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Compatibility of *Metarhizium anisopliae* with botanicals under laboratory condition

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Abstract
The investigation was carried out on "Compatibility of *Metarhizium anisopliae* with botanicals under laboratory condition" at College of Agriculture, Pune during 2021-22.

Studies on compatibility of *Metarhizium anisopliae* with botanicals by dual plate bioassay method revealed that Azadirachtin @ 1000 ppm recorded 1.80 per cent inhibition with *M. anisopliae*, which was at par with *M. anisopliae* with NSKE 5 per cent (6.31 %). Next best treatments in compatibility rating were *M. anisopliae* with Eucalyptus 1 per cent (32.87 %) which was on par with *M. anisopliae* with Karanj oil 5 per cent (43.24 %). *M. anisopliae* with Neem oil 5 per cent observed (59.45 %) reduction in germination. Therefore, Azadirachtin and NSKE were highly compatible with *M. anisopliae.* Eucalyptus oil and Karanj oil were compatible with *M. anisopliae.* Whereas, Neem oil was partially compatible with *M. anisopliae.*

Studies on compatibility of *M. anisopliae* with botanicals by dual liquid bioassay method revealed that *M. anisopliae* with Azadirachtin @ 1000 ppm recorded least per cent inhibition (9.50 %) which was at par with *M. anisopliae* with NSKE 5 per cent (23.15 %). Next best treatments were *M. anisopliae* with Eucalyptus 1 per cent which was on par with *M. anisopliae* with Karanj oil 5 per cent recorded 33.61 and 27.80 per cent reduction in germination, respectively. Maximum reduction in germination was observed in treatment with *M. anisopliae* with Neem oil 5 per cent (46.24 %). Thus, Azadirachtin and NSKE were highly compatible with *M. anisopliae.* Eucalyptus oil and Karanj oil were compatible with *M. anisopliae.* Whereas, Neem oil was partially compatible with *M. anisopliae.*

Keywords: *M. anisopliae*, compatibility, Azadirachtin, Neem oil, Karanj oil, Eucalyptus

Introduction
Poisonous pesticides are used to control pests which result into several dangerous side effects. The most frequent issues brought on by chemical *viz*., pesticide residues, pest resurgence and the emergence of pest resistance. Continuous parallel study is required to monitor these species because their ongoing evolution has resulted in pesticide resistance. Botanicals are an important component of biological approaches because they are widely available, easy to produce and create jobs in rural areas. Botanicals have been used for pest control since the Vedic era (Koul and Walia, 2009) [11].

More than 750 fungi from over 90 species are naturally entomopathogenic and these fungi can be easily incorporated into IPM strategies (Zare and Gams, 2001) [24]. Among the often accessible species are *Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecanicilium, Nomurea rileyi, Aschersonia, Hirsutella thompsoni* (Alves *et al*., 2008) [1].

The only disadvantage of these microbes is that they are slow to act, but this can be overcome by combining them with other chemicals in various strategies. Recent approaches demonstrated that "dual-attack" approach can result in higher pest mortality than their individual effect. Combinations of two products are frequently more effective, exhibiting greater effect than their 1+ 1 effect, which is technically known as synergism. Antagonism is the inverse phenomenon in which the toxicity of two compounds combined is less than the expected sum of their individual effects. The less-discussed phenomenon is that antagonism is also possible, but it is mostly hidden by the positive effects produced. Many terms, such as co-toxicity coefficient, synergistic ratio, per cent mortality and many sub lethal effects on pests that reduce yield loss, indicate synergy and antagonism. The combination of an insecticidal botanical or plant extract and an entomopathogen is a novel approach to combating resistance and resurgence issues caused by insect pests (Srivastava *et al*., 2011) [21]. These botanical biopesticide combinations (BBC) benefit organic agriculture by providing effective

management comparable to synthetic insecticides.

Metarhizium anisopliae was also found to be synergistic when combined with botanicals such as neem (Shoukat *et al.*, 2016) [19], pyrethrum (Fernández *et al.*, 2020) [6] and 1-chlorooctadecane (Hussain and Aljabr, 2020) [8]. *Metarhizium anisopliae* is an important EPF that causes the green muscardine disease in insects. It is broadly used for the biological control of many insect pest species (Reddy *et al.*, 2014). Some botanicals possess a different insecticidal activity. They are less harmful to environment as well as human so insect doesn't produces any resistance due to their uses. EPFs have a broad host range ability and are effective against a variety of insect pests (Ong and Vandermeer, 2014) [14].

The entomopathogenic fungi along with botanicals are potential bioagents found effective against almost all the life stages of insects. (Day *et al.*, 2017) [4]. Laboratory compatibility have the advantage of exposing the pathogen to the maximum activity possible of chemical products and or plant-based products, situation that does not occur under field conditions. Therefore, when a treatment is compatible *in vitro*, there is a strong evidence of its selectivity under field conditions. (Sahayaraj *et al.*, 2011) [18]. In Maize, *S. frugiperda* attack in all the stages of plant from seedling to tasseling and causing defoliation, killing the young plant, which shows grain damage and simultaneously reduces quantity and quality of yield (Chimweta *et al.*, 2019) [3]. Keeping this view in the present study was conducted to evaluate the synergetic and antagonistic effect of EPF with botanical against fall armyworm, *Spodoptera frugiperda* under laboratory condition

Materials and Methods

Present studies the compatibility of biopesticides were carried out under Biocontrol Laboratory, Department of Agricultural Entomology, College of Agriculture, Pune during the year 2021-2022 by completely randomize design with three replication and seven treatments

Materials

The material required for undertaking the present investigations are presented below.

Biopesticides for compatibility treatments

SN	Common Name	Trade Name
1	*Metarhizium anisopliae* 1x108 cfu/ml)	Phule *Metarhizium* (1x108cfu/ml)
2	Azadirachtin (1000ppm)	Multinemor
3	Neem oil (5 %)	-
4	NSKE (5 %)	-
5	Eucalyptus oil (1 %)	-
6	Karanj oil (5 %)	-

Preparation of medium for the study

Potato Dextrose Broth medium suggested by Kadam and Jaichakravarti (2013) [9] was used for the study.

Preparation of combination of *M. anisopliae* and botanicals by following method

al plate assay method:

Preliminary in vitro studies were undertaken in the laboratory to study the compatibility of *M. anisopliae* with the botanicals by adopting poisoned food technique (Olmert and Kenneth, 1974) [13]. Recommended field concentration of the plant products (5 per cent) and *M. anisopliae* were added to the sterilized PDA and poured to the Petri plate after proper agitation and allowed to solidify. Fungal disc from fully-grown 15 days old culture of *M. anisopliae* culture plate were transferred from the culture plate with the help of a sterilized cork borer of 8 mm size to the media. Seeded plates were incubated at 26 ± 0.1°C for 15 days. Then diameter of colony were recorded and per cent inhibition was calculated according to the method of (Kulkarni and Lingpa 2001) [12]. Formula for calculation of the diameter of colony is given below:

$$\text{Per cent inhibition} = \frac{\text{Growth in pure culture of } \textit{Metarhizium} - \text{Growth in treatment}}{\text{Growth in pure culture of } \textit{Metarhizium}} \times 100$$

In the case of plant extracts, after pouring the molten sterilized PDA into the sterile plates, 0.1 ml of 1 per cent of plant extract was added and the plate was shaken well in order to mix the plant products with the media. 100 ml of respective solvent was also used as control. The culture inoculation and growth inhibition bio-assay were followed as mentioned as per above method. Compatible inhibition effect of the plant extracts with *M. anisopliae* was calculating using the following formula:

$$\text{Compatible efficacy (CE)} = 100 - \frac{\text{Fungal colony growth in botanicals treatment}}{\text{Colony growth in sole } \textit{M. anisopliae}} \times 100$$

al liquid assay method

The dual liquid assay method was also used to determine the compatibility of *Metarhizium anisopliae* with botanicals. In this method 100 ml of PDA in 250 ml of conical flask was inoculated with 0.1 ml of the fungal spore suspension and the recommended dosage of available plant products separately. Control was maintained for each treatment and the inoculated flasks were incubated at 26 ± 0.1°C for 15 days in BOD incubator (Remi, Mumbai).

After 15 days, the mycelial mat was taken out from the flask by using sterile spatula and placed in the Petri dishes containing filter paper. The initial weight of the paper was recorded. The Petri dishes were kept in hot air oven at 50 ± 1° C for one hour and the dry weight of the fungal mycelia was recorded. The inhibitory activity was assessed by the difference between the dry weight of fungal mycelia in the control and the respective treatment.

Compatibility rating for biopesticides (Saindane *et al.*, 2007) [9]

Sr. No	Average growth and development (%)	Compatibility status
1	>70	HC
2	41-70	C
3	15-40	PC
4	<15	IC

Where,
HC = Highly compatible C = Compatible
PC = Partially compatible IC = Incompatible

Result and discussion

The laboratory investigations were carried out on compatibility of entomopathogenic fungi with botanicals at Biocontrol Laboratory of Department of Agricultural Entomology, College of Agriculture, Pune during year 2021-2022. The results obtained on distinct aspects under the studies have been presented herein.

Compatibility of *Metarhizium anisopliae* with botanicals laboratory condition.

The studies were carried out on compatibility of *M. anisopliae* with botanical under laboratory conditions during September to March, 2021-2022 at prevailing room temperature of 25 ± 2 °C and relative humidity of 65 ± 10 per cent. The results obtained in respect of growth of *M. anisopliae* are presented as below.

Effect of different botanicals on *M. anisopliae* by dual plate bioassay method

The data in respect of compatibility by dual plate bioassay method have been presented in Table 1.

The data on effect of botanicals on spore germination of *M. anisopliae* revealed that the sole *M. Anisopliae* (1x108cfu/ml) showed (100 %) spore germination. In treatment, *M anisopliae* with Azadirachtin @ 1000 ppm showed (98.20 %) which was at par with treatment of *M. anisopliae* with NSKE 5 per cent recorded 93.69 per cent spore germination. Next effective treatment was *M. anisopliae* with Eucalyptus oil 1 per cent (67.12%) which was at par with *M anisopliae* with Karanj oil 5 per cent observed 56.76 per cent spore germination. The minimum spore germination was recorded in *M. anisopliae* with Neem oil 5 per cent (40.54%).

Table 1: Effect of different botanicals on *M. anisopliae* by dual plate bioassay method

TN	Treatment	Doses	Spore Germination (%)	Mean colony radial growth (in mm) (15 DAT)	Per cent Reduction in germination over Sole *Metarhizium*	Compatibility Rating
T1	*M. anisopliae* (1x108 cfu/ml) + Azadirachtin (1000 ppm)	5 gm + 2 ml	98.20 (83.70)**	72.67 (8.55)*	1.80 (6.31)	Highly Compatible
T2	*M.anisopliae* (1x108 cfu/ml) + Neem oil (5 %)	5 gm + 1 ml	40.54 (39.54)	30.00 (5.52)	59.45 (50.46)	Partially Compatible
T3	*M. anisopliae* (1x108 cfu/ml) + NSKE (5 %)	5gm + 1 ml	93.69 (75.52)	69.33 (8.36)	6.31 (14.48)	Highly Compatible
T4	*M. anisopliae* (1x108cfu/ml) + Eucalyptus oil (1 %)	5gm + 1 ml	67.12 (55.02)	49.67 (7.08)	32.87 (34.98)	Compatible
T5	*M.anisopliae* (1x108 cfu/ml) + Karanj oil (5 %)	5gm + 1 ml	56.76 (48.89)	42.00 (6.52)	43.24 (41.11)	Compatible
T6	*M.anisopliae* (1x108 cfu/ml)	5gm + 1 ml	100 (90.00)	74.00 (8.63)	0.00 (0.00)	-
T7	Untreated control	-	0.00 (0.00)	0.00 (0.00)	-	-
	CD at 5 %		9.07	1.92	9.07	
	SE (m) ±		3.02	0.64	3.02	
	F Test		Sig	Sig	Sig	

*Figures in parenthesis are √x+0.5 transformed values. **Figures in parenthesis are arc sin transformed value

In case of mean colony radial growth all the treatments were superior over untreated control. Sole *M. anisopliae* (1x108cfu/ml) recorded 74.00 mm mean colony radial growth, as highest mycelium growth and treated as standard check followed by *M. anisopliae* with Azadirachtin @ 1000 ppm (72.67 mm) which were at par with *M. anisopliae* with NSKE 5 per cent (69.33 mm) and *M. anisopliae* with Eucalyptus oil 1 per cent (49.67 mm). It was followed by *M. anisopliae* with Karanj oil 5 per cent showed 42 mm radial growth which was at par with *M. anisopliae* with Neem oil 5 per cent (30 mm).

The data on per cent reduction in germination over sole *M. anisopliae* (1x108cfu/ml) observed zero per cent reduction in germination followed by *M. anisopliae* with Azadirachtin @ 1000 ppm (1.80%) which was at par with *M. anisopliae* with NSKE 5 per cent (6.31%). Next best treatment was *M. anisopliae* with Eucalyptus 1 per cent (32.87%) which was on par with *M. anisopliae* with Karanj oil 5 per cent (43.24%). *M. anisopliae* with Neem oil 5 per cent observed 59.45% reduction in germination. Compatibility rating over sole *M. anisopliae* showed that *M. anisopliae* with Azadirachtin @1000 ppm and *M. anisopliae* with NSKE 5 per cent was highly compatible followed by *M. anisopliae* with Eucalyptus oil 1 per cent and *M. anisopliae* with Karanj oil 5 per cent observed compatible. *M. anisopliae* with Neem oil 5 per cent was partially compatible with *M. anisopliae* because of maximum per cent reduction in germination of *M. anisopliae.*

The present studies on compatibility of *M. anisopliae* with botanicals was conformity with the Parjane *et al.* (2020) [15] who showed that Azadirachtin was highly compatible with *M. anisopliae* observed highest spore germination. The findings in respect of mean colony diameter, were similar line of observations recorded by Kakati *et al.* (2018) [10] who observed maximum mean colony diameter (25.90 mm) in

corroboration with the present results. The result obtained here in for Eucalyptus oil was in agreements with that reported by Ummidi and Vadalmani (2014) [22] found that different three concentration of Eucalyptus oil showed hazardous effect on *M. anisopliae* except 1 per cent Eucalyptus oil observed similar results with present finding. Effect of Neem oil on *M. anisopliae* denoted to be maximum inhibitory effect on germination in present findings and is in agreement with result obtained by Hirose *et al.* (2001) [7].

Effect of various botanicals on fungal growth of *M. anisopliae* by dual liquid bioassay method

Data in respect of compatibility by dual liquid bioassay method have been presented in Table 2.

The initial weight of Petri plate along with media was 109.21 gas untreated control. On transfer of mat from conical flask to petri plate, sole *M. anisopliae* recorded 119.11 g weight of mat with petri plate followed by *M. anisopliae* with Azadirachtin @ 1000 ppm (118.74 g), *M. anisopliae* with NSKE 5 per cent (117.37 g), *M. anisopliae* with Eucalyptus oil 1 per cent (116.80 g) followed by *M. anisopliae* with Karanj oil 5 per cent (116.24 g) and all the treatments were at par with each others. Whereas, *M. anisopliae* with Neem oil 5 per cent recorded least weight of mat with petri plate (112.37 g).

Data on initial weight of mat without petri plate in untreated control showed (0.00 g) followed by sole *M. anisopliae* recorded highest initial weight of mat without petri plate (9.90 g) and on par with *M. anisopliae* with Azadirachtin @ 1000 ppm (9.53 g). The next best treatment was *M. anisopliae* with NSKE 5 per cent recorded (8.17 g) followed by *M. anisopliae* with Eucalyptus oil 1 per cent (7.59 g), *M. anisopliae* with Karanj oil 5 per cent (7.03 g) at par with each others. The least weight of mat was observed in treatment *M. anisopliae* with Neem oil 5 per cent (5.16 g).

After dried in hot air oven for 1 hour dry weight of mat without petri plate was observed in untreated control (0.00 g). The sole *M. anisopliae* recorded 7.05 g dry weight which was significantly superior over all rest treatments. The next best treatment was *M. anisopliae* with Azadirachtin @ 1000 ppm showed (6.38 g) which was at par with *M. anisopliae* + NSKE 5 per cent (5.96 g) followed by *M. anisopliae* with Karanj oil 5 per cent (5.09 g) and *M. anisopliae* with Eucalyptus oil 1 per cent (4.68 g) and both the treatments were on par with each others.The minimum dry weight of mat recorded by *M. anisopliae* with Neem oil 5 per cent (3.79 g).

The data regarding per cent reduction in germination, the result revealed that the sole *Metarhizium anisopliae* (1x108cfu/ml) recorded zero per cent reduction. The next best treatment was *M. anisopliae* with Azadirachtin @ 1000 ppm (9.50 %) which was at par with *M. anisopliae* with NSKE 5 per cent (15.46 %). It was followed by *M. anisopliae* with Eucalyptus oil 1 per cent which was on par with *M. anisopliae* with Karanj oil 5 per cent recorded 33.61 and 27.80 per cent reduction in germination, respectively. Maximum reduction in germination was observed in *M. anisopliae* with Neem oil 5 per cent (46.24 %).

Data on compatibility showed that Azadirachtin @1000 ppm and NSKE 5 per cent were highly compatible with *M. anisopliae* followed by Eucalyptus oil 1 per cent and Karanj oil 5 per cent were compatible with *M. anisopliae.* The treatment with Neem oil 5 per cent was partially compatible with *M. anisopliae* because of maximum per reduction in germination of *M. anisopliae.*

Table 2: Effect of various botanicals on fungal growth of *M. anisopliae* by dual liquid bioassay method

TN	Treatment	Doses	Initial weight of mat with petri plate (gm)	Initial weight of mat without petri plate (gm)	Dry weight of mat without petri plate (gm)	Per cent Reduction of mat in dry weight over Sole *Metarhizium*	Compatibility Rating
T1	*M. anisopliae* (1x108 cfu/ml) + Azadirachtin (1000 ppm)	5 gm + 2 ml	118.74 (10.92)*	9.53 (3.17)	6.38 (2.62)	9.50 (17.95)**	Highly Compatible
T2	*M. Anisopliae* (1x108 cfu/ml) + Neem oil (5 %)	5 gm + 1 ml	112.37 (10.62)	5.16 (2.33)	3.79 (2.01)	46.24 (42.84)	Partially Compatible
T3	*M. anisopliae* (1x108 cfu/ml) + NSKE (5 %)	5gm + 1 ml	117.37 (10.85)	8.17 (2.94)	5.96 (2.54)	15.46 (23.15)	Highly Compatible
T4	*M.anisopliae* (1x10 8 cfu/ml) + Eucalyptus oil (1 %)	5gm + 1 ml	116.80 (10.83)	7.59 (2.84)	4.68 (2.27)	33.61 (35.43)	Compatible
T5	*M.anisopliae* (1x108 cfu/ml) + Karanj oil (5 %)	5gm + 1 ml	116.24 (10.80)	7.03 (2.75)	5.09 (2.36)	27.80 (31.82)	Compatible
T6	*M. anisopliae* (1x108 cfu/ml)	5gm + 1 ml	119.11 (10.93)	9.90 (3.23)	7.05 (2.75)	0.00 (0.00)	-
T7	Untreated control	-	109.21 (10.47)	0.00 (0.00)	0.00 (0.00)	-	-
	CD at 5 %		0.04	0.27	0.09	5.86	
	SE (m) ±		0.01	0.09	0.03	1.95	
	F Test		Sig	Sig	Sig	Sig	

*Figures in parenthesis are √x+0.5 transformed values. **Figures in parenthesis are arc sin transformed values.

The present results on compatibility of *M. anisopliae* with botanicals by dual liquid bioassay method are in agreement with the findings of Vyas *et al.* (1992) [24], observed that Neemark, a botanical pesticide of neem was compatible with *Metarhizium anisopliae*. In present investigation, karanj oil was next treatment, similar line of results were recorded by Devi and Prasad (1996) [5], found that neem and pongamia tolerated by *Nomuraea riley*. The results obtained in respect of Neem oil showed maximum per cent reduction in *M. anisopliae* is in accordance with Hirsoe *et al.* (2001) [7], Aguda *et al.* (1986) [2] and Rogerio *et al.* (2005) [17] showed more or less similar lines of results with neem + *M. anisopliae*

and azadirachtin with *B. bassiana* in present investigation, respectively.

Present results on compatibility of *M. anisopliae* with botanicals evidenced in the present study cannot be discussed due to paucity of literature.

Conclusion

The results obtained in respect to growth of *Metarhizium anisopliae* are presented as below.

Compatibility of *Metarhizium anisopliae* with botanicals under laboratory condition.

1. **Highly compatible:**
   M. anisopliae with Azadirachtin and *M. anisopliae* with NSKE
2. **Compatible:**
   M. anisopliae with Eucalyptus oil and *M. anisopliae* with Karanj oil
3. **Partially compatible:**
   M. anisopliae with Neem oil

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