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Band SS

Ph.D. Scholar, Department of Agriculture Entomology, Post Graduate Institute, MPKV, Rahuri, Maharashtra, India

Kabre GB

Professor of Entomology, Agriculture Entomology Section, College of Agriculture, Dhule, Maharashtra, India

Kulkarni SR

Professor of Entomology, Department of Agriculture Entomology, Post Graduate Institute, MPKV, Rahuri, Maharashtra, India

Saindane YS

Assistant Residue Analyst AINP on Residue, Post Graduate Institute, MPKV, Rahuri, Maharashtra, India

Navale AM

Professor of Plant Pathology and Microbiology, Department of Plant Pathology and Microbiology, Post Graduate Institute, MPKV, Rahuri, Maharashtra, India

Patil CS

Head, Department of Agriculture Entomology, Post Graduate Institute, MPKV, Rahuri, Maharashtra, India

Corresponding Author:

Band SS

Ph.D. Scholar, Department of Agriculture Entomology, Post Graduate Institute, MPKV, Rahuri, Maharashtra, India

Studies on compatibility of *Metarhizium anisopliae* with botanical and biorational pesticides

Band SS, Kabre GB, Kulkarni SR, Saindane YS, Navale AM and Patil CS

Abstract

The results on compatibility of *M. anisopliae* with various botanical and biorational pesticides revealed that highest mycelial growth (74.75 mm) was observed in azadirachtin 0.03% EC (300 ppm) and was significantly superior over rest of the pesticides tested. The minimum (16.94 per cent) growth inhibition of *M. anisopliae* was observed in pesticidal treatment with azadirachtin 0.03% EC (300 ppm) followed by pyriproxyfen 10% EC (18.61 per cent). The results on compatibility of botanical and biorational pesticides with *M. anisopliae* clearly indicates that the pesticides having better compatibility with *M. anisopliae* is having maximum mycelial growth in descending order are azadirachtin 0.03% EC (300 ppm) > pyriproxyfen 10% EC > spinosad 45% SC > eucalyptus oil > novaluron 10% EC > karanj oil.

Keywords: Compatibility, Vegetative growth, *Metarhizium anisopliae*, Botanicals, Biorational

Introduction

Insecticides have a negative impact on non-target predatory organisms in nature, including EPFs like *B. bassiana* and *M. anisopliae*, which are entomopathogenic fungi. However, there are interactions between insecticides and entomopathogens. Insecticides at low doses combined with an entomopathogenic fungus can work together to increase insect pest mortality. This combination is especially beneficial since it reduces the amount of insecticide used, reduces environmental contamination and reduces pest resistance (Abidin *et al.*, 2017) [1]. In order to conserve the ecofriendly biological microorganisms, biological control agents in nature, particularly within agricultural and horticultural ecosystems, must be protected from a wide range of harmful pesticides. As a result, determining their compatibility and interaction with pesticides, which is a key component of IPM programmes, is critical. In vitro studies have shown that various pesticides have selective effect on entomofungal infections, according to several researchers (Alves, 1986) [2], (Silva *et al.*, 1993) [13]. In integrated pest management, entomopathogenic fungi play a critical role. There are lots of cases where the use of a combination of several selective chemical insecticides and fungus enables efficient control of a variety of agricultural insect pests (Purwar and Sachen, 2006) [14]. Non-selective or incompatible chemical pesticides have the potential to stifle fungi's vegetative growth and development, resulting in a negative impact on IPM. (Malo, 1993) [8]. As a result, understanding the detrimental effects of various insecticides on entomopathogenic fungi is crucial.

Materials and Method

The investigation on compatibility of *Metarhizium anisopliae* with botanical and biorational pesticides were carried out in Biocontrol laboratory, Department of Agriculture Entomology, Post Graduate Institute, MPKV, Rahuri, during 2020-2021.

Poison food technique

Standard poison food technique was followed to assay the effect of botanical and biorational pesticides on *M. anisopliae*.

Quantity of pesticide required

The amount of toxicant (i.e. actual ingredient) in required quantity of PDA was calculated with the help of following formula,

$$\text{Amount of pesticide required} = \frac{\text{Percent of solution desired} \times \text{Quantity of solution required}}{\text{Strength of the formulation available}}$$

The insecticide doses were calculated for field application rate based on 500 litres/ha or with high volume sprayers. The pesticides were evaluated by poisoned food technique. Requisite quantity of botanical and biorational pesticides were added to the PDA medium in flask before solidification (medium temperature 46-48°C) to get desired concentration and mixed thoroughly. Then poured equally into the three petriplates and kept in laminar air flow. The medium was aseptically allowed to solidify under laminar air flow cabinet. After complete cooling of the PDA medium in petriplates the culture of *M. anisopliae* were inoculated under aseptic condition. Mycelial mat was cut with sterile cork borer (5 mm diameter) from 10 days old culture of *M. anisopliae* and placed aseptically in the centre of petriplates containing the poisoned media. For each botanical and biorational pesticides 4 replications were maintained in laboratory. Suitable check without poison was kept for comparison under same condition. Fungal colony diameter was measured at 3rd, 5th and 7th days after inoculation and compared with standard check to measure the degree of toxicity of different pesticides used in study. Inhibition of colony growth over untreated check was worked out for respective pesticides.

Vegetative growth of *M. anisopliae*

Radial mycelial growth of the fungus was measured after 3rd, 5th and 7th days after inoculation and compared with untreated control. The per cent reduction in radial growth was calculated by using formula,

$$R = C - T / C \times 100$$

Where, R - Per cent reduction of radial growth

C - Radial growth of fungi grown on untreated medium

T - Radial growth of fungi grown on pesticide treated medium

The experiments were carried out under laboratory condition in Completely Randomized Design during the year 2020-21 with the 4 replications & 7 treatments of botanical and biorational pesticides. The data so obtained was analyzed by standard statistical procedures.

Details of the botanical and biorational pesticides used to study compatibility of *M. anisopliae*

Sr. No.	Active Ingredient	Dose/liter	Dose (ml/g)/ha	
			a.i.(g)	g/ml
T ₁	Azadirachtin 0.03% EC (300 ppm)	4 ml/l	-	2000
T ₂	Karanj oil	4 ml/l	-	2000
T ₃	Eucalyptus oil	4 ml/l	-	2000
T ₄	Pyriproxyfen 10% EC	2 ml/l	50.0	500
T ₅	Spinosad 45% SC	0.4 ml/l	90	200
T ₆	Novaluron 10% EC	2 ml/l	100	1000
T ₇	Untreated check	-		

Result and Discussion

At 3rd day after inoculation

The data on effect of botanical and biorational pesticides on mean mycelial growth and percent growth inhibition of *M. anisopliae* at 3rd day after inoculation revealed that the highest (59.25 mm) mycelial growth was observed in untreated check and was significantly superior over the rest of the treatments. Among the various botanical and biorational pesticides tested for their compatibility with *M. anisopliae*, the highest (40.50 mm) mycelial growth was observed in pyriproxyfen 10% EC and was significantly superior over rest of the pesticides. The next superior treatment for their compatibility was azadirachtin 0.03% EC (300 ppm) (35.00 mm). The minimum (31.64%) growth inhibition of *M. anisopliae* was observed in pesticide

treatment with pyriproxyfen 10% EC and was significantly superior over rest of the pesticides which indicates its better compatibility with *M. anisopliae*. The next superior treatment in per cent growth inhibition was azadirachtin 0.03% EC (300 ppm) (40.86%) (Table 1).

At 5th day after inoculation

The Data on effect of botanical and biorational pesticides on mycelial growth and growth inhibition of *M. anisopliae* at 5th day after inoculation revealed that the highest (78.50 mm) mycelial growth was observed in untreated check and was significantly superior over the rest of the treatments. Among the various botanical and biorational pesticides tested for their compatibility with *M. anisopliae*, the highest (70.13 mm) mycelial growth was observed in pyriproxyfen 10% EC and was significantly superior over rest of the pesticides. The next superior treatment for their compatibility was azadirachtin 0.03% EC (300 ppm) (65.88 mm).

The minimum (10.61%) growth inhibition of *M. anisopliae* was observed in pesticide treatment with pyriproxyfen 10% EC and was significantly superior over rest of the pesticides which indicates its better compatibility with *M. anisopliae*. The next superior treatment in per cent growth inhibition was azadirachtin 0.03% EC (300 ppm) (16.03%) (Table 1).

At 7th day after inoculation

The Data on effect of botanical and biorational pesticides on mycelial growth and growth inhibition of *M. anisopliae* at 7th day after inoculation revealed that the highest (90.00 mm) mycelial growth was observed in untreated check and was significantly superior over the rest of the treatments. Among the various botanical and biorational pesticides tested for their compatibility with *M. anisopliae*, the highest (74.75 mm) mycelial growth was observed in azadirachtin 0.03% EC (300 ppm) and was significantly superior over rest of the pesticides. The next superior treatment for their compatibility was pyriproxyfen 10% EC (73.25 mm).

The minimum (16.94%) growth inhibition of *M. anisopliae* was observed in insecticidal treatment with azadirachtin 0.03% EC (300 ppm) and was significantly superior over rest of the pesticides which indicates its better compatibility with *M. anisopliae*. The next superior treatment in per cent growth inhibition was pyriproxyfen 10% EC (18.61%). The results on compatibility of pesticides with *M. anisopliae* clearly indicates that the pesticides having better compatibility with *M. anisopliae* as having maximum mycelial growth in descending order are azadirachtin 0.03% EC (300 ppm) > pyriproxyfen 10% EC > spinosad 45% SC > eucalyptus oil > novaluron 10% EC > karanj oil (Table 1).

The present finding on compatibility of *M. anisopliae* with botanical and biorational pesticides are in corroboration with Amutha and Banu (2012) [3] who reported that *Metarhizium anisopliae*, was hazardless with econeem on 14th and 30th days after inoculation. Parjane *et al.* (2020) [10] who noted that azadirachtin recorded the highest vegetative growth of *M. anisopliae* indicating their compatibility with *Metarhizium anisopliae*. Kakati *et al.* (2018) [6] who observed that maximum radial growth (25.90 mm) of *M. anisopliae* recorded with azadirachtin @ 0.075% (one fourth concentration of the recommended dose).

Gowrish *et al.* (2013) [4] who reported the minimum percent of growth inhibition (5.23 per cent) of colony diameter of *M. anisopliae* was found in samples treated with 0.0018 per cent concentration of spinosad 45% SC at 14th day after inoculation, while the maximum (26.75) percent inhibition was found in

treatment 0.0054 per cent of spinosad 45% SC. Spinosad 45% SC at the concentration of 0.0036 per cent recorded 10.44 percent inhibition diameter of the colony *M. anisopliae*.

Present finding are also in corroboration with Hirose *et al.* (2001) [5] who studied in vitro fungitoxic effect of neem oil

(*Azadirachta indica* A. Juss) on the entomopathogenic fungi *Metarhizium anisopliae*. Neem oil showed inhibitory effect on conidia germination with reductions of 21.28 and 17.26 per cent. In present studies also azadirachtin had shown 16.94 per cent growth inhibition.

Table 1: Effect of various pesticides on vegetative growth and growth inhibition of *M. anisopliae*

Treatment	Dose		3 DAI		5 DAI		7 DAI	
	ml/ha	ml/liter	Mean mycelial growth (mm)	Mean growth inhibition (%)	Mean mycelial growth (mm)	Mean growth inhibition (%)	Mean mycelial growth (mm)	Mean growth inhibition (%)
Azadirachtin 0.03 % EC (300 ppm)	2000	4	35.00 (5.96)*	40.86 (39.71)**	65.88 (8.15)	16.03 (23.55)	74.75 (8.67)	16.94 (24.30)
Karanj oil	2000	4	15.88 (4.05)	73.21 (58.83)	28.25 (5.36)	64.03 (53.15)	38.00 (6.20)	57.78 (49.48)
Eucalyptus oil	2000	4	12.88 (3.66)	78.26 (62.21)	31.88 (5.69)	59.37 (50.40)	45.63 (6.79)	49.31 (44.60)
Pyriproxyfen 10% EC	500	2	40.50 (6.40)	31.64 (34.23)	70.13 (8.40)	10.61 (18.93)	73.25 (8.59)	18.61 (25.53)
Spinosad 45% SC	200	0.4	30.25 (5.54)	48.93 (44.39)	48.00 (6.96)	38.82 (38.54)	63.25 (7.98)	29.72 (33.02)
Novaluron 10% EC	1000	2	19.63 (4.49)	66.87 (54.86)	33.38 (5.82)	57.47 (49.30)	45.25 (6.76)	49.72 (44.84)
Untreated check	--	-	59.25 (7.73)	0.00 (0.00)	78.50 (8.89)	0.00 (0.00)	90.00 (9.51)	0.00 (0.00)
S.E±			0.06	0.69	0.05	0.67	0.05	0.52
CD at 5%			0.16	2.04	0.16	1.98	0.14	1.54

*Figures in parenthesis are $\sqrt{x+0.5}$ transformed values. **Figures in parenthesis are arc sin transformed values.

Niassy *et al.* (2012) [9] who noted that azadirachtin was toxic to the fungus, *M. anisopliae* adversely affecting vegetative growth and sporulation.

Ummidi and Vadlamani (2014) [15] who observed that all three concentration of eucalyptus oil proved to be toxic to *Metarhizium anisopliae* except 1 per cent eucalyptus oil showed more reduction in conidiogenesis than germination and vegetative growth of *Metarhizium anisopliae*.

Kumar *et al.* (2008) [7] who noted that castor and pongamia oils, however both inhibited mycelial growth, but not significantly (1.90 cm). In present studies maximum per cent growth inhibition was recorded in treatment with karanj oil.

Rashid *et al.* (2010) [12] who studied the compatibility of *M. anisopliae* with pyriproxyfen 10% EC at varying doses of 10, 500 and 1000 ppm. To determine the vegetative growth of *M. anisopliae* based on colony diameter. Pyriproxyfen showing the reduction effect (24.59 per cent). In present investigation also pyriproxyfen had shown 18.61 per cent reduction effect.

Rachappa *et al.* (2007) [11] who reported that spinosad 45% SC were found safe to the fungus by inhibiting only 5.10 per cent growth. Present findings 29.72 per cent growth inhibition was observed in the treatment with spinosad 45% SC.

Conclusion

Azadirachtin 0.03% (300 PPM) showed better compatibility with *M. anisopliae* as having highest mycelial (vegetative growth) growth followed by Pyriproxyfen 10% EC.

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