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RESEARCH

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Effect of *Metarhizium anisopliae* on the nutritional physiology of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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Abstract

Background: The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), was recorded for the first time in Pakistan in 2019, and now it has spread in several regions, i.e., Punjab and Sindh, affecting maize production. Farmers are using widely synthetic pesticides to control the pest. Further, the resistance development in insects and the non-target effect of chemicals on the environment and humans pose serious threats of using insecticides. The use of entomopathogenic fungi (EPF) is being considered an important tool in integrated pest management program. The main objective of this study was to check the impact of different conidial concentrations of *Metarhizium anisopliae* fungus on the nutritional physiology of *S. frugiperda*.

Results: The dose-dependent effect of tested EPF was observed, and the highest concentration (1×10^9 conidia/ml) considerably affected nutritional parameters. Reduced relative consumption rate (21.7%), relative growth rate (19.5%), the efficiency of conversion of ingested food (24.2%), and approximate digestibility (16.3%) were observed in treated larvae compared to the untreated by using a higher concentration of EPF. Pupal weight was also found lower (77.9 mg and 84.2 mg, respectively), when larvae were treated by 1×10^9 and 1×10^8 conidia/ml concentrations of EPF. Further, 46.7% of larvae were found dead with this conidial concentration.

Conclusions: This study suggests that the application of a higher concentration of *M. anisopliae* could be an effective option to control *S. frugiperda*. The EPF can enhance the integrated pest management programs and could be useful in reducing the environmental impact of synthetic insecticides.

Keywords: Entomopathogenic fungus, *Spodoptera frugiperda*, Microbial control, Feeding indices

Background

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a migratory polyphagous insect pest and is native to the American continent, including tropical and subtropical regions (Montezano et al. 2018). It has invaded Africa, Australia, and Asia, and has a greater impact on global food security. It was first reported in West Africa (Goergen et al. 2016), and then in a short period, it was dispersed to sub-Saharan Africa (Stokstad 2017). In May 2018, *S. frugiperda* was observed in the Karnataka region of India (Kalleshwaraswamy et al. 2018). Occurrence of *S. frugiperda* has also been reported in southeastern Asian countries, including Bangladesh, Thailand, and Myanmar (Guo et al. 2018). In Pakistan, *S. frugiperda* species was reported in 2019 (Naeem-Ullah et al. 2019). There are two strains of this pest: the rice strain and the corn strain. The corn strain

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is more notorious for many crops than the rice strain (Dumas et al. 2015). In Pakistan, the rice strain of *S. frugiperda* has also been reported recently (Yousaf et al. 2021). Its population is spreading rapidly due to its biology, ability to migrate with air, and high fecundity rate (Prasanna et al. 2018).

Spodoptera frugiperda damages over 350 host plants worldwide, including major crops; corn, wheat, sugarcane, rice, cotton, cabbage, millets, and sorghum (Montezano et al. 2018). Although the use of insecticide is a quick tool to control the insect pests in the field, the insecticidal control of *S. frugiperda* is very difficult due to its feeding behavior, as the inner leaves of the plants with larval excrement protect them from toxicants (Paredes-Sánchez et al. 2021). Resistance development in insects is also another concern. The resistance in *S. frugiperda* against various insecticides has already been reported (Mota-Sanchez and Wise 2019). Thus, it is necessary to minimize the application of insecticides and find out some sustainable IPM technologies against this pest.

Microbial control is being considered a safer and eco-friendly approach to manage the population of insect pests. About 750 species of entomopathogenic microorganisms from about 85 genera have been identified (Norjmaa et al. 2019). The entomopathogenic fungi (EPFs) use enzymes to penetrate the cuticle of insects (Rajula et al. 2020). Various genera of fungi, *Beauveria, Isaria, Lecanicillium, Cordyceps, Metarhizium,* and *Nomuraea* have been utilized in the effective management of various aboveground and soil insect pests (Thaochan and Sausa-Ard, 2017). *Metarhizium* spp. have been studied against many insect pests due to its vast host range, environmental friendliness, and ease to produce (Greenfield et al. 2015). *Metarhizium anisopliae* species is being widely commercialized by companies and has the highest number of product registration (Mascarin et al. 2019). Although the mortality rate of insect pests by using EPFs have been studied, knowledge about their effect on consumption and growth rate of insect pests is limited. Keeping in view the increasing interest and successful use of entomopathogens to control insect pests, the present study aimed to determine the effect of different concentrations of *M. anisopliae* on the nutritional physiology of *S. frugiperda*.

Methods

Spodoptera frugiperda culture

Larvae and egg batches of *S. frugiperda* were collected from corn fields (32° 07′ 53.8″ N 72° 41′ 34.4″ E) in the Sargodha region, Pakistan. The collected population of larvae was brought to the laboratory of Entomology for rearing. Larvae of *S. frugiperda* were kept individually in small plastic Petri plates (90×15 mm). Fresh leaves of corn were provided to the larvae on daily basis. The diet was replaced daily, and the feces were removed from the Petri plates regularly. After the pupation, the pupae were placed in small plastic cages for adult emergence. The male and female adults were introduced in jars for mating and egg-laying. Honey solution (10%) as soaked cotton plugs was provided to adults. The stripes of muslin clothes were hung in jars for egg-laying. The culture was maintained under controlled conditions of 26 ± 2 °C and 65 ± 5% R.H. The culture was maintained up to F_3 generations to be used in the further experiment.

Entomopathogenic fungus

The EPF, *M. anisopliae* strain Met F52 (Earth BioSciences, New Haven, CT), was tested against 3rd instar larvae of *S. frugiperda*. Laboratory culture of EPF was grown on Potato Dextrose Agar (PDA) at 25 ± 2 °C, 70% RH, and 12:12 h (L:D) photoperiod. The conidia quality was determined using a Neubauer chamber hemocytometer. The germination was determined on PDA plates (based on the counts of 200 random conidia/plate), 18 h post-incubation at 25 ± 2 °C (Ayala-Zermeño et al. 2015), and in bioassay, suspension of 90% conidial germination was used. The conidial suspension was adjusted to different concentrations of 1×10^5, 1×10^6, 1×10^7, 1×10^8, and 1×10^9 conidia/ml in distilled water with 0.05% Tween 80. Sterile distilled water with Tween 80 was used as a positive control.

Nutritional physiology parameters

Thirty 3rd instar larvae were dipped in solution of each EPF concentration for 10 s. After application, larvae were placed in Petri plates. Fresh corn leaves were provided to larvae. Before and after 24 h of feeding, diet weight was recorded using weight balance. The larval length was recorded daily before and after the feeding of 24 h using a measuring scale. Similarly, the weight of each larva and feces was also recorded daily using digital weight balance. When the larva developed to a pupa, the pupal weight was recorded. The mortality data of larvae were also recorded daily. The parameters related to nutritional physiology such as RGR, relative growth rate; RCR, relative consumption rate; ECI, the efficiency of conversion of ingested food; and AD, approximate digestibility were calculated by the method suggested by Waldbauer (1968).

Data analysis

Data of all the nutritional physiology parameters including RCR, RGR, ECI, AD, and pupal weight, and larval mortality were analyzed by one-way analysis of variance (ANOVA). Means were separated by the least significant difference LSD test at a probability level of 5%. The analyses were performed using SPSS 20.0 software.

Results

The results showed that there was a significant ($F_{5,179} = 13.9$, $P < 0.001$) difference in relative consumption rate (RCR) of *S. frugiperda* after exposure to EPF concentrations. In the control treatment, the RCR value was 1.43 g^{-1} g^{-1} d^{-1}. With the exposure to 1×10^9 concentration, about 21.7% consumption was reduced than in the untreated larvae. Similarly, 16.7 and 13.9% consumption rates were reduced with the exposure to 1×10^8 and 1×10^7, respectively. Relative growth rate was also significantly ($F_{5,179} = 49.2$, $P < 0.001$) affected with EPF concentration. In comparison with the control treatment, the growth rate of larvae was reduced up to 19.5% with 1×10^9, 14.6% with 1×10^8, and 9.70% with 1×10^7. Efficiency of conversion of ingested food (ECI) was significantly ($F_{5,179} = 132.9$, $P < 0.001$) higher (35.6%) in control treatment. However, compared to control, EPF concentration of 1×10^9 reduced 24.2% ECI. With 1×10^8 concentration, 17.1% ECI was reduced. A significant ($F_{5,179} = 134.5$, $P < 0.001$) difference was recorded in approximate digestibility (AD) after EPF exposure. About 16.3% reduction occurred due to the application of 1×10^9 and 10.5% AD rate was reduced with 1×10^8 concentration. By using lower EPF concentration (1×10^5), 4.89% RCR, 4.87% RGR, 1.40% ECI, and 2.17% AD rate were reduced in comparison with control (Fig. 1).

A significant ($F_{5,143} = 17.5$, $P < 0.001$) difference was recorded in pupal weight after application of EPF concentration. Pupae gained the highest weight (93.6 mg) in the control treatment. However, pupal weight was reduced to 77.9 mg after exposure to 1×10^9 concentration followed by 84.2 mg after application of 1×10^8 concentration (Fig. 2).

Mortality rate of larvae was significantly ($F_{5,179} = 6.50$, $P < 0.001$) higher (46.7%) after application of 1×10^9 concentration. About 33.3% of larvae were dead with exposure to 1×10^8 concentrations. The least mortality of larvae (6.70%) was recorded when a lower concentration (1×10^5) of EPF was applied (Fig. 3).

Discussion

Use of entomopathogens as biocontrol agents is a promising and alternative tool in the integrated management of various insect pests (Deka and Baruah 2021). The impact of various concentrations of *M. anisopliae* was studied on *S. frugiperda*. During the penetration, *M. anisopliae* secretes the proteins such as trypsins, subtilisins, and chymotrypsins that digest the protein-rich procuticle of arthropods (Wang et al.

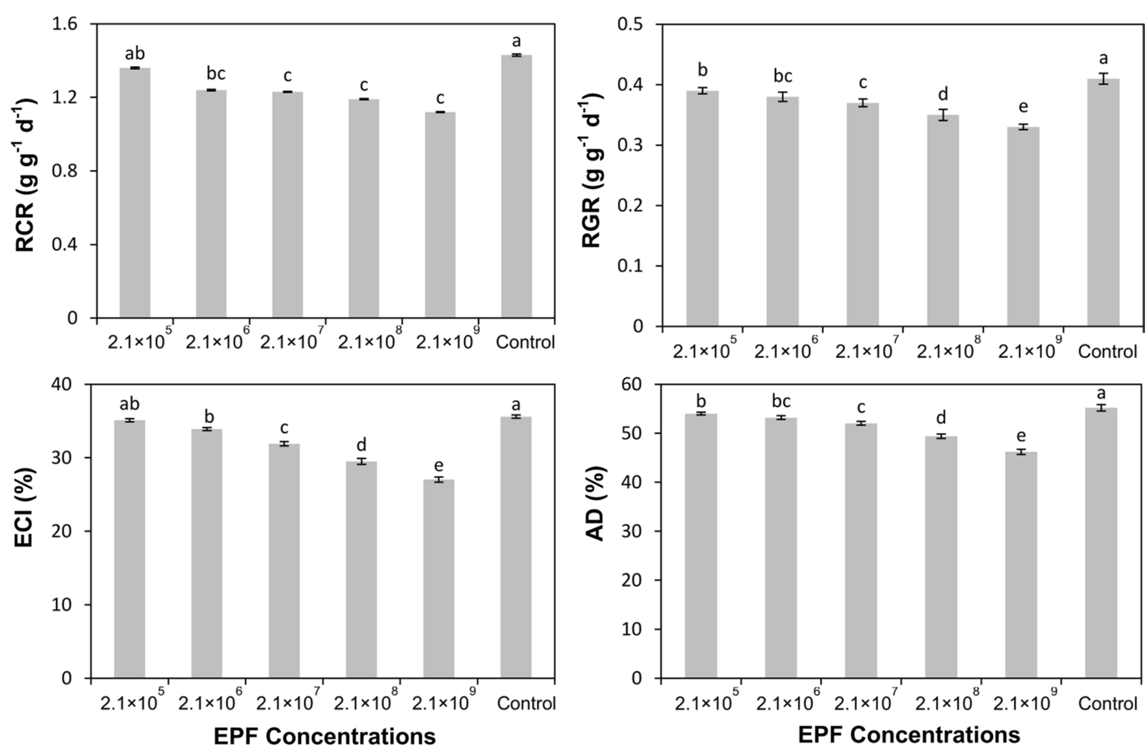

Fig. 1 Effect of various conidial concentration of *Metarhizium anisopliae* on nutritional physiology (means ± SE) of *Spodoptera frugiperda*, RCR = relative consumption rate, RGR = relative growth rate, ECI = efficiency of conversion of ingested food, AD = approximate digestibility, and means sharing similar letters are not statistically significant at $P > 0.05$

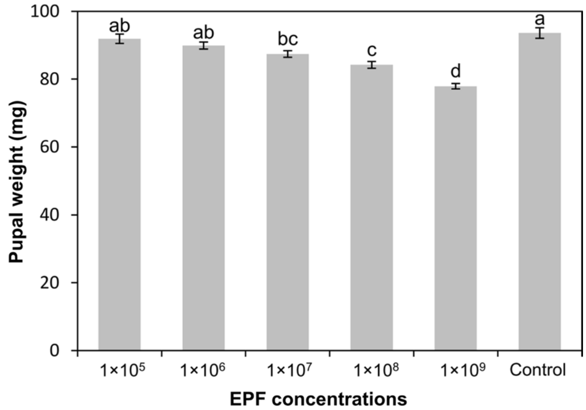

Fig. 2 Effect of various conidial concentration of *Metarhizium anisopliae* on pupal weight (mg ± SE) of *Spodoptera frugiperda*

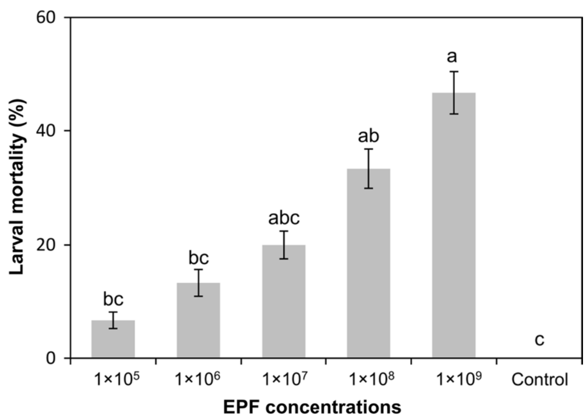

Fig. 3 Effect of various conidial concentration of *Metarhizium anisopliae* on larval mortality (% ± SE) of *Spodoptera frugiperda*

2008). Due to the penetration of EPF, the consumption of food biomass of larvae was reduced in terms of ECI and RCR. Obtained findings are supported by previous studies that reported a reduction in ECI and RCR values of treated insects than in untreated with the application of entomopathogens (Moorthi et al. 2015). The AD values were also reduced, and it could be due to indiscriminate food consumption or not retaining food to the intestine of larvae to get energy and nitrogen (Hussain et al. 2009). Obtained results are similar to the findings of Moorthi et al. (2015) who reported a reduction in feeding parameters including RCR, RGR, ECI, and AD of *Spodoptera litura* (Fabricius) after application of EPF, *Isaria fumosorosea*. Overall, the reduction in food consumption of insects is due to toxic substances secreted by EPF inside the host's body that ultimately disrupt the structural integrity (Tefera and Pringle 2003).

All the feeding indices parameters were reduced by increasing the concentration of EPF. Thus the effect of EPF is proportionally dependent on the conidial concentration (El Husseini 2019). Using higher concentrations is not challenging as the EPFs are safer for humans and the environment (Yasin et al. 2019). Lower values of RGR, RCR, and ECI possibly after exposure to EPF led to the formation of smaller pupa (Khosravi et al. 2010). de Souza et al. (2020) also reported that *B. bassiana* and *M. anisopliae* reduced the pupal weight of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). A higher concentration of EPF can be effective to manage the larval population of *S. frugiperda*. As Romero-Arenas et al. (2014) reported, 72.5% mortality of *S. frugiperda* larvae (3rd instar) with the application of *M. anisopliae*, was at the dose rate of 5.3×10^5 conidia/ml. Mahmood et al. (2019) also reported considerable mortality of *Sitobion avenae* (Fab.) (Hemiptera: Aphididae) with *B. bassiana* application. Due to exposure to a pathogen, the immunity of insects influences that led to a reduction in survival (Jensen et al. 2019). Effect of EPFs on the successive generation of *S. frugiperda* would be more helpful for designing an IPM technique of this pest.

Conclusions

The impact of *M. anisopliae* with various conidial concentrations was studied on the feeding indices parameters of *S. frugiperda*. The treated larvae with higher concentrations showed lower consumption and growth rate. Further, higher larval mortality (antifeedant effects) was observed using a higher concentration of EPF. With the benefits of environmental safety of microbial insecticides, *M. anisopliae* can be used in the IPM programs concerned with *S. frugiperda*. However, to better understand the biopesticidal effect of this EPF under field conditions, a more detailed study is needed before adding EPF into integrated management of *S. frugiperda*.

Abbreviations

EPF: Entomopathogenic fungi; RH: Relative humidity; PDA: Potato dextrose agar; RGR: Relative growth rate; RCR: Relative consumption rate; ECI: Efficiency of conversion of ingested food; AD: Approximate digestibility; ANOVA: Analysis of variance; LSD: Least significant difference.

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Author contributions

MIU planned and designed the project and experimental layout. NM, AK, LA, QT, AS and F performed the experiment. MA and NA performed the statistical analysis. The manuscript was prepared by MA and NA and reviewed by MIU. All authors read and approved the final manuscript.

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Availability of data and materials
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Declarations

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Not applicable.

Consent for publication
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Competing interests
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