In vitro compatibility of pesticides with *M. anisopliae* and its pathogenecity against *A. craccivora*

Abstract: An *in vitro* trial was conducted in the Department of Entomology, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra; to determine the compatibility of green muscardine fungus, *M. anisopliae* with pesticides and its pathogenicity against *A. craccivora*. Azadirachtin, Buprofezin and Sulphar were the most suitable pesticides as it showed significantly superior growth on potato dextrose agar media over the untreated control. However, the Emamectin benzoate, Azadirachtin, Profenophos, Indoxacarb, Thiophanate, Paraquat Dichloride and Glyphosate showed significantly higher mean mycelial weight over the untreated control on potato broth medium. At the same time, amongst pesticides used in the experiment only insecticides (Emamectin benzoate, Azadirachtin and Deltamethrin) reported synergistic increase in conidial count with the untreated control, while fungicides and herbicides reported drastic reduction in conidial count. The pathogenicity of the *M. anisopliae* harvested from the pesticide treatments against *A. craccivora* revealed that all the pesticides investigated had reduced its pathogenecity in comparison to untreated control. However Emamectin benzoate, Indoxacarb and Paraquat dichloride showed maximum per cent mortality and can be used in combination with *M. anisopleae*.

Keywords: Entomopathogenic fungi, pesticides, Bioassay, virulence, conidia

Comment [71]: It is convenient to mention the levels of the treatments, the number of repetitions and the design of the experiment used in the research.

Introduction

The genus *Metarhizium* is a parasite of a great number of arthropods, occurring in more than 200 species of insects and acaridae. These fungi are also adapted to live as saprophytes as well as symbionts in the plant rhizosphere (Gang and Leger, 2002), which make the soil their major habitat. Conidial survival can be effected by interaction with pesticides, environmental factor or by bio-pesticide and/or chemical product used to protect plants. The pesticides may antagonize or synergize efficacy and potential insecticidal activity of *M. anisopleae* and may disrupt natural epizootics of this pathogen.

The knowledge of the compatibility between the EPF and pesticides may facilitate the choice of these combinations in Integrated Pest Management (IPM) programs (Singh *et al.* 2014; Johnson *et al.*, 2020). Combined utilization of selective insecticides in association with fungal pathogens can increase the efficiency of control by reduction of the amount of applied insecticides, minimizing environmental contamination hazards and pest resistance (Quintela and McCoy 1998). There are numerous examples where the application of chemical pesticides has enhanced the efficacy of entomopathogens against insect pests (Kaakeh *et al.* 1997; Gardner and Kinard 1998). *Lecanicillium muscarium*, an insect pathogen that is used commercially to control greenhouse pests and is a candidate species for the control of *B. tabaci*, was suggested to be applied sequentially with imidacloprid. IPM Strategy (Duarte *et al*. 1992) pointed out the importance in considering the antagonistic effect of the pesticides on all developmental phases of entomopathogenic fungi since these products may affect the bio-insecticide potential as well as the occurrence of epizootics. Several studies have contributed information for the choice of pesticides with more selective action on the entomopathogens, and most of them were conducted under laboratory conditions (Silva *et al*. 1993).

Conidial germination is more important than vegetative growth and sporulation on cadavers, because the former corresponds to the first step that triggers an epizootic, and the fungus relies on it to infect the host successfully. Thereby, if a pesticide causes substantial decrease in conidial germination, it may reduce the effectiveness of the entomopathogen toward its target. As mycelial growth develops inside the insect host and the concentration of agrochemicals, especially those with systemic mode of action, are usually found in low titer in the hemolymph, there is little chance of this developmental stage to be negatively affected. Keeping this in view, a laboratory study was conducted to assess the compatibility between the different chemical pesticides with *M. anisopleae.*

Materials and methods

Comment [72]: It is necessary to mention for each experiment, the number of treatments and replications, as well as justify the design of experiments.

M. anisopleae was isolated from previously maintained lab culture on Agar plates and multiplied on Potato Dextrose Agar (PDA) media in petriplates as well as Potato Dextrose Broth media in conical flasks at post graduate laboratory, Department of Agricultural Entomology, Dapoli, Ratnagiri, Maharashtra, India. All *In vitro* studies were carried out aseptically in laminar air flow chamber. The Experiments were conducted under well-defined conditions of culture room maintained at 25±2^{0}C temperature, uniform light (1600 Lux) provided by fluorescent tubes (7200 K) over a light and dark cycle of 16/8 hours. The insecticides, fungicides and herbicides chosen for the compatibility study are the most frequently used by vegetable growers. The concentrations of all agrochemicals were tested following the label rates recommended by the IRAC. When the concentration of a product indicated minimum and maximum doses, the average concentration was used. The concentration tested for each product was calculated based on the spray volume rate of 500-1000 Lha^{-1}of water.

Effect of pesticides on radial growth of M. anisopliae

The effect of all pesticide preparations was tested on growth of *M. anisopliae* by poisoned food technique. With the help of sterile cork borer, discs of 3mm diameter were cut from actively growing culture of the fungus and transferred in the center of petridishes containing desired pesticides together with the medium. The inoculated dishes were incubated and observations on radial growth of colony (cm) were recorded on 4th, 6th, 8th, 10th and 12th day after inoculation on PDA medium.

Effect of pesticides on biomass of M. anisopliae (Mycelial dry weight)

The effect of pesticides was assessed on biomass of mycelial growth. Each insecticide concentration was mixed with 25 ml of Potato Dextrose Broth (PDB) media in conical flasks of 100 ml capacity. Circular bits of diameter 3mm were made by sterilized cork borer and the conical flasks were inoculated under laminar air flow. Each treatment (mixture of fungal conidia + pesticide) was replicated three times (three flasks per treatment). After the period of incubation, the mycelial growth was determined when control expressed mycelial mat covering complete surface of substrate. Afterwards, the entire mycelial mat was harvested on properly weighed handmade filter paper by filtration from the PDB medium and fresh weight of mycelial mat was recorded which was later kept in hot air oven at 55-60^{o}C until constant decrease in weight is observed .

Effect of pesticides on conidial germination of M. anisopliae

The appropriate concentrations of each pesticide were added to 25 ml of cooled (45^{0}C) PDB. This treated was directly inoculated with 1 ml conidial suspension of *M. anisopliae* containing10^{6} spore ml^{-1} that diluted in sterile distilled water amended with 0.01% Tween 80. The same aliquot of sterile distilled water, standard spore suspension and 0.01% Tween 80, without the pesticides, served as control. The same were incubated for 8 days. After incubation by haemo-cytometer conidia were counted. The data was used to calculate per cent (%) germinated or non-germinated spore.

Conidia production assessment

The sterile PDA medium cooled to 40 ± 5^{0}C and pre-established concentrations of insecticides were added with the antibiotic streptomycin (0.5 l^{-1}). Approximately 20 ml of each concentration was poured into 9 cm petridish. Medium without insecticide but containing streptomycin served as control. After solidification the medium was inoculated with *M. anisopliae.*

The dishes were incubated for 8 days. After 8 days, colony discs were selected randomly for conidial production assessment. Each disc was placed in a screw cap tube containing 10 ml of sterile water with (0.02%) Tween 20. The tubes were agitated until the conidia are completely released and the conidial concentration was counted using Neubauer Chamber.

Effect of pesticides on pathogenicity of compatible M. anisopliae against Aphid A. craccivora under laboratory conditions

Aphids were reared on brinjal plants grown in plastic pots. Twenty aphids of uniform growth and of same age were selected for experiment. The aphids were placed on a leaf disc in a Petri dish and sprayed with samples of suspension. Suspensions were prepared in distilled water by using 5g of aliquot per liter of water. The aphids were observed for mortality at an interval of 24 hrs. The data thus obtained on mortality was converted into per cent mortality. One control was maintained without any spray.

Data analysis

The data sets for each experiment were submitted to a two way analysis of variance (ANOVA) in Randomized Block Design and treatment means were compared by Fisher's least significant difference (LSD, α = 0.01) for statistical analysis.

Comment [73]: Reference should be made to the fulfillment of the basic assumptions (normality, homogeneity, additivity and independence) in the context of the validity of the results of the analysis of variance.

Results and Discussion

Effect of insecticides on *M. anisopleae*

The *in vitro* effect of insecticides on mean radial growth, mean mycelia weight, mean conidial germination and pathogenicity of the *M. anisopliae* culture compatible with insecticides was recorded

(Table 2). The pathogenecity test was conducted against cowpea aphid, A. craccivora *in vitro* and percent mortality was recorded.

Mean radial growth

4th day after inoculation

The results in the present investigations showed that there were significant differences among various treatments. The mean radial growth on 4th day after inoculation varied from 0.40cm (T_6-Deltamethrin) to 6.36cm (T_4-Buprofezin). The latter was at par with the treatments T_8- Azadirachtin (6.16cm) and T_{10}- untreated control (5.56cm) and significantly superior over rest of the treatments; also supported good growth of the EPF.

6th day after inoculation

The mean radial growth on 6th day after inoculation varied from 0.56cm (T_6-Deltamethrin) to 6.86cm (T_4-Buprofezin). The latter was at par with the treatments T_8- Azadirachtin (7.02cm) and T_{10}- untreated control (5.9cm) and significantly superior over rest of the treatments.

8th day after inoculation

The mean radial growth on 8th day after inoculation varied from 1.26cm (T_6-Deltamethrin) to 9.0cm (T_4-Buprofezin). The latter was at par with the treatments T_8- Azadirachtin (7.02cm) and T_{10}- untreated control (5.9cm) and significantly superior over rest of the treatments. The results of data 10th and 12th day after inoculation were similar to the readings after 8 days of inoculation.

In general the results indicated that amongst various insecticides tested, Buprofezin was found to be the most suitable for *M. anisopliae* followed by Azadirachtin. Mycelial growth was reduced in all insecticide treatments when compared to the untreated control except Buprofezin which allowed normal fungal growth. Lowest *M. anisopliae* mycelial growth was observed with Deltamethrin indicating high fungal toxicity. It is possible that those products compatible with *M. anisopliae*, especially the insecticides, might be used at sublethal dosages in combination with the fungus in further studies aimed at improving its virulence. Pachamuthu and Kamble (2000) also observed a significant interaction similar to present findings, between the EPF (*M. anisopliae*) and commercial pesticide (chlorpyrifos) at LT_{50} values. The present findings on mycelial growth at the recommended dose of insecticides were in contradictory with Puzari *et al*. (2006) who reported 100per cent of mycelial growth was observed when the entomopathogen was grown in presence of Dichlorvos at recommended dose. Cypermethrin and deltamethrin at recommended dose showed lowest growth inhibition against *M. anisopliae*, may be due species or strainal variation; although the biological activity of *M. anisopliae* can be enhanced by integrating it with sub lethal doses of chemical insecticides (Zurek *et al*., 2002). Schumacher and Poehling (2012) also mentioned similar findings that the insecticides fipronil, permethrin, imidacloprid, neemazal, and amitraz at different concentrations (0.32 to 200 ppm) found compatible against two strains of the entomopathogenic fungus *M. anisopliae* (MA) while only fipronil in the higher dose rates of 40 and 200 ppm as moderately toxic. The growth rate of *M. anisopliae* in presence of spinosad at low concentrations remains unaffected, but reduces at concentrations of 192 ppm or higher (Ericsson *et al*., 2007). The insecticide cypermethrin is slightly toxic to all the isolates of *M. anisopliae* and fungicides, carbendazim and hexaconazole are not compatible with the *M. anisopliae* isolates (Johnson *et al*., 2020).

Mean mycelial weight of *M. anisopleae*

Mycelial growth was reduced in all insecticide treatments when compared to the untreated control except T_8 (Azadirachtin 0.15EC), T_2 (Indoxacarb 14.5SC) and T_5 (Profenophos 50EC) which allowed normal fungal growth 6.211g, 3.787g and 3.784g respectively. White coloured unsporulated mycelial growth was observed with T_8 (Azadirachtin 0.15EC) which was different from the other treatments. The treatment T_7 (Dichlorovas 76EC) strongly inhibited the mycelial growth and recorded lowest mycelial weight 2.386g while other treatments *viz.*, T_4 (Buprofezin 25EC), T_1 (Cypermethrin 25EC), T_3 (Chlorantraniliprole 18.5SC) and T_6 (Deltamethrin 2.8EC) reported 3.484g, 3.292g, 3.187g and 3.105g of mycelial mat respectively. At the same time Control reported 3.605g weight of mycelial mat.

The present findings were similar with the findings of Singh *et al.* (2014) which reported that insecticides are compatible with EPF, though it shows noticeable reduction in biomass on broth media, they can be applied in combination with EPF at half of mean concentration on field evaluation. Babu *et al*. (2013) also found that *M. anisopliae* displays an enhancement in the vegetative growth with imidacloprid (2%) and HIT (2-18%). Among botanicals, Azadirachtin enhance the vegetative growth and spore germination of *B. bassiana* and *M. anisopliae* (Khan *et al*., 2012). Rashid *et al.* (2010) observed that the insecticides *viz*., Fipronil at concentration 10, 40 and 120 ppm, Pyriproxyfen at concentration 10, 500 and 1000 ppm and Hexaflumuron at concentration 20, 50 and 120 ppm on SDA medium; Hexaflumuron at the concentration of 120ppm reduce the vegetative growth of *M. anisopliae* to zero per cent compared to Pyriproxyfen (24.59%) and Fipronil (24.31%).

Mean conidial germination of *M. anisopleae*

All insecticides affected conidial germination in contrast to the untreated control. The highest (8.00) detrimental effect on conidial germination was observed in T_3 (Chlorantraniliprole 18.5SC). While other treatments *viz.*, T_7 (Azadirachtin 0.15EC), T_4 (Buprofezin 25EC), T_6 (Dichlorovas 76EC), T_2 (Indoxacarb 14.5SC) and T_1 (Cypermethrin 25EC) reported mean conidial germination 39.66, 29.33, 20.00, 19.00 and 13.00 respectively. The treatment T_9 (Emamectin benzoate 5SG) did not affected conidial germination and reported a maximum of mean conidial germination 92.00 while T_5 (Deltamethrin 2.8EC) with mean conidial germination 49.33 was at par with T_9 (Emamectin benzoate). At the same time Untreated Control reported 33.79 mean conidial germination.

The similar findings were also reported by James and Elzen (2001) reporting that insecticides slightly inhibit the conidial germination and the toxicity is dose dependent that it increases with increase in concentration of insecticides. The use of insecticides, in most cases, has no negative effect on conidia germination, conidia production and vegetative growth of *B. bassiana*, *M. anisopliae* and

Paecilomyces sp (Neves *et al.,* 2001). The application of the EPF *M. anisopliae var. acridum* and *M.anisopliae var. anisopliae* with the Neem oil on PDA medium still liquid (±45°C), inhibit conidial production (Santos *et al.*, 2009). Imidacloprid 17.8SL and neem oil 3 percent are significantly compatible and found safe to conidial germination and growth (Hemalatha *et al.*, 2015). No any reports were observed with the synergestic effect recorded by Emamectin benzoate and Deltamethrin.

Percent mortality of cow pea aphid (*A. craccivora*) by *M. anisopleae* suspension from different treatments

The highest (80.00%) per cent mortality was recorded in the treatment T_2 (Indoxacarb 14.5SC) and was significantly superior over other treatments. While other treatments *viz.,* T_7 (Emamectin benzoate 5SG), T_6 (Azadirachtin 0.15EC), T_4 (Buprofezin 25EC), T_3 (Chlorantraniliprole 18.5SC) and T_1 (Cypermethrin 25EC) reported 76.65 percent, 66.65 percent, 58.30 percent, 51.65 percent and 40.00 percent mortality respectively. The lowest (28.30%) per cent mortality was recorded in T_5 (Deltamethrin 2.8EC). At same time untreated control recorded 86.65 per cent mortality.

The present findings were in agreement with the findings of Araujo *et al.* (2009) which reported that neem based insecticides expressed 90 per cent mortality in comparison with untreated control against aphid *Lipaphis erysimi* (Kalt.). The synergism exists between the botanical insecticide, azadicarchtin and destruxin, a mycotoxin that extracted from *M. anisopliae* in a joint action against cotton aphid, *Aphis gossypii* (Fei Yi *et al*., 2012). The mortality of *B. tabaci* treated with comibination of *B. bassiana* and 3 EFAM fungicides is lower than or similar to the mortality with application of *B. bassiana* alone (Ling *et al*., 2016). The fungus-insecticide bait formulation (termiticide fipronil with a biocontrol agent *M. anisopliae*) shows the greatest compatibility and synergistic effect that increases subterranean termite (*C. curvignathus*) mortality as well as reduces the lethal time at a sublethal dose of fipronil (Jing *et al.,* 2016). The insecticide imidacloprid showes the best compatibility (95.2%) with a positive toxicity/virulence to the *Metarhizium* spp. (strain PDRL526); causes adult cotton mealy bug mortality after (LT_{50}) 13.8 and 19.6 days by using 6.3 $x10^{12}$ spores/acre, under screen house and field conditions, respectively (Ujjain *et al*., 2015).

Effect of fungicides on *M. anisopleae*

Similar to the insecticides, the *in vitro* effect of fungicides on *M. anisopliae* was recorded (Table 3).

Mean radial growth

4^{th} day after inoculation

The result showed that there were significant differences among various treatments. The highest (7.33cm) diameter was recorded in the treatment T_{10} (Untreated Control). The second best treatment was T_4 (Sulphar 80WDG) with diameter 7.16cm and was significantly superior over all other treatments. While other treatments *viz.*, T_9 *(*Metalaxyl 35WS) and T_5 (Thiophanate methyl 70WP) recorded mean diameter 2.53cm and 1.2cm respectively. On the same contrary the lowest or no growth was recorded in treatments *viz.,* T_1 (Carbendazim 50WP), T_2 (Mancozeb 75WP), T_3

(Copper oxychloride 50WP), T_6 (Hexaconazole 5EC), T_7 (Difenconazole 25EC) and T_8 (Captan 50WP) showing complete inhibition of germination and multiplication of *M. anisopleae*.

6th day after inoculation

The highest (9.0cm) diameter was recorded in the treatment T_4 (Sulphar 80WP) and was significantly superior over all other treatments. The second best treatment was T_{10} (Untreated control) with diameter 7.5cm and was significantly superior over all other treatments. While other treatments *viz.*, T_9 *(*Metalaxyl 35WS) and T_5 (Thiophanate methyl 70WP) recorded mean diameter 3.3cm and 1.26cm respectively. On the same contrary the lowest or no growth was recorded in treatments *viz.*, T_1 (Carbendazim 50WP), T_2 (Mancozeb 75WP), T_3 (Copper oxychloride 50WP), T_6 (Hexaconazole 5EC), T_7 (Difenconazole 25EC) and T_8 (Captan 50WP) showing complete inhibition of germination and multiplication of *M. anisopleae*. The results of data 8, 10 and 12 days after inoculation were similar to the readings after 6 days of inoculation except treatment T_9 (Metalaxyl 35WS) which showed slight increase in radial growth of fungi up to 4.23cm.

In general the results indicated that the fungicides were the most harmful products to all biological stages of *M. anisopliae* and they should not be applied together with this EPF. All fungicides negatively affected *M. anisopliae* growth compared to the controls except T_4 (Sulphar 80WP). The present findings were concurrent with Patil *et al*. (2014) and Kotwal *et al.* (2012) reporting that carbendazim and mancozeb resulted in complete growth inhibition (100%) of EPF. Sulphur displayed high compatibility to all isolates at higher concentration except for B57 and Copper oxy-chloride was compatible to all the isolates at lower concentration but showed toxicity at higher levels. This positive effect of sulphar on radial growth of EPF was also reported by Usha *et al.* (2014) and Kotwal *et al.* (2012). Similar findings were also reported by Autkar (2004) and Jayraman (2005). The fungicides (carbendazim and hexaconazole) have complete inhibitory action on the growth of *M. anisopliae* (Joshi *et al*., 2018). The copper fungicides are classified into the upper half of the highly toxic (T) category and are considered incompatible with the entomopathogenic fungus *B. bassiana* strain ATCC 74040 under *in vitro* conditions (Celar and Kos, 2020).

Mean mycelial weight of *M. anisopleae*

The result showed that there were significant differences among various treatments. The highest (3.787g) mycelial weight was recorded in the treatment T_5 (Thiophanate methyl 70WP). Treatments T_4-Sulphar 80WDG (3.651g) and T_9- Metalaxyl 35WS (3.568g) were at par with Thiophanate methyl 70WP. The treatments T_8 (Captan 50WP) and T_2 (Mancozeb 75WP) reported 1.970g and 1.809g mycelial weight respectively. The lowest or no growth was recorded in T_1 (Carbendazim 50WP), T_3 (Copper oxychloride 50WP), T_6 (Hexaconazole 5EC) and T_7 (Difenconazole 25EC). At the same time Control reported 3.763g weight of mycelial mat.

The present findings were in accordance with Niassy *et al.* (2012) who reported that amongst all the pesticides tested, Carbendazim and Copper fungicides were highly toxic to *M. anisopleae and B. bassiana* with lowest or nil biomass on broth media. Applications of the fungicides iprodione and tebuconazole, which are used routinely on *brassica* crops, are compatible with using *M. anisopliae* 389.93 against *D. radicum* under glasshouse conditions, even though these fungicides are inhibitory to fungal growth on SDA medium (Chandler *et al.*, 2005). The fungicides (carbendazim and mancozeb) produce complete growth inhibition (100%), whereas tridemefon and cyperconazole inhibit the growth of *N. rileyi* by (49.65% and 55.25%, respectively) *in vitro* at their recommended dosages (Patil *et al.,* 2014). The fungicides difenoconazole, propiconazole, trifloxystrobin and azoxystrobin are the most harmful products to all biological stages of *M. anisopliae* (strain CG 168) on PDA *in vitro* at recommended field dosage and they should not be applied together with this fungus in tank mixing (Silva, 2013).

Mean conidial germination of *M. anisopleae*

The result showed that there were significant differences among various treatments. The highest mean conidial germination 21.60 was recorded in the treatment T_2 (Thiophanate methyl 70WP) while other two treatments *viz.,* T_3 (Metalaxyl 35WS) and T_1 (Sulphar 80WDG) reported mean conidial germination 10.40 and 09.80 respectively and were at par with T_2 (Thiophanate methyl 70WP). At the same time Control recorded 92.08 mean conidial germination.

The present findings were in agreement with Silva *et al.* (2013) and Usha *et al.* (2014) reporting that fungicides caused more than 50 per cent reduction in conidial germination of EPF. The germination inhibition rate of autochthonous isolate of *Metarhizium* spp. increases with the concentration of fungicides Aby *et al.*, 2015).

Percent mortality of cow pea aphid (*A. craccivora*) by *M. anisopleae* suspension from different treatments

There were significant differences among various treatments. The highest (63.00%) percent mortality was recorded in the treatment T_2 (Thiophanate methyl 70WP) followed by 43.00 percent mortality in treatment T_3 (Metalaxyl 35WS). The lowest percent mortality 41.00 percent was observed in treatment T_1 (Sulphar 80WDG). At the same time Control reported 83.00 percent mortality.

The present findings were in agreement with Mutimura *et al.* (2010) which state that fungicides inhibited growth of mycelia and germination of the fungal conidia at all concentrations tested *in vitro,* and also reduced the efficacy of EPF in bioassays against arthropods. The pathogenicity of *Metarhizium anisopleae* in combination with fungicides shows highest mortality (33%) rate for adult weevils (*Cosmopolites Sordidus)* exposed to conidia produced on media containing high concentrations of Methyl thiophanate and Propiconazole; while 11 percent for Trifloxystrobin (Aby *et al.*, 2015).

Effect of herbicides on *M. anisopleae*

The *in vitro* effect of herbicides on *M. anisopliae,* in ways similar to the insecticides and fungicides was recorded (Table 4).

Mean radial growth

4th day after inoculation

The result showed that there were significant differences among various treatments. The highest (4.7cm) diameter was recorded in the treatment T_1 (Pendimethylene 30EC) and the treatments T_8 (Untreated control) (4.6cm), T_7 (Paraquat dichloride 24SL) (4.3cm) and T_6 (Glyphosate 41SL) (4.1cm) were at par with pendimethylene. The treatment T_5 (2,4-D Dimethyl amine Salt 58WSC) and T_2 (Pyrazosulfuron ethyl) recorded radial growth 3.5cm and 2.1cm respectively while other treatments *viz.,* T_3 (Oxadiargyl 80WP) and T_4 (Oxyflorofen) reported complete inhibition of germination and growth of *M. anisopleae*.

6th day after inoculation

The highest (5.6cm) diameter was recorded in the treatment T_6 (Glyphosate 41SL) (5.6cm) and the treatment T_1 (Pendymethylene 30EC) (5.3cm) was at par with glyphosate. The treatment T_7 (Paraquat dichloride), T_5 (2,4-D Dimethyl amine Salt 58WSC) and T_2 (Pyrazosulfuron ethyl) recorded radial growth 4.7cm, 3.8cm and 2.5cm respectively while other treatments *viz.,* T_3 (Oxadiargyl 80WP) and T_4 (Oxyflorofen) reported complete inhibition of germination and growth of *M. anisopleae*. At the same time untreated control recorded radial diameter of 6.8cm.

8th day after inoculation

The highest (8.8cm) diameter was recorded in the treatment T_6 (Glyphosate 41SL) (8.8cm) and was superior over all the treatments. The treatment T_7 (Paraquat dichloride), T_1 (Pendimethylene 30EC), T_5 (2,4-D Dimethyl amine Salt 58WSC) and T_2 (Pyrazosulfuron ethyl) recorded radial growth 5.3cm, 5.3cm, 4.5cm and 2.8cm respectively while other treatments *viz.,* T_3 (Oxadiargyl 80WP) and T_4 (Oxyflorofen) reported complete inhibition of germination and growth of *M. anisopleae*. At same time untreated control recorded radial diameter of 8.0cm. The results of data 10 and 12 days after inoculation were similar to the readings after 8 days of inoculation.

In general the results indicated that amongst various herbicides tested, glyphosate was found to be the most suitable for *M. anisopliae* followed by pendimethylene. The present findings are in contradictory with Silva *et al.* (2013) who reported a complete suppression of vegetative growth of *M. anisopliae* was caused by 2, 4-D as it expressed good mycelial growth during the present investigations. Most herbicides showed reduced mycelial growth in comparison with the control except for glyphosate. Similar findings were also reported by Silva *et al.* (2013) from Brazil.

Mean mycelial weight of *M. anisopleae*

The highest mycelial growth was recorded in the treatment T_6 (Glyphosate 41SL) (3.928g) and was significantly superior over all the treatments. The second best treatment was T_7 (Paraquat dichloride 24SL) (3.715g), followed by T_1 (Pendimethylene 30EC) (3.206) and T_2 (Pyrazosulfuron ethyl 10WP) (2.690). The other treatments *viz.,* T_3 (Oxadiargyl 80WP) and T_4 (Oxyflorofen 23.5EC) and T_5 (2,4-D Dimethyl amine Salt 58WSC) reported complete inhibition of germination and growth of *M. anisopleae*. At same time unrtreated control recorded radial diameter of 3.572g mycelial weight.

The present findings were in agreement with the results of Ambethgar *et al*. (2009) who reported that results obtained in some in vitro and in vivo experiments suggest a sensitivity of EPF to some herbicides and Celar and Kos (2020) who reported a strong inhibitory effect of some tested herbicides on mycelium growth of EPF in laboratory experiments. Herbicide 2,4-D is most harmful to *M. anisopliae* CG 168 as it completely inhibita conidial germination, vegetative growth and conidiation (Leipelt *et al*., 2001). The herbicides lambda cyhalothrin and theherbicides glyphosate, bentazon, and imazapic+ imazapyr are compatible with *M. anisopliae* (Silva *et al.*, 2013).

Mean conidial germination of *M. anisopleae*

The highest mean conidial germination 36.00 was recorded in the treatment T_1 (Pendimethylene 30EC). While other treatments T_5 (Paraquat dichloride 24SL), T_2 (Pyrazosulfuron ethyl 10WP) and T_3 (2,4-D Dimethyl amine Salt 58WSC) reported mean conidial germination 35.25, 30.25 and 20.50 respectively and were at par with the treatment T_1 (Pendimethylene 30EC). The lowest mean conidial germination 09.50 was recorded T_4 (Glyphosate 41SL). At same time unrtreated control recorded 92.25 mean conidial germination per 10µl of suspension.

The present findings were in line with Silva *et al.* (2013) who reported that all herbicides had a deleterious effect on conidial yield of EPF compared to the untreated control.

Percent mortality of cow pea aphid (*A. craccivora*) by *M. anisopleae* suspension from different treatments

The highest (73.75%) per cent mortality was recorded in the treatment T_5 (Paraquat dichloride 24SL) and was significantly superior over other treatments. While other treatments *viz.*, T_3 (2,4-D Dimethyl amine Salt 58WSC), T_1 (Pendimethylene 30EC) and T_2 (Pyrazosulfuron ethyl 10WP) reported 65.00 percent, 63.75 percent and 63.75 percent mortality respectively. The lowest (55.00%) per cent mortality was recorded T_4 (Glyphosate 41SL). At same time untreated control recorded 85.00 percent mortality. Most of the herbicides classified as toxic, but those formulated with imazapyr, glyphosate and metribuzin are considered compatible (Botelho, 2011).

Effect of pesticides on conidia production assessment of *M. anisopleae*

Since all the treatments showed germinated conidia and no any report of ungerminated conidia was noticed; the conidia production assessment was said to be 100 percent in all the treatments.

In summary, our findings from *in vitro* compatibility were in agreement with Silva *et al.* (2013) reporting that fungicides are more detrimental to conidial germination, mycelial growth and sporulation of *M. anisopliae* than herbicides and insecticides, and they should not be applied together with EPF. The insecticides exhibited the least degree of toxicity to fungal pathogen, whereas the herbicides had the greatest impact on mycelial growth.

After collecting published results of many different experiments on EPF and pesticides, we observed that fungicides have the highest mycelium growth inhibitory effect, while insecticides and herbicides have a fungistatic effect. Also reported by Klingen and Haukeland (2006). Although the results did not consider the effect of many variables associated with field use of pesticides, our findings are of paramount importance to guide and advice farmers to use safely this EPF in combination with pesticides without affecting fungal virulence and germination.

Future prospects

The present studies open an area of using EPF together with compatible insecticides as multiple mortality factors against target pests and also help in delaying expression of resistance to new insecticides.

Conclusions

In conclusion, our results indicated that the use of combination of *M. anisopliae* with lower dosage of insecticides might become an important component of IPM but at first, this approach must be tested under field conditions.

Integrating insecticides and entomopathogens has a few advantages:

1) Such approach will increase pest mortality as well as reduce the lethal time.

2) Prolong the use of a particular insecticide by reducing the total amount of insecticide used.

3) Minimizing environmental contamination and increasing human safety.

4) Accelerates the mode of action of fungus without compromising the fungus growth from cadavers that is crucial for inducing epizootic in house fly population particularly in larval bedding that humidity and temperature of bed supported the growth of muscardine on larval cadavers.

5) Sublethal dosage of synthetic insecticides can act as physiological stressors or behavioral modifiers, there by predisposing insects to diseases.

To decrease the dose of chemical pesticide used in agriculture, combination of chemical pesticides with biopesticides can be done so as to minimize the negative effect of former on the environment. Acetamaprid and Profenofos require further investigation. Also field evaluation of the interactions between *B. bassiana* and these pesticides should be undertaken to evaluate their effect on pest and beneficial insects. On the other hand, the high toxicity of a product *in vitro* not always means that the same will occur in the field, but is evidence that it may be possible to occur. Thus, when an IPM strategy is devised, 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.

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Table 1: Effect of insecticides on radial growth of *M. anisopleae*

Treat. No.	**Treatment**	**Concentration (%)**	**Mean Radial growth (cm)**			**Mean mycelial weight (g)**	**Mean Conidial germination (10 µl)**	**Mortality of test insect, (%)**
			4^{th} Day	**6^{th} Day**	**8^{th} Day**			
T_1	Cypermethrin 25EC	0.0075	2.93	3.43	4.16	3.292	13.00 (3.74)*	40.00 (39.23)**
T_2	Indoxacarb 14.5SC	0.01	3.83	4.26	4.63	3.787	19.00 (4.47)	80.00 (63.43)
T_3	Chlorantraniliprole *18.5 SC*	0.005	2.43	2.9	3.2	3.187	08.00 (3.00)	51.65 (45.95)
T_4	Buprofezin 25EC	0.015	6.36	6.86	9.0	3.484	29.33 (5.51)	58.30 (49.78)
T_5	Profenophos 50EC	0.15	1.73	2.16	2.4	3.784	49.33 (7.09)	28.30 (32.14)
T_6	Deltamethrin 2.8EC	0.002	0.40	0.56	1.26	3.105	20.00 (4.58)	66.65 (54.73)
T_7	Dichlorovas 76 EC	0.07	2.06	2.26	2.33	2.386	39.66 (6.38)	76.65 (61.10)
T_8	Azadirachtin0.15EC	0.05	6.16	7.03	7.2	6.211	92.00 (9.64)	86.65 (68.97)
T_9	Emamectin Benzoate 5SG	0.0016	4.3	4.53	4.9	4.052	33.79 (5.90)	40.00 (39.23)
T_{10}	Control		5.56	5.9	6.03	3.605	13.00 (3.74)	80.00 (63.43)
			SIG					
S.E. ±			1.05	0.99	1.13	0.43	2.63	1.10
C.D. at 1%			4.28	4.03	4.58	1.75	11.06	4.63

*Figures in the parentheses are square root transformed values

**Figures in the parentheses are arc sin transformed values

Table 2: Effect of fungicides on *M. anisopleae*

Treat. No.	Treatment	Concentration (%)	Mean Radial growth (cm)			Mean mycelial weight (g)	Mean Conidial germination (10 µl)	Mortality of test insect, (%)
			4th Day	6th Day	8th Day			
T_1	Carbendazim 50WP	0.1	0	0	0	0.00	-	-
T_2	Mancozeb 75WP	0.25	0	0	0	1.809	-	-
T_3	Copper oxychloride *50WP*	*0.25*	0	0	0	0.00	-	-
T_4	Sulphar 80WDG	0.2	7.16	9	9	3.651	09.80 (3.29)*	41.00 (39.82)**
T_5	Thiophanate methyl 70WP	0.1	1.2	1.26	1.26	3.787	21.60 (4.75)	63.00 (52.54)
T_6	Hexaconazole 5EC	0.05	0.0	0.0	0.0	0.00	-	-
T_7	Difenconazole 25EC	0.05	0.0	0.0	0.0	0.00	-	-
T_8	Captan 50WP	0.2	0.0	0.0	0.0	1.970	-	-
T_9	Metalaxyl 35WS	0.2	2.53	3.3	4.23	3.568	10.40 (3.38)	43.00 (40.98)
T_{10}	Control		7.33	7.5	7.5	3.763	92.08 (9.65)	83.00 (65.65)
			SIG					
S.E. ±			0.92	0.60	0.59	0.22	1.50	0.60
C.D. at 1%			3.75	2.43	2.41	0.88	5.50	2.46

*Figures in the parentheses are square root transformed values

**Figures in the parentheses are arc sin transformed values

Table 3: Effect of herbicides on radial growth of *M. anisopleae*

Treat. No.	Treatment	Concentration (%)	Mean Radial growth (cm)			Mean mycelial weight (g)	Mean Conidial germination (10 µl)	Mortality of test insect, (%)
			4th Day	6th Day	8th Day			
T_1	Pendimethylene 30EC	0.2	4.7	5.3	5.3	3.206	36.00 (6.08)*	63.75 (52.98)**
T_2	Pyrazosulfuron ethyl 10WP	0.003	2.1	2.5	2.8	2.690	30.25 (5.59)	63.75 (52.98)
T_3	*Oxadiargyl 80WP*	*0.016*	0.0	0.0	0.0	0.00	-	-
T_4	Oxyflorofen 23.5EC	0.03	0.0	0.0	0.0	0.00	-	-
T_5	2,4-D Dimethyl amine Salt 58 WSC	0.025	3.5	3.8	4.5	0.00	20.50 (4.64)	65.00 (53.73)
T_6	Glophosate 41SL	0.05	4.1	5.6	8.8	3.928	09.50 (3.24)	55.00 (47.87)
T_7	Paraquat dichloride 24SL	0.015	4.3	4.7	5.3	3.715	35.25 (6.02)	73.75 (59.18)
T_8	Control		4.6	6.8	8.0	3.572	92.25 (9.66)	85.00 (67.21)
			SIG					
S.E. ±			0.61	0.75	0.66	0.17	2.04	0.78
C.D. at 1%			2.55	3.16	2.80	0.72	8.51	3.19

*Figures in the parentheses are square root transformed values

**Figures in the parentheses are arc sin transformed values