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Effects of Common Pesticides Used in Rice Fields on the Conidial Germination of Several Isolates of Entomopathogenic Fungus, *Beauveria bassiana* (Balsamo) Vuillemin

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ABSTRACT

The integration of biological and chemical control approaches is very important for a successful Integrated Pest Management (IPM) program. The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (sensu lato) is one of the facultative insect pathogens with significant host range and host specificity. In this study, the effects of eight common pesticides used in rice fields were studied on the germination of *B. bassiana*. The experiments were carried out in the laboratory at 25±1°C, 80±5% RH and a 16h-photoperiod.The isolates Mcb 6, EUT 116, IRAN 429C and DEBI 005 of *B. bassiana* were treated with the field recommended rate of the pesticides. Experiments were performed according to the completely randomized design (CRD). The results showed that fungicides had the highest adverse effect on the germination of the isolates of *B. bassiana*. So it can be concluded that fungicides are limitation factors for activity of the fungus in the rice fields. The highest germination of fungal isolates was in the herbicide treatments, thus, they can be used in rice IPM programs.

Key words: *Beauveria bassiana*, pesticides, germination and integrated pest mangement.

INTRODUCTION

Rice is the most important staple food crop with more than half of the world's population relying on rice as the major daily source of calories and protein (Khanjani, 2006). Rice is also a major agricultural product in Iran where the area cultivated with rice is more than 600 thousand hectares (Anonymous, 2011).

The rice striped stem borer, *Chilo supprressalis* Walker is a cosmopolitan and destructive pest of rice in most places of the world (Khanjani, 2006). This pest was introduced to Iran in 1973 and has been widely distributed in all rice fields of north part of Iran and has caused economic damage for four past decades (Khanjani, 2006). Chemical compounds are the most effective tools for controlling the pests and are always required to suppress rapidly expanding insect pest populations (Ramazan-Asl *et al.,* 2010). Traditionally, chemical pesticides have been used for the protection of agricultural products from arthropod pests, but the indiscriminate use of these compounds can cause serious problems such as pest outbreaks (Metcalf, 1986).

On the other hand, the integrated pest management (IPM) programs can reduce the use of hazardous pesticides. Simultaneous use of biological control agents and chemical compounds may be needed for controlling the pest. Among the biological agents which can be use associated with these chemicals in pest control are entomopathogenic fungi (Hull and Beers, 1985). Entomopathogenic fungi have a considerable potential for efficacious suppression of a variety of arthropod pests. *B. bassiana* is one of the most important entomopathogenic fungi (Liu *et al.,* 2002; Al-maza *et al.,* 2006). This fungus is widely distributed in the world and has the potential to control over 70 insects' pest species (Hung and Boucias, 1992; Alizadeh *et al.,* 2007).

It was first reported as a pathogen of the silkworm, *Bombyx mori* L., by Agostino Bassi in 1834 (Feng *et al.,* 1994). In Iran *B. bassiana* was reported for the first time from the larva and pupae of *Chilo suppressalis* Walker in Guilan and Mazandaran provinces by Rezvani and Shah-Hosseini (1976). The most important factor in the insect contamination by pathogenic fungi is the high relative humidity. Majidi-Shilsar (2005) reported that this fungus showed more activity in the fall and winter months compared to July and August in Guilan province. Therefore, any changes in environmental conditions have to be taken into consideration to determine which one is effective on conidia germination and how its detrimental effect on conidia survival can be reduced (Hedimbi *et al.,* 2011). Because of the mentioned reasons, the effects of pesticides on pathogenic fungi should be considered and potential inhibitory effects of pesticides on entomopathogenic fungi cannot be ignored.

One of the most important subjects in the debate on the effects of pesticides on insect pathogenic fungi is the assessment of the effects of pesticides on germination rate of the fungus. Furthermore, effects of pesticides on conidial germination is the most important aspect in evaluation of insecticide compatibility (Neves *et al.,* 2001) since it is the first step of the infection process (Oliveira *et al.,* 2003). Majidi-Shilsar *et al.* (2005) reported that fungicides had the most adverse effect on the germination of *B. bassiana*.

The purpose of this study was to assess the effects of pesticides used in rice fields on the germination of different isolates of *B. bassiana*. Finally, after conducting the advanced field studies to determine the pesticides with least adverse effect on the germination of the fungal pathogen, the results would be used in integrated pest management in rice fields.

MATERIAL AND METHODS

Fungal Isolates

Four isolates of entomopathogenic fungus *B. bassiana* (followed by the host and obtaining area) were used in this study: EUT 116 (larvae of wax moths, Tehran); Mcb 6 (*Chilo suppressalis*, Fuman); IRAN 429C (*Chilo suppressalis*, Hassan rood); DEBI 005 (Tenebrionidae, Alfalfa fields).

Pesticides and Recommended Doses

The following pesticides (commercial products, followed by manufacturer recommended doses) were used in the experiments: A) Fungicides: tricyclazole (Beam® 75% WP, Shandong Kesai Eagrow Co., China - 0.5 Kg/ha), edifenphos (Hinosan® 50% EC, Bayer CropScience Co., Germany - 1 L/ha); B) Insecticides: malathion (MalathionGhazal® 57% EC, GhazalShimi Co., Iran - 2000 ppm) and diazinon (DiazinonAria® 50% EC, AriaShimi Co., Iran - 1500 ppm); C) Herbicides: butachlor (Machete® 60% EC, Monsanto Co., India - 4 L/ha), oxadiargyl (Raft® 3% EC, Bayer CropScience Co., Germany - 3.5 L/ha), cinosulfuron (Setoff® 20% WP, Syngenta Co., USA - 150 g/ha), pretilachlor (Krift® 50% EC, DuPont Co., India - 2 L/ha).

Culture of Fungus

B. bassiana was cultured on Saboraud's dextrose agar yeast extract (SDAY) in Petri dishes at 25±1°C, 80±5% RH and a 16h-photoperiod. After preparing the medium, a piece of the culture medium containing mycelium and conidia of fungus was removed by a sterile scalpel and transferred to Petri dishes containing the medium. After 15 days the medium was full of fungus growth and Petri dishes containing conidia of entomopathogenic fungus were used for preparing the suspensions for bioassay experiments.

Production of Suspension

For producing fungal suspension, conidia were transferred in tubes with lid containing sterile distilled water. For screening mycelium and medium, this suspension was passed through a mesh fabric. A haemocytometer (Paul Marienfeld GmbH and Co. KG, Germany) was used to determine the concentration of conidia in the initial suspension. After counting conidia, the main concentration was determined using the formula $Y= 5X\times10^4$. (X= number of conidia in five squares) (Erwin, 2002). Subsequent concentrations were determined using the logarithmic scale.

Effects of Pesticides on Conidial Germination

After preparing the desired concentration of each pesticide, appropriate amount of the solutions were added to the media. The concentration of each pesticide used in the media was the same as its field recommended rate. The concentrations were 1000, 2000, 2000, 1500, 8000, 7000, 300, and 4000 ppm for tricyclazole, edifenphos, malathion, diazinon, butachlor, oxadiargyl, cinosulfuron, and pretilachlor, respectively. The treated media were directly inoculated with 500 μl of the conidial suspension of *B. bassiana* containing 1×10^7 conidia/ml. The medium of the control plates was treated with a solution of Tween 80. This emulsifier (Merck, Darmstadt, Germany) was used in all dilutions as a spreader. After the treatment, Petri dishes containing conidia were sealed by Para film to prevent contamination by any other pathogens. Petri dishes containing media were transferred to an incubator (25±1°C, 80±5% RH and a photoperiod of 16-h for 18 hours. Then, 1ml of formaldehyde 0.5% was poured into Petri dishes to stop spore germination. Finally, spore germination was checked by using a microscope and data were recorded. Each experiment was replicated three times. The data were analyzed using SAS software (SAS, 2002).

RESULTS

The effects of field recommended concentrations of the insecticides on conidial germination of fungus isolates are summarized in Table 1. The results revealed that the chemicals affected the conidial germination of Mcb 6 ($F = 481.4$; $df = 8, 26$; $P < 0.0001$), EUT 116 ($F = 702.3$; $df = 8, 26$; $P < 0.0001$), IRAN 429C ($F = 1355.2$; $df = 8, 26$; $P < 0.0001$) and DEBI 005 ($F = 1089.5$; $df = 8, 26$; $P < 0.0001$) isolates significantly compared to control (Table 1). The results showed that the highest and lowest percentage of conidial germination occurred in the presence of oxadiargyl and tricyclazole, respectively (Table 1).

Table 1. Percentage of germination (±SE) of fungal isolates of *Beauveria bassiana* treated with the pesticides.

Pesticides	Fungus isolates			
	Mcb 6	EUT 116	IRAN 429C	DEBI 005
Tricyclazole	7.67 ± 0.67 e	5.67 ± 0.67 e	4.67 ± 0.33 f	5.67 ± 0.88 g
Edifenphos	3.33 ± 0.88 e	10 ± 0.58 de	9.67 ± 0.88 f	15.67 ± 1.45 f
Diazinon	17.33 ± 0.88 d	14 ± 2.89 cd	34 ± 2.08 e	35.67 ± 0.88 e
Malathion	21.33 ±2.85 d	18.33 ± 1.45 c	39.67 ± 1.76 d	43 ± 1.15 d
Butachlor	83 ± 3.06 c	90.33 ± 1.2 b	90 ± 0.58 c	88.67 ± 0.88 c
Cinosulfuron	87 ± 2.65 bc	91.33 ± 2.6 ab	93 ± 1.15 bc	93.33 ± 2.4 bc
Pretilachlor	89 ± 1.53 bc	92.67 ± 1.86 ab	94.33 ± 0.33 abc	94.67 ± 0.33 ab
Oxadiargyl	92.67 ± 1.86 ab	95.33 ± 0.88 ab	97.33 ± 0.33 ab	97 ± 0.58 ab
Control	99.67 ± 0.33 a	99 ± 0.58 a	99 ± 0.58 a	99.33 ± 0.33 a

Means in column followed by different small letters are significantly different.

DISCUSSION AND CONCLUSION

Entomopathogenic fungi were among the first organisms used for the biological control of pests (Ramzan-Asl *et al.,* 2010). Fungal species such as *Metarhizium anisopliae* and *B. bassiana* are well characterized in respect to pathogenicity to several insects and they have been used as agents for the biological control of different agricultural pests worldwide (Hung and Boucias, 1992). Germination of conidium is an important step in pest management, because the beginning of epizootic is conditioned to the capacity of these structures to germinate on the host (Oliveira *et al.,* 2003). On the other hand, the integrated pest management programs, which mean simultaneous use of biological control agents and chemical compounds are recommended (Hull and Beers, 1985). Therefore, the effects of pesticides on the entomopathogenic fungi should be studied. In this study, effects of common pesticides used in rice fields were studied on the germination of fungus *B. bassiana*. The results showed that tricyclazole and edifenphos had the greatest adverse effect on the germination of *B. bassiana*, thus they induced the lowest percentage of germination. The results showed that following the mentioned fungicides, the highest percentage of inhibition is dedicated to insecticides diazinon and malathion.

The highest percentage of germination was observed in butachlor, cinosulfuron, pretilachlor and oxadiargyl herbicide treatments (Table 1). The results of this study is consistent with results of Aguda *et al.*, (1988) that reported the fungicides, such as benomyl and edifenphos had a negative effect on germination and mycelial growth of entomopathogenic fungi *B. bassiana, M. anisopliae and Hirsutella citriformis*. Furthermore, Majidi-Shilsar *et al.,* (2005) reported that fungicides had the most adverse effect on the germination of *B. bassiana* which is similar with the results of this study. Various pesticide treatments displayed different potentials for growth inhibition and conidial germination of *B. bassiana* which is consistent with the previous findings of Mietkiewski and Gorski (1995) and Hedimbi *et al.,* (2008).

The potential inhibitory effects of pesticides on germination and mycelium growth of entomopathogenic fungi vary among taxa and strains (Vanninen and Hokkanen, 1988; Anderson *et al*., 1989; Hedimbi *et al.,* 2008). The observed variations in the inhibitory potential could be due to inherent diversity of chemical insecticides to entomopathogenic fungi (Gupta *et al.,* 1999). The results of this study showed that the highest and lowest adverse effects on the germination of entomopathogenic fungus *B. bassiana* were caused by fungicides and herbicides, respectively. Finally, herbicides may be used as compatible chemical materials with the entomopathogenic fungus in rice IPM programs.

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