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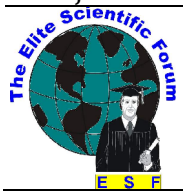

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Effects of Commercial Pesticides against Cotton Whitefly (*Bemisia tabaci* Genn.) and Mites (*Tetranychus urticae* Koch) on Growth and Conidial Germination of two species of Entomopathogenic Fungi

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ABSTRACT
The influence of nine commercial pesticides on conidial germination and mycelial growth of *Isaria fumosorosea* (Wize) Brown and Smith, and *Lecanicillium muscarium* (Zimmerman) Viegas were evaluated *in vitro*. The conidial germination and mycelial growth varied significantly by all tested pesticides depending upon the dose of pesticide and type of fungus. All pesticides inhibited conidial germination as well as mycelial growth significantly. Azocyclotin was proved to be highly toxic to germination of spores as well as mycelial growth followed by pyridaben, acetamiprid and propargite while buprofezin was the least toxic. *I. fumosorosea* proved to be more sensitive (0 - 75 % germination) at field recommended dose than *L. muscarium* (11 - 95 % germination) to all pesticides. The insecticides, among above mentioned pesticides (buprofezin, imidacloprid and diafenthuron), were more compatible to fungi than acaricides.

INTRODUCTION

Whitefly (*Bemisia tabaci* Genn.) is commonly encountered polyphagous insect pest of many field and horticultural crops throughout subtropical and tropical regions of the world including Pakistan (Amjad et al., 2009). Especially on cotton, this insect along with two spotted spider mites (TSSM) (*Tetranychus urticae* Koch.) has attained a status of serious cotton pest. The populations of both are mainly suppressed by the application of chemical pesticides and also alternatively by fungal biological control agents (BCAs) (Vidal et al., 1997; Wraight et al., 2000; Aslam et al., 2004).

It is evident that these fungal BCAs may be affected by the biotic and abiotic factors of the ambience. In our agro ecological conditions where farmers mainly rely on chemical pesticides for pest control, the effect of these chemicals on fungal BCAs may not be ignored. The negative effects by the chemical pesticides on the entomopathogenic fungi have been reported previously by many other researchers (Wilding and Brobyn, 1980; Poprawski and Majchrowicz, 1995; Yeo et al., 2003). Like all microorganisms, entomopathogenic fungi have specific biological characteristics that influence their activity in the environment (Parker et al., 2003). The strategies have been developed to combine the use of fungal BCAs and low doses of commercial pesticides for getting effective control of the target pests. The combined application of myco-insecticides and selective synthetic chemical pesticides is an attractive approache. The fungus and chemical insecticide may act synergistically allowing the reduction in the amount of pesticides applied, thus minimizing environmental contamination hazards and decrease the likelihood of resistance to either agent (Moino and Alves, 1998; Quintela and McCoy, 1998).

Maniania et al. (2003) studied the combined application of *M. anisopliae*and methomyl (Lannate®) on adults and larvae of western flower thrips, *Frankliniella occidentalis* and observed the significant reduction of both stages. Some researchers conducted bioassays on different crops and found some isolates of

entomopathogenic fungi to be highly effective against mites like *Isaria fumosorosea* and *Beauveria bassiana* etc. (Nugroho and Ibrahim, 2004; Draganova and Simova, 2010).

The successful application of these biological control agents (BCAs) depends on the extent of their compatibility with other crop protection tactics (Copping, 2004).

The combined application technique is especially more important when utilized in rotation with other control measures or within an IPM program. Therefore, in the present study, several commercial insecticides/ acaricides available in the market for the control of whitefly and mite pests were selected for evaluating their effects on the conidial germination and mycelial growth of *I. fumosorosea* and *L. muscarium in vitro* for efficient utilization of these fungi in an integrated pest manage- ment program.

MATERIALS AND METHODS

Sources of Fungus and Pesticides

All the pesticides tested (Table 1) were commercial products, used within the first year of manufacturing date at the time of test and held at 20±2°C. The concentrations employed were based on their field recommendations rate (RR), 0.5 × RR and 0.75 × RR. The two strains of entomopathogenic fungi which proved best against whitefly and TSSM used in this study were, *L .muscarium* (V17) and *I. fumosorosea* (n32). These strains were obtained from college of Natural Resources and Environment, South China Agricultural University, Guangzhou, China. The fungal strains were maintained on Sabouraud dextrose agar (SDA) in the Acarology laboratory, Department of Agri. Entomology, University of Agriculture, Faisalabad, Pakistan at 25°C for two weeks and then stored at lower temperature (4°C) until required.

Stock Solution Preparation and Effect of Pesticides on Conidial Germination

The SDA media was autoclaved for 20 minutes at 121ºC and poured on to sterilized Petri plates separately (Alves et al., 1998). After solidification these were inoculated with fungal spores and then put into incubator at 25 ± 2ºC, 80 ± 5% R.H and 16:8-h Light:Dark (L:D) photophase. Spore production was investigated after 2 weeks of culturing. Conidia from the plates in each treatment were harvested from 3 week old cultures gently by scraping with a sterile needle into 100 ml 0.01 % Tween 80 and vortexed for 10 minutes to get homogeneous mixture and filtered through a fine mesh sieve to remove conidial clumps and mycelial debris. The concentrations of conidia in the suspensions were quantified directly under the optical microscope, with a Neubauer® haemocytometer and subsequently adjusted to the required concentration by adding appropriate amount of distilled water.

Table 1: Pesticides used in bioassay for assessment of their effects on entomopathogenic fungi

S. No.	Pesticides tested		Recommended dose
	Brand name	Common name	g or ml/ acre
1	Confidor® 20SL	Imidacloprid	250
2	Mospilon® 20 SP	Acetamiprid	125
3	Byzin® 25 WP	Buprofezin	600
4	Polo® 50 SC	Diafenthiuron	200
5	Current® 15 EC	Pyridaben	500
6	Agrifol® 18.5 EC	Dicofol	1000
7	Unique-M® 5 SC	Fenpyroximate	200
8	Gallop® 25 WP	Azocyclotin	100
9	Somite® 57 EC	Propergite	100

The assays on conidial germination were carried out using 3 different concentrations of each pesticide viz. RR (Recommended Rate/acre as mentioned in Table 1), 0.5 × RR and 0.75 × RR with fungal stock solutions being diluted with water sufficient to make a final volume of 10 mL per concentration. Conidial suspensions were prepared in the pesticides solutions and 100 µL of each sample was used to inoculate each replicate dish. The lidsof the Petri plates were sealed with parafilm and placed in incubator at 25°C for 16 hrs in dark. The conidial suspensions without pesticides were used as control. Each treatment concentration of each pesticide for each fungal isolate was replicated three times. After 16 hours the Petri plates were examined for scoring germination of spores in each plate. The germination was assessed by placing cover slips on to the media inside the plates and were examined under the microscope at 40 × magnification, about 300 spores were examined in each plate and scored. Conidia having germ tubes greater than its width were considered to be germinated. The resultant data expressed as percent germination were subject to ANOVA followed by comparison of means using Tukey' HSD test ($\alpha = 0.05$). The analysis was carried out by using SPSS® version 16.0 computer software.

Effect of Pesticides on Mycelial Growth

The influence of pesticides on mycelial growth was evaluated at 3 different application rates for each pesticide as mentioned previously. Each pesticide concentration was mixed into SDA medium post autoclaving, while the medium was still liquid and hot (45-50°C) and ~ 20 mL of the mixture was poured into each 90 mm Petri dish and allowed to cool and solidify for 24 hrs. A 5 mm disc of mycelium plugs of each fungus isolate, cut aseptically from the growth of 1 week old colony with a sharp cork borer, were placed in the centre of each of three replicate plates per pesticide per concentration. The plates without pesticide were taken as control. The plates were incubated at 25±2°C. The diameter of the developing fungal colony was measured after 10 days of incubation at 10×

magnification using callipers. Radial growth in millimetres was used as the main parameter to assess the effects of pesticides on growth and effects of concentration of pesticide on radial growth.The ANOVA was carried out on growth measurements followed by comparison of means of total growth over 10 days using Tukey's HSD test ($\alpha = 0.05$). The analysis was carried out by using SPSS® version 16.0 computer software.

RESULTS

Effects on conidial germination

All the variables tested i.e. pesticides, test concentration and fungal species had a significant influence on the germination of conidia (Table 2). Both fungal species showed different sensitivity to the pesticides tested especially *I. fumosorosea* at the different concentrations used. Except the effects of imidacloprid and buprofezin insecticides on *L. muscarium*, which were non-significant with controls at each dose level, all other pesticides at their recommended doses showed negative effects on conidial germination of both the fungi under study. *I. fumosorosea* showed more sensitivity to all pesticides with 0.0 % germination also to one acaricide (100 % germination inhibition).

All pesticides affected conidial germination of both fungal species to varying degrees. Germination rates of conidia varied significantly among the pesticides at 50% dilution of recommended rate (0.5 × RR) for both *L.muscarium* and *I. fumosorosea* ($F = 492.80$, $df = 9,20$, $P<0.001$ and$F = 1510.88$, $df = 9,20$, $P<0.001$, respectively) (Table 2). At 75% of the recommended dose there were also significant differences among pesticides for causing germination inhibition. At the recommended level of pesticides (RR) again there were significant differences among pesticides for retarding germination of both *L. muscarium*and *I. fumosorosea* ($F = 1503.63$, $df = 9,20$, $P<0.001$ and $F = 1542.86$, $df = 9,20$, $P<0.001$, respectively). Except acetamiprid there were no significant differences among insecticides tested viz., imidacloprid, buprofezin and diafenthuron for retarding germination of both entomopathogenic fungi at RR dose level. For all other pesticides a single level of increase in pesticide concentration caused a significant reduction in the germination of both fungal species. Among all pesticides, azocyclotin caused 0.00 % germination of conidia of *I. fumosorosea* while 11.33-31.33 % in case of *L. muscarium* at all the tested doses. Among the tested insecticides, acetamiprid also exerted effects and caused germination of *I. fumosorosea* and *L. muscarium* varying from 10.33-32.66% and 81.0-82.0 %, respectively at tested concentrations. Generally, on overall basis, all acaricides caused germination of conidia from 11.33-70.66 % (~30-90% inhibition) in case of *L. muscarium* and 0.00-61.66 % (~40-100% inhibition) in case of *I. fumosorosea*.

Effects on mycelial growth

The radial growth studies showed that all pesticides caused significant reduction in mycelial growth to varying levels depending upon pesticides used, their concentrations and fungus as well. The growth of both fungi showed significant differences in pesticides at 0.5 × RR dose viz., *L. muscarium* ($F = 27.51$, $df = 9,20$, $P<0.001$) and *I. fumosorosea* ($F = 127.06$, $df = 9, 20$, $P<0.001$) and at 0.75 × RR ($F = 38.25$, $df = 9, 20$, $P<0.001$ and $F = 174.58$, $df = 9,20$, $P<0.001$, respectively) (Table 3). Similarly at RR level there is a mixed pattern of mycelial growth between insecticides and acaricides. At every level of increase among the concentration of pesticides used it caused a significant reduction in the growth of both fungi for most of the pesticides especially for acaricides. Except acetamiprid which was safer to *I. fumosorosea*, all other insecticides showed the same trend for both the fungi. Among all pesticides tested buprofezin was the safest showing mycelial growth 21.80-23.70mm over ten days in case of *L. muscarium* which is statistically at par with control while 18.40-19.23 mm in case of *I. fumosorosea* at different tested levels which is also very close to the control (21.56-25.20 mm) while azocyclotin was highly growth retarding showing mycelial growth between 11.23-16.30 mm and 4.60-11.81 mm for *L. muscarium* and *I. fumosorosea*, respectively.

DISCUSSION

Both entomopathogenic fungi showed almost similar pattern in terms of germination of conidia and mycelial growth but *I. fumosorosea* proved to be more sensitive to some pesticides than *L. muscarium*. Surprisingly the conidia of *L. muscarium* were not much affected when combined with insecticides and somewhat tolerated the poison effects. All the acaricides put their individual effects and caused less germination of conidia from 50% dilution to recommended rates of application. Some pesticides like azocyclotin proved to be highly detrimental to fungi causing (11-31 % conidia germination) (~30-90% inhibition) in case of *L. muscarium* while no germination at all even at 50 % dilution of *I. fumosorosea*. These results are in line with Asi et al. (2010) who reported that some new chemistry insecticides can be compatible with entomopathogenic fungi for pest control. Rachappa et al. (2007) also found similar results with chlorpyrifos and showed that it could cause extremely detrimental effects to various developmental stages of *Metarhizium*. Similarly, Li and Holdom (1994) demonstrated toxic effects of chlorpyrifos on growth and spores of *Metarhizium anisopliae* while in another study Spinosad was found to be potential and comparatively safer

Table 2: Average conidial germination (Mean % ± SEM) of *L. muscarium* and *I. fumosorosea* fungal isolates used with different concentrations of pesticides after 16 hrs at 25° C

	L. muscarium			*I. fumosorosea*		
	Pesticide concentration			Pesticide concentration		
Pesticides	0.5 × RR	0.75 × RR	RR*	0.5 × RR	0.75 × RR	RR
Imidacloprid 20 SL	95.66±0.88fg	95.66±0.33g	95.33±1.20ef	96.00±0.56h	85.33±0.88h	75.66±0.66f
Acetamiprid 20 SP	81.00±1.00e	81.0±0.57e	82.00±0.57d	32.66±0.66c	22.33±1.20c	10.33±0.88b
Buprofezin 25 WP	95.66±0.88fg	95.33±0.66fg	95.66±0.88ef	83.66±0.88f	75.00±0.57g	74.66±01.45f
Diafenthuron 50 SC	92.66±0.66f	91.66±0.88f	92.66±0.66e	91.66±0.88g	85.33±1.76h	73.33±1.76f
Pyridaben 15 EC	54.00±0.57b	41.0±0.57c	27.33±0.88b	27.66±1.20b	10.33±0.88b	11.00±0.57b
Dicofol 18.5 EC	61.66±0.33c	55.33±0.88d	47.66±0.88c	60.33±0.88e	48.66±0.88f	48.66±0.66e
Fenpyroximate 5 SC	59.66±0.33c	36.33±0.66b	12.00±1.15a	61.66±1.20e	42.33±1.20e	39.66±0.33d
Azocyclotin 25 WP	31.33±2.40a	21.66±0.88a	11.33±1.20a	0.00±0.00a	0.00±0.00a	0.00±0.00a
Propergite 57 EC	70.66±0.66d	56.33±1.20d	50.66±0.88c	42.00±1.15d	28.66±0.88d	28.33±0.33c
Fungus only (Control)	98.66±0.66g	99.66±0.33h	99.33±0.66f	99.33±0.33h	99.66±0.33i	100.00±0.00g

*Recommended rate. All the means within a column followed by the same letter are not significantly different by Tukey's HSD Test.

Table 3: Average radial growth (Mean growth in mm ± SEM) of *L. muscarium* and *I. fumosorosea* plugs when used in pesticide poisoned media at different concentrations after 10 days at 25°C.

	L. muscarium			*I. fumosorosea*		
	Pesticide concentration			Pesticide concentration		
Pesticides	0.5 × RR	0.75 × RR	RR*	0.5 × RR	0.75 × RR	RR
Imidacloprid 20 SL	22.64±0.85de	20.16±0.53bcd	17.37±0.72d	16.23±0.61de	15.26±0.34cd	12.66±0.34c
Acetamiprid 20 SP	14.23±0.66a	11.70±0,20a	11.53±0.96a	17.40±0.25ef	16.83±0.17de	15.50±0.37d
Buprofezin 25 WP	23.70±0.47de	23.10±0.68de	21.80±1.20e	19.23±0.46fg	18.50±0.32ef	18.40±0.20e
Diafenthuron 50 SC	20.66±0.71cd	21.06±0.90cd	17.90±0.40d	14.30±0.32cd	13.73±0.32c	13.33±0.40c
Pyridaben 15 EC	16.73±0.35ab	17.00±0.20b	15.10±.55bcd	11.70±0.32b	9.30±0.50ab	5.73±0.32ab
Dicofol 18.5 EC	20.83±0.82cd	19.06±1.23bc	13.43±0.39abc	8.76±0.47a	7.53±0.46a	5.26±0.20ab
Fenpyroximate 5 SC	22.65±0.80de	20.19±0.46bcd	16.82±0.49cd	20.66±0.70g	18.80±0.43f	11.60±0.51c
Azocyclotin 25 WP	16.30±0.62ab	13.10±0.52a	11.23±0.67a	11.81±0.33b	9.96±0.34b	4.60±0.35a
Propergite 57 EC	18.16±0.53bc	17.81±0.44bc	12.80±0.64ab	13.53±0.38bc	10.76±0.34b	6.86±0.51b
Fungus only (Control)	24.80±0.71e	24.80±0.71e	25.10±0.15e	25.20±0.21h	22.10±0.25g	21.56±0.43f

*Recommended rate. All the means within a column followed by the same letter are not significantly different by Tukey's HSD Test.

compound for fungi (Rachappa et al., 2007). There is inhibitory potential which varies both within and between chemical classes (Inglis et al., 2001). This shows that there may be inherent variability of chemical pesticide to entomopathogenic fungi.

The germination of conidia is the most important factor which should be considered while we are studying compatibility tests of any chemicals with entomopathogenic fungi (Neves et al., 2001; Hirose et al., 2001). According to our results the germination of conidia was more sensitive than mycelial growth of both the fungi. Oliveira et al. (2003) observed that out of many chemicals studied only 5 insecticides at field recommended doses promoted conidia viability higher than 60 % and showed that they could be employed in IPM program. Irigaray et al. (2003) conducted an experiment for the combined use of triflumuron and fungus and observed that mortality in mite eggs was increased significantly. Similarly, Cuthbertson et al. (2005) studied the combined effect of some insecticides and *V. lecanii* and found that all insecticides showed more or less effects on germination of conidia and mycelia growth except buprofezin which was acceptable and can be utilized in any IPM program.

The germination of conidia is more affected than mycelia growth with pesticides and lower concentration of pesticides may be employed for desirable results (Er and Gokce, 2004). Certain pesticides have potential to inhibit germination of entomopathogenic fungi in vitro but appear to have little or no effect on their virulence against target insects (Shah et al., 2009).

In our studies, it is evident that all the insecticides proved to be more compatible with both the entomopathogenic fungi and these could successfully be employed in the IPM program for the control of different pests. As such new chemicals were used in this study so previous record could not be encountered to compare with the results in a precise manner. The results of some chemicals like imidacloprid and buprofezin are similar to those of Cuthbertson et al. (2005). The results presented here are only laboratory experiments so there need to do some field or glasshouse experiments for further authentication of compatibility.

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