Journal of Entomology and Zoology Studies

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JEZS

Journal of Entomology and Zoology Studies

# Potential assessment of *Metarhizium anisopliae* and *Bacillus thuringiensis* against Brinjal insect pests *Amrasca bigutulla* (Jassid) and *Aphis gossypii* (Aphid)

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**E-ISSN: 2320-7078**
**P-ISSN: 2349-6800**
JEZS 2018; 6(2): 32-36
© 2018 JEZS
Received: 08-01-2018
Accepted: 09-02-2018

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## Abstract

The present study was conducted to investigate the pathogenicity of entomophathogenic fungi and bacteria against aphid and jassid at Youngwala, Entomological Research Area, University of Agriculture Faisalabad, Pakistan. Experiment performed on brinjal plants with field recommended doses of PACER® (*Metarhizium anisopliae*) at $1\times10^7$ conidia and LIPEL™ (*Bacillus thuringiensis*) at $1\times10^8$ spores to asses percent mortality under randomized complete block design (RCBD). The data regarding mortality were recorded after 12 hours, 24 hours, 48 hours, 72 hours, 7 days and then on weekly basis of exposure period of application treatment. Alone treatments of *Bacillus thuringiensis* (LIPEL™) showed maximum mortality 52.92% of jassid (*Amrasca bigutulla*), 52.46% of aphid (*Aphis gossypii*) and *Metarhizium anisopliae* (PACER®) gave 66.75% of jassid (*Amrasca bigutulla*), 58.66% of aphid (*Aphis gossypii*) after maximum days. Mortality in combined used of these treatments showed 83.33% of jassid and 72.45% of aphid. It is concluded that these bio-insecticides can efficiently be used against insect pests of brinjal and can also be integrated along with each other.

**Keywords:** Entomopathogenic fungi, *Metarhizium anisopliae*, *Bacillus thuringiensis,* Brinjal insect pests, Aphid, Jassid

## 1. Introduction

Brinjal (*Solanum melongena* L.) is one of the most important kharif season vegetable, belongs to the family Solanaceae [1] and common vegetable crops grown in Pakistan and probably native to India [2]. Eggplant generally recognized as brinjal, is an economically important common vegetable grown and consumed throughout the world [3]. The area cultivated under this crop was 9,000 hectares with production of 89,000 million tons in Pakistan which stands at 18th position in world ranking [4]. It is a good supply of minerals and vitamins particularly iron, as compared to other commonly used vegetables, and it is nutritionally comparable to tomato [5].

The most common sucking insect pests' attacks on brinjal are aphid (*Aphis gossypii*), jassid (*Amrasca biguttula*) and whitefly (*Bemisia tabaci*) [6]. The jassid inject the toxic substances and suck the cell sap of the leaves and causes 24.45% yield loss [7]. The damage of different insect pests varies from year to year depending upon intensity of insect pest attack [8]. Keeping in view, brinjal as a cheap source of nutrition for masses, it becomes important to increase yield by controlling the losses inflicted especially by insect pests [9].

These insect pests are controlled by using several fumigants and synthetic residual insecticides worldwide causing environmental hazards, insecticide resistance development and chemical residues in foodstuffs [10,11] Control of these insects using other alternative means have been attempted to overcome the undesirable side-effects associated with the frequent use of chemical control [12]. Due to development of this high level of resistance in insects and accumulation of pesticide residues in are investigating to shift over the use of safer biological control agents [13]. Biological control due to their low mammalian toxicity and high level of virulence against insect pests; entomopathogenic fungi have been acknowledged as worthy substitute to conventional fumigants and residual insecticides [14].

A group of fungi that kill an insect by attacking and infecting its insect host is called entomopathogenic fungi [15].

Life cycle of insect pathogenic fungi need an infectious spore phase that germinates on the host cuticle, later on a germ tube is formed penetrating into the host cuticle and eventually occupies the host [16]. Entomopathogenic fungi have been tested in both laboratory as well field and these experimentations showed a great success in control of various insect pest species [17-21]. The use of entomopathogenic fungi as alternative means to chemical control is a novel approach to control insect pests of vegetables [22]. Researchers made a focus on the assortment of virulent strains for the target insect pests and their improvement as biological control agents [23]. Entomopathogenic fungi have the ability to re-establish in the form of inoculum from the dead cadavers and can persist for longer time periods [24]. Additionally, the fungal conidia may be augmented with several carriers like mineral oils, inert dusts and botanical insecticides to improve their insecticidal activity [25].

Moreover a Gram-positive soil bacterium *Bacillus thuringiensis* also bear a high potential to control insect pests due to β-endotoxins that it produce and which act as an influential intestinal toxin for various insect pests [26]. Many studies involve *Bacillus thuringiensis* in which the bacteria produce spores and crystal toxins in the mid-gut of susceptible insect pests [27]. The pH value of fluid present in the gut of host is an important key factor [28]. Nevertheless, the triggered crystal toxin damages the gut cells, subsequently causing leakage of hemlymph into the midgut thus lowering pH, and permitting the spores to propagate [29]. Mixtures of Bt spore crystals are being used effectively as bio-insecticides against coleopteran, dipteran and lepidopterous insects [30].

Keeping in view these facts, the present study was conducted to achieve the following objectives; (a) to assess the individual performance of biopesticides *Beauveria bassiana* and *Bacillus thuringiensis* against brinjal pests *Amrasca bigutulla* and *Aphis gossypii* (b) combined effectiveness of these bio-pesticides (c) finding out that the bio-pesticides are the best alternative of conventional insecticides.

## 2. Materials and Methods

### 2.1 Experimental layout

Field experiment was conducted in Youngwala, Entomological Research Area at main campus, University of Agriculture Faisalabad, Pakistan, during 2016.The seeds of Dudhia variety of eggplant were sown in mid of June.

### 2.2 Pot Experiment

The nursery plants with 4-5 leaves were shifted to pots at the end of August. The pots were irrigated with 2-3 days interval. The experiment was laid out in RCBD with four treatments and three replications. The treatments were applied by foliar application method when pests were near to economic threshold level.

### 2.3 Source of Bio Insecticides

Below mentioned Bio insecticide formulations were imported from Agri Life Medak District (Hyderabad), Andhra Pradesh, India.

Fungal Formulation: *Metarhizium anisopliae* (PACER®)

Bacterial formulation: *Bacillus thuringiensis* var. *Kurstaki* (LIPEL TM)

### 2.4 Data Analysis

Insect's population data was collected by visual observation on per plant. The data was taken after 12, 24, 48, and 72 hours and then on weekly basis after application. The collected data was transformed into percent population reduction/increase by the following formula:

$$\text{Percent Population Reduction} = \frac{PBTA \times PATA}{\text{PBTA}} \times 100$$

PBTA = Population Before Treatment Application

PATA= Population After Treatment Application

### 2.5 Statistical Analysis

Data obtained in various treatments 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 Aphid

All post treatment of different biopesticides ANOVA table indicated that all treatments (biopesticides + control) have significant impact on population reduction percentage of aphid (*Aphis gossypii*). Maximum population reduction was observed after 72 hours (31.22%) of post application of plots treated with biopesticides (*Metarhizium anisopliae* + *Bacillus thuringiensis*) which was 10.69 times higher than control. *Metarhizium anisopliae* (25.12%) was 8.60 times while *Bacillus thuringiensis* (19.55%) 6.69 times higher that of control (Table 1). Maximum population reduction was observed after 21 days 72.45%, 58.66% and 52.46% of post application of plots treated with biopesticides *Metarhizium anisopliae* + *Bacillus thuringiensis*, *Metarhizium anisopliae* and *Bacillus thuringiensis* , respectively (Table 2).

**Table 1:** All-Pairwise Comparisons Test regarding population reduction percentage of aphid (*Aphis gossypii*) after 12, 24, 48 and 72 hours of post treatment application of different biopesticides

| Treatments | Population Change | | | |
|---|---|---|---|---|
| | 12 hrs | 24 hrs | 48 hrs | 72 hrs |
| *M. anisopliae & B. thuringiensis* | $2.55^{A}\pm0.51$ | $5.33^{A}\pm0.21$ | $18.22^{A}\pm0.43$ | $31.22^{A}\pm0.44$ |
| *Metarhizium anisopliae* | $1.48^{B}\pm0.43$ | $3.77^{B}\pm0.54$ | $12.32^{B} \pm 0.21$ | $25.12^{B}\pm0.22$ |
| *Bacillus thuringiensis* | $1.12^{C}\pm0.22$ | $2.61^{C}\pm0.15$ | $10.65^{C} \pm 0.22$ | $19.55^{C}\pm0.87$ |
| Control | $0.00^{D}\pm0.11$ | $0.00^{D}\pm0.76$ | $0.77^{D} \pm 0.76$ | $2.92^{D}\pm 0.32$ |

**Table 2:** All-Pairwise Comparisons Test regarding population reduction percentage of aphid (*Aphis gossypii*) after 7, 14, 21 and 28 days of post treatment application of different biopesticides

| Treatments | Population Change | | | |
|---|---|---|---|---|
| | 7 days | 14 days | 21 days | 28 days |
| *M. anisopliae & B. thuringiensis* | $52.21^{A}\pm0.35$ | $68.55^{A}\pm0.11$ | $72.45^{A}\pm0.11$ | $46.78^{A}\pm0.11$ |
| *Metarhizium anisopliae* | $43.43^{B}\pm0.22$ | $55.23^{B}\pm0.56$ | $58.66^{B}\pm0.56$ | $37.68^{B}\pm0.56$ |
| *Bacillus thuringiensis* | $38.85^{C}\pm0.77$ | $49.81^{C}\pm0.12$ | $52.46^{C}\pm0.12$ | $29.22^{C}\pm0.12$ |
| Control | $4.12^{D}\pm 0.23$ | $5.31^{D} \pm 0.33$ | $5.99^{D} \pm 0.33$ | $5.58^{D} \pm 0.33$ |

### 3.2 Jassid

All post treatment of different biopesticides ANOVA table indicated that all treatments (biopesticides + control) have significant impact on population reduction percentage of jassid (*Amrasca bigutulla*). Maximum population reduction was observed after 72 hours (22.55%) of post application of plots treated with biopesticides (*Metarhizium anisopliae* + *Bacillus thuringiensis*) which was 29.28 times higher than control. *Metarhizium anisopliae* (18.22%) was 23.66 times while *Bacillus thuringiensis* (14.88%) 19.32 times higher than control (Table 3). Maximum population reduction was observed after 21 days (83.33%) of post application of plots treated with biopesticides (*Metarhizium anisopliae* + *Bacillus thuringiensis*) which was 18.68 times higher than control. *Metarhizium anisopliae* (66.75%) was 14.96 times and *Bacillus thuringiensis* (52.92%) 11.86 times higher than control (Table 4).

**Table 3:** All-Pairwise Comparisons Test regarding population reduction percentage of jassid (*Amrasca bigutulla*) after 12, 24, 48 and 72 hours of post treatment application of different biopesticides

| Treatments | Population Change | | | |
|---|---|---|---|---|
| | 12 hrs | 24 hrs | 48 hrs | 72 hrs |
| *M. anisopliae* & *B. thuringiensis* | $2.12^{A}\pm0.88$ | $4.44^{A}\pm0.66$ | $12.44^{A}\pm0.67$ | $22.55^{A}\pm0.66$ |
| *Metarhizium anisopliae* | $1.43^{B}\pm0.45$ | $3.86^{B}\pm0.43$ | $9.43^{B}\pm0.11$ | $18.22^{B}\pm0.43$ |
| *Bacillus thuringiensis* | $1.05^{C}\pm0.21$ | $1.88^{C}\pm0.33$ | $8.22^{C} \pm 0.23$ | $14.88^{C}\pm0.89$ |
| Control | $0.00^{D}\pm0.01$ | $0.00^{D}\pm0.87$ | $0.15^{D}\pm0.32$ | $0.77^{D}\pm0.22$ |

**Table 4:** All-Pairwise Comparisons Test regarding population reduction percentage of jassid (*Amrasca bigutulla*) after 7, 14, 21 and 28 days of post treatment application of different biopesticides

| Treatments | Population Change | | | |
|---|---|---|---|---|
| | 7 days | 14 days | 21 days | 28 days |
| *M. anisopliae* & *B. thuringiensis* | $54.33^{A}\pm0.65$ | $79.43^{A}\pm0.89$ | $83.33^{A}\pm0.89$ | $55.22^{A}\pm0.89$ |
| *Metarhizium anisopliae* | $40.98^{B}\pm0.43$ | $63.65^{B}\pm0.25$ | $66.75^{B}\pm0.25$ | $42.26^{B}\pm0.25$ |
| *Bacillus thuringiensis* | $32.15^{C}\pm0.33$ | $48.98^{C}\pm0.87$ | $52.92^{C}\pm0.87$ | $39.44 \pm0.87$ |
| Control | $2.99^{D}\pm0.77$ | $3.12^{D}\pm0.03$ | $4.46^{D}\pm0.03$ | $3.47^{D}\pm0.03$ |

## 4. Discussion

The results revealed that combination of two treatments *Bacillus thuringiensis* (LIPEL™) and *Metarhizium anisopliae* (PACER®) were proved to be most effective against the particular insect pest after the time interval of 21 days showing mortality of aphid and jassid, 72.45% and 83.33%, respectively. These results are quite similar to Shafighi *et al*., 2014 [31] where they have used same chemical along with resistant varieties and reported that alone as well as in combination these chemicals caused maximum and reasonable population reduction of tested insect pests. Current research are also similar with the findings of Janaki *et al*., 2010 [32]; Sandhu *et al*., 2012 [33]; Erler and Ates 2015 [34]; Chinniah *et al*., 2016 [35]; Shahzad *et al*., 2016 [36]; where they have used same biocontrol agents and reported that these biocontrol agents have caused mortality for different life stages of insect pests of brinjal and gives maximum results for population reduction. Ursani *et al*., (2014) [37] also founded that the population of brinjal pests has been depressed by the use of different biopesticides. The findings of this research are in resemblance with present research. Srinivasan (2012) [38] performed an experiment to carry out the effects of biopesticides on pest management with collaboration of other management techniques such as resistant varieties, natural enemies. Integration of biopesticide can increase performance of IPM strategies. For instance, with the adoption of *Bacillus thuringiensis* and it based biopesticides, along with parasitoids such as *Cotesia plutellae*, *Diadegma semiclausum* and *Diadromus collaris* and reported that *Bacillus thuringiensis* and it based biopesticides caused maximum population reduction in form of mortality and proved as good control agents against pests on brassicas.

Sharma and tayde (2017) [39] founded that *Metarhizium anisopliae*, *Beauveria bassiana*, Neem oil, Spinosad, Emamectin benzoate, *Verticillium lecanii*, Cypermethrin were evaluated against fruit and shoot borer and found that these all chemicals in combination and alone as well caused maximum mortality of invested pests which supports present findings where used *B. thuringiensis* along with some botanicals against lepidopterous insect pests, provided mortality up to 85% and then reported that whenever all elements are combined they are proved to be helpful in controlling insect pests.

## 5. Conclusion

It is concluded that combination of *Bacillus thuringiensis* and *Metarhizium anisopliae* proved to be effective against the particular insect pest and can efficiently be used in integrated pest management strategies of brinjal insect pests.

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