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The use of promising entomopathogenic fungi for eco-friendly management of *Helicoverpa armigera* Hubner in chickpea

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Abstract*—Gram pod borer, Helicoverpa armigera Hubner is known to be a major constraint of chickpea production which causes serious economic loess. The management of this pest in any crop is always been challenge to the growers, famers and researcher. Thus, present study evaluated some promising entomopathogenic fungi for the sustainable management of H. armigera to minimize the economic loss in chickpea. Five different fungal isolates viz; Beauveria bassiana, Trichoderma virens, Trichoderma hamatum, Trichoderma koningii, and Paecilomyces sp. were used as entomopathogenic against gram pod borer, through dipping and poison food methods under laboratory conditions. The entomopathogenic potential of different fungal strains revealed significantly (P<0.05 = 0.0000) highest mortality with B. bassiana (46.67%) followed by T. koningi (23.33%), T. virens (11.11%) and T. hamatum (8.33%) through dipping method. In case of poison food method significantly highest mortality was recorded with T. koningi (20%) followed by B. bassiana (6.66%) after 24 h. The mortality with B. bassiana after 96 h was become higher (41.667%) compared with other strains. No mortality was recorded with Paecilomyces sp. and control (dipped in simple water) in both methods. It is obvious that microbial control agents are very effective and the promising entomopathogenic fungi of current study are hoped would be helpful for eco-friendly and alternative to chemical pesticides for sustainable management of H. armigera in chickpea.*

Keywords*— *Entomopathogenic fungi, Helicoverpa armigera, management, chickpea.

I. INTRODUCTION

Chickpea, *Cicer arietinum* L., locally known as gram, is an ancient cultivated plant with varying names in different countries **[1]**.It is believed that chickpea is a great source of biomolecules such as proteins, carbohydrates, dietry fibre, minerals and vitamins and its use has been increased for reducing risk of human diseases **[2]**. It is also rich source of balanced amino acids in human diet and is highly enriched with sulphur containing amino acids e.g. methionine and cysteine **[3]**.

Despite the fact that the chickpea has great economic and nutritional value, the production of chickpea is far below than other countries where chickpea is commonly cultivated, which likely due to several biotic and abiotic factors. Chickpea plant is highly susceptible to various insect pests at different critical growth stages from seedling stage to maturity. Around 60 species of insect pests belonging to orders Lepidoptera, Hemiptera, Diptera and Thysanoptera are commonly found in chickpea crop **[4, 5]**. However, the gram pod borer, *Helicoverpa armigera*Hubner (Lepidoptera: Noctuidae) is known to be a major constraint of chickpea production which reduces economic loess around 6 to 20% **[6, 7, 8, 9]**. Moreover, infestation of gram pod borer has increased globally in last 50 years due to diversification of crops in agro-ecosystem **[10]**. Gram pod borer is a highly polyphagous pest due to its nature of diverse nature feeding habit such as leguminous and vegetables etc. **[11, 12]**. Since it possesses polyphagy nature (approximately has 180 host crops mainly chickpea, tomato, cotton, pigeon pea, cowpea, some flowers, vegetables and forest trees), high mobility and fecundity **[13, 14, 15, 16, 17]** and capability and adaptability to different environments due its

facultative diapause, **[13, 18]**. Srivastava **[19]** reported that yield loss of chickpea can 10-60% in normal weather conditions and can increase upto 100% or complete crop failure **[20]**. In addition, a single larva of gram pod borer damaged 7- 10% pods, reduced 6.2% grains m^{-1} row^{-1} of the gram crop consequently overall declined 5.4% yield loss **[21]**.

The management of pests in any crop is always been challenge to the growers, famers and researcher. Various integrated pest management (IPM) practice for the control of insect pests of chickpea have been developed, tested and evaluated in farmers' fields **[22, 23, 24, 25, 26, 27].** In recent study **Ramesh and Rao [28]** advocated the IPM strategies. **Basha et al. [29]** compared IPM strategies such as resistant cultivars, intercropping, trap crop and border cropping for controlling insect pests of chickpea. They indicated that resistant cultivars, tillage practices, crop rotation, inter cropping and soil solarization were found effective measures for control of insect pest infestation on chickpea.

Nevertheless, the concern about the adverse effects of chemical pesticides on agriculture, human health and the environment has been increasing around the globe **[30, 31, 32, 33]**. It also has been reported about their adverse effects in many non-target organisms **[34, 35].** In the IPM programme, biological control with microbials is known to be a major component globally. Microbial control agents are believed an effective, environmental friendly and economic technique and alternative to chemical pesticides used for control of insect pest **[36, 37].** There are several microorganisms have been isolated for control of insect pests. Entomopathogenic fungi (EF) have been demonstrated to control Lepidopteran insect species **[37, 38]**. The EF play an important role in controlling the insect pests since there are 68% of EF based microbial pesticides **[39].** The EF has been recognized as important natural enemy of gram pod borer since long time. However, management is required a head of time prior to the onset of insect pest in field.

Based on aforementioned facts it was planned to use some promising entomopathogenic fungi for the sustainable management of *H. armigera* to minimize the economic loss in chickpea. To cater the need, present studies were conducted at Sindh Agriculture University Tandojam

II. MATERIAL AND METHOD

The evaluation of entomopathogenics was conducted in the Post Graduate laboratory of Department Plant Protection, Faculty of Crop Protection, Sindh Agriculture University, Tandojam, Pakistan. To evaluate the efficacy of entomopathogenic fungi (EPF) against the larval stages of H. armigera on chickpea crop series of experiments were conducted during 2015 and 2016under laboratory conditions

2.1 Fungal isolates

Five different fungal isolates viz; *Beauveria bassiana, Trichodermavirens, Trichoderma hamatum, Trichoderma koningii, Paecilomyces* sp. and *Penicillium* sp. were used as entomopathogenic against gram pod borer, *H. armigera* under laboratory conditions. The culture of all isolates were maintained by sub-culturing on Potato Dextrose Agar (PDA) medium and at the time of use freshly prepared culture was used as entomopathogenic.

2.2 Preparation spore suspension

The spores of all isolates were individually collected in 0.1 % Tween80 solution and final concentration was determined by hemocytometer. The stock solutions of different isolates were serially diluted to obtain the desired concentrations for bioassay. The desired amount of all strains conidia was put into distilled water and used to observe the efficiency against *H. armigera*.

2.3 Larval culture of *H. armigera*

The larvae of gram pod borer were collected before one day from the un-treated field of Pulses Sub-station, ARI, Tandojam, and culture was maintained in laboratory Plant Protection, Department, SAU, Tandojam on natural diet of chickpea tender shoots, leaves and pods. The five larvae were individually transferred in to plastic transparent cage.

2.4 Bioassay of entomopathogenic

The assessment of entomopathogenic fungi was conducted by two different methods such as: 1) Dip method and, 2) Poison Food method. All experiments were conducted with randomized complete block design under in vitro conditions. The details of methods are summarized here under:

2.4.1 Dip method

In this method different larval (2^{nd}, 3^{rd} and 4^{th}) instars were used to assess the efficacy of entomopathogenics against *H. armigera* under *in vitro* conditions. A total of five live larvae were dipped for 10 second in prepared concentration ($1.381X\ 10^{-5}$) first and then released in fresh chickpea food including tender leaves, shoots, and pods. The extra moisture of treated larvae was soaked on sterilized tissue paper. The experiments were conducted in the plastic transparent cage (measuring 30X30X30 cm) with randomized complete design with three replications. Moreover, experiments were repeated twice to further confirm the results.

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2.4.2 Food poising method

In this method poison food method similarly different larval (2^{nd}, 3^{rd} and 4^{th}) instar were used to assess the efficacy of entomopathogenics against. Initially, freshly collected food (chickpea tender leaves, shoots and pods) were sprayed individually with different entomopathogenic strains and then transferred in to plastic transparent cage (measuring 30X30X30 cm). The spore concentration was adjusted to $1.381X\ 10^{-5}$ by using distilled sterilized water before use. Five active larvae of different instar were released on to the contaminated food with fungal strains. The experiments were conducted under *in vitro* condition in randomized complete design with three replications. Moreover, experiments were repeated twice to further confirm the results.

2.5 Observations

After the application of entomopathogenics, observations were recorded on daily basis for mortality and survival of gram pod borer. Data was statistically analyzed by using the standard procedures for analysis of variance, ANOVA (linear model), by using the computer software Statistix 8.1 (Analytical Software, 2005). All differences described in the text were significant at the 5% level of probability.

III. RESULTS

The entomopathogenic potential of different fungal strains viz; *Beauveria bassiana, Trichoderma virens, Trichoderma hamatum, Trichoderma koningii* and *Paecilomyces* sp. were tested through two different methods, poison food and dipping methods in current study. The results of current study with five different entomopathogenic fungi (EPF) strains revealed significant differences among each other for their efficacy against the different larval stage (2^{nd}, 3^{rd} and 4^{th}) of gram pod borer, *H. armigera*. The results so far achieved are further discussed here:

3.1 Effect of EPF through dipping method

The effect of four different EPF used through dipping method exhibited varied responses at different post treatment time intervals. Significantly highest mortality percent was recorded with *B. bassiana* (46.67%) followed by *T. koningi* (23.33%), *T. virens* (11.11%) and *T. hamatum* (8.33%). However, no any mortality was notice in case of *Paecilomyces* sp. after 24 h of treatment through dipping method (Table 1). It was further observed that after 48 h of treatment the larval mortality was further increased with *B. bassiana* (55.57%); however, in case of other strains it was decreased. Moreover, after 72 and 94 hs the mortality was observed moderately with *B. bassiana;* while the response of *T. virens* (25.00%) was remained better compared to *T. koningi*, *T. hamatum* and *Paecilomyces* sp.

The mean mortality percent of *H. armigera* larvae treated with different fungal strain through dipping methods indicates the obvious response of all strains. The highest mortality was produced by *B. bassiana* followed by *T. virens* (Figure 1). No mortality was recorded with *Paecilomyces* sp. and control (dipped in simple water). While the mean mortality percent of *T. koningi* and *T. hamatum* was moderate through dipping method under laboratory conditions (Figure 1).

3.2 Effect of EPF through poison food method

The efficacy of all tested fungal strains through poison food method was lower compared to dipping method. However, the trend of mortality was correlated with dipping method. After 24 h, significantly highest mortality percent was recorded with *T. koningi* (20%) followed by *B. bassiana* (6.66%). While, no any mortality was recorded with *T. virens, T. hamatum* and *Paecilomyces* sp. after 24 h of treatment through dipping method (Table 2).

It was further observed that after 48 h of treatment the larval mortality was increased with *B. bassiana* (46.667%); however, in case of *T. koningi* it was decreased (8.33%). Moreover, after 72 h mortality of larva was also observed with *T. virens* and *T. hamatum*. The mortality of larva with *B. bassiana* after 96 h was higher (41.667%) compared with other strains. Moreover, no any mortality was notice in case of *Paecilomyces* sp. after up to 120 h of treatment through dipping method (Table 2).

The mean mortality percent of *H. armigera* larvae treated with different fungal strain through poison food methods indicates the obvious response of all strains. The highest mortality was produced by *B. bassiana* followed by *T. koningi* (Figure 2). No mortality was recorded with *Paecilomyces* sp. and control (dipped in simple water); while the mean mortality percent of *T. virens* and *T. hamatum* was moderate through dipping method under laboratory conditions. It was further observed that mortality of larva with *T. virens* in case of poison food method was lower compared to dipping method (Figure 2).

IV. DISCUSSION

It is obvious that insect pest management is always dominated by the use of synthetic pesticides; thus results several health and environmental hazards that ultimately

impacting all living beings. The entomopathogenic fungi (EPF) are fungal species that are pathogenic to insects. These fungal pathogen species play a vital role in reducing insect population dynamics comparatively earlier than other measures **[40].** The EPF has been recognized as important natural enemy of gram pod borer since long time. In the current study, different fungal strains viz; *Beauveria bassiana, Trichoderma virens, Trichoderma hamatum, Trichoderma koningii* and *Paecilomyces* sp. were tested through two different methods, poison food and dipping methods. Significant difference for the efficacy against the different larval stage (2^{nd}, 3^{rd} and 4^{th}) of gram pod borer, *H. armigera* was observed among each other. Highest mortality percent was recorded with *B. bassiana* followed by *T. koningi*, *T. virens* and *T. hamatum*. However, no mortality was noticed in case of *Paecilomyces* sp. after 24 h of treatment through dipping method.The mean mortality percent of *H. armigera* larvae treated with different fungal strain through dipping methods has indicates the obvious response of all strains. The highest mortality was produced by *B. bassiana* followed by *T. virens*. No mortality was recorded with *Paecilomyces* sp. and control (dipped in simple water). While the mean mortality percent of *T. koningi* and *T. hamatum* was moderate through dipping method under laboratory conditions. In case of poison food method, lower mortality has been noticed compared to dipping method. Almost same trend of mortality was observed with dipping method. It was further observed that mortality of larva with *T. virens* in case of poison food method was lower compared to dipping method. In the previous study, fifteen fungal species, *Metarhizium anisopliae* (Metsch.), *M. flavoviride* (Metsch.), *Nomuraea rileyi* (Farlow) Samson, *Beauveria bassiana* (Balsamo) and *Paecilomyces farinosus* have been found which could be promising myco-insecticides **[41].** However, several studies focused on the use of *Beauveria bassiana* and their promising control on different insect pests. **Ebrahimi et al. [42]** conducted study on effect of entomopathogenic nematode, *Steinernema feltiae*, on survival and plasma phenoloxidase activity of *Helicoverpa armigera* (Hb) (Lepidoptera: Noctuidae) in laboratory conditions. Another study conducted by **Majeed et al. [43]** on pathogenicity of indigenous soil isolate of *Bacillus thuringiensis* to *Helicoverpa armigera* Hübner 1809 (Lepidoptera: Noctuidae). Specifically, **Mishra and Sobita [44]** evaluated the efficacy of *Beauveria bassiana* Balsamo against *Helicoverpa armigera* (Hubner) in field condition and revealed significant mortality of *H. armiger* at 1 and 5% level with different doses. In our study we also observed *Beauveria bassiana* as most promising EPF; however, our study also explored *T. koningi* and *T. virens*s trains that maybe further study through different methods. The bio-efficacy of *B. bassiana* studied by **Prasad et al. [45]** against *H. armigera* (Hubner) with four different concentrations which were sprayed topically against the most damaging 4^{th} instar larvae and found up to 76.70 percent mortality with highest dose of 0.25ml $x10^8$ spores/ml. Our study is consistent with findings of these lines and we found *Beauveria bassiana* as most effective EPF through both methods. **Savita et al. [46] (2015)** evaluated the effectiveness of *Metarhizium anisopliae, Beauveria bassiana, Nomuraea rileyi* with different concentration against *Helicoverpa armigera* (Hub.) infestation on chickpea under field conditions. They found *Metarhizium anisopliae* as most effective with minimum larval survival. However, our student is not in agreement with finding of this study; in our study we found *Beauveria bassiana* as most effective against different larval instar through both observed methods. The study of Aneela et al. **[47]** also supported our finding with reference to *Beauveria bassiana*. However, in addition to *Beauveria bassiana*, they also used jasmonic acid and the chlorantraniliprole (insecticide) either alone or combined form against gram pod borer. Significant decline was observed for larval population of gram pod borer and which was further reduced with increase in time of application. Moreover, our study also explored *T. koningi* and *T. virens* strains that maybe further explored for their entomopathogenic potential through different methods. These strains may also be explored to develop new myco-insecticides to be used against gram pod borer and other serious insect pests.

V. CONCLUSION

The use of entomopathogenic fungi (EPF) has been believed to be safe and an alternative to synthetic pesticides. Since many years, EPF has been recognized as important natural enemy of gram pod borer, *H. armigera*. In the current study, five different fungal strains viz; *Beauveria bassiana, Trichoderma virens, Trichoderma hamatum, Trichoderma koningii* and *Paecilomyces* sp. were tested through two different methods, poison food and dipping methods. Significant difference for the efficacy against the different larval stage (2^{nd}, 3^{rd} and 4^{th}) of gram pod borer, *H. armigera* was observed among each other. Highest mortality percent was recorded with *B. bassiana* followed by *T. koningi*, *T. virens* and *T. hamatum*; however, no any mortality was noticed in case of *Paecilomyces* sp. through dipping and poison food methods under laboratory conditions. In our study we observed *Beauveria bassiana* as most promising EP; however, other strains such *T. koningi* and *T. virens* maybe further explored for their entomopathogenic potential through different methods. These strains may

also be explored to develop new myco-insecticides to be used against gram pod borer and other serious insect pests.

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Table 1 Effect of different fungal strains on the mortality of H. armigeraused through dipping methods under laboratory conditions

Hours	Mortality Percent of *H. armigera*											
	B. bassiana		***T. koningi***		***T. hamatum***		***T. virens***		***Paecilomyces*sp.**		**Control**	
24	46.67	ab	23.33	abcd	8.33	cd	11.11	bcd	00.00	d	00.00	d
48	55.57	a	13.33	bcd	6.67	cd	00.00	d	00.00	d	00.00	d
72	25.00	abcd	00.00	d	6.67	cd	25.00	abcd	00.00	d	00.00	d
96	24.44	abcd	8.33	cd	11.11	bcd	8.33	cd	00.00	d	00.00	d
120	40.00	abc	24.44	abcd	8.33	cd	16.67	bcd	00.00	d	00.00	d
SE	18.370											
LSD	36.745											

Note: Figures following the similar letter within a column are not significantly varied according to the LSD (least significant difference) test at $P<0.05$.

Table 2 Effect of different fungal strains on the mortality of H. armigeraused through poison food methods under laboratory conditions

Hours	Mortality Percent of *H. armigera*											
	B. bassiana		***T. koningi***		***T. hamatum***		***T. virens***		***Paecilomyces*sp.**		**Control**	
24	6.6667	bc	20.00	abc	00.00	c	00.00	c	00.00	c	00.00	c
48	46.667	a	8.3333	bc	00.00	c	00.00	c	00.00	c	00.00	c
72	17.778	abc	00.00	c	6.6667	bc	6.6667	bc	00.00	c	00.00	c
96	41.667	a	6.6667	bc	8.3333	bc	00.00	c	00.00	c	00.00	c
120	33.333	ab	16.667	abc	6.6667	bc	6.6667	bc	00.00	c	00.00	c
SE	15.851											
LSD	31.707											

Note: Figures following the similar letter within a column are not significantly varied according to the LSD (least significant difference) test at $P<0.05$.

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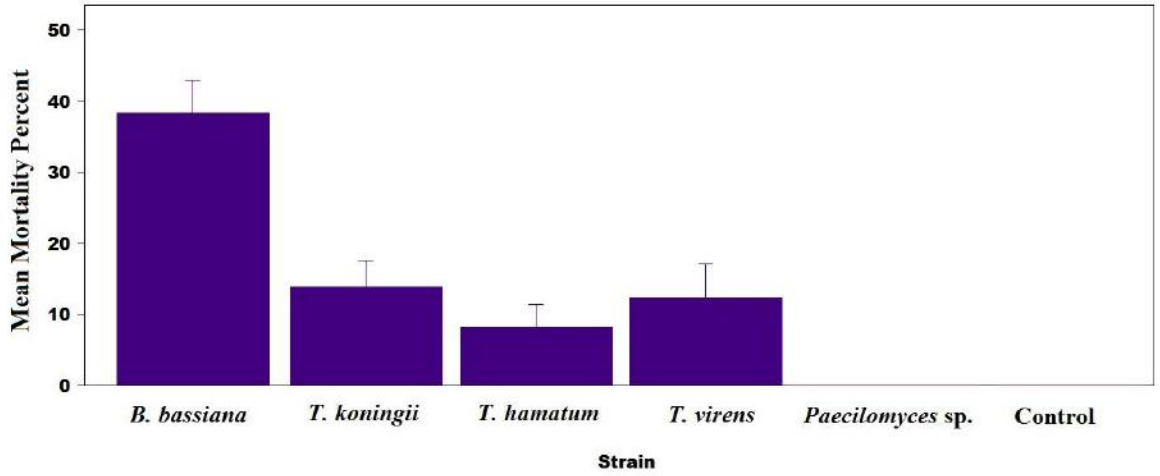

Fig. 1 Mean mortality percent of H. armigera treated with different fungal strain through dipping methods under laboratory conditions

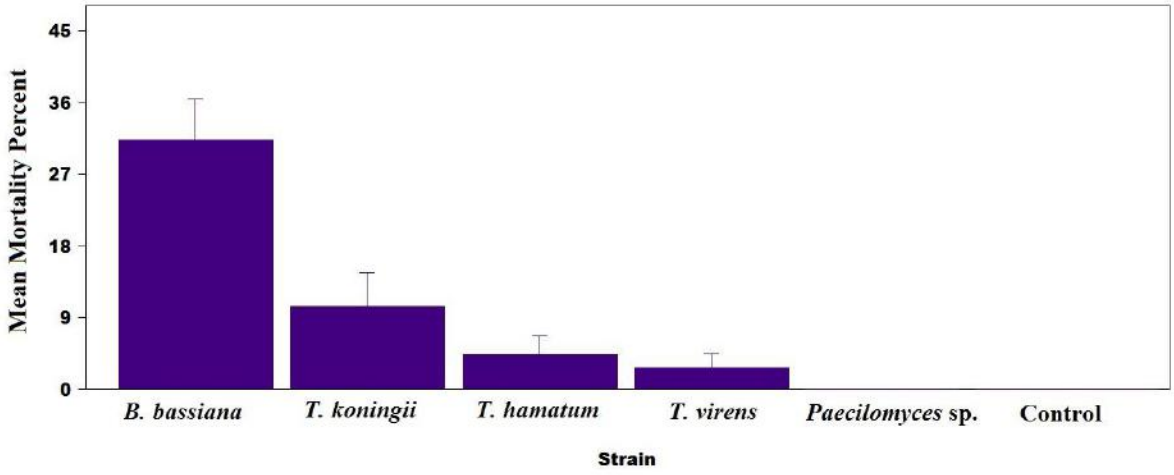

Fig. 2 Mean mortality percent of H. armigera treated with different fungal strain through poison food methods under laboratory conditions