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Compatibility of selective insecticides with Metarhizium anisopliae (Metchnikoff) Sorokin (1883)

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Abstract : Potent of entomopathogenic fungi as alternative pest control agents are increasing even though chemical pesticides have been used as the main control agent for pest management. An *in vitro* compatibility study was conducted to evaluate the effect of ten insecticides on the germination and mycelial growth of Metarhizium anisopliae using the poisoned food technique. The insecticide doses were calculated for a field application rate based on 500 li./ha. All ten insecticides tested, significantly inhibited mycelial growth and conidial germination of the fungal pathogen at varying levels. Among the insecticides tested for comptibility, Profenophos, Quinalphos and Chlorpyriphos were harmful where as Thiodicarb and Buprofezin were found to be safe for conidial germination and mycelial growth of M.anisopliae. Indoxacarb, Acetamiprid, Triazophos and Imidacloprid were slightly harmful to M. anisopliae. The result of the present study suggested that except Profenophos, Quinalphos, Chlorpyriphos and Thiomethaoxam, the rest of the insecticides tested can be safely used along with the M.anisopliae.

Keywords : Compatibility, entamopathogens, insecticides

Entomopathogenic fungi have been considered as potential candidates for the effective suppression of a variety of arthropod pests. However, their field application may give inconsistent control because infections of hosts with entomopathogenic fungi are easily affected by factors like temperature, humidity, microorganisms or bio-pesticides and other chemical products used to protect plants. The pesticides may antogonise or synergise the efficacy and potential insecticidal activity of entomopathogenic fungi and may disrupt natural epizootics. Fungal biological control agents and selective insecticides may act synergistically to increase the efficiency of the control, allowing lower doses of insecticides and the preservation of natural enemies, minimizing environmental pollution and decreasing the likelihood of development of resistance to either agent (Ambethgar *et al.*, 2009). By contrast, the use of incompatible insecticides may inhibit the growth and reproduction of the pathogens and adversely affect integrated pest management. Knowledge of compatibility between entomopathogenic fungi and insecticides is crucial for selecting appropriate compounds for scheduling treatments in order to minimize the deleterious effects on biocontrol efficiency and to incorporate entomopathogenic fungi into the Integrated Pest Management Programme of Cotton.

Most fungus insecticide compatibility studies have dealt with the effects of insecticides on mycelial growth and sporulation of fungi. Conidail germination is the first step of the infection process by entomopathogenic fungi (Oliveria *et al.*, 2003). The effects of insecticides on conidial germination is frequently overlooked. (Neves *et al.*, 2001; Hirose *et al.*, 2001 The potential inhibitory effects of pesticides on germination and mycelial growth of entomopathogenic fungi often vary among fungal species and strains (Vanninen and Hokkanen, 1988; Anderson *et al.*, 1989). Therefore, keeping this in view, the present investigation was aimed at assessing the compatibility of *M. anisoplea* (CICR-Ma) with some selected insecticides under in vitro conditionsto predict compatible

combinations to exploit for the management of insect pests in cotton.

MATERIALS AND METHODS

Fungal isolate

The isolate of Metarhizium anisopliaeused in the present study was isolated from mealy bug, Paracoccus marginatus cadavers from ICAR-Central Institute for Cotton Research, Regional station, Coimbatore, and identified at USDA-ARS, USA. The fungus was cultured on SDAY (Sabouraud Dextrose Agar with yeast extract). and incubated at 27 ± 1Ë% C, 80 ± 5 pre cent relative humidity and photophase of 12 hours. The conidia were harvested gently by scraping the surface of a 15 days old culture with an inoculation needle. The conidia were suspended in distilled water containing 0.1 pre cent Tween-80. The mixture was stirred on a magnetic shaker for 10 minutes. The hyphal debris was removed by filtering the mixture through fine mesh sieve, and the conidial concentration was determined by using a haemocytometer. Suspension of the desired concentration (1x106 conidia ml^{-1}) was prepared in distilled water containing 0.1 pre cent Tween-80 and was preserved at 5° C.

Growth inhibition test

Ten chemical insecticides recommended for pest management in cotton were selected for this study. Each insecticide, based on field application rate, was added to the SDAY medium(100 ml) in the flask before solidification to get the desired concentration and was mixed thoroughly. The medium was then evenly distributed among the five Petri plates. Small disc (10 mm dia.) of fungal mycelium from 15 days old culture was cut with a sterile cork borer and placed aseptically in the centre of each plate containing the insecticide amended medium. For each treatment three replications were maintained. Untreated control s without insecticides were kept for comparison under the same conditions. Fungal colony diameter was measured at 7 and 14 days after inoculation. Percent growth inhibition of fungus over untreated control was worked out for the respective insecticides.

Germination inhibition test

Each insecticide, based on field application rate,was added to water (100 ml) in a flask to get the desired concentration and was mixed thoroughly. The effect of insecticides on the germination of the conidia was determined by placing 10 microliter of each insecticide suspension containing fungal spores at a concentration of 1 x 106 conidia/ml on a thin film of SDAY medium in a Petri plate. The conidia in distilled water suspension served as a control. The experiment was conducted under CRD with three replications for each treatment. Inoculated petri plates were incubated at 27 ± 1° C, 80 ± 5 pre cent relative humidity in the dark for 24 hours. After staining with cotton blue lacto-phenol, germination was checked under a microscope. Only conidia with a germ tube as long as the conidia widths were considered to have germinated. Inhibition of conidial germination over untreated control was worked out for each insecticide.

Data analysis

Hassan's classification scheme (Hassan, 1989) was used to analyse the fungus-insecticide compatibility data. The data was expressed as percentage growth inhibition of fungus by insecticide (Hokkanen and Kotiluoto, 1992).

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

where X, Y, Z represents the percentage of growth inhibition, the radial growth of fungus in untreated control and radial growth of fungus in poisoned medium, respectively.

The insecticides were further classified on a 1-4 scoring: .1 = harmless (<50% reduction) 2 = slightly harmful (50 - 79%) 3 = moderately harmful (80 - 90%) 4 = harmful = (>90%).

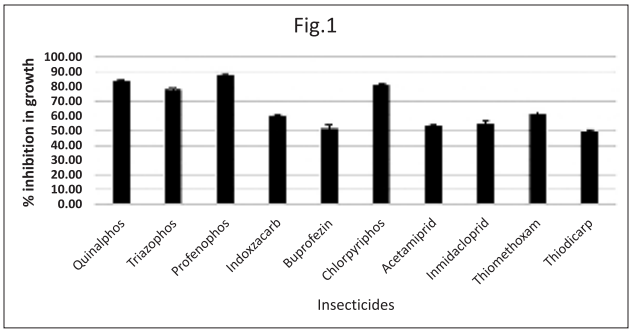

Fig. 1. Effect of various insecticides on mycelial growth of *M. anisopliae* at 7 Days after inoculation. Bars represent standard error of means based on three replications.

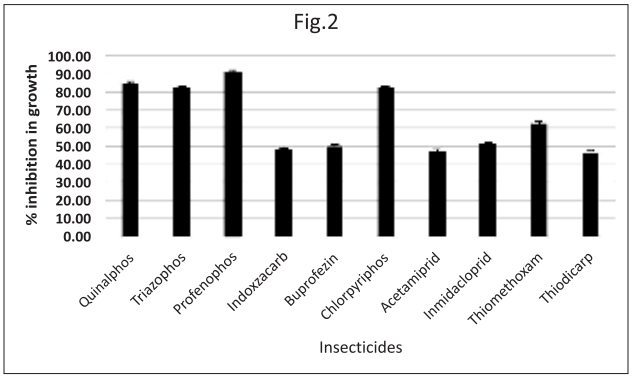

Fig. 2. Effect of various insecticides on mycelial growth of *M. anisopliae* at 14 Days after inoculation. Bars represent standard error of means based on three replications.

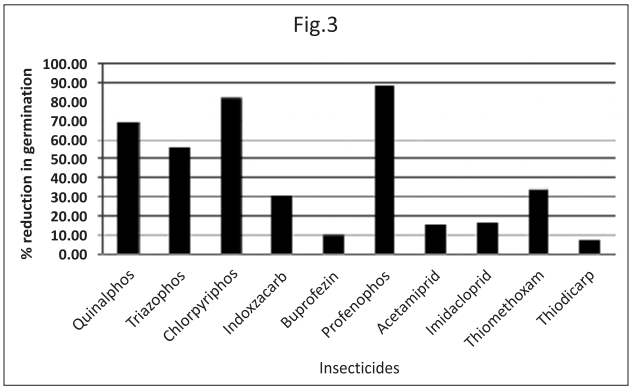

Fig. 3. Effect of various insecticides on germination of *M. anisopliae* .

RESULTS AND DISCUSSION

Effect of insecticides on mycelial growth

All the insecticides tested caused a significant reduction in the growth of *M. anisopliae* which ranged from 49.43 to 87.51 percent at 7 DAI (Fig.1). Profenophos inhibited the growth of *M. anisopliae* the most, with a maximum growth reduction of 87.51 per cent, followed by Quinalphos and Chlorpyriphos. All three of these insecticides were classified as moderately harmful as the per cent inhibition in growth ranged from 80-90. Triazophos, Thiamethoxam, Indoxacarb, Imidacloprid, Acetamiprid and Buprofezin inhibited the growth of *M. anisopliaeby* 50-79 pre cent and are classified as slightly harmful. Thiodicarb was classified as harmless as the percent inhibition in growth of *M. anisopliae* was less than 50 per cent.

The effect of insecticides on the growth of *M. anisopliae* at 14 DAI revealed that all insecticides tested significantly inhibited the growth of *M. anisopliae* at varying levels(Fig.2). Among them, Profenophos recorded a maximum of 91.28 per cent reduction over control, which was followed by Quinalphos, Triazophos and Chlorpyriphos. These three insecticides are classified as moderately harmful to *M. anisopliae* under *in vitro* conditions. Thiamethoxam and Imidacloprid recorded 62.48 and 51.13 percent reduction over control, respectively, and they are slightly harmful to M.anisopliae. Buprofezin, Indoxacarb, Acetamiprid and Thiodicarb recorded less than 50 per cent reduction in growth.

Effect on germination

The toxicity of insecticides to conidial germination is given in Fig.3. The per cent reduction in germination in different insecticide treatments ranges from 7.05 to 88.71, and it varies significantly among the insecticides tested. Profenophos recorded maximum of 88.71 per cent inhibition in germination which was followed by Chlorpyriphos. Imidacloprid, Acetamipride and Buprofezin recorded less than 20 per cent reduction in germination. Thiodicarb was found to be safer to M.anisopliae germination which recorded 7.05 per cent reduction in germination.

All tested insecticides displayed varying degrees of potential to inhibit growth and conidial germination of entomopathogenic fungi corroborating previous findings of Mietkiewski and Gorski (1995) , Gupta *et al.*, (1999), Asi *et*

al., 2010 , Amutha *et al.*, 2012 and Sain *et al.*, 2019. They observed variations in the toxicity responses of entomopathogenic fungi from synergistic to antagonistic to neutral to insecticides. Hassan and Charnley (1989) also reported inconsistent interactions between fungus and insecticides.

A synergistic interaction of imidocloprid with M.anisopliae was reported by Balista Filho *et al.*, 2001. Khan *et al.*, 2012 reported that Acetamipride (0.004%), Thiomethoxam (0.005%), Imidocloprid (0.005%), Quinolphos (0.005%), Profenophos (0.05%) and Dimethoate (0.05%) were found to be compatible and safer for *B. bassiana* and *M. anisopliae* whereas chlorpyriphos showed the highest reduction in vegetative growth. The compatibility of Indixacarb with different isolates of *M. anisopliae* was reported by Akbar *et al.*, 2012.In the present study, Profenophos was found to be harmful to *M. anisopliae* germination and growth. Profenophos was reported to be detrimental to the growth of *M. anisopliae* (Akbar *et al.*, 2012), *B. bassiana* (Amutha *et al.*, 2012) and *Fusarium pallidoroseum* (Monga *et al.*, 2011). Extreme detrimental effects of Chlorpyriphos to various developmental stages of *M. anisopliae* were reported by Mohammad *et al.*, (1987), Li and Holdom (1994) and Rachappa *et al.*, (2007). At their registered concentrations, formulated Trichlorfon, Acephate and Indoxacarb were compatible with *M. anisopliae* (Khun *et al.*, 2021).

Although the different insecticides tested in the present investigations inhibited the growth of *M. anisopliae* under in vitrocondition, the combined use of the fungus and insecticides cannot be completely ruled out. Inglis *et al.*, 2001 observed that the inhibitory potential of insecticides varies both between and within chemical classes. Fungitoxic effects of insecticides vary as a function of the chemical nature of the products and the interacting microbial species (Antonio *et al.*, 2001; Kumar *et al.*, 2008). A given insecticide may have different fungitoxic effects on various developmental stages of the fungus (Li and Holdom, 1994). The potential inhibitory effects of pesticides on germination and mycelial growth of entomopathogenic fungi vary among taxa and strains (Vanninen and Hokkanen, 1988; Anderson *et al.*, 1989).

The laboratory results on artificial media may not be reproducible under the field as there will be degradation of toxicants. Moreover, the fungus is directly exposed to the insecticides under in vitro conditions which are altered by several factors under field conditions. Some fungi may recover after some chemical pesticides are decomposed on plant leaves. But this basic study will be helpful when this fungus is mixed with a sub lethal dose of insecticides for the management of insects under field conditions.

Studies on the effect of insecticides on conidial germination should be the key factor to be considered as the attachment of conidia and its germination is the first step in the infection of the host by the entomopathogenic fungus. The present investigations showed complex and varying effects of insecticides on conidial germination and growth of *M .anisopliae* under in vitro conditions. In the presence of pesticides, the germination of conidia is more severely affected than the growth of entomopathogenic fungi (Hall, 1981; Er and Gokce, 2004: Asi *et al.*, 2010). The differences observed between germination and growth inhibition are probably due to some reduction over time of the insecticide's effect in the medium, since germination was assessed one day post treatment while radial growth was measured later (Asi *et al.*, 2010). Griffen (1994) also observed that the effects of many insecticides on fungus growth decline gradually over time.The potential inhibitory effects of pesticides on germination and mycelia growth of biocontrol fungi vary among taxa and strains (Anderson *et al.*, 1989).

A combination of sub lethal concentrations of chemical insecticides and entomopathogenic fungi can causeincreased stress, immune compromise, and a consequentalteration in insect physiology and behaviour, especially in survival

and reproduction; thus leading to more favourable results in an insect control programme (Boucias *et al.*, 1996). A pairing of sublethal concentrations of insecticides with fungal entomopathogenscan increase pest mortality as well as reduce the killingtime compared to the use of either agent alone (Foster *et al.*, 1996). Sub lethal effect of these insecticides are to be tested in future, as well as their actual effects on the fungi at cellular level. Long term effect of insecticides on entomopathogenic fungi are to be studied to understand whether the effects are permanent or temporary and also improving the knowledge of entomopathogenic fungi insecticide interaction and mechanism involved. The results obtained in this study clearly indicated that Thiodicarb was found to be compatible with *M. anisopliae* and can be used in Cotton IPM.

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