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In vitro Assessment of Compatibility of Selected Pesticides with Metarhizium anisopliae

Parjane Nitin
Mahatma Phule Krishi Vidyapeeth
Sojwal Shinde
Parul University
Sawant C.G.
dr.cgsawant@cau.ac.in

Central Agriculture University, India
Kabre Ghansham
Mahatma Phule Krishi Vidyapeeth

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Abstract

Background: - Assessing the compatibility of chemical pesticides with *Metarhizium anisopliae*, an entomopathogenic fungus, is essential to effectively implementing sustainable pest management strategies. Understanding how different pesticides influence the fungal growth, spore production and pathogenicity can help to identify suitable combinations of chemical and microbial control agents for sustainable integrated pest management.

Results:- In the present study, the compatibility of ten pesticide formulations with *Metarhizium anisopliae* was evaluated under in vitro conditions by recording radial mycelial growth and conidial production. The investigated formulations included insecticidal mixtures and a fungicidal mixture. Substantial variation was observed in the response of *M. anisopliae* to the different formulations. Spirotetramat 11.01% + imidacloprid 11.01% SC was the most compatible formulation, supporting the highest radial mycelial growth (83.20 mm) and conidial production (3.88×10^8). In contrast, metalaxyl-M 4% + mancozeb 64% WP exerted the greatest inhibitory effect, with radial mycelial growth of 14.00 mm and conidial production of 0.72×10^8. Based on radial mycelial growth, compatibility decreased in the order spirotetramat 11.01% + imidacloprid 11.01% SC > thiamethoxam 12.6% + lambda-cyhalothrin 9.5% EC > beta-cyfluthrin 8.49% + imidacloprid 19.81% OD > fipronil 40% + imidacloprid 40% WG > bifenthrin 3% + chlorpyriphos 30% EC > chlorpyriphos 50% + cypermethrin 5% EC > profenofos 40% + cypermethrin 4% EC > azoxystrobin 18.2% + difenoconazole 11.4% SC > metalaxyl-M 4% + mancozeb 64% WP.

Conclusion: - The marked differences among formulations indicated that pesticide selection may influence the successful incorporation of *M. anisopliae* into integrated pest management programmes. The formulations showing comparatively lower inhibitory effects warrant further evaluation in combination with *M. anisopliae*under greenhouse and field conditions to determine their effects on fungal establishment, sporulation and biocontrol efficacy.

1. Background

For more than five decades, synthetic insecticides have served as the primary means of managing insect pests because of their rapid action and broad-spectrum effectiveness. Nevertheless, their intensive, unwise and indiscriminate application has led to numerous ecological and agricultural concerns, including the development of insecticide resistance, pest resurgence, secondary pest outbreaks, contamination of soil and water resources, and detrimental effects on non-target organisms, biodiversity, and human health (Lal, 2001). The increasing severity of these challenges under intensive agricultural systems has accelerated the global transition towards environmentally sustainable pest management practices and the development of microbial biopesticides (Wang et al., 2024; de Sousa et al., 2025). In 1962, Rachel Carson's landmark book Silent Spring drew the attention of world environmentalists towards the indiscriminate application of pesticides like Dichlorodiphenyltrichloroethane (DDT). The book highlighted the harmful effects of DDT on ecosystems and played a pivotal role in shaping public awareness of environmental conservation. Since its publication, there has been a gradual shift in crop protection from heavy dependency on synthetic pesticides to more sustainable approaches that integrate biological control with other pest management practices to reduce environmental impacts.

Integrated Pest Management (IPM) is widely recognized as one of the most effective and sustainable approaches for managing insect pests by integrating biological, cultural, mechanical and chemical control measures to maintain insect pest populations below economically damaging levels while minimizing environmental risks. Biological control agents constitute an indispensable component of IPM because they help conserve natural enemies, maintain ecological balance, reduce pesticide dependence and delay the evolution of insecticide resistance. Among these agents, microbial pesticides including entomopathogenic fungi, bacteria, viruses and entomopathogenic nematodes have received considerable attention due to their high target specificity, environmental compatibility and suitability for sustainable agricultural production (Brar and Dhiruga, 2004). Recent progress in microbial biotechnology, strain improvement, fermentation processes and formulation technologies has substantially enhanced the commercialization and field performance of microbial biopesticides, making them increasingly attractive alternatives to conventional insecticides (Emaru et al., 2024; de Sousa et al., 2025).

Among the available microbial biocontrol agents, entomopathogenic fungi (EPF) are one of the most promising groups because they infect insect hosts through direct penetration of the cuticle, eliminating the requirement for ingestion. The infection process begins with the attachment of fungal conidia to the insect cuticle followed by germination, enzymatic degradation of the cuticle, penetration into the haemocoel, multiplication within the host, secretion of toxic metabolites and ultimately host mortality. This distinctive infection mechanism enables EPF to successfully infect insects with piercing and sucking mouthparts as well as concealed and soil inhabiting developmental stages that are often difficult to control using conventional insecticides. To date, approximately 1,200 fungal species belonging to nearly 85 genera have been identified as insect pathogens, with more than 750 species reported worldwide. Among these, *Metarhizium*, *Beauveria*, *Cordyceps*, *Isaria*, *Lecanicillium*, and *Nomuraea* have been extensively exploited for the management of economically important insect pests affecting agricultural and horticultural crops (Thaochan and Sausa-Ard, 2017). Recent advances in fungal genomics, strain selection, mass production, fermentation technology and formulation development have further expanded the practical application of EPF as environmentally safe biopesticides within sustainable crop protection programmes (Wang et al., 2024; Shehzad et al., 2025).

The integration of entomopathogenic fungi with selective insecticides has emerged as a promising strategy for improving pest management while reducing dependence on synthetic chemicals. When compatible, these combinations may exhibit additive or synergistic interactions that enhance pest mortality, lower insecticide application rates, conserve beneficial arthropods, reduce environmental contamination, and delay the evolution of insecticide resistance (Ambethar, 2009). Selective insecticides can also provide immediate suppression of pest populations during the comparatively slower infection cycle of entomopathogenic fungi, thereby improving overall pest management and their efficiency. Conversely, incompatible insecticides may adversely affect fungal germination, mycelial growth, sporulation, conidial viability, infectivity, and persistence, ultimately reducing their effectiveness in IPM programmes (Duarte et al., 1992; Malo, 1993). Consequently, recent studies have emphasized that compatibility assessments should encompass multiple biological characteristics, including vegetative growth, conidial germination, sporulation, virulence, persistence and formulation stability before fungal biocontrol agents are integrated with chemical insecticides (Wang et al., 2024; Angelo et al., 2025).

Although numerous studies have investigated the compatibility of entomopathogenic fungi with individual insecticides applied at recommended or sublethal concentrations (Er and Gokce, 2004; Alizadeh et al., 2007; Rachappa et al., 2007), comparatively little information is available regarding their interaction with commercially available ready-mix insecticide formulations. The adoption of these premixed insecticides has increased substantially in modern agriculture because they provide broad spectrum pest control, improve operational efficiency, reduce labour and application costs and contribute to insecticide resistance management. However, the combined effects of multiple active ingredients on fungal growth, sporulation, viability, and pathogenicity remain poorly understood. Furthermore, environmental variables such as temperature, relative humidity, ultraviolet radiation, and formulation characteristics may significantly influence the field performance and compatibility of entomopathogenic fungi, highlighting the need for comprehensive compatibility evaluations under standardized conditions (Emaru et al., 2024; Wang et al., 2024; Shehzad et al., 2025).

Despite the increasing use of commercial ready-mix insecticides, information regarding their compatibility with *Metarhizium anisopliae* under *in vitro* conditions remains limited. Therefore, the present study was undertaken based on the hypothesis that commercially available ready-mix insecticides differ in their compatibility with *M. anisopliae* and that suitable combinations can be incorporated into Integrated Pest Management programmes without adversely affecting fungal growth and sporulation. Accordingly, the objective of this study was to evaluate the *in vitro* compatibility of selected ready-mix insecticides with *Metarhizium anisopliae* strain IPL/KC/44 (Accession No. ITCC 6895) by assessing their effects on mycelial growth, conidial germination and sporulation. The outcomes of this investigation are expected to identify compatible insecticide fungus combinations that can be effectively integrated into sustainable IPM programmes, thereby reducing reliance on conventional insecticides while preserving the biological efficacy and viability of this important entomopathogenic fungus.

2. Material and Methods

2.1 Collection and Maintenance of Pure Culture

A pure culture of *Metarhizium anisopliae* IPL/KC/44 (Strain No. ITCC 6895) was procured from the Biocontrol Laboratory, Department of Entomology, Postgraduate Institute, Mahatma Phule (Agriculture) Vidyapeeth (University), Rahuri, Maharashtra, India. The fungus was routinely grown on Potato Dextrose Agar (PDA) medium in sterilized petri plates. The inoculated plates were incubated at 25 ± 2 °C under a 12:12 h light and dark photoperiod with relative humidity maintained between 70 and 80%. The cultures were allowed to grow for 10–14 days to obtain adequate sporulation for further experimental study.

To ensure continued viability and purity, the fungal culture was transferred to fresh PDA medium at 30 day intervals. For preservation between experimental periods, actively growing cultures were kept at 4 ± 1 °C in a refrigerator. Before each experiment, the preserved culture was revived by transferring it onto fresh PDA medium. All transfers and subculturing procedures were performed aseptically inside a laminar airflow cabinet to minimize the possibility of microbial contamination.

2.2 Calculation of Pesticide Concentration for PDA Medium

The quantity of each pesticide added to the Potato Dextrose Agar (PDA) medium was determined from its recommended field dose. The required amount was adjusted according to the volume of PDA used in each treatment. The calculation was made following the method suggested by Vincent (1947), using the equation given below:

$$\text{Amount of pesticide} = \frac{\text{(Required concentration} \times \text{Volume of PDA medium)}}{\text{Formulation strength (\% a.i.)}}$$

In this equation, the required concentration (%) indicates the final pesticide concentration intended in the PDA medium. The volume of PDA medium refers to the amount of molten medium used for preparing the respective treatment. The formulation strength (% a.i.) represents the percentage of active ingredient present in the commercial pesticide formulation.

For the compatibility experiment, the amount of individual pesticides was calculated according to their recommended field dose for high-volume application. The calculated dose was then proportionately adjusted to match the quantity of PDA medium used in each treatment. The final amounts of the pesticides incorporated into the PDA medium for the compatibility experiment are given in Table 1.

2.3 Poison Food Technique

The compatibility of selected insecticides with *M. anisopliae* 1.15 % WP was evaluated using the standard poison food technique described by Moorhouse et. al. 1992. The required quantity of each pesticide was incorporated into sterilized molten Potato Dextrose Agar (PDA) Medium (Cooled to approximately 45-48 ^{o}C) to obtain the desired concentration. The poison medium was thoroughly mixed and poured aseptically into sterile 90 mm Petri plates (approximately 20 mL per plate). PDA medium without pesticide serves as an untreated control (De Olivera and Neves 2004). After solidification, the center of each plate was inoculated with 5 mm diameter mycelial discs cut from the actively growing margin of a 7-day-old culture of *M. anisopliae.* The plate was incubated at 25 ± 2 °C for around 10-14 days. Each treatment was replicated three times in a Completely Randomized Design (CRD). Fungal compatibility was assessed by recording radial mycelial growth and sporulation. The percent inhibition of mycelial growth over the untreated control was calculated using Vincent's (1947) formula:

Percent inhibition (I) = C−T/ C ×100

Where:

I = percent inhibition of mycelial growth,

C = radial growth (mm) in the untreated control

T = radial growth (mm) in the pesticide-treated medium

2.4 Recording Observations on Mycelial Growth of *Metarhizium anisopliae*

Observation on mycelial growth: Radial mycelial growth of *M. anisopliae* was recorded by measuring the colony diameter (excluding the 5mm inoculum disc) in two perpendicular directions on the 3rd, 5th, 7th, 9th, and 11th days after inoculation (DAI) (Moorhouse et al 1992). The average colony diameter (mm) for each Petri plate was calculated, and the mean value of three replicates was used for statistical analysis. The radial growth of each treatment was compared with that of the untreated control to assess the effect of different pesticides on fungal growth.

Table 1. Details of the chemical pesticides used to study the compatibility of *Metarhizium anisopliae*

Sr. No.	Common Name	IUPAC Name	Formulation	Trade name	Dose (g or ml ha^{-1})	Company Name
1.	Bifenthrin + Chlorpyriphos	(2-methylbiphenyl-3-yl)methyl (Z)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate + O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate.	3% + 30 % EC	Lethal Gold	1000 ml/ha	Insecticide India Limited
2.	Chlorpyriphos + Cypermethrin	O, O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate + (RS)-α-cyano-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	5 % + 50 % EC	Googly-505	1000 ml/ha	Crop Care
3.	Profenofos + Cypermethrin	O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate + (RS)-α-cyano-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	40 % + 4 % EC	Dyna	1000 ml/ha	Shree Ram Agro
4.	Thiamethoxam + Lambda-cyhalothrin	(EZ)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine + rac-(R)-cyano(3-phenoxyphenyl) methyl (1S,3S)-3-[(Z)-2-chloro-3,3,3-trifluoroprop-1-en-1-yl]-2,2-dimethylcyclopropane-1-carboxylate	12.6 % + 9.5 % EC	Commander	200–250 ml/ha	United Insecticides
5.	Fipronil + Imidacloprid	5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-4-(trifluoromethyl)sulfinylpyrazole + (E)-1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine	40% + 40% WG	Attack	80–100 g/ha	United Insecticides
6.	Spirotetramat + Imidacloprid	cis-4-(ethoxycarbonyloxy)-8-methoxy-3-(2,5-xylyl)-1-azaspiro[4.5]dec-3-en-2-one + (E)-1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine	11.01 (w/w) + 11.01 (w/w) SC	Movento Energy	500 ml/ha	Bayer CropScience Ltd.
7.	Beta-cyfluthrin + Imidacloprid	S)-α-cyano-4-fluoro-3-phenoxybenzyl (1R,3R)-3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropane-1-carboxylate + (E)-1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine	8.49 (w/w) + 19.81 (w/w) OD	Solomon	200 ml/ha	Bayer CropScience Ltd.
8.	Metalaxyl-M + Mancozeb	methyl N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alaninate +manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt	4% (w/w) + 64 % (w/w) WP	Ridomil Gold	2000 g /ha	Syngenta Crop Protection Ltd.
9.	Azoxystrobin + Difenoconazole	Methyl(E)-2-{2-[6-(2-cyanophenoxy)-4-pyrimidinyl]oxy}-α-(methoxymethylene)benzeneacetate + 1-[2-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-4- methyl[1,3]	18.2 % (w/w) + 11.4 % (w/w) SC	Amistar Top	300–400 ml/ha	Syngenta Crop Protection Ltd.
10.	Untreated control	--	-	-	-	-

2.5 Assessment of Conidial Production:

After 11 days of incubation, conidial production was quantified following the method described by Marzieh et. al. (2010). The 5-mm diameter agar was aseptically excised from the actively growing central region of each fungal colony to obtain a representative sample for conidial estimation.

The discs were transferred individually into sterile glass tubes containing 10 mL of sterile distilled water suspended with 0.05% Tween 80 and vortexed thoroughly to dislodge the conidia, resulting in a uniform conidial suspension. The conidial concentration was determined using a Neubauer

hemacytometer under the light microscope and expressed as conidia mL^{-1}

2.7 Experimental Design and Statistical Analysis

The experiment was conducted in a Completely Randomized design (CRD) comprising 10 treatments with five replications each. The data on radial Mycelial growth and conidial production were subjected to analysis of variance (ANOVA) using standard statistical procedure. The data on mycelial growth were converted in to square root and angular (Arc sin) transformation before analysis. The treatment means were compared using the Least Significant Difference (LSD) test at the 5 % level of significance (P = 0.05).

3. Results

3.1 Mycelial Growth and Percent Growth Inhibition of *Metarrhizium anisopliae*

Assessment at Three (03) Days After Inoculation (DAI)

Radial mycelial growth of *Metarrhizium anisopliae* differed significantly among the pesticide treatments at three (03) DAI (Table 2 and Fig. 1). The untreated control exhibited the greatest colony growth (32.60 mm) and was significantly superior to all pesticide amended media. Among the evaluated pesticides combination, thiamethoxam 12.6% + lambda-cyhalothrin 9.5% EC recorded the highest mycelial growth (26.80 mm), which was statistically comparable with beta-cyfluthrin 8.49% + imidacloprid 19.8% OD (26.60 mm) and fipronil 40% + imidacloprid 40% WG (25.20 mm). Conversely, metalaxyl-M 4%+ mancozeb 64% WP exhibited the greatest suppressive effect, recording the lowest mycelial growth (10.60 mm) and the highest growth inhibition (67.49%). In comparison, thiamethoxam 12.6% + lambda-cyhalothrin 9.5% EC caused the least inhibition (17.83%), indicating greater compatibility with *M. anisopliae.*

Assessment at Five (05) Days After Inoculation (DAI)

The radial mycelial growth of *M. anisopliae* varied considerably among the pesticide treatments at five (05) days after inoculation (DAI) (Table 2 and Fig. 1). The untreated control recorded the maximum colony growth of 44.60 mm, which was significantly superior to that observed in all pesticide treated media. Among the pesticides, thiamethoxam 12.6% + lambda-cyhalothrin 9.5% EC supported the greatest mycelial growth (40.20 mm) and was statistically at par with spirotetramat 11.01% + imidacloprid 11.01% SC (39.40 mm), beta-cyfluthrin 8.49% + imidacloprid 19.81% OD (38.60 mm), and fipronil 40% + imidacloprid 40% WG (37.80 mm). In contrast, the minimum mycelial growth (11.20 mm) was recorded with metalaxyl-M 4% + mancozeb 64% WP.

The pattern of growth inhibition corresponded with the observed mycelial growth. The lowest inhibition (9.45%) was recorded with thiamethoxam 12.6% + lambda-cyhalothrin 9.45% EC, indicating its comparatively higher compatibility with *M. anisopliae*. Conversely, metalaxyl-M 4% + mancozeb 64% WP resulted in the highest growth inhibition (74.77%), indicating a strong inhibitory effect on fungal growth.

Assessment at Seven (07) Days After Inoculation (DAI)

A clear variation in the radial growth of *M. anisopliae* was evident among the pesticide treatments at seven (07) days after inoculation (DAI) (Table 2 and Fig. 1). The fungus exhibited the greatest colony development in the untreated control with a radial growth of 59.20 mm, which was significantly higher than recorded in the pesticide-treated media. Among the evaluated pesticides combination, spirotetramat 11.01% + imidacloprid 11.01% SC supported the highest growth (53.40 mm) and remained statistically at par with thiamethoxam 12.6% + lambda-cyhalothrin 9.5% EC (52.40 mm) and beta-cyfluthrin 8.49% + imidacloprid 19.81% OD (49.80 mm). The minimum fungal growth 11.80 mm was recorded in the medium amended with metalaxyl-M 4%+ mancozeb 64% WP (11.80 mm).

The inhibitory response of the fungus followed a contrasting trend. The spirotetramat 11.01% + imidacloprid 11.01% SC treatment produced the lowest growth inhibition (9. 77%), whereas the highest inhibition (80.02 mm) was recorded with metalaxyl-M 4% + mancozeb 64% WP. These results indicate that the former treatment was comparatively less inhibitory to *M. anisopliae*, while the latter exerted a pronounced suppressive effect on fungal growth.

Assessment at Nine (09) Days After Inoculation (DAI)

The marked difference in the results of radial mycelial development of M. *anisopliae* was recorded among the pesticide treatments at nine (09) days of inoculation (DAI) (Table 2 and Fig. 1). The untreated control supported the maximum fungal growth (82.40 mm) and was significantly superior to all the pesticide treatments.

Among the pesticides evaluated, thiamethoxam 12.6% + lambda-cyhalothrin 9.5% EC recorded the highest mycelial growth (73.60 mm), which was statistically at par with spirotetramat 11.01% + imidacloprid 11.01% SC (72.80 mm). In contrast, the minimum mycelial growth (13.80 mm) was recorded with metalaxyl-M 4% + mancozeb 64% WP.

The percentage inhibition of fungal growth showed an inverse trend. The lowest inhibition (10.62%) was observed with thiamethoxam 12.6% + lambda-cyhalothrin 9.5% EC, indicating comparatively lower suppression of *M. anisopliae.* Conversely, metalaxyl-M 4% + mancozeb 64% WP (83.21%), reflected its strong inhibitory effect on fungal growth.

Assessment at 11 Days After Inoculation (DAI)

At 11 days after inoculation (DAI), substantial differences in the radial mycelial growth of *M. anisopliae* were observed among the pesticide treatments (Table 2 and Fig. 1). The untreated control attained the maximum colony diameter of 90.00 mm and was significantly superior to all the pesticide treated media. Among the evaluated pesticides, spirotetramat 11.01% + imidacloprid 11.01% SC supported the highest fungal growth (83.20 mm), which was statistically at par with thiamethoxam 12.6% + lambda-cyhalothrin 9.5% EC (82.00 mm). Conversely, the lowest mycelial growth (14.20 mm) was recorded with metalaxyl-M 4% + mancozeb 64% WP, which remained statistically at par with azoxystrobin 18.2% + difenoconazole 11.4% SC.

The percentage inhibition of mycelial growth showed a reverse pattern. The lowest inhibition (7.56%) was recorded with spirotetramat 11.01% + imidacloprid 11.01% SC, indicating its high compatibility with *M. anisopliae*. In contrast, metalaxyl-M 4% + mancozeb 64% WP caused the highest inhibition (84.44%), demonstrating a pronounced effect on fungal growth.

The results on pesticide compatibility with *M. anisopliae* clearly demonstrated that the pesticides with the best compatibility with *M. anisopliae* resulting in the greatest mycelial growth were found with spirotetramat 11.01% + imidacloprid 11.01% SC followed by thiamethoxam 12.6% + lambda-cyhalothrin 9.5% EC, beta-cyfluthrin 8.49% + imidacloprid 19.81% OD, fipronil 40% + imidacloprid 40% WG, bifenthrin 3% + chlorpyriphos 30% EC, chlorpyriphos 50% + cypermethrin 5% EC, profenofos 40% + cypermethrin 4% EC, azoxystrobin 18.2% + difenoconazole 11.4% SC and metalaxyl-M 4% + mancozeb 64% WP,, respectively.

3.2 Effect of Pesticides on Sporulation of *Metarhizium anisopliae* after 11 Days of Inoculation

Conidial production of *M. anisopliae* varied significantly among the treatments after 11 days of incubation (Table 3, Fig. 2 and Plate 1). The untreated control supported the greatest sporulation, recording a conidial count of 6.75×10^8 conidia $mL^{-1,}$ which was significantly higher than that observed in all pesticide-amended media.

Pesticide treatments differed significantly in their effect on the conidial production of *M. anisopliae.* The greatest conidial production was obtained with spirotetramat 11.01% + imidacloprid 11.01% SC (3.88×10^8 conidia mL^{-1}), which did not differ significantly from fipronil 40% + imidacloprid 40% WG (3.67×10^8 conidia mL^{-1}) and thiamethoxam 12.6% + lambda-cyhalothrin 9.5% EC (3.57×10^8 conidia mL^{-1}). In contrast, metalaxyl-M 4% + mancozeb 64% WP caused the greatest reduction in conidial production, recording only 0.72×10^8 conidia mL^{-1}.

Table 2
Mycelial growth and growth inhibition of *Metarhizium anisopliae* with chemical pesticides

Tr.	Name of the Pesticide	Dose	3 days		5 days		7 days		9 days		11 days	
		(g/ml)/ha	Mycelial growth (mm) Mean	G.I (%) Mean	Mycelial growth (mm) Mean	G.I (%) Mean	Mycelial growth (mm) Mean	G.I (%) Mean	Mycelial growth (mm) Mean	G.I (%) Mean	Mycelial growth (mm) Mean	G.I (%) Mean
T_1	Bifenthrin 3% + Chlorpyriphos 30% EC	1000 ml/ha	13.20 (3.70) *	59.53 (50.50) **	18.80 (4.39) *	57.65 (49.40) **	26.00 (5.14) *	56.10 (48.51)**	38.20 (6.22) *	53.63 (47.08) **	46.40 (6.84) *	48.44 (44.11)**
T_2	Chlorpyriphos 50% + Cypermethrin 5% EC	1000 ml/ha	13.40 (3.73)	58.87 (50.11)	17.00 (4.18)	61.71 (51.77)	21.60 (4.70)	63.52 (52.85)	27.60 (5.30)	66.49 (54.63)	35.00 (5.96)	61.11 (51.42)
T_3	Profenofos 40% + Cypermethrin 4% EC	1000 ml/ha	11.80 (3.51)	63.79 (53.01)	15.00 (3.94)	66.20 (54.46)	19.20 (4.44)	67.58 (55.30)	27.20 (5.26)	66.98 (54.93)	34.20 (5.89)	62.00 (51.94)
T_4	Thiamethoxam 12.6% + Lambda-cyhalothrin 9.5% EC	200–250 ml/ha	26.80 (5.22)	17.83 (24.83)	40.20 (6.38)	9.45 (17.84)	52.40 (7.27)	10.77 (18.93)	73.60 (8.60)	10.62 (18.67)	82.00 (9.08)	8.89 (17.29)
T_5	Fipronil 40% + Imidacloprid 40% WG	80–100 g/ha	25.20 (5.07)	22.64 (28.36)	37.80 (6.19)	14.84 (22.58)	47.40 (6.92)	19.92 (26.49)	68.60 (8.31)	16.72 (24.11)	75.40 (8.71)	16.22 (23.74)
T_6	Spirotetramat 11.01% (w/w) + Imidacloprid 11.01 (w/w) SC	500 ml/ha	24.80 (5.03)	23.84 (29.15)	39.40 (6.31)	11.71 (19.96)	53.40 (7.34)	9.77 (18.06)	72.80 (8.56)	11.63 (19.87)	83.20 (9.15)	7.56 (15.93)
T_7	Beta-cyfluthrin 8.49 (w/w) + Imidacloprid 19.81 (w/w) OD	200 ml/ha	26.60 (5.20)	18.42 (25.26)	38.60 (6.25)	13.05 (21.14)	49.80 (7.09)	15.88 (23.43)	68.60 (8.31)	16.73 (24.13)	79.80 (8.96)	11.33 (19.66)
T_8	Metalaxyl-M 4% (w/w) + Mancozeb 64% (w/w) WP	2000 g /ha	10.60 (3.33)	67.49 (55.26)	11.20 (3.42)	74.77 (59.87)	11.80 (3.48)	80.02 (63.65)	13.80 (3.77)	83.21 (65.87)	14.00 (3.81)	84.44 (66.78)
T_9	Azoxystrobin 18.2% (w/w) + Difenoconazole 11.4 (w/w) SC	300–400 ml/ha	12.80 (3.65)	60.67 (51.17)	13.40 (3.73)	69.82 (56.69)	13.60 (3.75)	77.02 (61.36)	14.20 (3.83)	82.76 (65.48)	14.20 (3.83)	84.22 (66.60)
T_{10}	Untreated control	-	32.60 (5.75)	0.00 (0.00)	44.60 (6.72)	0.00 (0.00)	59.20 (7.72)	0.00 (0.00)	82.40 (9.10)	0.00 (0.00)	90.00 (9.51)	0.00 (0.00)
	S.E.±	**-**	**0.05**	**1.02**	**0.07**	**0.64**	**0.10**	**1.02**	**0.07**	**0.88**	**0.06**	**0.39**
	CD at 1%	**-**	**0.15**	**2.92**	**0.21**	**1.85**	**0.31**	**2.92**	**0.22**	**2.52**	**0.17**	**1.11**

Note: * Figures in parentheses are √ X + 0.5 transformed values. ** Figures in parentheses are arc sin transformed values.

Table 3
Spore count of *Metarhizium anisopliae* after 11 days of inoculation

Tr.	Name of the Pesticide	Dose (g/ml)/ha	Spore count Mean ($\times 10^8$)
T_1	Bifenthrin 3% + Chlorpyriphos 30% EC	1000 ml/ha	1.90 (1.55) *
T_2	Chlorpyriphos 50% + Cypermethrin 5% EC	1000 ml/ha	1.40 (1.38)
T_3	Profenofos 40% + Cypermethrin 4% EC	1000 ml/ha	2.23 (1.65)
T_4	Thiamethoxam 12.6% + Lambda-cyhalothrin 9.5% EC	200–250 ml/ha	3.57 (2.02)
T_5	Fipronil 40% + Imidacloprid 40% WG	80–100 g/ha	3.67 (2.04)
T_6	Spirotetramat 11.01% (w/w) + Imidacloprid 11.01 (w/w) SC	500 ml/ha	3.88 (2.09)
T_7	Beta-cyfluthrin 8.49 (w/w) + Imidacloprid 19.81 (w/w) OD	200 ml/ha	2.20 (1.64)
T_8	Metalaxyl-M 4% (w/w) + Mancozeb 64% (w/w) WP	2000 g /ha	0.72 (1.10)
T_9	Azoxystrobin 18.2% (w/w) + Difenoconazole 11.4 (w/w) SC	300–400 ml/ha	0.88 (1.18)
T_{10}	Untreated control	-	6.75 (2.69)
	S.E.±	-	**0.03**
	CD at 1%	-	**0.09**

Note

* Figures in parentheses are √ X + 0.5 transformed value.

4. Discussion

Our current results on mycelial growth and growth inhibition corroborate with those of Sahoo et al. (2023), who reported that imidacloprid 17.8% SL at sub lethal concentrations exhibited the least inhibition of fungal growth and the highest radial mycelial growth. Similarly, Nawaz et al. (2022) demonstrated that imidacloprid was highly compatible with *M. anisopliae* and showed synergistic efficacy when combined with the fungus against *Aphis gossypii*. Likewise, our findings also corroborate with earlier reports by Parjane et al. (2020), who demonstrated that neonicotinoid insecticides, particularly imidacloprid and thiamethoxam, are compatible with *M. anisopliae*. Similarly, Akbar et al. (2012), who also reported that imidacloprid which were relatively less toxic to mycelial growth and spore production (P = 0.05) of *Metarhizium anisopliae*. A similar compatibility of imidacloprid and thiamethoxam based insecticidal combinations was observed in the present investigations.

Further, current findings are in accordance with the results of Khan et al. (2012), who stated that among the pesticides tested, imidacloprid (0.005%) was the safest and most compatible with *M. anisopliae*. Niassy et al. (2012) tested the compatibility of *M. anisopliae* (Metschnikoff) Sorokin isolate ICIPE 69 with pesticides thiamethoxam and imidacloprid and reported that, they were found to be compatible with *M. anisopliae*.

Present findings of compatibility of imidacloprid with *M. anisopliae* are also supported by Parjane et al. (2020) who demonstrated least inhibitory effect of imidacloprid and fipronil on vegetative growth of *M. anisopliae*. Likewise, Lavanya and Matti (2020) found that fipronil based insecticides exhibited high compatibility with *M. anisopliae*, as evidenced by low growth inhibition and high spore production. Consistent with these reports, fipronil + imidacloprid 40% WG exhibited good compatibility with *M. anisopliae* in the present study, recording mycelial growth of 75 .40 mm at 11 days after inoculation.

The present findings are in agreement with those of Silva et al. (2013), who reported the compatibility of thiamethoxam and lambda-cyhalothrin with *M. anisopliae*. A similar observation was reported by Parjane et al. (2020), who found that imidacloprid and pyrethroid based insecticides exerted minimal inhibitory effect on *M. anisopliae*. Consistent with these reports, in our present investigation thiamethoxam + lambda-cyhalothrin 9.5% EC showed high compatibility with *M. anisopliae*.

The present findings are supported by Nawaz et. al. (2022), who reported that both spirotetramat and imidacloprid were highly compatible with *M. anisopliae* and showed positive integration with the fungus. Similar observations were reported by Band and Kabare (2022), on compatibility of imidacloprid with *M. anisopliae* under in vitro conditions. In the current investigation, spirotetramat 11.01% (w/w) + imidacloprid 11.01 (w/w) SC demonstrated the highest mycelial growth (83.20 mm) with the least growth inhibition (7.56%).

These results are further supported by Nawaz et al. (2022), who demonstrated that *M. anisopliae* remained compatible with selective insecticides, including a thiamethoxam based combination and that their interaction enhanced pest suppression without compromising fungal performance. More recently, Patil et al. (2025) also reported that thiamethoxam 12.6% + lambda-cyhalothrin 9.5% was classified as harmless and compatible with *M. anislopliae* under laboratory conditions.

The present results are in agreement with those of Dhanya et al. (2019), who reported that *M. anisopliae* was least affected by fipronil, indicating its compatibility with the entomopathogenic fungus. Similarly, in the present investigations, the combination of fipronil 40% + imidacloprid 40% exhibited high compatibility with *M. anisopliae*, causing only 16.22% mycelial growth inhibition and supporting radial mycelial growth of 75.40 mm.

The current findings are not consistent with Lavanya and Matti (2020) research on the effectiveness of insecticides against the entomopathogenic fungus *M. anisopliae*. Imidacloprid + Fipronil exhibited (39.84%) growth suppression in vitro, while in the current findings, imidacloprid + fipronil merely showed (16.22%) growth inhibition, indicating its compatibility.

The present findings demonstrated that the insecticide combinations thiamethoxam 12.6% + lambda-cyhalothrin 9.5% EC (82.00 mm), fipronil 40% + imidacloprid 40% WG (75.40 mm), spirotetramat 11.01% + imidacloprid 11.01%. SC (83.20 mm) and beta-cyfluthrin 8.49% + imidacloprid 19.81% OD (79.80 mm) supported substantial radial mycelial growth of *Metarrhizium anisopliae*, indicating their high compatibility with the entomopathogenic fungus. The superior fungal growth observed in these treatments suggests that this insecticides mixture did not significantly inhibit fungal development and, therefore have potential for combined use with *M. anisopliae* in IPM strategies to achieve effective and sustainable pest management.

5. Conclusion

The present study demonstrated differential compatibility of the tested chemical pesticides with *Metarhizium anisopliae*, as reflected by variations in mycelial growth and conidial production. Among the evaluated formulations, spirotetramat 11.01% + imidacloprid 11.01% SC exhibited the greatest compatibility, supporting maximum radial mycelial growth (83.20 mm) and conidial production (3.88×10^8), whereas metalaxyl-M 4% + mancozeb 64% WP showed the strongest inhibitory effect, with mycelial growth and conidial production of 14.00 mm and 0.72×10^8, respectively.

Declarations

Author Contribution

Nitin Parjane and Kabre Ghansham conducted the study and collected the data during the course of investigation. Nitin Parjane, Shinde Sojwal and Sawant C.G wrote the manuscript. Shinde Sojwal and Sawant C.G helped to prepare the manuscript along with technical assistance and shared their experience about compatibility of entomopathogenic fungus with different pesticides and its biological control.

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Plate

Plate 1 is available in the Supplementary Files section.

Figures

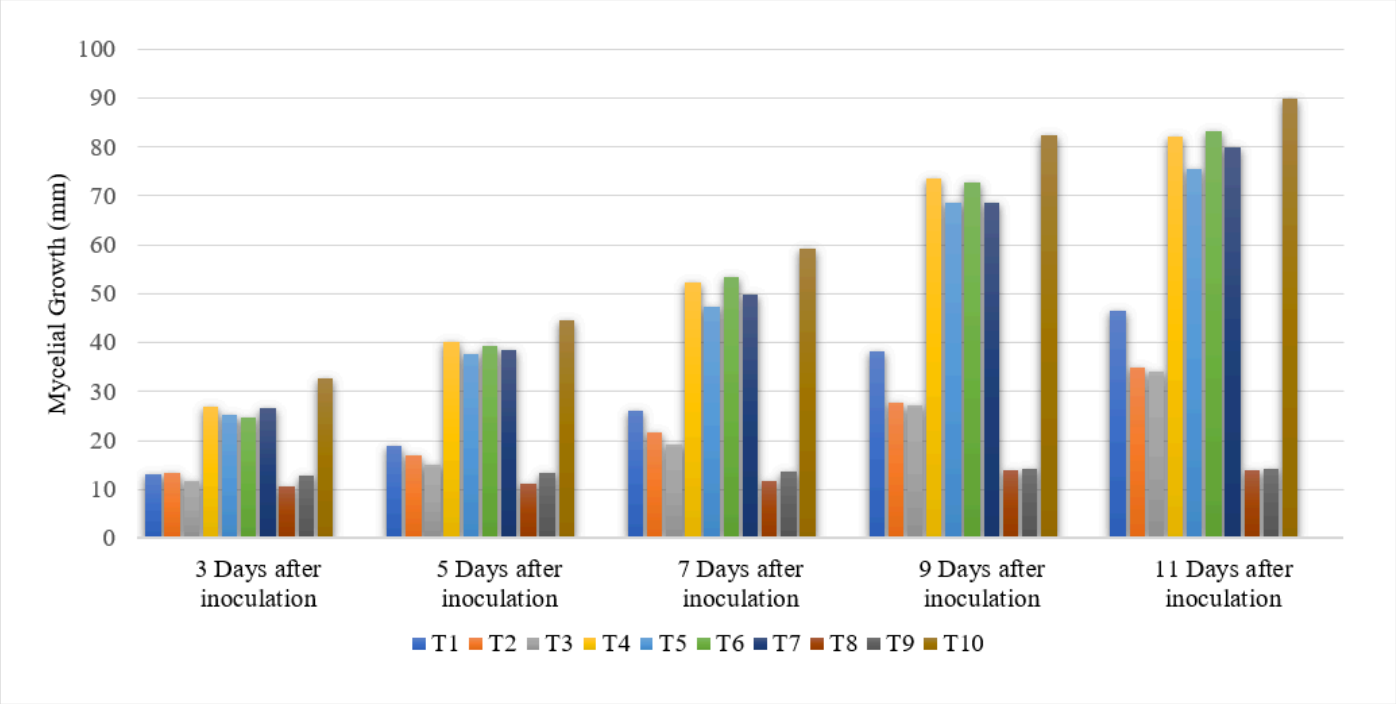


Figure 1

Vegetative growth of *Metarhizium anisopliae* 1.15 % WP with selective pesticides at 3rd, 5th, 7th, 9th and 11th day after inoculation

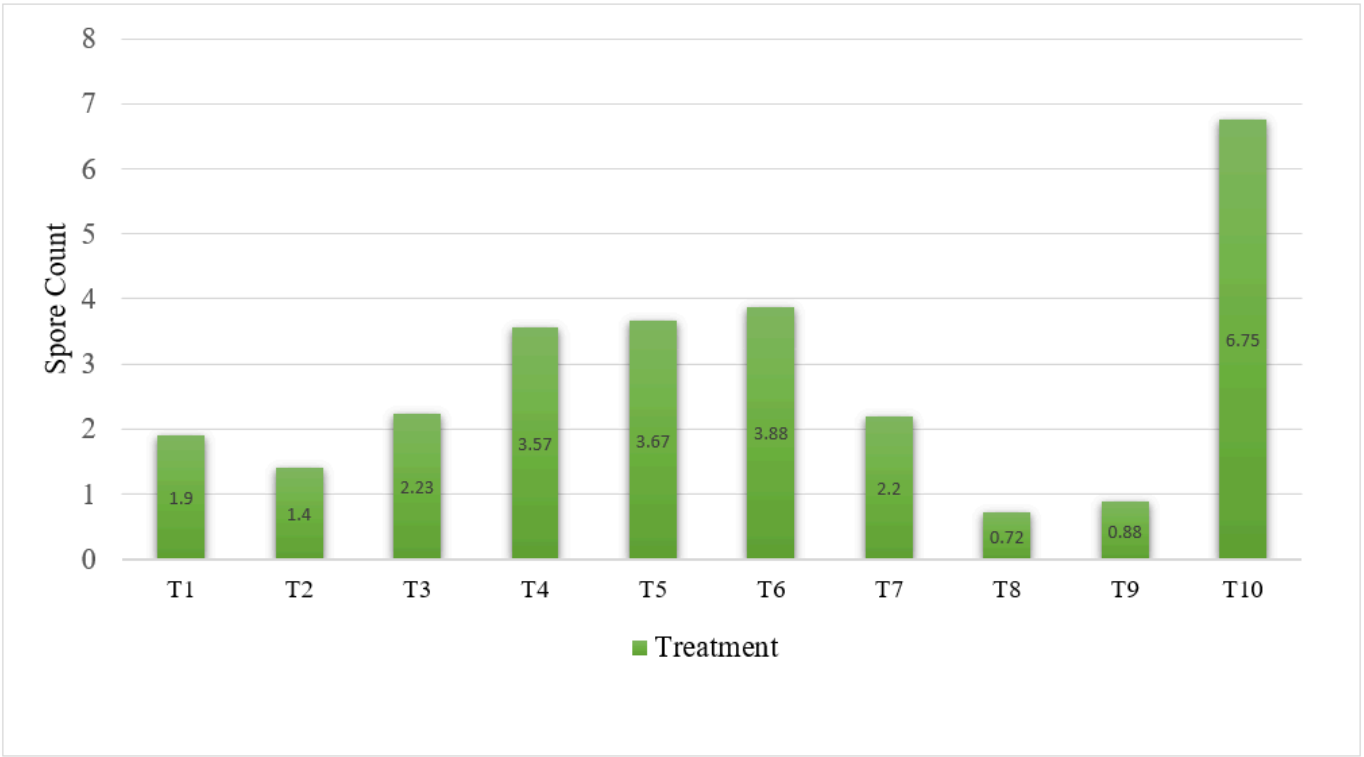


Figure 2

Spore count of *Metarhizium anisopliae* 1.15 % WP at 11th day after inoculation

Supplementary Files

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- plate1.png