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In-vitro evaluation of biopesticides (Beauveria bassiana, Metarhizium anisopliae, Bacillus thuringiensis) against mustard aphid Lipaphis erysimi kalt. (Hemiptera: Aphididae)

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Abstract

In this study efficacy of bio-pesticides was evaluated against mustard aphid Lipaphis erysimi *in-vitro* condition at the Department of Entomology, University of Agriculture Faisalabad from July 2015 to May 2016. Mustard aphid Lipaphis erysimi (Homoptera: Aphididae) is the limiting factor of qualitative and quantitative losses by attacking and hurting the leaves and pods in growing areas of Pakistan and also develop resistance to some synthetic insecticides. Biopesticides are target specific, retard insect growth, metabolic process and has a less adverse toxicity to mammals. Five concentrations with three replications of each insecticide were used in several bioassays. The mortality data was recorded over a period of three days at 12h interval. Among entomopathogenic biopesticides M. anisopliae (83.23%) found most effective against mustard aphid followed by B. bassiana (78.33%) and B. thuringiensis (73%). Bio-pesticides can be used as a potential candidate for integrated pest management against mustard aphid after field efficacy.

Keywords: mustard aphid, biopesticides, integrated pest management, *Brassica* species, *Bacillus thuringiensis*

1. Introduction

Brassicaceae family comprises of approximately 375 genera and 3200 species of plants in which *Brassica* species considered an important oilseed crops [1-3]. Canola used for three *Brassica* spp. and preferred due to low level of erucic acid and glucosinolates [4,5]. Pakistan Agricultural Research Council introduced mustard in Pakistan during 1980-81 and now widely grown [6, 7].

Insect pests are an important qualitative and quantitative yield limiting factors of *Brassica* crop [8-10]. Nearly 92 spp. of aphids are in Pakistan and cause stunting, distortion and discoloration of plant leaves [11-15]. The yield losses of *Brassica* crop due to insect pests have been reported in Pakistan 70-80% and aphid caused 23 to 57% [16, 17]. Crinkling and blistering type distortion of leaves occur due to colony formation of aphids on underside of leaves [19,20], serving as the vector of viral disease [21] and also act as a medium for the growth of sooty fungus, known as sooty mold [22].

To control the aphid, growers of *Brassica* crops, blindly use the conventional insecticides of different groups which posed the several ecological changes like resistance development, bio control agent’s equilibrium disturbance, environmental pollution and accumulation of toxic substance in food commodities that lead to the health hazards like cancer, kidney and liver failure and genetic disorders in human beings [23-27]. These issues come into the control by developing the user safe and eco-friendly approaches like biopesticides that are host specific and less toxic to the environment and mammals [28].

Microorganisms are active ingredient in biopesticides and some isolated from soil [29, 30]. *B. bassiana* and *M. anisopliae* cause disease in target insect known as white and green muscardine, respectively [31-33]. These fungi are environmental friendly, safe for user and have no residual effects [34]. The road-shape, spore forming gram-positive entomopathogenic bacterium *Bacillus thruingiensis* are capable to produce crystal protein [35] and available commercially with different formulations and brand names [36].

Keeping in view these facts, the present study was conducted to achieve the following objectives; (a) to access the individual performance of biopesticides *Beauveria bassiana*, *Metarhizium anisopliae* and *Bacillus thuringiensis* against *L. erysimi* (b) evavaluation the optimal application rates and duration of activity for biopesticides (c) finding out that the biopesticides are the best alternative of conventional insecticides.

2.1 Insect collection
Mustard aphids were collected from *brassica* fields, placed in ventilated plastic jars and *brassica* leaves were used as food for aphids of *Brassica* crop. After checking for disease and parasitism, healthy individuals were used in pathogenicity assays.

2.2 Biopesticides

Table 1: The following biopesticides were used in research

Active ingredient	Trade name	Formulation
Bacillus thuringiensis	Lipel ®	Wettable Powder
Beauveria bassiana	Racer ™	Wettable Powder
Metarhizium anisopliae	Pacer ®	Spray able Powder

2.3 Concentration preparation
Five conidial suspensions (dilutions) *i*.e., 5, 10, 15, 20 and 25% of each bio-pesticide were prepared. The determined quantity of each was mixed in water up to the required volume to prepare 5, 10, 15, 20 and 25% dilutions. The Colony-forming unit CFU counted by using hemocytometer.

2.4 Calculation of colony forming unit of bacteria and fungi
Colony-forming unit (CFU) is a measure of viable bacterial or fungal cells. Serial dilutions, plating and counting of live bacteria was used to determine the number of bacteria and fungi in a given population. Serial dilutions were made of bacteria and fungi and compared them to the dilution factor. Each colony forming unit represents a bacterium and fungus that were present in the diluted sample. The numbers of colony forming units (CFU's) divided by the product of the dilution factor and the volume of the plated diluted suspension to determine the number of bacteria and fungi per mL that were present in the original solution.

2.5 Calculating the number of bacteria per mL of serially diluted bacteria
The number of bacteria and fungi per mL of diluted sample was calculated by using the following equation:

$$\frac{\text{Number of CFU}}{\text{Volume plated (mL) x total dilution used}} \longrightarrow \frac{\text{Number of CFU}}{\text{mL}}$$

Table 2: calculated colony forming unit of *Bacillus thuringiensis*

Concentrations	*Bacillus thuringiensis* colony	Calculated CFU
5%	128	1.28×10^7
10%	258	2.58×10^7
15%	390	3.90×10^7
20%	521	5.21×10^7
25%	649	6.49×10^7

Table 3: calculated colony forming unit of *Beauveria bassiana*

Concentrations	*Beauveria bassiana* colony	Calculated CFU
5%	95	0.95×10^8
10%	188	1.88×10^8
15%	286	2.86×10^8
20%	382	3.82×10^8
25%	478	4.78×10^8

Table 4: calculated colony forming unit of *Metarhizium anisopliae*

Concentrations	*Metarhizium anisopliae* colony	Calculated CFU
5%	104	1.04×10^8
10%	210	2.10×10^8
15%	321	3.21×10^8
20%	428	4.28×10^8
25%	539	5.39×10^8

2.6 Experimental layout
The experiment was laid out in completely randomized design having three repeats under *in vitro*. For each treatment, 50mm diameter leaf disc was cut out of a healthy *Brassica* crop and dipped into 5ml of conidial suspension for 10 seconds while excess suspension was removed by placing the leaf discs on sterile filter paper for few minutes, while control leaf discs was treated with 0.05% Tween 80. These discs were placed on moist filter paper in plastic petri plates. Healthy aphids were distributed with the camel hair brush per replication on treated and untreated leaf disc and incubated at 23±2°C with 16:8 L: D. The mortality data was recorded over a period of three days at 12h interval. Cadavers were shifted to petri dishes with moist filter paper to promote fungal development and sporulation in order to confirm that death is due to fungal infection.

2.7 Statistical analysis
The percentage mortality of insects was calculated by the Henderson and Tiltion formula [37].

$$\text{Corrected \%} = \left(1 \; \frac{n \text{ in Co before treatment} \times n \text{ in T after treatment}}{n \text{ in Co after treatment} \times n \text{ in T before treatment}}\right) \times 100$$

Data obtained in various treatments of different concentrations were compared by ANOVA technique, Tukey's Honestly Significant Difference (HSD. For the analysis of data statistical software (8.1) was used.

3. Results
3.1 Mortality effect of *Beauveria bassiana*
Analysis of Variance indicated that effects of all concentrations of *Beauveria bassiana* were significantly different against adults of *Lipaphis erysimi*. The maximum mortality (78.33%) was obtained at 25% concentration of *B. bassiana* followed by 20%, 15%, 10%, and 5% with 60%, 50%, 40.30% and 25% mortality, respectively as compared to control (11%) as shown in Table 5.

Table 5: Percent mortality of *L. erysimi* adults after post treatment of *B. bassiana*

B. bassiana	Mean Percent Mortality					
Concentration	**12 hours**	**24 hours**	**36 hours**	**48 hours**	**60 hours**	**72 hours**
25%	16.00a	28.00a	36.00a	45.00a	60.00a	78.33a
20%	12.45b	20.50b	25.33b	38.33b	48.33b	60.00b
15%	5.00c	15.00c	20.33c	28.33c	40.00bc	50.00c
10%	1.66d	10.00d	15.00d	21.66d	33.33c	40.30d
5%	0.00e	1.00e	6.67e	11.66e	15.00d	25.00e
Control	0.00e	0.00e	4.00f	5.40f	7.20e	11.00f

3.2 Mortality effect of *Metarhizium anisopliae*

Analysis of Variance showed that the effects of all concentrations of *Metarhizium anisopliae* were significantly different against adults of *Lipaphis erysimi.* The highest mortality (83.23%) was obtained at 25% concentration of *M. anisopliae* followed by 20%, 15%, 10%, and 5% to 66.67%, 58.56%, 46.96% and 28.33% mortality, respectively as compared to control (10.90%) as shown in Table 6.

Table 6: Percent mortality of *L. erysimi* adults after post treatment of *M. anisopliae*

M. anisopliae	Mean Percent Mortality					
Concentration	**12 hours**	**24 hours**	**36 hours**	**48 hours**	**60 hours**	**72 hours**
25%	19.66a	31.00a	43.33a	56.66a	70.00a	83.23a
20%	14.33b	26.00b	38.33b	50.00b	61.76b	66.67b
15%	10.33c	20.00c	33.33c	45.00c	53.34c	58.56c
10%	6.66d	13.00d	20.00d	31.33d	38.00d	46.96d
5%	1.66e	10.00e	13.00e	16.33e	22.30e	28.33e
Control	0.00e	3.00f	5.00f	6.00f	8.70f	10.90f

3.2 Mortality effect of *Bacillus thuringiensis*

Analysis of Variance resulted that effects of *Bacillus thuringiensis* were significantly differing against adults of *Lipaphis erysimi.* The supreme mortality (73%) was obtained at 25% concentration of *B. thuringiensis* followed by 20%, 15%, 10%, and 5% to 57.2%, 45%, 34.8% and 20% mortality, respectively as compared to control (9%) as shown in Table 7.

Table 7: Percent mortality of *L. erysimi* adults after post treatment of *B. thuringiensis*

B. thuringiensis	Mean Percent Mortality					
Concentration	**12 hours**	**24 hours**	**36 hours**	**48 hours**	**60 hours**	**72 hours**
25%	8.33a	21.66a	33.00a	40.00a	56.33a	73.00a
20%	5.00b	13.40b	25.10b	33.00b	46.34b	57.20b
15%	3.33c	10.00c	18.80c	25.00c	32.30c	45.00c
10%	1.66d	5.00d	12.00d	16.00d	28.33d	34.80d
5%	0.00e	0.00e	5.33e	6.00e	13.80e	20.00e
Control	0.00e	0.00e	2.00f	2.70e	6.10e	9.00f

4. Discussion

The effect of different biopesticides, mortality of mustard aphid after the application of all concentrations showed that all the biopesticides at the highest concentration (25%) and 72 hours provided the maximum mean percent mortality, *M. anisopliae* (83.23%), *B. bassiana* (78.33%), and *B. thuringiensis* (73%), all treatments showed the varying degree of control. *M. anisopliae* (83%) proved most affective while *B. thuringiensis* (73%) was less effective against mustard aphid. Our results are comparable to some earlier researchers as reported by Ujjan *et al*., (2012) [38] that *B. bassiana*, *M. anisopliae* have been effective and virulent in controlling the mustard aphid provided 88% mortality after 3 days and *M. anisopliae*. Araujo *et al*., (2009) [39] have reported 90% mortality with high concentration (10^7 spore per ml) of *B. bassiana* after 4.4 days, while the present study provided 78% mortality after 3 days with high concentration of *B. bassiana* (25%), differences in results may be due to duration. Saranya *et al*., (2010) [40] recorded percent morality with 12 hour interval up to seven days and concluded that mortality of aphid was increased with the increase in concentration; at high concentration the mortality was obtained after 72 hours, ranging between 53 to 60 percent, however in current study mortality was recorded upto 78% of *B. bassiana* at high concentration and similarly in case of *M. anisopliae* aphid mortality was obtained 60 to 70% while in present study the mortality was 83%. Loureiro *et al.,* (2006) [41] reported 100% mortality of turnip aphid through *M. anisopliae* and *B. bassiana* at 10^7 and 10^6 spore/ml respectively. *B. bassiana* and *V. lecanii* (4x 106 cfu/ml) were more effective than any other entomopathogenic fungi against aphids on lucerne crop [42]. Anuradha *et al.,* (2015) [43] resulted that *V.lecanii* was proved to be the best biopesticide to control spotted alfalfa aphid on lucerne. According to Ahmad *et al*., (2007) [44] least mortality of aphid was monitored during the treatment of BtA after 48 and 72 hours of treatment application, it reduced the aphid population 70% while in current study the mortality of aphid was observed 73% after 72 hours of application of *B. thuringiensis* treatments. Khan *et al*., (2015) [45] compared the effectiveness of a biopesticide (BtA) with synthetic insecticides (Confidor, trend and megamos) against *M. persicae* (tobacco aphid) and found that yield of tobacco was significantly higher in confidor (2368 Kg ha-1) and lower in BtA (1815 Kg ha-1).

Bio pesticides can be used against mustard aphid. M. *anisopliae* provided highest mortalities of *L. erysimi* than the other treatments and control. Biopesticides can be a promising and alternate contestant against chemical pesticides in integrated pest management. The results of present experiments might help in better control of *L. erysimi* on *Brassica* crop.

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