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Compatibility of different Insecticides with Entomopathogen *Metarhizium anisopliae* (Metchnikoff) Sorokin

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ABSTRACT

The most notable entomopathogenic fungus species is *Metarhizium anisopliae*. The most adaptable and ecologically secure biological control agents are the entomopathogenic fungi, which are frequently implicated in high levels of epizootics in the natural world. The present study was carried out in the Entomology laboratory, School of Crop Protection (SCP), College of Post Graduate Studies in Agricultural Sciences (CPGS-AS), CAU (Imphal), Umiam, (25°40'52.32"N, 91°54'41.04"E) Meghalaya during the academic year 2021-2022. The compatibility of six different insecticides with entomopathogen *Metarhizium anisopliae* was evaluated using the 'Poisoned food technique' at both lethal (LD) and sub-lethal dosages (SLD). *In vitro* evaluation of compatibility of six different chemical insecticides with entomopathogen *M. anisopliae*, revealed that Imidacloprid17.8% SL (SLD) showed the best compatibility with *M. anisopliae* showing lowest per cent inhibition and highest radial growth, followed by Pymetrozyne 50%WG (SLD), Thiamethoxam25% WG (SLD) and Diafenthiuron50% WP (SLD). Deltamethrin 2.8% EC (LD) was most detrimental to entomopathogen showing lowest radial growth and highest per cent inhibition followed by Acephate75% SP (LD).

1. Introduction

Metarhizium anisopliae is a fungus that naturally occurs in soils all over the world and causes disease in a variety of insects. It is an asexual diasporic fungus that was previously included in the form class Hyphomycetes of the phylum Deuteromycota (also often called Fungi Imperfecti). As biological control agents for insects, including gregarious insect pests, many entomopathogenic fungi, including *Metarhizium anisopliae*, are utilised.

According to Carruthers and Hural (1990), entomopathogenic fungi are significant natural biological control agents of numerous insects, including a number of pests. Achievement of entomopathogens is one of the techniques that should be taken into consideration as a significant pest reduction element in Integrated Pest Management (IPM) programmes. IPM may be impacted by the use of incompatible insecticides because they may impede the growth, development, and germination of these pathogens (Anderson and Roberts, 1983, Duarte,1992 and Malo,1993). An essential aspect in reducing pest population density in Integrated Pest Management (IPM) is biological control using entomopathogens. Therefore, if entomopathogens exist naturally, are treated, or are introduced with the intention of reducing pests, their conservation is required. In other hand combined application of compatible insecticides with entomopathogens can increase the control efficiency, decrease the amount of insecticides required and minimize the risks of environmental contamination and pests resistance expression (Moino,1998 and Quintela and McCoy, 1998).

Insecticide overuse may hasten resistance and result in the resurgence of herbivore pests, as well as negative effects on natural predators and pollinators and concerns about environmental pollution. Future efforts should be directed toward the development of alternative approaches to

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reduce the use of chemical agents and their effects on non-target organisms. Microbial control is a safe and effective method used in integrated pest management programs, with significant potential for insect pest control. Entomopathogenic fungi are important biological pest control agents that pose little risk to the environment. Resistance to entomopathogenic fungi does not develop quickly in insect pests. So simultaneous use of fungal and chemical agents to control insects may result in much higher mortality than using either control strategy alone and may be beneficial to the farming community in terms of quality food production with low environmental risk.

2. Materials and Methods

The experiment was initiated in the month of June 2021 in the Entomology laboratory, School of Crop Protection, College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University, Umiam (25°40’52.32"N,91°54’41.04"E), Meghalaya.

Culture of *Metarhizium anisopliae*

The fungus inoculum of *M. anisopliae* was taken from the Biopesticide production unit, CPGS-AS, CAU, Umiam, Meghalaya. The fungus was multiplied and grown in autoclaved potato dextrose broth medium, as suggested by Kadam and Jaichakravarthy (2003).

Poison Food Technique

The standard poison food technique (Nene and Thapliyal, 1993) was followed to assay the effect of pesticides on *Metarhizium anisopliae*.

Quantity of insecticides required

The amount of insecticides used was determined by the rate of application in the field using high volume sprayers. Insecticides were filtered through micro filters and added to the PDA medium (100ml) in flask before solidification (medium temperature 46-48 ºC) to achieve the desired concentration and thoroughly mixed. The medium was allowed to solidify aseptically in a laminar airflow cabinet. The mixture was then evenly distributed among the five petri plates, which were kept in laminar air flow. The culture of *M. anisopliae* was inoculated under aseptic conditions after the PDA medium in Petri plates had completely cooled. A small disc fungus of 5 mm diameter was taken from pure culture of *M. anisopliae* and mycelium was cut with a sterile cork borer (5mm diameter) and aseptically placed in the centre of petri plates containing the poisoned medium. Three replications of each treatment were kept. A suitable check, free of poison, was kept for comparison under the same conditions. These plates were incubated in the BOD incubators at 25±1ºC. To assess the degree of toxicity of different insecticides used in the study, fungal colony diameter was measured on the ninth day after inoculation and compared to a standard check by following the method of Depieri *et al.* (2005). For each chemical, the inhibition of colony growth over an untreated control was determined.

Table 1. Details of insecticides used for the compatibility study

Sl. No.	Name of the chemical	Trade name	Lethal dosage	Sub-lethal dosage
1.	Pymetrozyne 50%WG	Pymate (Godrej Agrovet. Ltd.)	0.5g/L of water	0.25g/L of water
2.	Deltamethrin 2.8% EC	Decis (Bayer Crop Sciences Pvt. Ltd.)	1.5 ml/L of water	0.75 ml/L of water
3.	Thiamethoxam 25% WG	Eco champ (TM-Trademark of Isagro Agrochemical Pvt. Ltd.)	0.25 g/L of water	0.125 g/L of water
4.	Acephate 75% SP	Chetak (GSP Crop Sciences Pvt. Ltd.)	1 g/L of water	0.5 g/L of water
5.	Imidacloprid 17.8% SL	Imidacel (Sumitomo Chemical India Ltd.)	0.3 ml/L of water	0.15 ml/L of water
6.	Diafenthiuron 50% WP	Jimmy Deluxe (ANU Product Ltd.)	1 g/L of water	0.5 g/L of water

Vegetative growth of *M. anisopliae*

The fungus's radial growth was measured on the 9th day after inoculation and compared to an untreated control.

Formula of percentage inhibition:

Percent inhibition of radial growth = $\left[\frac{(C-T)}{C}\right] \times 100$

Where, C = Colony diameter of BCA in control (mm)

T = Colony diameter of BCA in treatment (mm)

BCA: Biocontrol agent

Statistical Analysis

The experimental design for all trials was completely randomized. The data were analysed using the analysis of variance (ANOVA) and the mean values were compared by using the Turkey multiple range test (P = 0.05) using the statistical package for the social sciences (SPSS) package 11.5 version.

3. Results and Discussion

Radial growth

Figure 1 shows the radial growth recorded on 9thday after inoculation (DAI) in PDA poisoned with insecticides. The radial growth of fungus is expressed as mean colony diameter, as indicated in Table 2.

At Half the Recommended Dose

The mean colony diameter on 9th DAI, it was observed that, in poisoned media, the highest radial growth of 90 mm observed in T1 treatment *i.e.*, control. The highest mean radial growth of 63.26 mm was recorded in T11 treatment combination *i.e.* Imidacloprid 17.8 % SL @ 0.1-0.15 ml/L (SLD) with *M. anisopliae* followed by T3 treatment *i.e.*, combination of Pymetrozyne 50 % WG @0.25 g/L (SLD) with *M. anisopliae* (61.67 mm), T7 i.e., Thiamethoxam25 % WG @ 0.125 g/L+ *M. anisopliae* (52.53 mm), T13 *i.e.*, Diafenthiuron 50 % WP @ 0.4-0.5 g/L +*M. anisopliae* (47.06 mm), T9 *i.e.*, Acephate75 % SP @0.5g/L+*M. anisopliae* (36.33 mm) and the lowest mean colony diameter at half the recommended dose was observed in T5 treatment combination *i.e.* Deltamethrin2.8 % EC @ 0.75 ml/L (sub-lethal dose) with *M. anisopliae* which gave 33.73 mm.

At half the recommended dose, Imidacloprid 17.8 % SL exhibited the highest radial growth followed by Pymetrozyne 50 % WG, Thiamethoxam25 % WG, Diafenthiuron 50 % WP, Acephate75 % SP. The least radial growth was observed in Deltamethrin2.8 % EC.

At Recommended Dose

The highest radial growth of 90.00 mm on 9 DAI was observed in T1 treatment *i.e.*,control. Among recommended dose of chemical insecticides, the highest radial growth was observed in T10 treatment combination *i.e.*, Imidacloprid 17.8 % SL @ 0.2-0.3 ml/L (LD) with *M. anisopliae* (53.93 mm) followed by T2 *i.e.*, Pymetrozyne 50 % WG @ 0.5 g/L (LD) with *M. anisopliae* (51.20 mm),T6 treatment *i.e.*, Thiamethoxam @ 0.25 g/L (LD) with *M. anisopliae* (49.73 mm), T12 treatment *i.e.*, Diafenthiuron @ 0.8-1 g/L of water (lethal dose) with *M. anisopliae* (42.53 mm) and T8 treatment *i.e.*, Deltamethrin2.8 % EC @ 1.5 ml/L (lethal dose) with *M. anisopliae* (26.00 mm).

At recommended dose, Imidacloprid 17.8 % SL observed highest radial growth followed by Pymetrozyne 50 percent WG, Thiamethoxam25 % WG, Diafenthiuron 50 % WP and Acephate75 % SP. The least radial growth was observed in Deltamethrin2.8 % EC.

Per cent inhibition

Per cent inhibition was calculated over radial growth which is presented in the Table 2.

At Half the Recommended Dose

Evaluation of per cent inhibition of radial growth of entomopathogenic fungus showed that, highest per cent inhibition of 62.51 % was in T5 (Deltamethrin 2.8 % EC@ 0.75 ml/L (SLD) with *M. anisopliae* followed by T9 (Acephate75 % SP @ 0.5 g/L (SLD) + *M. anisopliae*), T13 (Diafenthiuron50 % WP @ 0.4-0.5 g/L (SLD) +*M. anisopliae*), T7 (Thiamethoxam25 % WG@0.125 g/L (SLD) + *M. anisopliae*) , T3 (Pymetrozyne 50 % WG @ 0.25 g/L (SLD) + *M. anisopliae*, which gave per cent inhibition of 59.62, 47.70, 41.62, 31.48 %, respectively. The lowest per cent inhibition of 0.00 % was observed in Control treatment T1 (only *M. anisopliae* without ant insecticides) followed by T11 treatment (Imidacloprid @ 0.1-0.15 ml/L (SLD) with *M. anisopliae)* of 29.70 %.

At half the recommended dose, Deltamethrin 2.8 % EC was exhibited highest per cent inhibition followed by Acephate75 % SP, Diafenthiuron50 % WP, Thiamethoxam25 % WG and Pymetrozyne 50 % WG. The least per cent inhibition was observed in Imidacloprid 17.8 % SL.

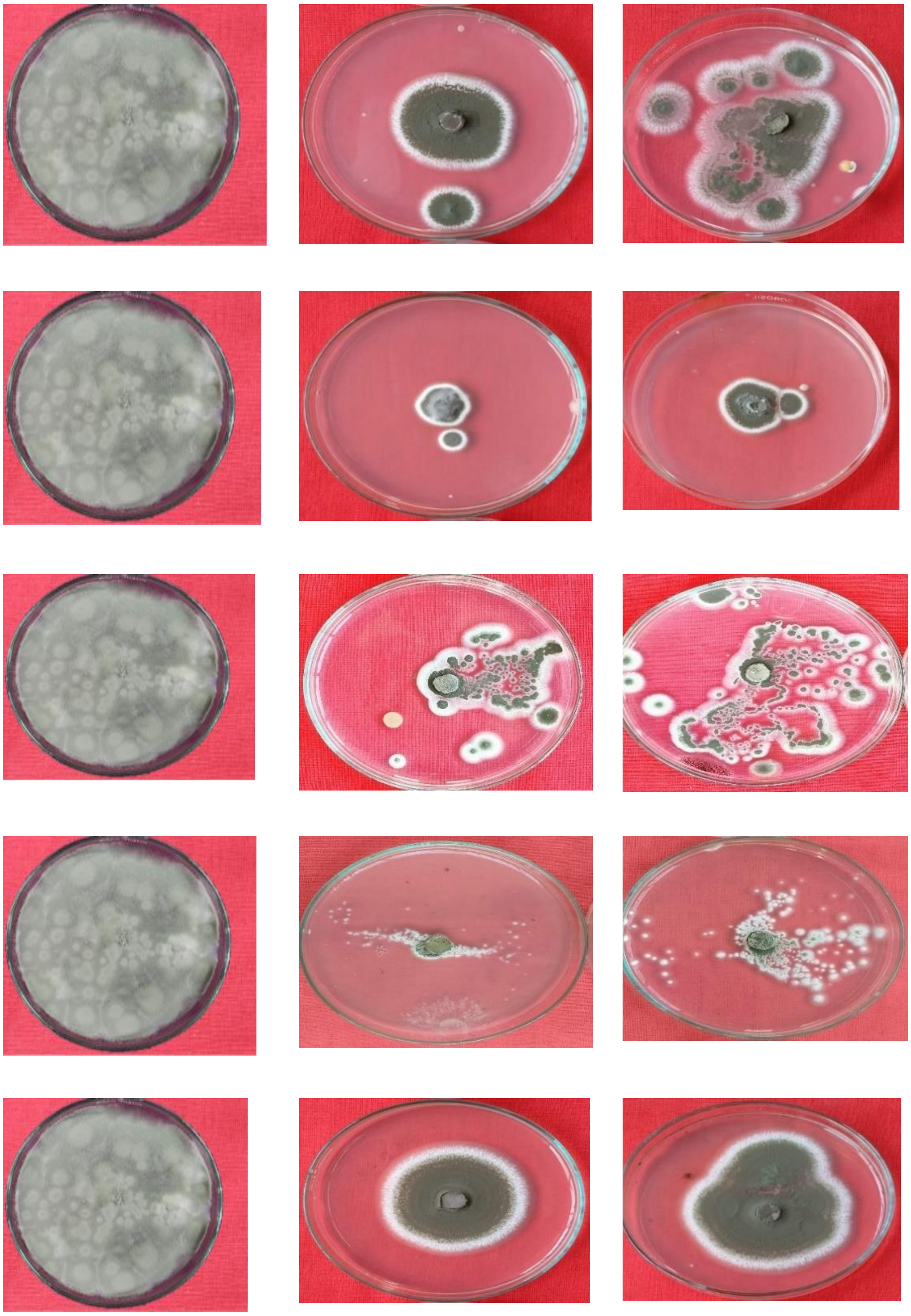
At Recommended Dose

Evaluation of per cent inhibition of radial growth of the *M. anisopliae* at recommended dose of chemical insecticides showed the highest per cent inhibition of 71.11 % in T4 treatment *i.e.*, Deltamethrin 2.8 % EC @ 1.5 ml/L (lethal dose) with *M. anisopliae* followed by T8 (Acephate75 % SP @ 1 g/L (lethal dose) with *M. anisopliae*), T12 (Diafenthiuron50 % WP @ 0.8-1 g/L lethal dose) + *M. anisopliae*), T6 (Thiamethoxam25 % WG @ 0.25 g/L (lethal dose) + *M. anisopliae*), T2 (Pymetrozyne50

% WG @ 0.5 g/L (lethal dose) + *M. anisopliae*) of 62.81, 52.74, 44.74, 43.11 % respectively. The lowest per cent inhibition of 0.00 % was observed in the T1 treatment (*M. anisopliae* without any insecticides) followed by T10 treatment combination *i.e.,* Imidacloprid17.8 % SL @ 0.2-0.3 ml/L (lethal dose) with *M. anisopliae* of 40.07 %.

At recommended dose, highest per cent inhibition was observed in Deltamethrin2.8 % EC followed by Acephate75 % SP, Diafenthiuron50 % WP, Thiamethoxam25 % WG and Pymetrozyne 50 % WG. The least per cent inhibition was observed in Imidacloprid 17.8 % SL

Chemicals	Control	Lethal dose (LD)	Sub-lethal dose (SLD)
a. Pymetrozyne 50 % WG			
b. Deltamethrin 2.8 % EC			
c. Thiamethoxa m 25 % WG			
d. Acephate 75 % SP			
e. Imidacloprid 17.8 % SL			



f. Diafenthiuron 50 % WP

Figure 1. (a-f) *In vitro* studies of compatibility of six different chemical insecticides with *M. anisopliae*: a) Pymetrozyne, b) Imidacloprid, c) Thiamethoxam, d) Acephate e) Deltamethrin, f) Diafenthiuron

Table. 2 *In vitro* studies of compatibility of six different chemical insecticides with *M. anisopliae* based on radial growth and per cent inhibition

Tr. No.	Treatments	Radial growth in mm	Per cent inhibition
T_1	Control (*M. anisopliae*)	90.00±0.00 (9.49)	0.00±0.00 (0.00)
T_2	Pymetrozyne @ 0.5 g/L of water (LD) + *M. anisopliae*	51.20±7.80 (7.16) *	43.11±8.67 (6.57)
T_3	Pymetrozyne @ 0.25 g/L of water (SLD) + *M. anisopliae*	61.67±3.97 (7.85)	31.48±4.41 (5.61)
T_4	Deltamethrin @ 1.5 ml/L of water (LD) + *M. anisopliae*	26.00±6.05 (5.10)	71.11±6.73 (8.43)
T_5	Deltamethrin @ 0.75 ml/L of water (SLD) + *M. anisopliae*	33.73±18.90 (5.81)	62.51±21.00 (7.91)
T_6	Thiamethoxam @ 0.25 g/L of water (LD) + *M. anisopliae*	49.73±3.91 (7.05)	44.74±4.35 (6.69)
T_7	Thiamethoxam @ 0.125 g/L of water (SLD) +*M. anisopliae*	52.53±16.80 (7.25)	41.62±18.67 (6.45)
T_8	Acephate @ 1 g/L of water (LD) +*M. anisopliae*	33.46±15.06 (5.79)	62.81±16.73 (7.93)
T_9	Acephate @ 0.5 g/L of water (SLD) +*M. anisopliae*	36.33±3.00 (6.03)	59.62±3.34 (7.72)
T_{10}	Imidacloprid @ 0.2-0.3 ml/L of water (LD) +*M. anisopliae*	53.93±12.22 (7.34)	40.07±13.57 (6.33)
T_{11}	Imidacloprid @ 0.1-0.15 ml/L of water (SLD) +*M. anisopliae*	63.26±15.77 (7.95)	29.70±17.52 (5.45)
T_{12}	Diafenthiuron @ 0.8-1 g/L of water (LD) +*M. anisopliae*	42.53±5.30 (6.52)	52.74±5.89 (7.26)
T_{13}	Diafenthiuron @ 0.4-0.5 g/L of water (SLD) +*M. anisopliae*	47.06±8.54 (6.86)	47.70±9.49 (6.91)
	SEm (±)	3.58	3.98
	CD	10.41	11.57

*Data in parenthesis are square root transformed values

These findings are in conformity with the earlier work of Faraji *et al.,* (2016) who reported that imidacloprid at lower concentration significantly allowed the germination, mycelial growth and sporulation of *M. anisopliae*. Nawaz *et al.* (2022) reported that the greatest radial growth of the fungi with any insecticide treatment was observed with Flonicamid with a colony diameter of 4.74 mm at the lowest concentration followed by Imidacloprid (4.37 mm). Niassy *et al.* (2012) found similar results, who reported that imidacloprid and thiamethoxam among different insecticides found more compatible and lower growth inhibition than other insecticides. Neves *et al.* (2001) presented similar results, who found that Imidacloprid treatments had no negative effect on germination, vegetative growth and conidia production of *M. anisopliae*. The present findings are in conformity with the results of Khan *et al.* (2012) who stated that neonicotinoids (Acetameprid, Imidacloprid and Thiamethoxam) were compatible and safer to *B. bassiana* and *M. anisopliae*.

Several workers (*e.g.*, Quintela and McCoy, 1998; Jaramillo *et al.*, 2005) have also shown a stimulation of *M. anisopliae* virulence by imidacloprid, indicating compatibility similar to the present findings. However, Batista *et al.* (2001) described imidacloprid as moderately toxic to *M. anisopliae* at the maximum “recommended dose rate” of 400 g/ha (w4000 ppm) and very toxic at the minimum dosage of 70 g/ha (w700 ppm) which is not in agreement with the present findings which might be due to different strains of fungus.

Many authors (*e.g.*, Moino *et al.*, 1998; Loureiro *et al.* 2002) tested commercial products containing imidacloprid to be compatible with *M. anisopliae* which is in conformity with the present investigations.

4. Conclusion

Among different treatment combinations of entomopathogen *M. anisopliae* with chemical insecticides at sub-lethal doses in laboratory and field conditions showed that *M. anisopliae* was compatible with imidacloprid. Therefore, Imidacloprid in combination with *M. anisopliae* can be recommended as a component of IPM. Furthermore, it can be popularized among farmers for managing insect pests of different crops.

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