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# Compatibility of *Metarhizium anisopliae* with new generation insecticide *in vitro* condition

**KD Tekam, NM Kelwatkar and SB Das**

**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 new generation insecticides. The effect of new generation insecticide on the mycelial growth of *Metarhizium anisopliae* was conducted in *In vitro*. Among the new generation insecticides tested the emamectin benzoate 5 SG was found to be most compatible with least inhibition percentage followed by flubendiamide 20 SC and rynaxypyr 20 SC. Although the different new insecticide tested in the present investigations slightly inhibited the growth of *M. anisopliae* in poisoned media, the combined use of the fungus and new generation insecticide cannot be completely ruled out. All the insecticides tested have been combined at half recommended dose with entomopathogenic fungi to explore the possibility of increasing the efficacy of the combinations for pest control.

**Keywords:** Compatibility, *M. anisopliae,* new generation insecticides, *in vitro*

## 1. Introduction
The entopathogenic fungus *Metarhizium anisopliae* (Metsch) Sorokin (Deutromycotina: Hyphomycetes) is exploited in greenhouse and outdoor crops as a tool for the control of many agricultural arthropod pests, including whiteflies, aphids, thrips, psyllids, weevils, caterpillars and mealy bugs [1-4]. It is a common soil borne entopathogenic fungus include its portal of entry, which is by contact instead of ingestion [5], its wide host range, replication in target insects [6,7] and safety to non target organisms [8].
Microbial organisms such as *M. anisopliae* are sustainable programs through their dynamic relationship with insects. In some cases, compatible products may be associated with entopathogenic fungi, increasing the control efficiency, decreasing the amount of insecticides required and minimizing the risks of environmental contamination and pest resistance expression [9, 10]. The knowledge of the compatibility between entomopathogenic fungi and pesticides may facilitate the choice of proper products for integrated pest management (IPM) program considering the fungus as an important pest control agent. Combined utilization of new insecticides in association with fungus pathogens can increase the efficiency of control by reduction of the amount of applied insecticides, minimizing environmental contamination hazards and pest resistance [11]. When an IPM strategy is desired, it is important to take into account the compatibility of products sprayed on the crop avoiding the use of most toxic or using them during seasons when the effect over a natural control agent is minimized. The integration of microbial pesticides with chemical pest management practices requires detail compatibility studies. Keeping the fact in background the present investigation are undertaken on compatibility of *Metarhizium anisopliae* with new generation insecticides under laboratory condition.

## 2. 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 three new generation insecticides *viz.* Rynaxypyr 20 EC, Flubendiamide 20 EC and Emamectin benzoate 5 SG 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. 1ml of *M.anisopliae* solution which contained $10^4$ spores / ml was inoculated at the center of the petriplate containing PDA. Growth medium (PDA) without insecticide but inoculated with fungus, served as an untreated check. The plates were incubated in BOD at $25^0$C and were replicated five times. The diameter of the growing culture was measured at 2 days interval after inoculation and continued upto $10^{th}$ day. The data were expressed as percentage growth inhibition of *M. anisopliae* on insecticides treated PDA [12] was computed with the following formula:

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

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 and new generation insecticides (*in vitro*)

| Treatment Code | Treatments | Dose |
|---|---|---|
| $T_1$ | *Metarhizium anisopliae* + Rynaxypyr 20 EC | $10^4$ spores /ml + 9 g *a.i.*/ ha |
| $T_2$ | *M anisopliae* + Flubendiamide 20 EC | $10^4$ spores /ml + 30 g *a.i.* / ha |
| $T_3$ | *M anisopliae* + Emamectin benzoate 5 SG | $10^4$ spores /ml + 5.5g *a.i.*/ ha |
| $T_4$ | *M. anisopliae* | $1\times10^4$spores / ml |

**2.1 Statistical Analysis:** All the data were subjected to statistical analysis after appropriate transformation as suggested by Snedecor [13].

### 3. Results

An experiment was conducted in the laboratory to screen some new generation insecticides 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 insecticide media are presented in Table 2 and depicted in Fig. 1.

**Table 2:** *In vitro* studies on compatibility of *Metarhizium anisopliae* with new insecticides

| Treatment no. | Treatment | Dose | Performance of *Metarhizium anisopliae* in different media (at DDAI) | | | | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Growth (mm) | | | | | | Growth inhibition (%) | | | | | | |
| | | | 2 | 4 | 6 | 8 | 10 | Mean | 2 | 4 | 6 | 8 | 10 | | |
| $T_1$ | *M. anisopliae* + Rynaxypyr 20 EC | $10^4$ spores /ml + 9 g *a.i.*/ha | 20.80 | 29.40 | 35.20 | 48.80 | 53.60 | 37.56 | 39.87<br>(39.13) | 39.25<br>(38.76) | 39.73<br>(39.03) | 39.46<br>(38.89) | 39.36<br>(38.84) | | 39.50<br>(38.90) |
| $T_2$ | *M. anisopliae* + Flubendiamide 20 EC | $10^4$ spores /ml + 30 g *a.i.*/ha | 21.60 | 30.40 | 36.40 | 51.20 | 55.60 | 39.04 | 37.56<br>(37.77) | 37.19<br>(37.57) | 37.67<br>(37.83) | 36.48<br>(37.13) | 37.10<br>(37.50) | | 37.11<br>(37.50) |
| $T_3$ | *M. anisopliae* + Emamectin benzoate 5 SG | $10^4$ spores /ml + 5.5g *a.i.*/ha | 22.40 | 31.20 | 37.40 | 52.40 | 57.60 | 40.20 | 35.23<br>(36.36) | 35.52<br>(36.55) | 35.96<br>(36.82) | 34.99<br>(36.25) | 34.84<br>(36.13) | | 35.24<br>(36.39) |
| $T_4$ | Control (*M. anisopliae*) | $1\times10^4$spores/ml | 34.60 | 48.40 | 58.40 | 80.60 | 88.40 | 62.08 | - | - | - | - | - | | - |
| | SEm± | | 0.23 | 0.28 | 0.23 | 0.32 | 0.24 | 0.14 | 0.57 | 0.41 | 0.12 | 0.21 | 0.27 | | 0.15 |
| | CD at 5% | | 0.70 | 0.85 | 0.70 | 0.95 | 0.73 | 0.43 | 1.74 | 1.26 | 0.38 | 0.65 | 0.84 | | 0.46 |

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 parenthesis are arcsin transformed values.

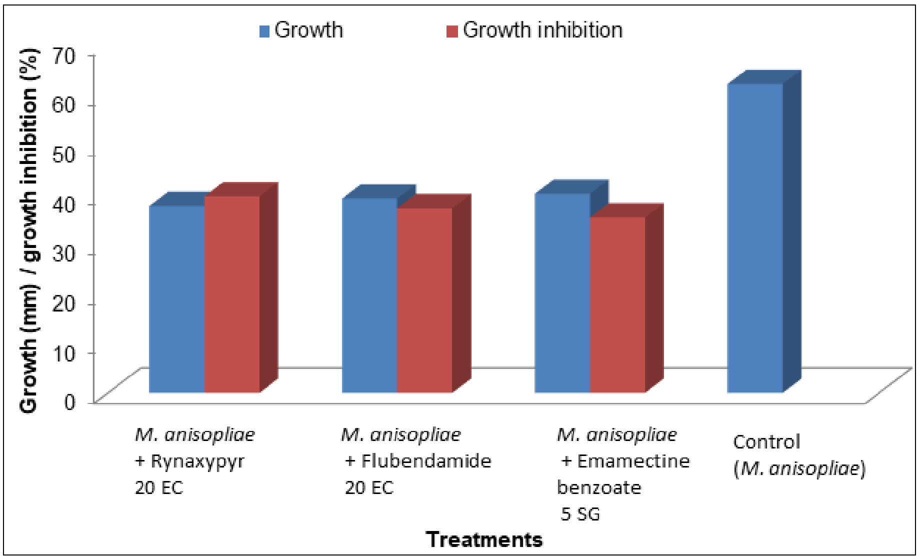


**Fig 1:** Growth and growth inhibition of *Metarhizium anisopliae* on different media

**2.2 Two days after inoculation (DAI)**

At two days after inoculation, the differences in the mean growth of *M. anisopliae* on different media were significant. Among the treatments, emamectin benzoate showed maximum growth (22.40 mm) at recommended half dose @ 5.5g *a.i.* /ha and was found to be the most compatible insecticide. This was followed by flubendiamide 20 SC @ 30 g *a.i.* /ha and rynaxypyr 20 SC @ 9 g *a.i.* /ha, and they recorded a growth of 21.60 mm and 20.80 mm, respectively. While in the control plate maximum growth of 34.60 mm was observed.

**2.3 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, emamectin benzoate showed maximum growth (31.20 mm) at recommended half dose @ 5.5g *a.i.*/ha and was found to be the most compatible insecticide followed by flubendiamide 20 SC @ 30 g *a.i.*/ha and rynaxypyr 20 SC @ 9 g *a.i.*/ha (30.40 mm and 29.40 mm, respectively). The control plate recorded maximum growth of 48.40 mm.

**2.4 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, emamectin benzoate showed maximum growth (37.40mm) at recommended half dose @ 5.5g *a.i.*/ha and was found to be the most compatible insecticide followed by flubendiamide 20 SC @ 30 g *a.i.*/ha and rynaxypyr 20 SC @ 9 g *a.i.*/ha (36.40 mm and 35.20 mm, respectively). The control plate recorded maximum growth of 58.40mm.

**2.5 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, emamectin benzoate showed maximum growth (52.40 mm) at recommended half dose @ 5.5g *a.i.*/ha and was found to be the most compatible insecticide followed by flubendiamide 20 SC @ 30 g *a.i.*/ha and rynaxypyr 20 SC @ 9 g *a.i.*/ha (51.20 mm and 48.80 mm, respectively). The control plate recorded maximum growth of 80.60mm.

**2.6 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, emamectin benzoate showed maximum growth (57.60 mm) at recommended half dose @ 5.5g *a.i.*/ha and was found to be the most compatible insecticide and was again followed by flubendiamide 20 SC @ 30 g *a.i.*/ha and rynaxypyr 20 SC @ 9 g *a.i.*/ha (55.60 mm and 53.60 mm, respectively). The control plate recorded maximum growth of 88.40 mm.

**2.7 Mean**

On overall basis among the insecticides tested, emamectin benzoate showed maximum growth (40.20 mm) at recommended half dose @ 5.5g *a.i.* /ha and was found to be the most compatible insecticide followed by flubendiamide 20 SC @ 30 g *a.i.*/ha and rynaxypyr 20 SC @ 9 g *a.i.*/ha (39.04 mm and 37.56 mm, respectively). The control plate recorded maximum growth of 62.08 mm.

**(ii) Growth inhibition of *M. anisopliae* in different insecticide media**

The data on effects of insecticides on the growth inhibition of *M. anisopliae* are presented in Table 2 and depicted in Fig. 1.

**2.8 Two days after inoculation**

All the treatments showed significant differences in growth inhibition of *M. anisopliae* in different insecticide treated media. Among the insecticides tested, emamectin benzoate showed least growth inhibition (35.23%) at recommended half dose @ 5.5g *a.i.*/ha and was found to be the most compatible insecticide, followed by flubendiamide 20 SC @ 30 g *a.i.*/ha and rynaxypyr 20 SC @ 9 g *a.i.*/ha, which recorded growth inhibition of 37.56 % and 39.87%, respectively.

**2.9 Four days after inoculation**

All the treatments showed significant differences in growth inhibition of *M. anisopliae* in different insecticide treated media. Among the insecticides tested, emamectin benzoate showed least growth inhibition (35.52 %) at recommended half dose @ 5.5g *a.i.*/ha and was found to be the most compatible insecticide, followed by flubendiamide 20 SC @ 30 g *a.i.*/ha and rynaxypyr 20 SC @ 9 g *a.i.*/ha, which recorded growth inhibition of 37.19% and 39.25 %, respectively.

**2.10 Six days after inoculation (DAI)**

All the treatments showed significant differences in growth inhibition of *M. anisopliae* in different insecticide treated media. Among the insecticides tested, emamectin benzoate showed least growth inhibition (35.96 %) at recommended half dose @ 5.5g *a.i.*/ha and was found to be the most compatible insecticide, followed by flubendiamide 20 SC @ 30 g *a.i.*/ha and rynaxypyr 20 SC @ 9 g *a.i.*/ha, which recorded growth inhibition of 37.67% and 39.73%, respectively.

**2.11 Eight days after inoculation (DAI)**

All the treatments showed significant differences in growth inhibition of *M. anisopliae* in different insecticide treated media. Among the insecticides tested, emamectin benzoate showed least growth inhibition (34.99 %) at recommended half dose @ 5.5g *a.i.*/ha and was found to be the most compatible insecticide, followed by flubendiamide 20 SC @ 30 g *a.i.*/ha and rynaxypyr 20 SC @ 9 g *a.i.*/ha, which recorded growth inhibition of 36.48% and 39.46%, respectively.

**2.12 Ten days after inoculation (DAI)**

All the treatments showed significant differences in growth inhibition of *M. anisopliae* in different insecticide treated media. Among the insecticides tested, emamectin benzoate showed least growth inhibition (34.84 %) at recommended half dose @ 5.5g *a.i.*/ha and was found to be the most compatible insecticide, followed by flubendiamide 20 SC @ 30 g *a.i.*/ha and rynaxypyr 20 SC @ 9 g *a.i.*/ha which recorded growth inhibition of 37.10% and 39.36%, respectively.

**2.13 Mean**

All the treatments showed significant differences in growth inhibition of *M. anisopliae* in different insecticide treated media. Among the insecticides tested, emamectin benzoate showed least growth inhibition (35.24 %) at recommended half dose @ 5.5g *a.i.*/ha and was found to be the most compatible insecticide, followed by flubendiamide 20 SC @ 30 g *a.i.*/ha and rynaxypyr 20 SC @ 9 g *a.i.*/ha which recorded growth inhibition of 37.11% and 39.50%, respectively.

## 4. Discussion
The effect of insecticides on the mycelia growth of *M. anisopliae* was conducted in *in vitro*. All the treatments showed significant mycelial growth, but less than control. Among insecticides tested emamectin benzoate 5SG @ 5.5 g *a.i.* /ha (half recommended dose) was found more compatible with least inhibition in the growth and it differed significantly than the other two chemicals flubendiamide 20SG @ 30 g *a.i.* /ha and rynaxypyr 20SG @ 9 g *a.i.* /ha. The present findings confirm the findings of Malik [14]. They also reported that emamectin benzoate was relatively less toxic to mycelial growth and spore of *M. anisopliae*. Similarly Malik [14] Dhar [15] Asi [16] and Naissy [17] reported insecticide acetamiprid, tracer, indoxacarb and imidacloprid to be quite safe for the growth and sporulation of *M. anisopliae*, respectively.
Although the different insecticides tested in the present investigations slightly inhibited the growth of *M. anisopliae* in poisoned media *in vitro*, the combined use of the fungus and insecticides cannot be completely ruled out. All the insecticides 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 insecticides at sub lethal dose with fungus for an enhance result over pest control. Laboratory and field studies on combined application of insecticides with entomopathogenic fungi and their compatibility might provide valuable data usefully for the development of strategies for handling plagues in organic agriculture.

## 5. Conclusions
The effect of insecticides on the mycelial growth of *Metarhizium anisopliae* was conducted *in vitro*. Among the insecticides tested, half recommended dose of fungus and emamectin benzoate 5 SG were found to be most compatible with least growth inhibition followed by flubendiamide 20SC and rynaxypyr 20SC. Although the different insecticides tested in the present investigations slightly inhibited the growth of *Metarhizium anisopliae* in the poisoned media, the combined use of the fungus and insecticides cannot be completely ruled out. All the insecticides tested have been combined at half recommended dose with entomopathogenic fungi to explore the possibility of increasing the efficacy of the combinations for pest control.

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## 7. References
1. Shah PA, Goettel MS. Directory of microbial control products and services. 2nd edition. Society for Invertebrate Pathology, Division of Microbial Control, Gainesville, USA, 1999, 81.
2. Sahayaraj K, Borgio JF. Tri-tropic interaction of cotton, red cotton bug and green muscardine fungi under *in vitro* condition. Journal of Bio pesticides. 2008; 1(1):41-46.
3. Jagadeesh Babu CS, Venkata Chalapatty CM, Amitha CN. Evolution of locally available substrates for mass multiplication of ento pathogenic bio pesticides. Journal of Bio pesticides. 2008; (2):146-147.
4. Mehta J, Dhaker JK, Kavia A, Sen P, Kaushal N, Datta S. Biomass production of Entomopathogenic fungi using various Agro Products in Kota Region, India. International Research Journal of Biological Sciences. 2012; 1(4):12-16.
5. Fuxa JR. Ecological consideration for the use of entomopathogens in IPM. Annual Review of Entomology. 1987; 32:225-251.
6. Ferron P. Biological control of insect pest by entomopathogens fungi. Annual Review of Entomology. 1978; 23:409-442.
7. Roberts DW, Humber RA. Entomogenous fungi. In Cole, G.T. and B Kendrick (eds.). The biology of conidial fungi. Academic Press New York, USA, 1981, 201-236.
8. Hokkanen H, Lynch JM. Biological control: Benefits and Risks. Cambridge University Press, UK, 1998, 304.
9. Moino Jr A, Alves SB. Efeito de Imidaclorprid e Fipronil sobre *Beauveria bassiana* (Balsamo) Vuil. E *Metarhizium anisopliae* (Metschnikoff) Sorok eno compor ramento de limpeza de Heteroterwes tenis (Hagen). Anais da Sociedade Entomológica do Brasil. 1998; 27:611-619.
10. Quintela ED, McCay CW. Synergetic effect of imidacloprid and two entomopathogenic fungi on the behaviour and survival of larvae of *Diapreper abbreviates* (Coleoptera: Curculionidae) in soil. Journal of Economic Entomology. 1998; 91:110-122.
11. Neves PMOJ, Hirose E, Tchujo PT. Compatibility of entomopathogenic fungi with neonicotinoid insecticides. Neotrop Entomol. 2001; 30:263-268.
12. Hokkanen HMT, Kotiluoto R. Bioassay of the side effects of pesticides on Beauveria bassiana and Metarhizium anisopliae: standardized sequential testing procedure. IOBC/WPRS Bulletin. 1992; 11(3):148-151.
13. Snedecor GW, Cochran WG. Statistical Methods. Oxford and IBH Publishing Company, New Delhi, 1967, 1-292.
14. Malik Akmal, Muhammad MN, Khan MB. Compatibility of *Metarhizium anisopliae* with different insecticides and fungicides. African Journal of Microbiology Research. 2012; 6(17):3956-3962.
15. Dhar Priyanka, Kaur Gurvinder. Compatibility of the entomopathogen fungi, *Beauveria bassiana* and *Metarhizium anisopliae* with neonicotinoid insecticide, Acetamiprid. Journal of Entomological Research. 2009; 33(3):195-202.
16. Asi MR, Bashir MH, Sahi ST. Compatibility of entomopathogenic fungi of *Metarhizium anisopliae* and *Paecilomyces fumosorosesus* with selective insecticides. Pakistan Journal of Botany. 2010; 42(6):4207-4214.
17. Naissy S, Maniania NK, Subramanian S, Gitonga ML, Maranga R, Obonyo AB, *et al*. Compatibility of *Metarhizium anisopliae* isolate ICIPE 69 with agrochemicals used in French bean production. International Journal of Pest Management. 2012; 58(2):131-137.