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Insecticidal Potential of Eco-friendly Mycoinsecticides for the management of fall armyworm (*Spodoptera frugiperda*) under *in vitro* condition

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Abstract

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The invasive polyphagous pest Fall armyworm (FAW) *Spodoptera frugiperda*, (Lepidoptera: Noctuidae) is a migratory pest of maize crops in Asia and other countries including Pakistan. It is mostly fed on leaves at the larval stage this pest can be controlled by various types of pesticides globally but now resistance has come in pest against insecticides. So, we can also control this pest by other control strategies including physical, biological, and mechanical Eco-friendly Mycoinsecticides fungus *Beauveria bassiana, Trichoderma,* and *Metarhizium anisopliae* instead of chemical control method. By this, we can save our ecosystem and nature from the hazardous effects of chemicals. According to *in vitro* bioassay of entomopathogenic fungi like *Beauveria bassiana Trichoderma* and *Metarhizium anisopliae* under control conditions *Metarhizium anisopliae* was found very effective on fall armyworm 1st 2nd 3rd and 4th instar larvae and mortality percent was recorded 85.5% on 1st 2nd and 3rd instar larvae at 40ppm dose while minimum mortality was recorded 18.6% on 10ppm dose of Trichoderma during bioassay under control condition. During the second instar larvae of bioassay entomopathogenic fungi, *Metarhizium anisopliae* was recorded as very effective and showed mortality of FAW up to 83.3% at a dose of 40ppm after exposure of three days at 25°C approximately. While minimum mortality of FAW was recorded at 10 ppm after exposure for three days. In 2nd instar larvae, exposer *Metarhizium anisopliae* was found to have entomopathogenic potentials and Couse highest mortality of FAW the minimum mortality was recorded at 48% on 10ppm dose exposure of *M. anisopliae* after three days at 25°C and approximately 53% humidity. The studies show that the entomopathogenic potentials of fungi decrease with increasing larval instars. On 3rd and 4th instar larvae only *M. anisopliae* was recorded as effective and mitigated the larvae population 78.5% at 40 ppm and 55.8% mortality was observed on 4th instar larvae after exposure of three days among all *Metarhizium anisopliae* was found potential insecticidal properties as compare to *Beauveria bassiana and Trichoderma.* This comparative study shows that *M. anisopliae* was found highest insecticidal properties as compared to *Beauveria bassiana* and *Trichoderma.* So, *M. anisopliae* was found broad spectrum insecticidal potentials on FAW multiple larval stages under control conditions. Among all only *Metarhizium anisopliae* was found with exophytic sporulation characters and dynamic insecticidal potentials while *Beauveria bassiana* and *Trichoderma* were recorded as endo sporulation characters.

Keywords: Insecticidal potential, *Mycoinsecticide*s, endosporulation, FAW, Exophytic sporulation

Introduction

The fall armyworm (FAW) *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) is an invasive polyphagous pest, particularly damaging to corn. Although native to the American continent, it has spread to various African and Asian countries including Pakistan. However, no confirmed report of its presence has been made from Pakistan as it has already been reported from its neighbors India and China also distributed in tropical and subtropical regions (Pogue, 2002); This insect pest causes damage to several host plants (maize, peanuts, cotton, soybean, and forage grasses); however, the maize (Zea mays L.) constitutes the main agricultural crop preferred by *S. frugiperda* (Virla et al., 2008). The most serious damage produced by this pest is the continued consumption of the young shoots reducing the photosynthetic area of the plant. Currently, the most employed method to reduce populations of *S. frugiperda* is the spraying of chemical insecticides, but despite its fast mode of action, the larvae have developed resistance as an effect of this method of control, and it causes environmental pollution (Berón & Salerno, 2006); Furthermore, *S. frugiperda* larvae remain feeding inside the plant shoots reducing the contact with insecticides applied for their control (Braga et al., 2013).

Like other moths in the genus *Spodoptera*, FAW moths have both a migratory habit and a more localized dispersal habit. In the migratory habit, moths can migrate over 500 km (300 miles) before oviposition. When the wind pattern is right, moths can move much larger distances: for example, a flight of 1,600 km from the southern U.S. state of Mississippi to southern Canada in 30 hours has been recorded (Ramos et al., 2010).

Due to its rapid spread and distinctive ability to inflict widespread damage across multiple crops, FAW poses a serious threat to the food and nutrition security and livelihoods of hundreds of millions of farming households in SSA – particularly when layered upon other drivers of food insecurity (Paiva, 2013);

Pakistan to determine the presence and damage status of FAW on fodder and grain corn along with sorghum and millet. The presence of FAW was confirmed in all corn-growing districts of Sindh except the Jacobabad, Larkana, and Shikarpur districts of upper Sindh. The identification of FAW was done based on its morphological characters. More damage was recorded in fodder corn than in grain corn. The presence of FAW was also recorded from sorghum and millet. 100% damage was recorded on fodder corn in Shaheed Benazir Abad district. Although this is the first record of FAW from Sindh, Pakistan, a country-wide study along with molecular identification of FAW should be conducted to confirm its presence in corn-growing areas of Pakistan (Glial, 2020);

FAW is an invasive pest that can cause severe economic losses because of its high reproductive potential and intensive migratory behavior with high dispersal capability as adults can fly up to 100 km in a night. Therefore, an early and integrated management approach is required to reduce the damage of FAW. Moreover, FAW has several generations in a year and temperature has a significant role in its development. Its life cycle is as low as 30 days in summer but can be extended up to 60 days in winter. The eggs are laid in masses of 100-200 eggs on the upper surface of leaves (Ganiger et al., 2018).

The biological control use to reduce pest insect populations is an alternative to the use of synthetic insecticides technique. The use of entomopathogenic fungi and microbial control has some advantages over the use of chemical insecticides, which emphasizes the high specificity of the host and low environmental contamination. However, the production of biological control agents such as *Beauveria bassiana* Trichoderma and *Metarhizium anisopliae* was evaluated in this research, the mortality of fall armyworm (*Spodoptera frugiperda* J. E. Smith) in laboratory conditions by using concentrations of conidium from a native strain of *M. Beauveria bassiana* and *Trichoderma on* FAW multiple instars. Different concentrations of conidium 10% 20% and 40% were conducted where 96.6% and 86% were obtained and with 20% and 40% concentrations of a native strain of *M. anisopliae* and 36%, 70%, mortality was recorded from 10% of commercial of *Metarhizium* (Frank, 2019).

Therefore, keeping in view, the losses caused by the fall armyworm of maize the aim of the study is to find out the most effective and eco-friendly approach to minimize the losses and manage the insect pest in the field of Maize crop by using eco-friendly *Mycoinsecticides* for the management of Fall armyworm (S*podoptera frugiperda*) Invitro condition.

Material Method

Survey of infected fields

A survey of maize fields was conducted of districts Islamabad carried out to record the occurrence of FAW.

Disease incidence

During the survey, observations were recorded on the incidence of the FAW of Maize fields. The infection incidence was calculated according to the incidence formula

$$\textbf{Disease incidence (\%)} = \frac{\textbf{Number of effected plants}}{\textbf{Total Number of plants}} \times \textbf{100}$$

Identification of fall armyworm

It has been observed that females lay eggs in clusters; brownish yellow in color on the underside of the leaves and close to the stem and the same has been confirmed by (Osabutey et al., 2022). After emergence, young larvae are light greenish in color with a dark black head. The full-grown larvae are dark brownish having characteristic inverted 'Y' shaped marking in front of the head, elevated dim shaded dark spots (pinacula) all over their body along with spines, and a significant trapezoidal pattern of prominent four pinacula on the 9th abdominal segment. The pupae of *S. frugiperda* are ruddy dark colored having a normal cremaster with two spines at the end. The adults are greyish brown. The forewings of males are greyish brown, covered with oval or oblique orbital spots (marked with red) with a small distinct sideways v-shaped marking (marked with green) along with white patches at outer margins, whereas, hind wings are white with dark trimmings at edges (marked with blue). The forewings of females are uniformly greyish brown lacking distinct markings. The examination of male genitalia showed quadrate and broad valve; short clavus; costal process elongated, narrow, and straight with hair-like structure at its tip, juxta concave at the base with a dorsal process; ampulla slightly curved; a single lobed.

Bioassay of Entomopathogenic fungi on the different instar of FAW

Three different entomopathogenic fungi were tested under in-vitro conditions for their efficacy against the predominant pest Fall armyworm (*Beauveria bassiana Trichoderma* and *Metarhizium anisopliae*). The standard (aqueous) solution of these fungi was preparing Randomized block design (RBD) in 4 treatments and 3 replications briefly by (Steel et al., 1997). The doses of the entomopathogenic fungi were kept the same (10, 20, 40 ppm) from a stock solution of 10% 20% and 40% one of each dose of the entomopathogenic fungi was sprayed on maize stem, and kept with FAW larvae into Petri dishes at 25℃ and 52% humidity the growing margin of 6 days old culture of entomopathogenic fungi with the help of sterilized cork borer. Petri dishes containing only FAW and fresh maize stem and leaves without entomopathogenic fungi were used as control. All the Petri dishes were then transferred to an incubator at 25℃ for about 3 days. Mycelial growth of the fungus on insects was recorded on infected FAW larvae after 24, 48, and 72 hours of inoculation till 3 days of inoculation (Muhammad et al., 2019).

Inoculation of *Beauveria bassiana Trichoderma* and *Metarhizium anisopliae* the larvae of armyworm (*S. frugiperda*) First, second, third, and fourth stages used in this research brought from a CABI Biocontrol lab. The methodology treatments of inoculation consisted of placing 10 larvae in immersion in a spread petri dish, then the excess inoculum on larvae 30 was removed and placed in each plastic container with a semitransparent cover 25 mL capacity with lid, which has a circular window in the center to facilitate gas exchange and placed inside 5 g of diet 24,48 and 72 hours incubated at 25°C. In the case of the control, sterile distilled water was used. The design for this study was a randomized block, where three conidial concentrations (10, 20, and 40 ppm) with strains of *Beauveria bassiana Trichoderma* and *Metarhizium anisopliae* were compared. A total of 12 treatments were evaluated including control, each treatment had 36 replicates; thus, having a total of 432 each instar larvae of fall armyworm. Larvae were evaluated every 24 hrs. Starting from the first day after the experiment began. The dead larvae were selected separately in trays and were observed until it was detected sporulation of *M. anisopliae, Beauveria bassiana,* and *Trichoderma*. During the monitoring period was registered the number of live larvae and dead with sporulation was due to the effect of the entomopathogenic fungus among all only *Metarhizium anisopliae* were found with exophytic sporulation characters while *Beauveria bassiana* and *Trichoderma* were recorded endo sporulation characters. The mortality expressed in percent was determined by the following formula:

$$\% M = \frac{Lv}{P0} \times 100$$

In which: % M = Percentage of larval mortality
P0 = Initial Population
Lv = Dead larvae per treatment

Table 1. Doses of different Entomopathogenic fungi

No.	Entomopathogenic fungi	Dose in %
1.	*Metarhizium anisopliae*	i. 1 ppm 100 ml ii. 2 ppm 100ml iii. 4 ppm 100 ml
2.	*Beauveria basssiana*	i. 1 ppm 100 ml ii. 2 ppm 100ml iii. 4 ppm 100 ml
3.	*Trichoderma*	i. 1 ppm 100 ml ii. 2 ppm 100ml iii. 4 ppm 100 ml
4.	Control	Distil water 10 ml

Results and Discussion

A Survey of different fields of district Islamabad, Chak Shahzad, and NARC was carried out to observe the incidence of FAW in the maize fields of Islamabad. During the survey, it was observed that almost all the maize fields were suffering from severe attacks of pests like maize stem borer

and Spodoptera specie. Among all of them, the Fall armyworm was found most dominating insect throughout all the maize fields visited. The maximum incidence was recorded from Chakshahzad (60.0%) whereas, the incidence in NARC was a bit low as compared to Chak Shahzad 20% maximum incidence of FAW recorded from Chakshahzad was (60.0%). Fig 1.

Bioassay on FAW larvae by using entomopathogenic fungi

The study confirmed the *S. frugiperda* based on its morphological characters from various cornfield of Islamabad, Pakistan as comparatively more damage was recorded on fodder than grain corn crop. According to *in vitro* bioassay of entomopathogenic fungi like *Beauveria bassiana Trichoderma* and *Metarhizium anisopliae* under control conditions *Metarhizium anisopliae* was found very effective on Fall armyworm 1st 2nd 3rd and 4th instar larvae and mortality per-

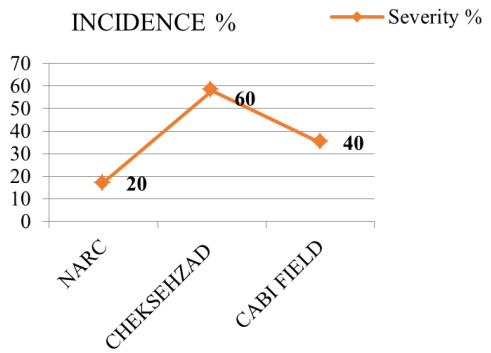

Fig 1. Infection incidence of the FAW in Maize fields from different localities of Islamabad recorded by CABI Plant Pathology laboratory Pakistan

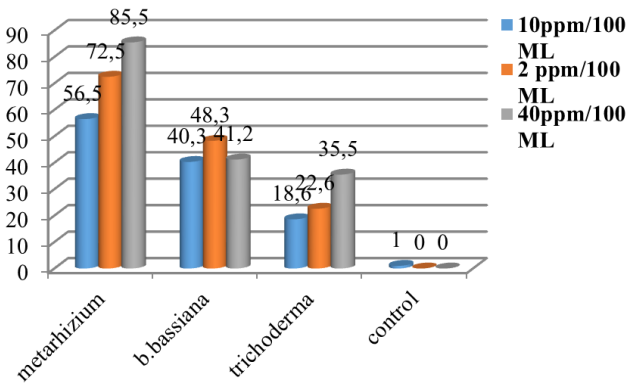

Fig 2. % Infestation Entomopathogenic fungi on FAW first instar larvae under control condition recorded by CABI plant pathology laboratory Pakistan

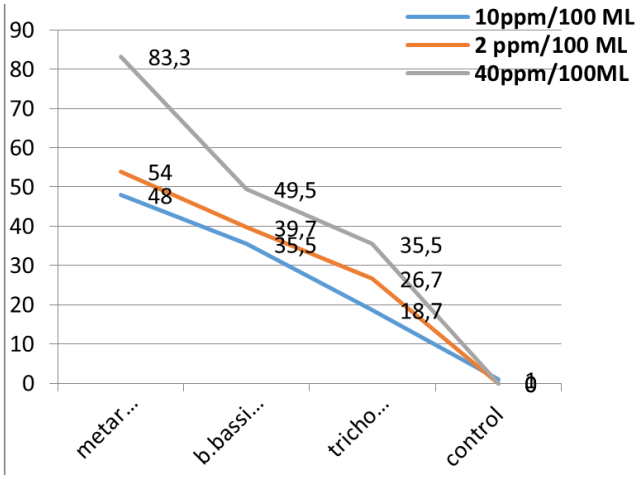

Fig. 3. %Infestation Entomopathogenic fungi on FAW 2nd instar larvae under control condition recorded by Riaz Mehmood Insect Biological Control Laboratory CABI-Regional Bioscience Centre Pakistan

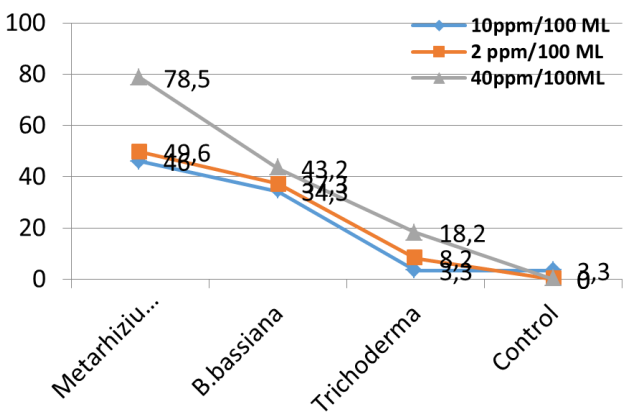

Fig. 4. %Infestation Entomopathogenic fungi on FAW 3rd instar larvae under control condition recorded by by Riaz Mehmood Insect Biological Control Laboratory CABI-Regional Bioscience Centre Pakistan

cent was recorded 85.5% on 1st 2nd and 3rd instar larvae at 40ppm dose while minimum mortality was recorded 18.6% on 10ppm dose of *Trichoderma* during bioassay under control condition Fig 2. During the second instar larvae of bioassay entomopathogenic fungi, *Metarhizium anisopliae* was recorded as very effective and showed mortality of FAW up to 83.3% at a dose of 40ppm after exposure of three days at 25°C approximately. While minimum mortality of FAW was recorded at 10 ppm after exposure for three days. In 2nd instar larvae, exposure to *Metarhizium anisopliae* was found

entomopathogenic potentials and Couse highest mortality of FAW the minimum mortality was recorded at 48% on 10ppm dose exposure of *M. anisopliae* after three days at 25°C and approximately 53% humidity. Show in Fig 3. The studies show that the entomopathogenic potentials of fungi decrease with increasing larval instars. On 3rd and 4th instar larvae only *M. anisopliae* was recorded as effective and mitigated the larvae population 78.5% at 40 ppm and 55.8% mortality was observed on 4th instar larvae after exposure of three days among all *Metarhizium anisopliae* was found potential insecticidal properties as compare to *Beauveria bassiana* and *Trichoderma* Fig 3, 4. These comparative studies show that *M. anisopliae* was found highest insecticidal properties as compared to *Beauveria bassline* and *Trichoderma.* So, *M. anisopliae* was found broad spectrum insecticidal potentials on FAW multiple larval stages under control condition.

Isolation and identification of Entomopathogenic fungi

Identification of *Metarhizium* anisopliae

The morphological identification of the strain was performed in order to confirm the taxonomic classification of the genre indicated by (Eberlein, 2010); for which were used the proposed keys by (Barnett and Hunter 1998). The CP-MA1 strain showed white and cottony colonies, that started green yellow and became dark green olive and crusted areas with abundant aerial mycelium white and deep yellow reverse; also presents conidiophore with whorls 2-3 branches each, with dark olive green tones that clarified to the apex, conidiogenous cells 6.0-10.0 (8,1µ) x 2.0 2.5 (2,1µ), subhead line to slightly green conidia, cylindrical to slightly ellipsoidal, 5.0-8.5 (6,6µ) x 2.0-3.0 (2,4µ). Among all the isolated fungi *Beauveria bassiana Trichoderma* and *Metarhizium anisopliae* were recognized on the bases of their size and shapes color, the shape of spores mentioned in the book ―identification of the fungi written by (Frank, 2005); and with the help of an electronic microscope, help from the senior Professors of the department Plant Pathology Arid and Agriculture University Rawalpindi was also taken in this regard (Fig. 5, 6, 7).

Efficacy of different entomopathogenic fungi on first second third and fourth instar of Fall armyworm (FAW) Spodoptera frugiperda

During the study, three different entomopathogenic fungi *Beauveria bassiana, Trichoderma,* and *Metarhizium anisopliae* were tested against *Spodoptera frugiperda the* invasive pest of maize for their efficacy under in-vitro conditions at different doses. Data were recorded on the regular basis from 24, 48, and 72 hours of inoculation till

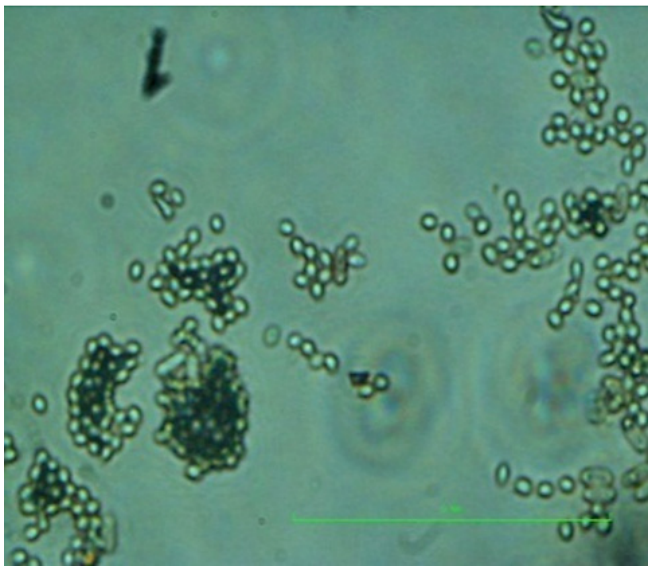

Fig. 5. Microscopic identification of *Metarhizium anisopliae* at 100MX lens at Aflatoxin Testing Laboratory CABI-Regional Bioscience Centre Pakistan

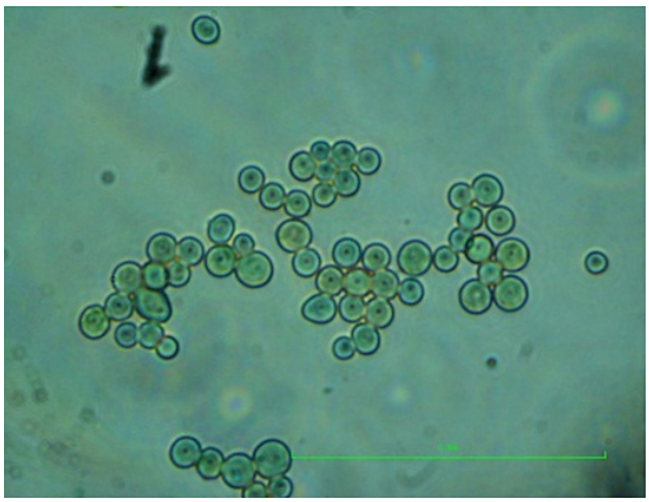

Fig. 6. Microscopic identification of *Beauveria basssiana* at 100MX lens at Aflatoxin Testing Laboratory CABI-Regional Bioscience Centre Pakistan

3 days of incubation at 25°C an average humidity of 42. Data were analyzed by using the Statistics 8.1 application of the computer which shows that all the used mycoinsecticides significantly reduced the pest of maize *S. frugiperda* ($p<0.000$). Among them, *Metarhizium anisopliae* was found more efficient in reducing the population of the FAW instars at their highest dose (96.6%) as compared to its lowest dose (33.33%) respectively, followed by *Beau-*

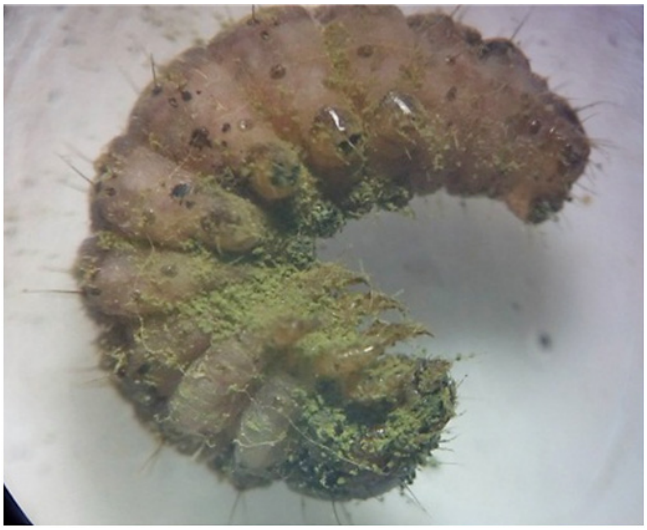

Fig. 7. *Metarhizium anisopliae* infected FAW larvae after three days inoculation at Aflatoxin Testing Laboratory CABI-Regional Bioscience Centre Pakistan

veria bassiana which reduce the population of FAW at its highest dose (90.33 mm) and at the lowest dose (50.00 %) whereas, *Trichoderma* was less effective in a population of the FAW as compared to *Beauveria bassiana* and *Metarhizium anisopliae* which decrease the FAW population at the highest dose (96.6%) and at the lowest dose (70.3%), while, *Trichoderma* at its highest dose mitigate the population of FAW (15.66%) and at the lowest dose (3.33%). All the entomopathogenic fungi at their respective doses significantly retarded the population of FAW as compared to the control (Muhammad et al., 2019).

Conclusions and Recommendations

The study confirmed the insecticidal potential of entomopathogenic fungi *Metarhizium anisopliae* against *S. frugiperda* Based on its morphological and insecticidal characteristics recorded on 1st 2nd 3rd and 4th instar larvae under control conditions at CABI plant pathology lab Rawalpindi Pakistan. *M. anisopliae* comparatively showed more damage and the highest mortality was recorded on all instars of FAW. However, further studies and molecular identification should be done for more potential properties of Metarhizium. Among all *Metarhizium anisopliae* was found potential insecticidal properties as compared to *Beauveria bassiana and Trichoderma.* So, *M. anisopliae* was found broad spectrum insecticidal potentials on FAW multiple larval stages under control condition

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Data analysis

Data were analyzed by using the Statistics 8.1 application of the computer which shows that all the used entomopathogenic fungi significantly reduced the fall armyworm population ($p < 0.000$).

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