



## In Vitro Compatibility and Toxicity of *Metarhizium anisopliae* with Insecticides against *Spodoptera litura*

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### Abstract

The present investigation was conducted during June, 2022 to January, 2023 at Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (MH) to examine the compatibility of *M. anisopliae* with insecticides by poison food technique in completely randomized design. The data on colony diameter of *M. anisopliae* in each treatment was collected at 10 days after inoculation. Among the insecticides tested, Chlorantraniliprole 18.50% SC (0.006%) exhibited minimum inhibitory effect on *M. anisopliae* and proved most compatible insecticide tested against *M. anisopliae*, stood at par with *M. anisopliae* alone. It was followed by treatment Chlorantraniliprole 9.30%+λ-cyhalothrin 4.60% ZC (0.007%), Thiamethoxam 25% WG (0.003%), Thiamethoxam 12.60%+λ-cyhalothrin 9.50% ZC (0.009%), λ-cyhalothrin 5% EC (0.005%). However, all the insecticidal treatments proved “harmless” as per Hassan (1989) and were compatible with *M. anisopliae*. Besides, the experiment was performed to assess the toxicity of *M. anisopliae* alone and in combination with insecticides at half the recommended concentration against third instar larvae of *Spodoptera litura* by leaf dip bioassay. The observations on mortality of larvae of *S. litura* were recorded at 3, 7 and 10 days after treatment. At 10 days after treatment, Chlorantraniliprole 18.50% SC (0.003%)+*M. anisopliae* and Chlorantraniliprole 9.30%+λ-cyhalothrin 4.60 % ZC (0.0035%)+*M. anisopliae* caused promising larval mortality of cent per cent each. The combination of *M. anisopliae* with insecticides at half the recommended dose led to higher mortality of the *S. litura*.

**Keywords:** Compatibility, insecticides, *Metarhizium anisopliae*, *Spodoptera litura*, toxicity

### 1. Introduction

*Spodoptera litura* is a serious polyphagous pest in the Indian subcontinent. It causes severe damage to more than 115 plant species including pulses, cotton, cabbage, cauliflower, castor, groundnut and oilseed crops (Atwal and Dhaliwal, 2009). In cabbage, maximum head infestation (54%) was recorded by average larval population of *S. litura* (Sahu et al., 2020). It continues to inflict heavy losses in several cotton growing regions of India. Moreover, the intensity of *S. litura* is likely to further increase under the potential climate change, as it has been found to consume more than 30% cotton leaves at elevated CO<sub>2</sub> levels (Kranthi et al., 2009). Outbreaks of *S. litura* were also noticed in major sunflower growing areas of Central and Southern India. During 2005, the outbreak of *S. litura* led to more than 90% defoliation of sunflower cultivar germplasm (Sujatha and Lakshminarayana, 2007). When its control and management strategies are discussed, findings come forward that, the exhaustive use of insecticides for its management has

led to the problem of resistance. Moreover, growing public concern over potential health hazards of synthetic pesticides has changed the research towards more environment friendly insect pest management tactics (Dhar et al., 2019).

Use of biological inputs in plant protection started gaining importance as an alternative to chemical pest management practices. Entomopathogenic fungus like *Beauveria bassiana*, *Metarhizium anisopliae*, *Nomuraea rileyi*, *Lecanicillium lecanii* and *Paecilomyces* have been widely researched among entomopathogenic fungi for their bioefficacy and based on that many commercial products have been developed (Faria et al., 2007). Fungi are the most widespread group of entomopathogens closely linked to agriculture. Many entomopathogenic fungi, particularly *Metarhizium anisopliae*, are used as biological control agents against insects, including gregarious pest species (Moorhouse et al., 1992, 1993a, 1993b; Booth and Shanks, 1998; Bruck, 2005; Bruck and Donahue, 2007; Freed et al., 2012). There



are numerous examples where the combination of different selective chemical insecticides and fungi has proven effective in controlling various agricultural insect pests (Quintela and McCoy, 1998; Dayakar et al., 2000; Serebrove et al., 2005; Purwar and Sachen, 2006).

The integrated use of entomofungal pathogens with full or reduced doses of chemical insecticides presents a promising pest control strategy. The inclusion of synergists can improve both the cost-effectiveness and environmental sustainability of insecticides by minimizing the required dosage and prolonging their residual activity. By targeting pests through different modes of action, synergists also play a crucial role in resistance management. The outcome of insecticides on the growth of fungi can be different due to the chemical nature of products and the fungal species that are interacting with it (Antonio et al., 2001; Kumar et al., 2000). As per the study of Akbar et al. (2012) insecticides significantly inhibited mycelial growth and spore production of the *M. anisopliae*. But on the contrary, some were significantly compatible and were found safe to conidial germination and growth of the fungi. In connection to this, various scientists have urged that effect of insecticides on EPF needs further exploration and research for the collective application of insecticides and fungi in order to control insect pests.

Farmers and officials in the Department of Agriculture frequently inquire about the compatibility of *M. anisopliae* with the insecticides available in the market. Therefore, by taking the need of further research into consideration, attempt have been made in the present study to find out *in vitro* compatibility of *M. anisopliae* with pesticides and to assess their efficacy against *S. litura*.

## 2. Materials and Methods

The present investigation was conducted during June, 2022 to January, 2023 at Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (MH) to examine the compatibility of *M. anisopliae* with insecticides by poison food technique in completely randomized design.

### 2.1. Fungus culture

The *Metarhizium anisopliae* used in the study was sourced from the culture collection at the Centre for Organic Agriculture Research and Training (COART) at Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The fungus was mass multiplied using Sabouraud's dextrose medium and subsequently employed in the research.

### 2.2. *In vitro* compatibility of *M. anisopliae* with insecticides

The desired quantity of recommended concentrations of insecticide for 100 ml SDA media was incorporated separately in warm autoclaved SDA media just before pouring the petri plates. The plates with SDA media were kept inside the laminar air flow chamber for solidification. The plates after solidification were inoculated centrally with 6 mm disc

of young sporulating culture of *M. anisopliae* with the help of sterilized cork borer and fungal inoculating needle. As such four replications were maintained for each treatment of insecticides of recommended concentration. A medium without insecticides served as control. The inoculated plates were incubated at  $25 \pm 1^\circ\text{C}$  in the incubator. The observations on colony diameter in each plate were recorded at 10 days after treatment. The percentage inhibition of *M. anisopliae* was determined based on the colony growth diameter, using the formula provided by Hokkanen and Kutluoto (1992).

The pesticides were then categorized for evaluation using a 1 to 4 scoring index: 1-harmless (<50% reduction), 2-slightly harmful (50–79% reduction), 3-moderately harmful (80–90% reduction), and 4-harmful (>90% reduction), following Hassan's classification (Hassan, 1989).

### 2.3. Toxicity of *M. anisopliae* alone and in combination with insecticides against *Spodoptera litura*

The laboratory-reared third instar larvae were used for this experiment, following the leaf dip bioassay. The measured quantity of pesticide was added in sterile distilled water to get the desired concentration. Separate plastic container was used for each treatment. The stock solution of *Metarhizium anisopliae* was prepared from 20 days old sporulating culture plates. Treatment solution were prepared separately for leaf dip bioassay. Fresh castor leaves sterilized with 0.01% sodium hypochlorite were used for leaf dip bioassay. The castor leaves were dipped for 10 seconds in treatment solution and were air dried at room temperature. Ten larvae of third instar per treatment per replication were exposed to the treated leaves. As such three replications were maintained at  $27 \pm 2^\circ\text{C}$  and  $78 \pm 2\%$  RH. The untreated control was maintained by using distilled water.

The observations were recorded on larval mortality at 3<sup>rd</sup>, 7<sup>th</sup> and 10<sup>th</sup> days after treatment. The moribund larvae were prodded with the blunt needle and those unable to move were considered as dead.

### 2.4. Statistical analysis

All experiments employed a completely randomized design (CRD). The collected data were transformed as needed, analyzed using ANOVA, and mean values were compared using the critical difference method with a significance level of  $p=0.01$  (Gomez and Gomez, 1984).

## 3. Results and Discussion

### 3.1. Compatibility of *M. anisopliae* with insecticides

The data presented in the Table 1 on compatibility of *M. anisopliae* with insecticides by poison food technique revealed that among the insecticides tested Chlorantraniliprole 18.50% SC exhibited minimum inhibitory effect on *M. anisopliae* on the SDA media plates as it showed 5.70% mycelial growth inhibition and 59.25 mm radial mycelial growth. Moreover, it proved as the most compatible and least toxic



Table 1: Compatibility of *Metarhizium anisopliae* with insecticides (Poison food technique)

Sl. No.	Treatment	Conc. (%)	<i>M. anisopliae</i> (10 DAI)		
			Mean of radial mycelium growth (mm)	Growth inhibition (%)	Grade*
1.	Chlorantraniliprole 9.30%+λ-cyhalothrin 4.60% ZC (combi product)	0.007	57	9.28	1
2.	Thiamethoxam 12.60%+λ-cyhalothrin 9.50% ZC (combi product)	0.009	40.5	35.54	1
3.	Chlorantraniliprole 18.50% SC	0.006	59.25	5.70	1
4.	λ-cyhalothrin 5% EC	0.005	34	45.88	1
5.	Thiamethoxam 25% WG	0.003	44.88	28.56	1
6.	<i>Metarhizium anisopliae</i> (Control)	–	62.83	–	
	'F' test		Sig.		
	SEm±		1.19		
	CD ( $p=0.01$ )		4.84		
	CV		4.78		

Note: DAI: Days after inoculation; \*Grade 1: harmless (<50% reduction in beneficial capacity); 2: slightly harmful (50–79%), 3: moderately harmful (80–90%), 4: harmful (>90%) in *in vitro* toxicity tests (Hassan, 1989)

insecticide tested against *M. anisopliae* and stood at par with *M. anisopliae* alone with 62.83 mm radial mycelial growth. It was followed by treatment Chlorantraniliprole 9.30%+λ-cyhalothrin 4.60% ZC (combi product) which recorded 9.28% growth inhibition with radial mycelial growth of 57 mm. The insecticidal treatments Thiamethoxam 25% WG and Thiamethoxam 12.60%+λ-cyhalothrin 9.50% ZC (combi product) exhibited relatively less compatibility with *M. anisopliae*. These treatments showed 44.88 and 40.50 mm radial mycelial growth and exhibited 28.56 and 35.54% mycelial growth inhibition, respectively. Moreover, these treatments were found statistically at par with each other. The insecticidal treatment λ-cyhalothrin 5% EC appeared to be least compatible with *M. anisopliae* which recorded 34 mm radial mycelial growth and highest mycelial growth inhibition i.e. 45.88%. However, all the insecticidal treatments showed less than 50% mycelial growth inhibition and proved harmless as per the classification scheme given by Hassan (1989).

The present findings on the compatibility of *M. anisopliae* with insecticides are in corroboration with the results of Kiruthiga et al. (2022) who reported that Chlorantraniliprole had synergistic activity at lower and field doses with less effect on vegetative growth, sporulation and biomass production. Furthermore, Dhanya et al. (2020) stated that Chlorantraniliprole have high compatibility with *M. anisopliae* with less than 25% growth inhibition over control. Band and Kabre (2022) recorded 27.78% growth inhibition of *M. anisopliae* in Chlorantraniliprole 18.5 SC indicated its better compatibility with *M. anisopliae*.

The results of the present studies pertaining to compatibility of *M. anisopliae* with Thiamethoxam are in the line of research

work of Naissy et al. (2012) revealed that Thiamethoxam did not have deleterious effect on vegetative growth and conidia production of *M. anisopliae* and found to be compatible. Gulsar and co-workers (2014) categorized Thiamethoxam 25 WG as hazardless in relation to isolate of a native entomopathogenic fungus, *M. anisopliae* (ARSEF-9613).

The earlier worker, Silva et al. (2012) reported that Thiamethoxam and λ-cyhalothrin were found compatible with *M. anisopliae* (strain CG 168), while Thiamethoxam+λ-cyhalothrin was classified as moderately toxic. However, as per Anderson et al. (1989) the potential inhibitory effects of pesticides on germination and mycelia growth of biocontrol fungi vary from taxa and strains.

### 3.2. Toxicity of *M. anisopliae* alone and in combination with insecticides against *Spodoptera litura*

The results displayed in Table 2 revealed that all the treatments were significantly superior over untreated control in recording larval mortality of *S. litura* at 10 days after treatment. The treatment Chlorantraniliprole 18.50% SC (0.003%)+*M. anisopliae* and Chlorantraniliprole 9.30%+λ-cyhalothrin 4.60% ZC (0.0035%)+*M. anisopliae* caused promising larval mortality of cent per cent each. These were closely followed by λ-cyhalothrin 5% EC (0.0015%)+*M. anisopliae* (96.67% larval mortality), Chlorantraniliprole 9.30%+λ-cyhalothrin 4.60% ZC (0.007%), Thiamethoxam 12.60%+λ-cyhalothrin 9.50% ZC (0.0045%)+*M. anisopliae* and Chlorantraniliprole 18.50% SC (0.006%) caused 93.33% larval mortality each. The remaining treatments viz., Thiamethoxam 12.60%+λ-cyhalothrin 9.50% ZC (0.009%), λ-cyhalothrin 5% EC (0.003%) (90% larval mortality each), Thiamethoxam 25% WG (0.005%) and Thiamethoxam 25% WG (0.0025%)+*M.*



Table 2: Toxicity of *Metarhizium anisopliae* and insecticides against *Spodoptera litura*

Sl. No.	Treatment	Conc.	<i>S. litura</i> larvae (% mortality )		
			3 DAT	7 DAT	10 DAT
1.	Chlorantraniliprole 9.30%+λ-cyhalothrin 4.60% ZC (combi product)	0.007%	73.33 (58.91)*	90.00 (71.57)	93.33 (75.04)
2.	Chlorantraniliprole 9.30%+λ-cyhalothrin 4.60% ZC (combi product)+ <i>M. anisopliae</i>	0.0035%+1×10 <sup>8</sup> (conidia ml <sup>-1</sup> )	66.67 (54.74)	96.67 (79.48)	100.00 (89.09)
3.	Thiamethoxam 12.60%+λ-cyhalothrin 9.50% ZC (combi product)	0.009%	73.33 (58.91)	90.00 (71.57)	90.00 (71.57)
4.	Thiamethoxam 12.60%+λ-cyhalothrin 9.50% ZC(combi product)+ <i>M. anisopliae</i>	0.0045%+1×10 <sup>8</sup> (conidia ml <sup>-1</sup> )	53.33 (46.91)	86.67 (68.58)	93.33 (75.04)
5.	Chlorantraniliprole 18.50% SC	0.006%	76.67 (61.12)	90.00 (71.57)	93.33 (75.04)
6.	Chlorantraniliprole 18.50% SC+ <i>M. anisopliae</i>	0.003%+1×10 <sup>8</sup> (conidia ml <sup>-1</sup> )	63.33 (52.73)	100.00 (89.09)	100.00 (89.09)
7.	λ-cyhalothrin 5% EC	0.003%	76.67 (61.12)	90.00 (71.57)	90.00 (71.57)
8.	λ-cyhalothrin 5% EC+ <i>M. anisopliae</i>	0.0015%+1×10 <sup>8</sup> (conidia ml <sup>-1</sup> )	73.33 (58.91)	93.33 (75.04)	96.67 (79.48)
9.	Thiamethoxam 25% WG	0.005%	56.67 (48.83)	66.67 (54.74)	73.33 (58.91)
10.	Thiamethoxam 25% WG+ <i>M. anisopliae</i>	0.0025% +1×10 <sup>8</sup> (conidia ml <sup>-1</sup> )	46.67 (43.09)	53.33 (46.91)	73.33 (58.91)
11.	<i>M. anisopliae</i>	1×10 <sup>8</sup> (conidia ml <sup>-1</sup> )	13.33 (21.42)	33.33 (35.26)	66.67 (54.74)
12.	Untreated control	–	0.00 (0.91)	6.67 (14.96)	10.00 (18.43)
	'F' test		Sig.	Sig.	Sig.
	SEm±		2.06	4.94	4.29
	CD (p=0.01)		8.16	19.56	16.96
	CV		7.55	13.46	10.72

DAT– Days after treatment; \*Figures in parentheses are corresponding arcsine transformed values; The value of 0% is substituted by (1/4n) and the value of 100% by (100–1/4n), where n is the number of units upon which the percentage data is based (i.e. the denominator used in computing the percentage)

*anisopliae* (73.33% each) found at par with each other. The mean larval mortality of *S. litura* was lowest in treatment of *M. anisopliae* alone (i.e. 66.67%) but superior over untreated control (10% larval mortality).

The results of present study find support of the research work carried out by earlier worker like Batool et al. (2022) evaluated the insecticides and EPF against 3<sup>rd</sup> instar larvae of *S. litura* using diet incorporation method. Among the insecticides, Chlorantraniliprole exhibited maximum larval mortality with minimum LC<sub>50</sub> and LT<sub>50</sub> values. Whereas, among the EPF, *M. anisopliae* showed significantly higher entomopathogenicity and caused 20–53% larval mortality in 3–10 days' post exposure. Moreover, observed that binary combination of *M. anisopliae* and Chlorantraniliprole exhibited synergistic effect against *S. litura* larvae. Further concluded that these pesticides can be employed together against *S. litura* infestations and it will also aid to mitigate the insecticides resistance. The

combined application of an insecticide along with an EPF may synergize their effectiveness as the mycopathogen makes the host more susceptible to insecticides and the insecticide in turn makes the pest more vulnerable to disease establishment (Charnley and Collins, 2007; Jia et al., 2016).

Whereas, Negi and Shrivastava (2019) revealed that Chlorantraniliprole+λ-cyhalothrin was most effective showing better contact and stomach action against *S. litura* and also was effective in reducing the feeding. The results on the efficacy of insecticides against *S. litura* are in accordance with Barrania (2013) who studied the antifeedant, growth inhibitory and toxicity effect of Chlorantraniliprole, Thiamethoxam and Novaluron at different rates against *Spodoptera littoralis*. The result revealed that feeding the *Spodoptera littoralis* larvae on treated cotton leaves decreased the food consumption, relative growth rate and showed effective larval mortality. It is likely that the increased toxicity could be due to less–





widely-used status of this insecticide against *S. litura* and the different and unique mode of action.

The results pertaining to toxicity of *M. anisopliae* finds support in the research work carried out by Sahayaraj et al. (2018) revealed that the *M. anisopliae* altered the feeding behaviour and subsequent food consumption and weight gain, fecal pellet production, and relative growth rate of *S. litura*. Amer et al. (2008) reported that *M. anisopliae* gave the highest mortality (60 and 55%) to the 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *S. littoralis* with lethal time (LT<sub>50</sub>) 7 and 10 days, respectively. Whereas, Hu et al. (2007) observed that larvae of *S. litura* were repelled by *M. anisopliae* toxins (destruxins, depsi peptides), and their death was presumably due to starvation as opposed to toxicosis.

#### 4. Conclusion

Among the insecticides tested, Chlorantraniliprole 18.50% SC (0.006%) exhibited minimum inhibitory effect on *M. anisopliae* and proved most compatible insecticide tested against *M. anisopliae*. Furthermore, in toxicity experiment, Chlorantraniliprole 18.50% SC (0.003%)+*M. anisopliae* and Chlorantraniliprole 9.30%+λ-cyhalothrin 4.60% ZC (0.0035%)+*M. anisopliae* caused promising larval mortality of cent per cent each. Using these combined applications can improve control effectiveness by reducing the amounts used, minimizing the risk of environmental pollution, and decreasing the likelihood of pest resistance development.

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