Biological and Clinical Sciences Research Journal
eISSN: 2708-2261; pISSN: 2958-4728
www.bcsrj.com
DOI: https://doi.org/10.54112/bcsrj.v2024i1.1036
*Biol. Clin. Sci. Res. J., Volume, **2024**: 1036*
Original Research Article

BIO MANAGEMENT OF FALL ARMYWORM USING *METARHIZIUM ANISOPLIAE*

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(Received, 2nd April 2024, Revised 8th July 2024, Published 20th July 2024)

Abstract *Agriculture is the backbone of Pakistan. However, the recent threat to cash crops of Pakistan is fall armyworm (Spodoptera frugiperda). The farmers used various types of insecticides for the effective management of fall armyworm. Due to the environmental hazards associated with the pesticides, it is necessary to reduce the use of synthetic pesticides and develop novel strategies for the management of insect pests especially fall armyworm. The diseases caused by fungi in insects commonly; reduce populations significantly, demonstrating that bio-insecticides may be a viable option for solving the problems of insect pests in agriculture. Among the fungi used as biological insecticides Metarhizium anisopliae has been widely used. Therefore, the current research study was conducted to manage the fall armyworm using Metarhizium anisopliae. The fungus was cultured under lab conditions and various concentrations were prepared. The four concentrations with three replications were applied on the larval or pupal stages of fall armyworm by using dip and foliar application methods. The mortality was recorded after 24, 48, 72 and 96 hours of application. The data were arranged and analyzed using suitable statistical software. The homogenous mean was analysed using Tuckey HSD (p<0.05). The results showed that the higher concentration of M. anisopliae was more effective as compared to lower concentrations both in lab and field conditions. The highest percentage mortality of 3rd, 4th, 5th and late instar larvae of fall armyworm was recorded as 98.663±0.66, 93.550±0.22, 87.333±0.14 and 84.440±0.11 %, respectively, after the treatment of 1×108 conidia/ml and 12 days of exposure period. After 12 days the maximum reduction in FAW population was recorded (0.500±0.28 larvae/five plants) by the treatment of EPF (1×108 conidia/ml) after 12 days of exposure, which was significantly different form other treatments. The minimum reduction of fall armyworm larvae was recorded (3.00±1.22 larvae/ five plants) after the treatment of EPF at 1×106 conidia/ml. In conclusion, the Metarhizium anisopliae considered as ecofriendly approach to manage the fall armyworm in maize crop and can be used in integrated pest management programs.*

Keywords: *fall armyworm; bio-insecticides; Metarhizium anisopliae; mortality; ecofriendly; integrated pest management*

Introduction

For the production of maize (*Zea mays*) crop the farmers faced significant challenges in the form of biotic and abiotic stresses. The fall armyworm (*Spodoptera frugiperda*) (FAW) is considered as the polyphagous insect pest that causes substantial damage to maize plants. It is native to subtropical and tropical areas of America and now distributed to approximately 70 countries of the world including Africa, Europe, Australia and Asia (Yainna et al., 2021). It was first time reported in Pakistan in 2019. The fall armyworm not just attack the maize crop, it also feeds on the other economically important crops like cotton, soybean, sorghum and rice and causes yield losses (Helen et al., 2019).

The fall armyworm feeds on the reproductive parts of plants, stem and leaves (Tefera et al., 2019). Infestations and symptoms begin with the larval stage, which produces various-sized papery holes in leaflets. This causes severe loss of foliage of plant. The infestation of fall armyworm can cause yield losses up to 70%. In recent years, *S. frugiperda* has severely damaged corn in Africa and India, producing economic hardship to commercial and small-scale farmers.

Mainly, various types of pesticides are used to manage the FAW. The highest use of pesticides causes pest resistance, as well as impact the beneficial wildlife (Romero-Arenas et al., 2014). It is suggested that fewer traditional pesticides be used and that new IPM techniques, primarily based on biological methods, be developed instead. This is because biological control is a more sensible and environment friendly way to control pests (Badii et al., 2006). As over use of pesticides causes serious problems to the natural environment. There is a need to select elective techniques for example in organic farming use of entomopathogenic fungi, use of new chemistry pesticide as potential management strategies for FAW (Mas et al., 2019).

For control of forest insect pests and agricultural pests entomopathogenic fungi have been widely used (Maniania, 1993). Entomopathogenic fungi are available all over the world as the natural controller mediator (Wraight et al., 2001). The effective application of these microbial agents depends on extent of the techniques that are compatible with the other tactics of crop protection. Against various types of insect's pest, the EPF considered as the sustainable approach when used with some novel chemistry insecticides in IPM control strategies. Among the fungi used as biological insecticides are included *Beauveria bassiana* and *Metarhizium anisopliae* (Monzon et al., 2008). The M. anisopliae have significant effect against approximately 200 species of insect's pests related to orders Coleoptera, Dermaptera, Hemiptera, Hymenoptera and Orthoptera and others (Panfilio, 2008). This species is widely used for the management of different kinds of insect pests in soil and above the ground (Thaochan and Sausa-Ard, 2017). Because of its wide variety of potential hosts, friendly to the environment, and simplicity of production, Metarhizium spp. have been examined against several insect pests (Greenfield et al., 2015). The Metarhizium (an insect pathogenic fungus) is highly effective and used in different biological control strategies (Clifton et al., 2018). Considering the active role of EPF against insect pests, the current study was conducted for the "Bio management of fall armyworm using *Metarhizium anisopliae*".

The objectives of current studies were;

- To evaluate the impact of Metarhizium anisopliae against the fall armyworm.
- To test the effectiveness of fungus to control the fall armyworm

Materials and methods

The current study was conducted to evaluate the impact of Metarhizium anispliae in the control of fall armyworm.

Mass Culture of Fall Armyworm

Culture of *S. furgiperda* larvae was taken from the fields, flushed, cleaned, and moved onto young leaves of maize seedlings. The larvae feeding on maize leaves were transferred into a growth chamber at a temperature of 24°C and a relative humidity of 75-10% (Fig. 1). Pupae were taken and placed on a petri dish. For sexual maturity, petri plates having pupae were placed in jars. Adults were given open plastic jars with white net covering on top, which contained a food source consisting of wet cotton soaked in a 10% (v/v) honey solution. After two days of adult emergence egg-laden was recorded and egg-laden netting were exchanged. This process was continued unless egg production decreased there was no more eggs. Smaller patches were cut from netting and deposited into 200 ml circular plastic containers with a plastic lid to provide a safe environment until the eggs hatch. The eggs were gathered and maintained to see if they would hatch. For all bioassays, a homogeneous population were used (Yan et al., 2020).

Figure 1: Rearing of Fall armyworm under Laboratory conditions (a & b)

Metarhizium anisopliae

Pacer (India) (*Metarhizium anisopliae*) was used against fall armyworm. Different concentration of Metarhizium anisopliae was applied to assess its effect against fall armyworm.

For the commercial use of *Metrhizium anisopliae* in order to make a specific product, strains of the fungus were collected and cultured from different infected insect pests. Through a process called biofermentation, the product was prepared. To make the fungus into a liquid form (sprayable), the conidia (spores) of *Metrhizium anisopliae* were extracted (Fig. 2). The concentration of substainable spores of *Metrhizium anisopliae* in the liquid spray should be 2.5×10. The action and infection of the fungal spores (conidia) become increased with high rate of humidity and water. The solar rays have adverse effect on the action of fungal spores.

Mode of Action

When the insect pests come into contact with the spores (conidia) of *Metarhizium anisopliae*, it kills them. The contact between the fungus and the insect pest was made by different methods. The most operative and common method was that the droplets of the fungus which were in spray form falls on to the insect pest or the pest itself walked to the surface of plats be treated by the fungus. As the spore of fungus (conidia) adhered to the cuticle of the pest, germination of fungal spores started and the protruded hyphae of the fungus penetrated into the body of pest and then spreaded to the whole body of the insect.

Figure 2: Preparation of Metarhizium anisopliae solution for application

Death of the insect pest infected with fungus occured within three to five days. Second proliferation of the fungus occured as the dead insect pest played role as a source of spread. The male infected adult insect also played role in the transmission of fungus while mating. As, the action and infection of the fungal spores (conidia) become increased with high rate of humidity and water. The solar rays had adversely effect on the action of fungal spores. For field application, the plants infested with insect pests were sprayed in the dawn and dusk considering cool and moderate temperature (Butt and Goettel et al., 2000).

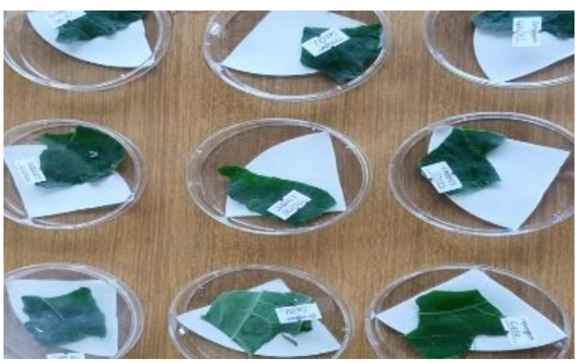

Figure 3: Lab trial of the effect of EPF on fall armyworm

The application of the fungus (Metarhizium anisopliae) in the in vivo (field) conditions was done on that parts of the plant where pests frequently occur such as on the undersides of the leaves and on the top of leaves (Fig. 4). The fungus was applied in a way that all parts of the plant containing pests were covered. The life cycle of the spores is comparatively short, so the spray should be enough to contact properly with the insect pest.

The efficacy of the fungus to cause mortality of the insect pests depends upon the environmental conditions, vulnerability of the insect pest, age of the pest and number of conidia to which the insect pest makes contact properly.

Bioassays under lab conditions

The data regarding the mortality of FAW was recorded under the laboratory conditions at 3rd, 4th and late-stage larval instar after the 3, 6, 9 and 12 days of Metarhizium anisopliae application. Three of Metarhizium anisopliae were tested against larval armyworm. The three concentrations of Metarhizium anisopliae were prepared with three replications. The larvae of FAW were placed in plastic cup with artificial diet and the Metarhizium anisopliae solution were sprayed of the diet. The mortality of FAW was recorded after 3, 6, 9 and 12 days of treatments. The mortality data were subjected to statistical analysis at significance level of 5%. The percentage mortality was calculated using formula.

Mortality (%) = (Number of dead learve after treatment)/(Total numbers of larvae) ×100

Bioassay in Field

The data regarding the field application and mortality of FAW under the field/in vivo conditions was recorded on 10 days after the first application and secondly after 7 days of 2nd EPF conidial application. The field application was done using Knap-sack sprayer. The dose rate of applied conidial suspension was 2.5×10.

Figure 4: Application of Metrhizium anisopliae on maize crop and late-stage data collection.

Statistical analysis:

The data was analysed statistically by statistical software. The mortality was recorded after 24, 48, 72 and 96 hours of application. The data were arranged and analyzed using suitable statistical software. The homogenous mean was analysed using Tuckey HSD ($p<0.05$).

Results

The current research study was conducted to check the effect *Metarhizium anisopliae* on fall armyworm. The fungus was cultured under lab conditions and various concentrations were prepared. The three concentrations with three replications were applied on the different larval stages of fall armyworm by using leaf dip and foliar application methods. The mortality was recorded after 24, 48, 72 and 96 hours of application. The data were arranged and analyzed using suitable statistical software. The homogenous mean was analysed using Tuckey HSD (p<0.05).

Mortality of 3rd instar larvae of fall armyworm after 3, 6, 9 and 12 days of *Metarhizium anisopliae* application

The analysis of variance showed that the entomopathogenic fungus *Metarhizium anisopliae* had significant effect against fall armyworm after 3, 6, 9 and 12 days of treatment (Table 1). All the treatments were significantly different from each other's at each interval (Table 2). The results furthermore showed that higher concentrations of entomopathogenic fungi (*Metarhizium anisopliae*) caused high mortality as compared to lower concentrations.

The maximum percentage mortality was observed (98.663±0.14 %) after the treatment of 1×10^8 and 12 days of exposure period, which was significantly different from other treatments. The mortality was observed as (81.33±0.28 %) after the treatment of 1×10^7 conidia/ml followed by mean percentage mortality 71.997±0.28 % by the treatment of *Metarhizium anisopliae* at 1×10^6 conidia/ml. The mortality was recorded 6.660 % in control after 12 days of interval.

Mortality of 4th instar larvae of fall armyworm after 3, 6, 9 and 12 days of *Metarhizium anisopliae* application

The ANOVA from table (3) showed that the different concentrations of entomopathogenic fungi (*Metarhizium anisopliae*) had significant effect on fall armyworm larvae after 3, 6, 9 and 12 days of treatment. All the treatments were significantly different from each other's (Table 4). The results further exhibited that higher the concentrations of EPF higher the mortality. The maximum mortality was recorded (93.550±0.22 %) by the treatment of EPF at the rate of (1×10^8 conidia/ml), after 12 days of exposure which was significantly different form other treatments. The mortality was noticed (87.770±1.11 %) after the treatment of EPF at 1×10^7 conidia/ml, followed by the 78.670±1.33 % by the treatment of EPF @ of 1×10^6 conidia/ml concentrations. The mortality was recorded 13.333 % in control after 12 days of exposure period.

Mortality of 5th instar larvae of fall armyworm after 3, 6, 9 and 12 days of *Metarhizium anisopliae* application

The ANOVA from table 5 exhibited that the different concentrations of entomopathogenic fungi (*Metarhizium anisopliae*) had significant effect on fall armyworm larvae after 3, 6, 9 and 12 days of treatment. All the treatments were significantly different from each other's (Table 6). The results further revealed that higher the concentrations of EPF higher the mortality. The maximum mortality was recorded (87.333±0.14 %) by the treatment of EPF at the rate of (1×10^8 conidia/ml), after 12 days of exposure which was significantly different form other treatments. The mortality was noticed (76.000±0.43 %) after the treatment of EPF at 1×10^7 conidia/ml, followed by the 68.888±0.47 % by the treatment of EPF @ of 1×10^6 conidia/ml concentrations. The mortality was noticed as 6.667 % in control after 12 days of period.

Table 1: Mean sum of squares of percentage mortality of late-stage 3rd instar larvae of fall armyworm to different concentrations of *Metarhizium anisopliae* at 3, 6, 9 and 12 days after exposure (DAE)

Source	DF	3 DAE	6 DAE	9 DAE	12 DAE
Treatments	3	1113.17**	1690**	3302.58**	5256.94**
Error	8	5.55	12.44	5.03	3
Total	11				

Table 2: Pair wise comparison of mortality (%) of late-stage 3rd instar larvae of fall armyworm larvae to different concentrations of *Metarhizium anisopliae* (Tukey HSD, p<0.05) at 3, 6, 9 and 12 days after exposure (DAE)

Treatments	Mean (%) mortality after 3DAF	Mean (%) mortality after 6DAF	Mean (%) mortality after 9DAF	Mean (%) mortality after 12DAF
T0 Control	0.000±0.00D	0.000±0.0C	6.660±0.57D	6.660±0.00D
T1 (1×10^6)	25.327±1.33C	34.663±0.28B	62.220±0.47C	71.997±0.28C
T2 (1×10^7)	36.663±1.92B	46.660±0.81A	71.997±0.28B	81.333±0.28B
T3 (1×10^8)	44.000±1.38A	53.330±0.57A	80.000±0.0A	98.663±0.14A

Table 3: Mean sum of squares of percentage mortality of 4th instar larvae of fall armyworm to different concentrations of *Metarhizium anisopliae* at 3, 6, 9 and 12 days after exposure (DAE)

Source	DF	3 DAE	6 DAE	9 DAE	12 DAE
Treatments	3	2031.66**	3076.73**	2767.9**	4344.94**
Error	8	2.67	4.03	17.05	1.81
Total	11				

Table 4: Pair wise comparison of mortality (%) of late-stage 4th instar larvae of fall armyworm larvae to different concentrations of *Metarhizium anisopliae* (Tukey HSD, p<0.05) at 3, 6, 9 and 12 days after exposure (DAE)

Treatments	Mean (%) mortality after 3DAF	Mean (%) mortality after 6DAF	Mean (%) mortality after 9DAF	Mean (%) mortality after 12DAF
T0 Control	0.0000±0.00D	0.0000±0.00D	6.666±0.00D	13.333±0.00D
T1 (1×10⁶)	31.111±2.11C	48.888±2.22C	56.667±0.70C	78.670±1.33C
T2 (1×10⁷)	45.333±1.33B	52.444±0.88B	68.883±2.22B	87.770±1.11B
T3 (1×10⁸)	52.663±0.66A	65.333±1.33A	76.663±1.92A	93.550±0.22A

Table 5: Mean sum of squares of percentage mortality of 5th instar larvae of fall armyworm to different concentrations of *Metarhizium anisopliae* at 3, 6, 9 and 12 days after exposure (DAE).

Source	DF	3 DAE	6 DAE	9 DAE	12 DAE
Treatments	3	1841.31**	3301.75**	2849.37**	3734.58**
Error	8	8.83	17.31	5.12	4.03
Total	11				

Table 6: Pair wise comparison of mortality (%) of 5th instar larvae of fall armyworm larvae to different concentrations of *Metarhizium anisopliae* (Tukey HSD, p<0.05) at 3, 6, 9 and 12 days after exposure (DAE)

Treatments	Mean (%) mortality after 3DAF	Mean (%) mortality after 6DAF	Mean (%) mortality after 9DAF	Mean (%) mortality after 12DAF
T0 Control	0.0000±0.00D	0.0000±0.00D	0.000±0.00D	6.667±0.00D
T1 (1×10⁶)	38.440±2.81C	46.667±2.13C	51.111±0.47C	68.888±0.47C
T2 (1×10⁷)	46.667±0.11B	53.330±1.12B	62.666±2.03B	76.000±0.43B
T3 (1×10⁸)	56.663±1.92A	68.883±2.22A	76.888±0.22A	87.333±0.14A

Mortality of late instar larvae of fall armyworm after 3, 6, 9 and 12 days of *Metarhizium anisopliae* application

The ANOVA from table 7 exhibited that the different concentrations of entomopathogenic fungi (*Metarhizium anisopliae*) had significant effect on fall armyworm larvae after 3, 6, 9 and 12 days of treatment. All the treatments were significantly different from each other's (Table 8). The results further revealed that higher the concentrations EPF higher the mortality. The maximum mortality was observed (84.440±0.11 %) by the treatment of EPF at (1×10^8 conidia/ml) after 12 days of exposure, which was significantly different form other treatments. The mortality was noticed (75.550±2.22 %) after the treatment of EPF at 1×10^7 conidia/ml followed by the 66.667±0.57 % by the treatment of EPF @ the 1×10^6 conidia/ml concentrations. The mortality was recorded 13.33% in control after 12 days of period.

Field application of *Metarhizium anisopliae*

Population of fall armyworm (FAW) in maize field before treatment

The survey was done at the entomological research area, University of Agriculture, Faisalabad to check the population status and for the division of field into blocks and sub blocks to using randomized complete block design (RCBD) layout. The random sampling method was used and numbers of FAW larvae per plant were counted. In block one the numbers of FAW larvae were recorded 4.10±0.11 larvae/ five plant, inside whorl and infesting cob etc. In block two the numbers of average larvae were recorded as 2.80±0.57 larvae/ five plant and in block 3 the numbers of average larvae were recorded as 3.00±0.75 larvae/ five plant (Figure 5).

Table 7: Mean sum of squares of percentage mortality of late instar larvae of fall armyworm to different concentrations of *Metarhizium anisopliae* at 3, 6, 9 and 12 days after exposure (DAE)

Source	DF	3 DAE	6 DAE	9 DAE	12 DAE
Treatments	3	2510.8**	3317.58**	3692.39**	3781.02**
Error	8	1.63	17.94	7.88	11.15
Total	11				

Table 8: Pair wise comparison of mortality (%) of late instar larvae of fall armyworm larvae to different concentrations of *Metarhizium anisopliae* (Tukey HSD, p<0.05) at 3, 6, 9 and 12 days after exposure (DAE)

Treatments	Mean (%) mortality after 3DAF	Mean (%) mortality after 6DAF	Mean (%) mortality after 9DAF	Mean (%) mortality after 12DAF
T0 Control	0.0000±0.00D	0.0000±0.00D	6.660±0.00D	13.33±0.00D
T1 (1×10^6)	44.440±2.22C	55.550±0.47C	64.440±0.47C	66.667±0.57C
T2 (1×10^7)	54.440±1.11B	62.667±0.43B	72.220±0.62B	75.550±2.22B
T3 (1×10^8)	62.222±2.11A	71.111±2.02A	82.222±0.94A	84.440±0.11A

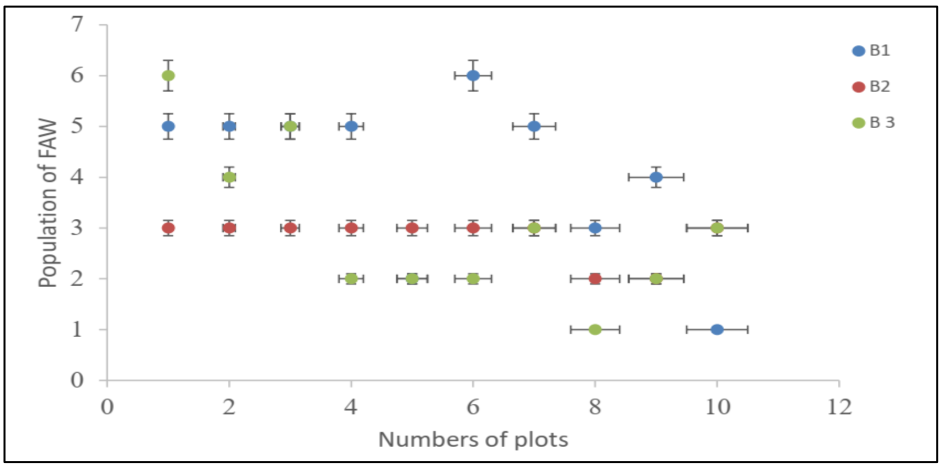

Figure 5: Mean ± S.E of fall armyworm population at different spots in maize field.

Application of *Metarhizium anisopliae* on maize

The ANOVA from table 9 exhibited that the different concentrations of entomopathogenic fungi (*Metarhizium anisopliae*) had significant effect on fall armyworm larvae after 7 and 10 days of treatment. All the treatments were significantly different from each other's.

The maximum reduction in FAW population was recorded (0.800±0.28 larvae/five plants) by the treatment of EPF at (1×10^8 conidia/ml) after 7 days of exposure, which was significantly different form other treatments. The highest numbers of FAW larvae were noticed (3.30±1.22 %) after the treatment of EPF at 1×10^6 conidia/ml (Fig. 6).

The maximum reduction in FAW population was recorded (0.500±0.28 larvae/five plants) by the treatment of EPF at (1×10^8 conidia/ml) after 10 days of exposure, which was significantly different form other treatments. The highest numbers of FAW larvae were noticed (3.00±1.22 %) after the treatment of EPF at 1×10^6 conidia/ml (Fig. 7).

Table 9: Analysis of variance of numbers of larvae of FAW after 10 days of exposure to different concentrations of *Metarhizium anisopliae* under field conditions.

Source	DF	7 DAE	10 DAE
Replications	9	0.5778	0.163
Treatments	2	15.63**	17.43**
Error	18	0.4111	0.6185
Total	29		

[Citation: Rehman, H.U., Sahi, G.M., Ali, Q., Sagheer M (2024). Bio Management of Fall Armyworm using *Metarhizium anisopliae*. *Biol. Clin. Sci. Res. J.*, **2024**: https://doi.org/10.54112/bcsrj.v2024i1.1036]

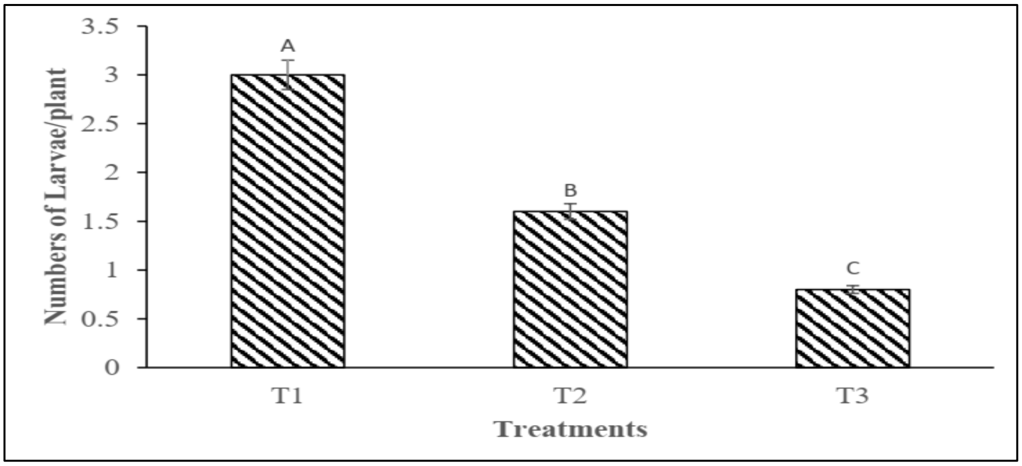

Figure 6: Mean ± S.E of mortality percentage showed that effect of Metarhizium anisopliae on fall armyworm after 7 days of exposure period under filed conditions, bar with uppercase letter of are significantly different from each other's Tukey HSD (P<0.05). The vertical bars indicated standard error. Whereas T0: control, T1: 1×10^6 conidia/ml, T2: 1×10^7 conidia/ml and T3: 1×10^8 conidia/ml.

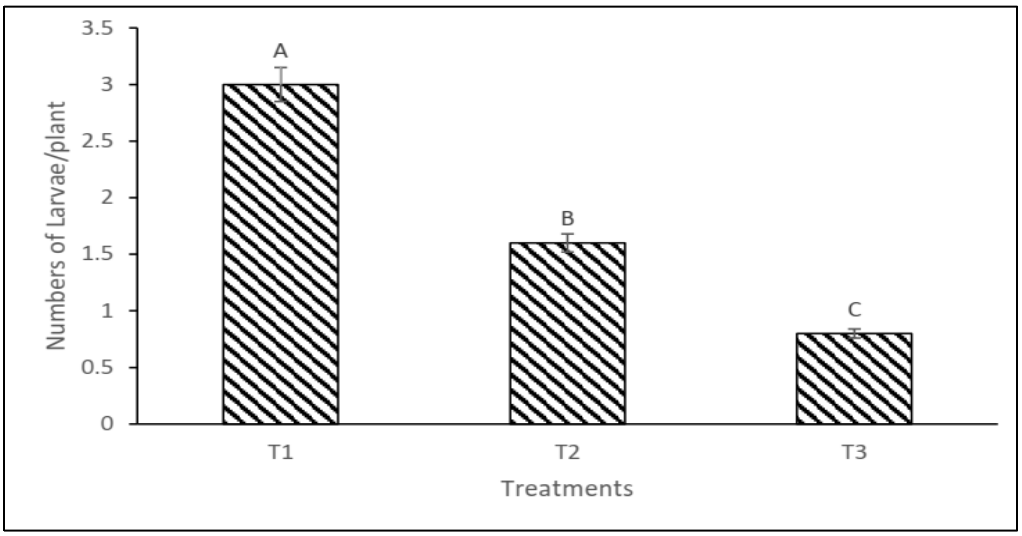

Figure 7: Mean ± S.E of mortality percentage showed that effect of *Metarhizium anisopliae* on fall armyworm after 10 days of exposure period under filed conditions, bar with uppercase letter of are significantly different from each other's Tukey HSD (P<0.05). The vertical bars indicated standard error. Whereas T0: control, T1: 1×10^6 conidia/ml, T2: 1×10^7 conidia/ml and T3: 1×10^8 conidia/ml.

Discussion

The *Spodoptera frugiperda* considered as the destructive insect pest related to Nuctuidae family as well as Order Lepidoptera. It feed on approximately every kind of cereal crop (Baudron *et al*., 2019) and produce economic loses. It mostly effects the maize, cotton, rice, sorghum and different vegetable crops. This pest is also considered as one of the among other pests which ultimately effect the food security (Bateman *et al.*, 2018). As maize plants are attacked by this insect in their early stages, the output of the crop might decrease by up to 70% (Ayala *et al*., 2013; Hruska, 2019). Mainly, various types of pesticides are used to manage the FAW, meanwhile, the highest use of pesticides causes pest resistance, as well as impact the beneficial wildlife (Romero-Arenas *et al*., 2014). It is suggested that fewer traditional pesticides be used and that new IPM techniques, primarily based on biological methods, be developed instead. This is because biological control is a more sensible and environmentally friendly way to control pests (Badii *et al*., 2006).

Entomopathogenic fungi are a group of microorganisms with over 700 species within 90 genera that can infect insects (Goettel *et al*., 2005; Roy *et al*., 2006). The *M. anisopliae* have significant effect against approximately 200 species of insect pests related to orders Coleoptera, Dermaptera, Hemiptera, Hymenoptera and Orthoptera and others (Panfilio, 2008). This species is widely used for the management of different kinds of insect pests in soil and above the ground (Thaochan and Sausa-Ard, 2017). Because of its wide variety of potential hosts, friendly to the environment, and simplicity of production, *Metarhizium spp*. have been examined against several insect pests (Greenfield *et al*., 2015). Thus, to keep in mind the role of EPF against insect pests, the current study was conducted with the aim Bio management of fall armyworm using *Metarhizium anisopliae*.

The result of present study showed that higher mortality was recorded 44.000±1.38, 53.330±0.57, 80.000±0.0 and 98.663±0.66 % after the treatment of *Metarhizium anisopliae* at 1×10^8 conidia/ml after 3, 6, 9 and 12 days of exposure period. The 3rd and 4th stage larvae were more susceptible to EPF than the higher/ late stages. The results further revealed that higher the concentration of entomopathogenic fungi higher the percentage mortality. The current study agreement with Kumar *et al*., (2021) who state that the best mortality of FAW was recorded 67.67 percent after the treatment of EPF M. anisopliae @ of 5ml/l concentration. The present study exhibited that the rate of mortality directly proportional to exposure period and time. The Karthi *et al*. (2019) also found that fungal mycotoxins of M. anisopliae and B. bassiana effectively decreed the growth rate of lepidopterans pests and it is depending on exposure time. The results obtained were in agreement with Al-Deghairi (2008) who also use M. anispliae and B. bassiana against whitefly, while the plant and insect species vary form present study. Similarly, Poprawski et al., (2000) evaluated that the 3rd instar larvae of Trialeurodes vaporariorm were highly vulnerable to EPF strains B. bassiana and M. anisoplae on cucumber plants. However, Cuthbertson et al. (2005) reported that these same species of pathogenic fungus are more effective against 2nd instar than other stages. The rate of infection also redends on the concentration of EPF conidia, higher the concentration of conidia higher the mortality percentage (Samuel et al., 2002; Al-Deghairi, 2008). The present results further showed that use of entomopathogenic fungi against fall armyworm is a good alternative approach to manage the lepidopterans pests both under laboratory and field conditions. Similarly, Denisse *et al*. (2016) stated that the treatment of EPF such as *Beauveria bassiana* conceded as an effective and sustainable approach against different insect pests and also a good alternative of chemical pesticides.

Even though there were some signs of a fungal infection as early as the third day following inoculation, the number of dead larvae kept rising until 12 days later. Twelve days following the application, there was no more rise in mortality. The conclusion implies that a lengthy incubation period had to be used prior to evaluating the results. An essential stage in the fungal infection process is the development and infiltration of fungal spores on insect cuticles (Abdel-Baky *et al*., 2005). Variations in the production of enormous amounts of cuticle lipids, particularly long chain wax esters, may account for changes in mortality or interspecific variance in fungus (James, 2001). The fungal spore penetrated and inhibited the thick waxy layer of insect cuticle. Brownbridge *et al* (2001) shown that B. bassiana's virulence is increased upon invasion of Bemisia argentifolli Bellows and Perring, and that it becomes less virulent upon repeated sub-culturing on synthetic medium.

The current study showed that the field trial of EPF showed that different concentrations of entomopathogenic fungi (*Metarhizium anisopliae*) had significant effect on fall armyworm larvae after 5 days of treatment. All the treatments were significantly different from each other's. The maximum reduction in FAW population was recorded (0.700±0.28 larvae/five plants) by the treatment of EPF at (1×10^8 conidia/ml) after 5 days of exposure, which was significantly different form other treatments. The highest numbers of FAW larvae were noticed (3.30±1.22 %) after the treatment of EPF at 1×10^6 conidia/ml. The present study is in the agreement with Dhobi *et al*. (2020) who evaluated the effectiveness of various biopesticides against fall armyworm on maize crop. They reported that the lower numbers of larval population were noticed 1.81 larvae/ 10 plants and lowest plant damage were recorded as 17.70 percent and cob damage were recorded 15.19 % by the use of biopesticides. The effectiveness of EPF in field mainly supported to some extent by the laboratory bioassay for example some labs tested *M. anisopliae* and *B. Bassiana* isolates were proved to be the maximum pathogenicity to adult female and eggs laying ability of lepidopterans. Similarly, the studies exhibited that eggs of stable fly, *Stomoxys calcitrans* and fall armyworm were highly susceptible to insect pathogenic fungus *M. anisopliae* as compared to *Paecilomyces javanicus*, respectively (Moraes et al., 2008). The trial conducted by Shi and Feng, (2004) seated that egg laying ability of spider female reduced by the application of EPF isolates (Shi *et al*., 2008).

Conclusion

The study demonstrated that *Metarhizium anisopliae* is an effective biological control agent against the fall armyworm (*Spodoptera frugiperda*), with significant mortality observed at higher concentrations and extended exposure periods. Third and fourth instar larvae were particularly susceptible, supporting the potential of this entomopathogenic fungus in integrated pest management (IPM) strategies. Field trials confirmed the efficacy of different concentrations of *M. anisopliae*, showing substantial reduction in FAW populations. Overall, the use of *M. anisopliae* presents a sustainable and environmentally friendly alternative to chemical pesticides for managing lepidopteran pests.

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Declaration

Ethics Approval and Consent to Participate
Not applicable.

Consent for Publication
The study was approved by authors.

Funding Statement
Not applicable

Conflict of Interest
There is no conflict of interest among the authors regarding this case study.

Authors Contribution
All authors contributed equally.