			3.25		

RC: Recommended Concentration, *Figures in parenthesis are arcsine transformed values

Table 8. Mean effect of insecticides on growth inhibition of *Metarhizium anisopliae* at weekly intervals

Sl. No.	Insecticides	Mean growth inhibition (%)		
		7DAI	14DAI	21DAI
1	Chlorantraniliprole 9.3+Lambda-cyhalothrin 4.6 ZC	11.29	6.00	3.29
2	Spinetoram 11.7 SC	34.58	20.51	12.00
3	Chlorantraniliprole 18.5 SC	19.54	15.10	11.76
4	Thiodicarb 75 WP	22.75	15.96	15.88
5	Emamectin benzoate 5 SG	13.46	7.45	5.69
6	Novaluron 5.25+Emamectin benzoate 0.9 SC	12.75	6.86	4.31

DAI: Days after incubation

Similar results were recorded by Bagchi et al. (2016), who reported Lorsban as most toxic insecticide to mycelial growth and conidial germination, followed by Lannate, Larvin, and Pirate. At same time, Cascade, Match, Steward, and Proclaim were comparatively less toxic to mycelial growth (36.78-48.67% inhibition) and conidial germination (40.32-49.97% inhibition) of fungal pathogen.

4. CONCLUSION

The compatibility of entomopathogenic fungus, *M. anisopliae* with various insecticides was evaluated, and the results demonstrate that most insecticides, at lower concentrations, exhibited a high degree of compatibility with the fungus. The mycelial growth inhibition by the insecticides ranged from 0.71% to 51.88%, depending on the insecticide and concentration. Notably, the insecticides Chlorantraniliprole 9.3 + Lambda-cyhalothrin 4.6 ZC followed by Novaluron 5.25 + Emamectin benzoate 0.9 SC and Emamectin benzoate 5 SG showed minimal inhibition, even at higher concentrations, suggesting they are highly compatible with *M. anisopliae*. In contrast, Spinetoram 11.7 SC and Thiodicarb 75 WP exhibited higher levels of inhibition, particularly at their recommended concentrations, indicating that they are partially harmful to the fungus. Over time, the fungal growth inhibition was less

pronounced, with lower concentrations of the insecticides showing negligible effects on fungal growth.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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