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Insecticidal Potential of *Beauveria bassiana* Strain PDRL1187 and Imidacloprid to Mustard Aphid (*Lipaphis erysimi*) Under Field Conditions

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Abstract.- We studied the insecticidal potential of the Hypocreales entomopathogenic fungal (EPF) strain of *Beauveria bassiana* (Bals.-Criv) Vuill., and insecticide Imidacloprid to mustard aphid (MA) at field conditions. The *Beauveria bassiana* strain PDRL1187 caused 50% mortality (LT_{50} value = median lethal time) of adult MA population after 6.4 days, when sprayed on insect population $6.3x10^{12}$ spores per acre. The insecticide, 10 g per acre, caused the insect mortality with LT_{50} value 3.2 days. Along with the pest mortality, the seed yield of mustard plant (canola), increased with application of EPF and insecticide, at field trials. The study confirmed that strain is effective against mustard aphid under field conditions.

Key words: Entomopathogenic fungi, Canola (*Brassica napus*) and mycoinsecticides.

INTRODUCTION

Mustard aphid, *Lipaphis erysimi* Kalt., (Homoptera: Aphididae), is the important insect pest of mustard plants. It feeds through phloem and transmits plant pathogens. Mustard oil crops are its favorite host. Infested plants limit their flowering, growth and even die (Singhvi *et al.*, 1973). Insecticides are rapid remedy to economic losses in developing countries like Pakistan (Razaq *et al.*, 2011). World without pesticides would loss 78% of fruits, 54% of vegetables and 32% of cereal crops (Cai *et al.*, 2009). Pesticides are notorious for hazards to the environment. Therefore, the health concerns demand food free from synthetic insecticide. Development of pest resistance to pesticides has forced the farmers to use extensive doses of insecticide, which is worldwide concern in term of pesticide residue (Pimental *et al.*, 1992). Organic farming copes with alternatives to insecticides by using pest and disease resistant varieties, bio-pesticides, cultural practices, and judicial use of insecticides or altogether (Aktar *et al.*, 2010); since the field of biological control serves insect pest issues of field crops. Entomopathogenic fungi are contemporary to the groups of bioinsecticides, predators, and parasitoids. The genera *Beauveria* has pivotal place in mycoinsecticides. *Beauveria* (Bals.) Vuill. (Ascomycota: Hypocreales) is cosmopolitan genus. It is a significant broad spectrum entomopathogenic fungus (Bassi 1836; Vago, 1963; Steinhaus, 1949; Ferron, 1978; McCoy *et al.*, 1990; Feng *et al.*, 1991; Brand *et al.*, 2012); its pathogenicity was also reported against various aphid species (Akmal *et al.*, 2013). It is one of the most important mycoinsecticidal agent registered in the world. The *B. bassiana* is broad-spectrum insect pathogen (Faria and Wraight, 2007).

The *B. bassiana* strain PDRL1147 reported for insecticidal efficiency to mustard aphid (*L. erysimi*) under laboratory and screen house conditions (Ujjan and Shahzad, 2012). The team followed the same lines and studied the mycoinsecticidal potential of the strain under field conditions. A compatible insecticide Imidacloprid (Ujjan, 2013) in a formulation with the fungal spores, sprayed for its synergized toxicity to mustard aphid was also studied.

MATERIALS AND METHODS

The strain PDRL1187 of *B. bassiana* was received from abroad, Wahat Al Sehra Nurseries Desert Group Dubai, UAE. The fungal culture regularly maintained and revived on mycological

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media according to the line suggested by Goettel and Inglis (1997). The test crop canola (*Brassica napus* L.) was cultivated in blocks of 6x12 feet for each test. The block-to-block distance was 10 ft. The soil and seedbed was prepared according to agronomic protocol and procedures (Anonymous, 2009) at the agronomic field of Department of Agriculture and Agribusiness Management, University of Karachi. After sowing, 30 seedlings were maintained in each block with 1.2 ft between seedlings and 2 ft between rows by thinning excessive plants after 28 day. The plants were artificially infested with 05 wingless female aphids per plant before 15 days of spray treatments. Each block sprayed with fungal spores using low volume sprayer of 06L capacity. Separate hands carry with spray machines < 200 µ pores size used as prevention of cross contamination. A plot was applied with the insecticide formulation (10 g a.i. +100 L water/acre or 16.5 mg +1.65 L/plot). Another plot was sprayed with spore ($6.30x10^{12}$ spores+100 L water/acre or $1.0x10^{10}$ spores +1.65 L/plot) of EPF spores formulation in aqueous solution of 0.2% Tween 20. Third plot was sprayed with the aforementioned spore + insecticide combination (16.5 mg + $1.0x10^{10}$ spores/plot) in 1.65 L aqueous solution of 0.2% Tween 20. A plot sprayed with the same volume of sterilized aqueous solution of 0.02% Tween80 as control. The numbers of insects in the population was randomly counted on apical racemes of five plants in each block before spray (day 1) and after 05, 10, 20 and 30 days. The differences in populations recorded between control and treatments. Percent mortality was corrected by using a formula (Henderson and Tilton, 1955). The seed yield of each plant compared to control treatments. The median lethal time (LT_{50}) analyzed by using probit analysis on IBM-SPSS 19 as keeping constant dose and variable times (days).

The died insects were taken to the laboratory and inoculated on PDA plates after surface sterilization with 1% sodium-hypochlorite aqueous solution, and confirmed the *B. bassiana* sporulations. The insect that was grown over by the fungi during incubation was studied for scanning electron microscopy. The insect was fixed over night at 4ºC with 2% (v/v) glutaraldehyde, 2% (v/v) paraformaldehyde in 0.1 M sodium cacodylate buffer at pH 7.2. Post-fixation was carried out in 1% (w/v) osmium tetroxide in the same buffer. The specimens were rinsed in buffer, dehydrated in a series of 30–100% alchohal solutions, dried at the critical point in CO_2 and coated with gold in a sputter-coater and observed under scanning electron-microscope at central science laboratory, University of Karachi.

RESULTS AND DISCUSSION

Strain PDRL1187 *B. bassiana* treatment caused 50% mortality of mustard aphid after 6.4 days (LT_{50}) under field conditions (Table I). The mortality caused by the strain was lower at field than greenhouse bioassays (Ujjan, 2013). During greenhouse bioassay, the LT_{50} was 4.0 days. At field, conditions the canola seed yield was 10.2g as it was higher 12.0g per plant recorded at green house bioassay (Fig. 1) (Ujjan, 2013).

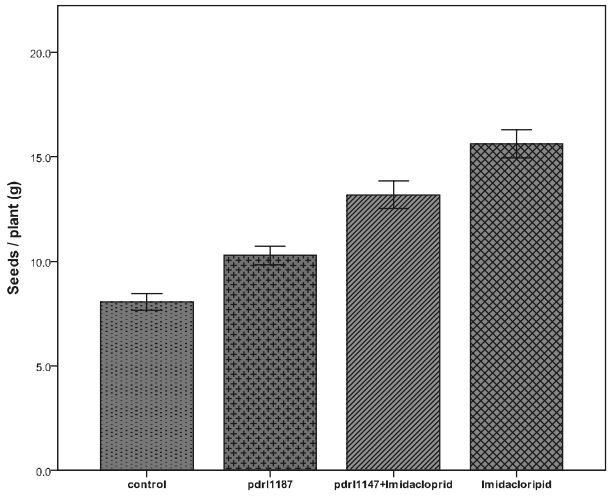

Fig. 1. Yield of canola seed per plant after spray of the Imidacloprid(10 g a.i. per acre +100 L water) and the *Beauveria bassiana* strain PDRL 1187 alone or in combination with spore formulation ($6.3x10^{12}$ spores /acre+100 L water) at final harvest (24 December to 15 March, 2011).

Imidacloprid caused 50% mortality of mustard aphid after (LT_{50}) 3.2 days (Table I). The combined application showed additive effects and increased insect mortality by lowering LT_{50} value 2.69 days. The combined application of Imidacloprid

Table I.- The median lethal time (LT_{50}) value of *Beauveria bassiana* strain PDRL1187 applied with ($6.30x10^{12}$ per acre) or in combination of Imidacloprid (10 g a.i. per acre) in the field trials on mustard aphid.

Strains	Life stages	LT_{50} (days)	95% Conf. Limit		Estimate-intercept	Z-intercept	Chi^2 (df^a)
			Lower	Upper			
PDRL1187	Adults	6.497	2.536	12.500	0.9-0.8	18.1-13.9	564.6(23)*
Imidacloprid	Adults	3.222	1.401	5.287	1.6-0.8	26.5-13.6	661.0(23)*
PDRL1187 + Imidacloprid	Adults	2.809	1.311	4.523	2.0-0.9	29.8-14.6	785.0(23)*

[a] Statistics based on individual cases differ from statistics based on aggregated cases.
[*] Since the significance level is less than 0.150, a heterogeneity factor is used in the calculation of confidence limits.

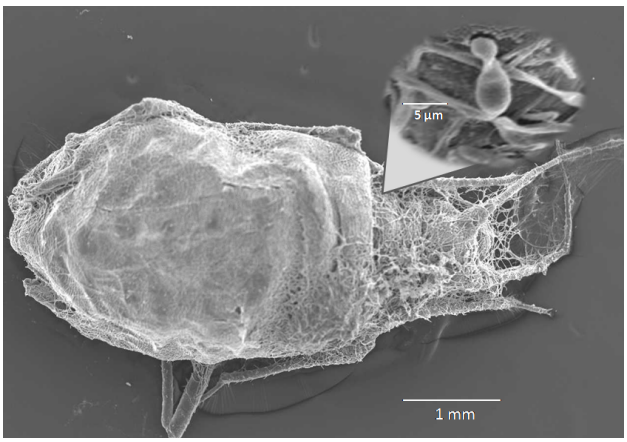

Fig. 2. The scanning electron micrograph shows the *B. bassiana* hyphal growth on the treated insect's body.

and *B. bassiana* strain reported by Parmar and Kapadia (2007), therein the additive effects in mustard aphid mortality was absent. The insect dead bodies from field transferred to incubation chambers for hyphal growth and sporulations of the EPF *B. bassiana* strain PDRL 1187, and the microscopic examination confirmed the pathogenesis of the *B. bassiana* strain (Fig. 2). The alone application of the insecticide promoted the highest yield 15.6 g seeds per plant among treatments. The synergistic treatment showed median yield (14.8 g) between the single treatments of the EPF (10.6 g) and the insecticide (15.6 g). The yield showed weak additive effects of the insecticide and *B. bassiana* strain PDRL 1187 EPF in terms of plant protection as compare to control treatments 8.2 g/plant (Fig. 1). It was perceived that the combined application of the insecticide and EPF spores might boost the plant yield as these controlled the insect population in the experiment. However, the results were unexpected in canola seed yield (Fig. 1).

During preliminary investigation, the strain PDRL1187 found effective with higher insecticidal attributes to mustard aphid. It assured the virulence *in vitro* and *in vivo* conditions (Ujjan and Shahzad, 2012).

The *Beauveria bassiana*, strain PDRL1187, bioassay on mustard aphid at field conditions confirmed its presence of mycoinsecticide potential. The strain's virulence varied between field and green house bioassays. Insecticide Imidacloprid found more effective than EPF strains when applied as the single doses at recommended rate. The insecticide 10 g *a.i.* mixed with EPF strains $6.3x10^{12}$ spores/acre, suspension caused synergistic effects and increased mortality of mustard aphid population (Table I). Rawat *et al.* (2008) reported a *B. bassiana* strain induced 48% mortality after 3 days of mustard aphid population under field conditions; which is consistent with our study. Filho *et al.* (2011) reported a *B. bassiana* strain and Imidacloprid spray regimes controlled 50 and 100% population after 14 days, respectively against *Myzus persicae* on cabbage; it supports our study. When the insecticide Imidacloprid (50 mL a.i./acre) was applied it reduced 96.1% mustard aphid population after 7 days (Rana *et al*., 2007). In other report, the dose of 24 g a.i./acre of Imidacloprid was 86% toxic to mustard aphid (*L. erysimi*) on canola (*B. napus*) after 7 days (Devee and Bruah, 2012). Aslam and Munir (2001) reported that Imidacloprid 8 g *a.i.* /acre dose reduced the aphid (*L. erysimi*) population by 89.2% after 10 days. During the present study, Imidacloprid 10g /acre caused 89.9% mortality after 10 day, which showed a similarity of insecticide efficacy as reported by Aslam and Munir (2001); Parmar and Kapadia (2007). Combined use of the

EPF strain with Imidacloprid gave increase in plant seed yield as compared to control (Fig. 1).

Hence, our study confirms that the EPF strain has efficient virulence against the mustard aphid. The strain has no lethal effect on host plant (*B. napus*). The spores of the strain are utilizable to the mustard aphid with combination of insecticide Imidacloprid. Therefore, the strain (*B. bassiana* PDRL1187) has mycoinsecticidal potential and needs further probes for biological control strategies in sustainable agriculture practices.

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