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# Compatibility of *Metarhizium anisopliae* with neem derivatives *in vitro* condition

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**Abstract**
The present investigation was carried out during *rabi* 2013-2014 at the Entomology laboratory, College of Agriculture JNKVV, Jabalpur at Completely Randomized Block Design (CRBD). The experiment on compatibility studies was undertaken on four treatments and five replication with neem derivatives. The effect of neem derivatives on the mycelial growth of *M. anisopliae* was conducted in *In vitro*. Among the neem derivatives tested Neem soap 0.5% (w/v) (half recommended dose) was found to be most compatible with least inhibition growth followed by Neem oil 2.5% (v/v) and NSKE 2.5% (w/v). Although the different neem derivatives tested in the present investigations slightly inhibited the growth of *M. anisopliae* in poisoned media, the combined use of the fungus and neem derivatives cannot be completely ruled out. All the neem derivatives tested have been combined with entomopathogenic fungi for obtaining better control of pod borer in chickpea considering this it is worth exploring the effect of these neem derivatives at sub lethal dose with fungus for an enhance result over pest control.

**Keywords:** Compatibility, *M. anisopliae,* Neem derivatives, *in vitro*

## Introduction
*Metarhizium* causes a disease known as 'green muscardine' in insect hosts because of the green colour of its conidial cells. In 1883, Metschnikoff commenced mass culturing of fungus and carried out the first experiment with two beetle pests. *Metarhizium anisopliae* (Metschnikoff) Sorokin is the second most widely exploited Entomopathogenic fungus in biocontrol trials. Species within the genus *Metarhizium* are pathogenic fungi having broad ranges of insect hosts. *M. anisopliae* was found to be a species complex composed of nine species based on multilocus phylogeny [1]. It is known to attack over 200 species of insects belonging to orders Coleoptera, Dermaptera, Homoptera, Lepidoptera and Orthoptera [2].
With the current thrust on sustainable agriculture and organic farming, the use of botanical products as pesticides has acquired greater significance. Implementation of environmental friendly agricultural practices is essential for the preservation of the quality of life on earth. This has evoked a search for eco-friendly and indigenous botanical pesticides and they will decrease the dependence on the unsafe insecticides [3-7]. Among so many plants investigated, the Indian neem tree, *Azadirachta indica* is a promising source of botanical insecticides [8]. Neem extracts make the ideal insect control for impoverished farmers worldwide by providing a safe, inexpensive and very effective insect control for both ends of the agricultural spectrum. When the microscopic spores of the fungus comes into contact with the body of an insect host, they germinate, penetrate the cuticle, and grow inside, killing the insect within a matter of days [9-10]. Biopesticides made from neem are biodegradable, non-toxic, eco-friendly and have no residual effect on agriculture produce. Keeping the fact in background the present investigation are undertaken on compatibility of *Metarhizium anisopliae* with neem derivatives under laboratory condition.

## Materials and Methods
The experiments were carried out during *rabi* 2013-2014 at the Entomology laboratory, College of Agriculture JNKVV, Jabalpur under Completely Randomized Block Design (CRBD). The experiment on compatibility studies was undertaken on four treatments and five replications with neem derivatives (Table 1)**.**
Studies on compatibility of neem derivatives with *M. anisopliae* were evaluated by poisoned food technique in Potato Dextrose Agar (PDA) medium. For this purpose, 20 ml of PDA medium having neem derivatives of required concentration were poured into petriplates

aseptically and allowed to solidify under laminar flow cabinet. A 5 mm disc of fungus was taken from 10th day old culture of *Metarhizium anisopliae* and placed at the center of the petriplate containing PDA. Growth medium (PDA) without neem derivatives but inoculated with mycelial disc served as untreated check. The plates were incubated in BOD at $25^0$C and periodic observations were taken at 2 days interval. Each treatment was replicated five times. The diameter of the growing culture was taken at 2 days interval and it continued upto 10th day. The data were expressed as growth inhibition of *Metarhizium anisopliae* in neem derivatives treated PDA [11].

$$X = \frac{Y - Z}{Y} \times 100$$

Where X, Y, Z stand for percentage of growth inhibition, radial growth of fungus in untreated check and radial growth of fungus in poisoned medium, respectively.

**Table 1:** Treatment details of compatibility of *Metarhizium anisopliae* with neem derivatives - *in vitro* studies

| Treatment Code | Treatments | Dose |
|---|---|---|
| $T_1$ | *Metarhizium anisopliae* + NSKE | $1\times10^4$ spores/ml + 2.5% w/v |
| $T_2$ | *M. anisopliae* + Neem oil | $1\times10^4$ spores/ml + 2.5% v/v |
| $T_3$ | *M. anisopliae* + Neem soap | $1\times10^4$ spores/ml +0.5% w/v |
| $T_4$ | *M. anisopliae* | $1\times10^8$ spores/ml |

**Statistical Analysis**
All the data were subjected to statistical analysis after appropriate transformation as suggested by [12].

**Results and Discussion**
An experiment was conducted in the laboratory to screen some of the neem derivatives recommended to control gram pod borer in chickpea, for their toxic effect on the growth of *Metarhizium anisopliae*.

**(i) Growth performance of *Metarhizium anisopliae* in different neem derivatives media:**
Data on growth performance of *M. anisopliae* in different neem derivative media are presented in Table 2 and depicted in Fig. 1.

**Two days after inoculation**
At two days after inoculation, the differences in the mean growth of *Metarhizium anisopliae* on different media were significant. Among the treatments, Neem soap showed maximum growth (25.40 mm) at recommended half dose @ 0.5% (w/v) and was found to be the most compatible biopesticide. This was followed by Neem oil @ 2.5% (v/v) and NSKE @ 2.5% (w/v) and they recorded a growth of 23.20 mm and 22.80 mm, respectively. While in the control plate maximum growth of 35.60 mm was observed.

**Four days after inoculation**
At fourth day after inoculation, the differences in the mean growth of *M. Anisopliae* among different treatments were significant. Among the treatments, Neem soap showed maximum growth (32.80 mm) at recommended half dose @ 0.5% (w/v) and was found to be the most compatible biopesticide followed by Neem oil @ 2.5% (v/v) and NSKE @ 2.5% (w/v) (32.60 mm and 32.20 mm, respectively). The control plate maximum growth of 52.80 mm.

**Six days after inoculation:**
At sixth day after inoculation, the differences in the mean growth of *M. Anisopliae* among different treatments were significant. Among the treatments, Neem soap showed maximum growth (39.80 mm) at recommended half dose @ 0.5% (w/v) and was found to be the most compatible biopesticide followed by Neem oil @ 2.5% (v/v) and NSKE @ 2.5% (w/v) (39.60 mm and 39.20 mm, respectively). The control plate recorded maximum growth of 69.20 mm.

**Eight days after inoculation**
At eighth day after inoculation, the differences in the mean growth of *M. Anisopliae* among different treatments were significant. Among the treatments, Neem soap showed maximum growth (49.80 mm) at recommended half dose @ 0.5% (w/v) and was found to be the most compatible biopesticide followed by Neem oil @ 2.5% (v/v) and NSKE @ 2.5% (w/v) (48.60 mm and 47.80 mm, respectively). The control plate recorded maximum growth of 84.80 mm.

**Ten days after inoculation**
At tenth day after inoculation, the differences in the mean growth of *M. Anisopliae* among different treatments were significant. Among the treatments, Neem soap showed maximum growth (61.80mm) at recommended half dose @ 0.5% (w/v) and was found to be the most compatible biopesticide followed by Neem oil @ 2.5% (v/v) and NSKE @ 2.5% (w/v) (61.40 mm and 61.20 mm, respectively). The control plate recorded maximum growth of 87.60 mm.

**Mean**
On the overall basis among the neem derivatives tested, Neem soap showed maximum growth (41.92 mm ) at recommended half dose @ 0.5% (w/v) and was found to be the most compatible biopesticide followed by Neem oil @ 2.5% (v/v) and NSKE @ 2.5% (w/v) (41.08 mm and 40.64 mm, respectively). The control plate recorded maximum growth of 66.00 mm.

**(ii) Growth inhibition percentage of *M. anisopliae* in different neem derivative media**
The data on effects of neem derivatives on the growth inhibition of *M. anisopliae* are presented in Table 2 and depicted in Figure 1.

**Two day after inoculation**
All the treatments showed significant differences in growth inhibition of *M. anisopliae* in different neem derivatives treated media. Among the neem derivatives tested, Neem soap showed least growth inhibition (28.62%) at recommended half dose @ 0.5% (w/v) and was found to be the most compatible biopesticide, followed by Neem oil @ 2.5% (v/v) and NSKE @ 2.5% (w/v) which recorded growth inhibition of 34.86% and 35.92%, respectively.

**Four days after inoculation**
All the treatments showed non-significant differences in growth inhibition of *M. anisopliae* in different neem derivatives treated media. Among the neem derivatives tested, Neem soap showed least growth inhibition (37.86%) at recommended half dose @ 0.5% (w/v) and was found to be

the most compatible biopesticide, followed by Neem oil @ 2.5% (v/v) and NSKE @ 2.5% (w/v) which recorded growth inhibition of 38.23% and 39.00%, respectively.

**Six days after inoculation**

All the treatments showed non significant differences in growth inhibition of *M. anisopliae* in different neem derivatives treated media. Among the neem derivatives tested, Neem soap showed least growth inhibition (42.44%) at recommended half dose @ 0.5% (w/v) and was found to be the most compatible biopesticide, followed by Neem oil @ 2.5% (v/v) and NSKE @ 2.5% (w/v) which recorded growth inhibition of 42.76% and 43.32%, respectively.

**Eight days after inoculation**

All the treatments showed non-significant differences in growth inhibition of *M. anisopliae* in different neem derivatives treated media. Among the neem derivatives tested, Neem soap showed least growth inhibition (41.26%) at recommended half dose @ 0.5% (w/v) and was found to be the most compatible biopesticide, followed by Neem oil @ 2.5% (v/v) and NSKE @ 2.5% (w/v) which recorded growth inhibition of 42.68% and 43.63%, respectively.

**Ten days after inoculation**

All the treatments showed non significant differences in growth inhibition *M. anisopliae* in different neem derivatives treated media. Among the neem derivatives tested, Neem soap showed least growth inhibition (29.45%) at recommended half dose @ 0.5% (w/v) and was found to be the most compatible biopesticide, followed by Neem oil @ 2.5% (v/v) and NSKE @ 2.5% (w/v) which recorded growth inhibition of 29.91% and 30.14%, respectively.

**Mean**

All the treatments showed significant differences in growth inhibition of *M. anisopliae* in different neem derivatives treated media. Among the neem derivatives tested, Neem soap showed least growth inhibition (36.48%) at recommended half dose @ 0.5% (w/v) and was found to be the most compatible biopesticide, followed by Neem oil @ 2.5% (v/v) and NSKE @ 2.5% (w/v) which recorded growth inhibition of 37.76% and 38.28%, respectively. The effect of neem derivatives on the mycelial growth of *M. anisopliae* was conducted in *In vitro*. All the treatments showed significant mycelial growth, but less than control. Among neem derivatives tested, Neem soap 0.5% (w/v) (half recommended dose) was found more compatible with least growth inhibition percentage followed by Neem oil 2.5% (v/v) and NSKE 2.5% (w/v). The present findings are in conformity with the findings of [13-17]. They also reported that neem oil less than 0.25% or neem derivatives less than 5% were relatively less toxic for mycelial growth and spores of *M. anisopliae.*

Although the different neem derivatives tested in the present investigations inhibited the growth of *M. anisopliae* in poisoned media *In vitro*, the combined use of the fungus and neem derivatives cannot be completely ruled out. All the neem derivatives tested in this study have been combined at half recommended dose with entomopathogenic fungi for obtaining better control of pod borer in chickpea considering this, it is worth exploring the effect of these neem derivatives at sublethal dose with fungus for an enhance result over pest control.

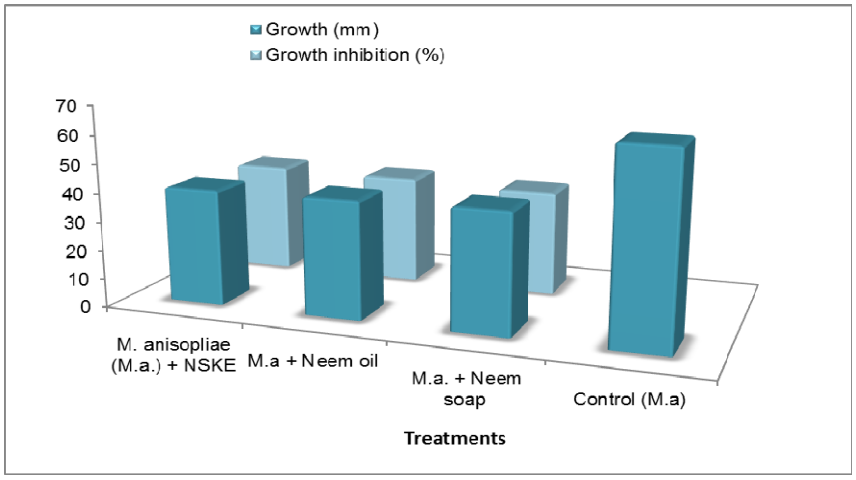


**Fig 1:** Growth and growth inhibition of *Metarhizium anisopliae* on different media

**Table 2:** *In vitro* studies on compatibility of *Metarhizium anisopliae* with neem derivatives

| Treatment nos. | Treatment | Dose | Performance of *M. anisopliae* in different media (at DDAI) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Growth (mm) | | | | | | Growth inhibition (%) | | | | | |
| | | | 2 | 4 | 6 | 8 | 10 | Mean | 2 | 4 | 6 | 8 | 10 | Mean |
| $T_1$ | *M. anisopliae*+ NSKE | $10^4$ spores /ml + 2.5% (w/v) | 22.80 | 32.20 | 39.20 | 47.80 | 61.20 | 40.64 | 35.92 (36.76) | 39.00 (38.75) | 43.32 (41.13) | 43.63 (41.31) | 30.14 (33.27) | 38.28 (38.25) |
| $T_2$ | *M. anisopliae*+ Neem oil | $10^4$ spores /ml + 2.5% (v/v) | 23.20 | 32.60 | 39.60 | 48.60 | 61.40 | 41.08 | 34.86 (36.15) | 38.23 (38.17) | 42.76 (40.81) | 42.68 (40.75) | 29.91 (33.11) | 37.76 (37.76) |
| $T_3$ | *M. anisopliae*+ Neem soap | $10^4$ spores /ml + 0.5% (w/v) | 25.40 | 32.80 | 39.80 | 49.80 | 61.80 | 41.92 | 28.62 (32.39) | 37.86 (37.95) | 42.44 (40.61) | 41.26 (39.93) | 29.45 (32.84) | 36.48 (37.12) |
| $T_4$ | Control | $1\times10^4$spores/ml | 35.60 | 52.80 | 69.20 | 84.80 | 87.60 | 66.00 | -- | -- | -- | -- | -- | --- |
| | | SEm± | 0.52 | 0.38 | 0.67 | 0.54 | 0.52 | 0.27 | 1.04 | 0.59 | 0.69 | 0.48 | 0.42 | 0.29 |
| | | CD at 5% | 1.57 | 1.14 | 2.00 | 1.63 | 1.57 | 0.82 | 3.21 | NS | NS | NS | NS | 0.89 |

Max. temp. 38.7± 2.8°C; Min. temp. 30.2 ± 5.8 °C; Morning RH(%) 51 ± 19; Evening RH(%) 24.5 ± 4.5

DDAI =Different days after inoculation

*= Figures in parentheses are arcsin-transformed values

## Conclusions

*In vitro* studies on compatibility of *M. anisopliae* with neem derivatives showed that neem soap was most compatible with least inhibition in mycelial growth.


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