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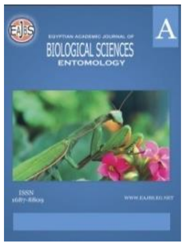

Appraisal Role of Two Entomopathogenic Fungi for Management of Fall Armyworm, *Spodoptera frugiperda* (Smith.) (Lepidoptera: Noctuidae) under Field Conditions

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ABSTRACT

Maize (*Zea mays* L.), along with rice and wheat, is a vital important cereal crop worldwide. Fall armyworm *Spodoptera frugiperda*, a significant agricultural pest, has caused substantial economic losses to maize crops globally. This study evaluated the efficacy of two commercial bioinsecticides, Bio-Magic® and Bio-Power®, against the fall armyworm, *Spodoptera frugiperda*, in field conditions over two growing seasons 2023 and 2024. Larval mortality was assessed three days post-treatment. Both bioinsecticides demonstrated efficacy, with Bio-Magic® consistently exhibiting higher mortality rates. Importantly, the virulence of both compounds persisted for up to ten days. Bio-Power® was less toxic to *S. frugiperda* compared to Bio-Magic®. Sublethal doses of both bioinsecticides led to increased chitinase and alkaline phosphatase activities in *S. frugiperda* larvae, while acid phosphatase activity was slightly elevated. These findings highlight the potential of bioinsecticides as sustainable alternatives to synthetic insecticides for *S. frugiperda* management. Entomopathogenic fungi stand out as targeted insect pest control solutions, minimizing harm to beneficial insects compared to broad-spectrum insecticides. Their integration into IPM programs is crucial for sustainable pest management.

INTRODUCTION

The fall armyworm (*Spodoptera frugiperda*) first appeared in Africa in early 2016 (Goergen *et al.,* 2016). The Ministry of Agriculture's Agricultural Pesticides Committee (APC) in Egypt confirmed the initial detection of fall armyworm in maize crops in the town of Kom Ombo within Aswan Governorate in May 2019. (FAO 2019; Dahi *et al.,* 2020). On August 6, 2021, the fall armyworm began to invade the maize crop in Assiut Governorate, Upper Egypt, causing more severe damage to sorghum plants (Mohamed *et al.*, 2022).

The widespread devastation caused by *S. frugiperda* in Africa looms over the food security of 300 million people. This destructive pest has a remarkably wide host range, with the ability to feed on 353 plant species from 76 different plant families, including important crops from the Poaceae (grasses like maize and sorghum), Asteraceae (sunflowers), and Fabaceae (legumes) families (Casmuz Augusto *et al.*, 2010; CABI 2018; Montezano *et al.*, 2018). As a result, *S. frugiperda's* voracious appetite significantly reduces crop yields, leading to substantial financial losses for farmers (Idrees *et al.*,2022).

Resistance to multiple insecticides makes *S. frugiperda* a pest that is difficult to control with chemical insecticides (Abd El-Samei *et al.* 2019). Due to the risk of insecticide resistance, in addition to negative impacts on the environment and human health, there is a need for other effective, sustainable and cost-effective control alternatives. Entomopathogenic fungi emerge as a beacon of hope, offering a compelling alternative due to their remarkable effectiveness, exceptional host specificity, and minimal environmental and human health impacts Fakeer *et al.* (2024).

In a comprehensive field study spanning two growing seasons, researchers investigated the efficacy of two entomopathogenic fungi against *S. frugiperda*. This study not only assessed the lethal effects of the biopesticides on *S. frugiperda* but also delved into their biochemical impact. By examining the influence of these fungi on the activity of chitinase, acid phosphatase (ACP), and alkaline phosphatase (ALP) enzymes, we gained a deeper understanding of their potential mechanisms of action and their broader physiological effects on the target insect.

MATERIALS AND METHODS

1. Insect Source and Rearing Conditions:

Egg masses of a laboratory strain of *S. frugiperda* (Smith) were acquired from the Plant Protection Research Institute in Egypt. These eggs were incubated in plastic cups under controlled conditions (25 ± 2 °C, 70 ± 10% relative humidity. until hatching

2. Bio-Pesticides Selection and Application:

Two commercial bio-pesticides were utilized in this study:

- Bio-Power®: A 1.15% wettable powder (WP) formulation of *Beauveria bassiana* (Balsamo) Vuillemin. Applied at a rate of 1.5 kilograms per feddan.
- Bio-Magic®: A 1.75% WP formulation of *Metarhizium anisopliae* (Metchnikoff) Sorokin. Applied at a rate of 1.5 kilograms per feddan.

Both biopesticides were obtained from the Gaara Establishment (Import and Export).

3.Field Application:

This study was carried out on infested corn plants with the *S. frugiperda* larvae during two consecutive planting seasons in 2023 and 2024. Field trials using the same treatments were carried out each year at the same location .The site experiment was conducted in the village of Tahla, central Banha, Qayubiya Province. All experiments used a maize variety (Hi Tech 2031) and were grown until mid-April in both seasons. The site area is 262.5 m2 (1/16 f.) and a randomized complete block design is used. Each treatment was assigned four plots, and the two rows of plants separating these plots were also designated as untreated areas. The application is done with an 80-liter capacity motorized knapsack sprayer. The test compounds were applied at recommended concentrations based on the guidelines provided by the Agricultural Pesticides Committee (APC) of the Egyptian Ministry of Agriculture and Land Reclamation. The untreated plot received a water-only spray. A total of ten plants/plots/treatments were evaluated at the following time points: before the initial application and one, three, five, seven- and ten days post-application of all compounds. The effectiveness of the test treatment was assessed by determining the percentage reduction in S. frugiperda larval infestation density, using the method described by Henderson and Tilton (1955).

4. Determining LC Values for the Tested Bio-Pesticides on *S. frugiperda* (Smith):

The larvicidal efficiency of the studied compounds was estimated on newly moulted 2nd of *S. frugiperda* larvae. Fresh corn leaves were immersed in each of the prepared bio-pesticides concentrations. Subsequently, allowed to dry at room temperature prior to being made an offer to the Fourth instar larvae preserved in ice cube packs. Larvae were awarded contaminant leaves for 48 hours. Each handling involved 50 larvae which were replicated five times. The control comprised similar numbers of larvae, and given Corn leaves immersed in distilled water. The mortality percent of the larvae was calculated post-exposure by 48 h. and adjusted using Abbott's formula from (1925). The obtained results were expressive graphically and LC_{50} values were calculated using a computerizing LDP line.

5. Enzymatic Changes:

- Insect Sample Preparation and Enzyme Extraction:

Insect samples were prepared following the methodology outlined by Amin (1998). Second-instar larvae were treated with the LC_{50} concentration of the tested compounds for 48 hours. Surviving larvae as 6th instar were weighed (1 gram each) and homogenized in distilled water (50 mg/1 ml). The resulting homogenates were centrifuged at 8000 rpm for 15 minutes at 4 °C using a cooling centrifuge. The supernatant, referred to as the enzyme extract, was retained and could be stored for up to one week at 50 °C without significant loss of activity.

-Determining Enzyme Activities:

The effect of the LC_{50} of the tested compounds on the chitinase enzyme in 6th instar larvae pretreated as 2nd instar larvae was assessed following the method described by Bade and Stinson (1981). The activity of both acid phosphatase (ACP) and alkaline phosphatase (ALP) was evaluated using the procedure outlined by Powell and Smith (1954).

6. Statistical Data Analysis:

All assessed toxicity and physiological parameters were evaluated using three replicates, with results expressed as the mean ± standard error. The data from each experiment were analyzed independently using analysis of variance (ANOVA) with SPSS 17.0 software. Mean comparisons were conducted according to the methods described by Snedecor and Cochran (1989), and differences were considered significant at $P \leq 0.05$. LC_{50} values were determined using regression lines as outlined by Finney (1971) with the "LdPLine®" software. The percentage reduction for each treatment was calculated using the Henderson and Tilton formula (Henderson & Tilton, 1955).

RESULTS AND DISCUSSION

The findings presented in the following tables highlight the potential of Entomopathogenic fungi as eco-friendly and effective alternatives to chemical insecticides for *S. frugiperda* management in corn cultivation.

The presented Table (1), effectively demonstrates the efficacy of two entomopathogenic fungi, Bio-Power® and Bio-Magic®, in controlling fall armyworm (*S. frugiperda*) larvae in corn fields during the 2023 growing season. The data highlights the remarkable ability of these fungi to suppress *S. frugiperda* populations, offering a promising alternative to conventional insecticides. Prior to the implementation of treatment, the average number of larvae within a specified area ranged between 14.25 and 14.5. Following the treatment, there was a significant decrease in the mean number of larvae across all treatment groups. Notably, the most substantial reduction was observed with Bio-Magic®, which achieved a reduction rate of 76.97% when compared to the control group. The **table (1)** also shows the mean number of larvae and reduction percentage at different days after treatment. This information is useful for understanding the duration of the entomopathogenic fungi effect. For both Bio-Power® and Bio-Magic®, the reduction percentages were highest at 7 days after treatment and remained relatively high at 10 days after treatment. This suggests that these insecticides have a long-lasting effect on *S. frugiperda* larvae.

Table (2), presents the average number and percentage reduction of *S. frugiperda* larvae, the condition of the corn field both prior to and following the application of the tested treatment was examined entomopathogenic fungi through the 2024 agricultural season. Both Bio-Power® and Bio-Magic® exhibited exceptional efficacy in reducing *S. frugiperda* larval numbers. Bio-Power® achieved an impressive 76.12% reduction, while Bio-Magic® demonstrated a remarkable 77.27% reduction.

Bio-Power® and Bio-Magic® outperformed the untreated control group, which experienced a substantial increase in larval numbers. This underscores the significant contribution of these fungi in controlling *S. frugiperda* infestations. These findings support the results of Kumari *et al*. (2020), who observed a reduction in *S. frugiperda* larvae populations after spraying with Cypermethrin and Emamectin benzoate. Our results also align with those of Karina da Silva *et al.* (2020), who reported a significant decrease in *S. frugiperda* larvae following the application of Teflubenzuron, Flubendiamide, and Emamectin benzoate. Furthermore, our findings support the conclusions of Mintesnot and Ebabuye (2019), who estimated that Profenophos, Cypermethrin, and Spinosad achieved maximum mortality rates among sixth-instar *S. frugiperda* larvae. Additionally, the studies by Sileshi *et al.* (2022) demonstrated that plots treated with, Deltamethrin, and Lambda-cyhalothrin experienced a significant reduction in *S. frugiperda* larvae populations within three days compared to the control. Amein and Abdelal (2023) also found that plots treated with the insecticides Alpha-Cypermethrin and Emamectin benzoate experienced a substantial reduction in *S. frugiperda* larvae populations within just three days. These results were notably different from the control groups that were not treated with any insecticides. These findings corroborate the earlier observations of Satyanarayana *et al.* (2010), who identified Emamectin benzoate as the most effective insecticide for reducing *Spodoptera litura* larval populations." Our results also align with those of Fakeer *et al.* (2024), Who first identified the suitability of entomopathogenic fungi and essential oils for controlling *Spodoptera frugiperda***.**

The median lethal concentration (LC_{50}) is a measure of the toxicity of a substance to an organism. In table (3), the LC_{50} values for Bio-Power® and Bio-Magic® are 0.1621 g/ml and 0.0920 g/ml, respectively. This means that Bio-Power® is less toxic to *S. frugiperda* than Bio-Magic®.

These discoveries are consistent with the results demonstrated by Karina da Silva *et al.* (2020), who assessed larval mortality in two *S. frugiperda* populations exposed to different insecticides (teflubenzuron and Emamectin benzoate). Their study revealed that both insecticides exhibited high levels of mortality against *S. frugiperda.* Additionally, our results corroborate the findings of Zhuo-Kun Liu *et al.* (2022), who investigated the toxicity of Emamectin benzoate on *S. frugiperda* and discovered that even low, sublethal concentrations of Emamectin benzoate could significantly impact the life cycle of both parental and first-generation *S. frugiperda.* Furthermore, our results are consistent with those of Amein and Abdelal (2023), who observed that the laboratory strain of *S. frugiperda* is particularly susceptible to Emamectin benzoate."

The presented Table (4), sheds light on the impact of two entomopathogenic fungi, Bio-Power® and Bio-Magic®, on the enzymatic activity of *S. frugiperda* larvae. The data suggests that these fungi induce significant alterations in enzyme levels, potentially contributing to their insecticidal efficacy.

The results explore how two entomopathogenic fungi, affect the enzyme activity levels in fall armyworm *S. frugiperda* larvae. The data suggests that these fungi can significantly alter enzyme levels, potentially contributing to their ability to kill the larvae (insecticidal efficacy).

Bio-Power® and Bio-Magic® treatments significantly increased chitinase activity compared to the control. Chitinase is an enzyme that breaks down chitin, a major component of insect exoskeletons. Increased chitinase activity might disrupt the larvae's ability to molt (shed their exoskeleton) and grow properly (Soderstrom, *et al.* 2000).

Both fungi also caused a substantial rise in ALP activity. ALP plays a role in various physiological processes in insects, and its elevation may indicate cellular stress or disruption within the larvae (Kuramoto. *et al.* 2013).

ACP (Acid Phosphatase): Treatment with both fungi caused a slight, increase in ACP activity compared to the control group. ACP is involved in various metabolic processes in insects, and its altered levels could suggest metabolic disturbances in the larvae (Rani, *et al.*,2009).

Table 1: The average number and percentage reduction of *S. frugiperda* larvae in the corn field were observed before and after treatment with the tested Bio-pesticides during the 2023 agriculture season.

Treatments	Before treatment Mean ±SE	Mean and reduction percentage after treatment				
		Initial effect		Residual effect		
		1 DAY Mean ±SE	3 DAYS Mean ±SE	5 DAYS Mean ±SE	7 DAYS Mean ±SE	10 DAYS Mean ±SE
Bio-Power®	14.25 ± 0.1 [a]	13.75 ± 1.5 [b] (13.90) *	12.25 ± 0.5 [b] (29.78) *	8.25 ± 0.5 [b] (56.39) *	7.75 ± 0.9 [b] (61.53) *	5.25 ± 0.5 [b] (75.44) *
Bio-Magic®	13.75 ± 0.5 [a]	12.25 ± 1 [b] (20.50) *	10.25 ± 0.8 [b] (39.10) *	8.75 ± 0.5 [b] (52.07) *	6.5 ± 0.6 [b] (66.56) *	4.75 ± 2.4 [b] (76.97) *
Untreated area	14.5 ± 0.6 [a]	16.25 ± 0.5 [a]	17.75 ± 0.5 [a]	19.25 ± 0.8 [a]	20.5 ± 0.8 [a]	21.75 ± 1.2 [a]

Means followed by the same lowercase letter within a column are not significantly different at the 5% level of probability.

* Numbers in parentheses show the percentage reduction.

Table 2: The average number and percentage reduction of *S. frugiperda* larvae in the corn field were observed before and after treatment with the tested Bio-pesticides during the 2024 agriculture season.

Treatments	Before treatment Mean ±SE	Mean and reduction percentage after treatment				
		Initial effect		Residual effect		
		1 DAY Mean ±SE	3 DAYS Mean ±SE	5 DAYS Mean ±SE	7 DAYS Mean ±SE	10 DAYS Mean ±SE
Bio-Power®	13.5 ± 06 [a]	12± 0.9 [b] (23.16) *	11.25 ± 0.8 [b] (32.54) *	10.5 ± 0.6 [b] (38.97) *	8.75 ± 0.8 [b] (53.44) *	5.5 ± 0.6 [b] (76.12) *
Bio-Magic®	12.25 ± 0.5 [a]	11.75 ± 1 [b] (17.09) *	10.5 ± 0.6 [b] (30.61) *	8.75 ± 0.5 [b] (43.96) *	7.5 ± 0.6 [b] (56.02) *	4.75 ±1 [b] (77.27) *
Untreated area	12.75 ± 1 [a]	14.75 ± 1 [a]	15.75 ± 0.8 [a]	16.25 ± 0.5 [a]	17.75 ± 0.8 [a]	21.75 ± 1 [a]

Means followed by the same lowercase letter within a column are not significantly different at the 5% level of probability.

* Numbers in parentheses show the percentage reduction.

Table 3. The LC_{50} values for the tested Bio-pesticides against the six instar larvae pretreated as the second instar of *S. frugiperda* under laboratory conditions.

Tested compounds	Median lethal concentration (LC_{50}) (gm/m)	Fiducial limits (C.I. 95%) (gm/ml)		Slope
		Lower	Upper	
Bio-Power®	0.1621	0.1126	0.2451	1.2448 ± 0.2071
Bio-Magic®	0.0920	0.0079	0.0126	1.6199 ± 0.2390

Table 4: Enzyme activity in *S. frugiperda* larvae following Bio-pesticides treatment with LC_{50}.

Tested compounds	Chitinase (µg NAGA/min/gmb.w.) (Mean ± S. E.)	ALP activity (U x10^3 / gm. b.w.) (Mean ± S.E.)	ACP activity (U x10^3 / gm. b.w.) (Mean ± S.E.)
Bio-Power®	209.33 ± 2.22 c	250.3 ± 1.2 c	112.6 ± 1.2 c
Bio-Magic®	224.3 ± 2.37 b	261± 0.6 b	115.6 ± 1.8 b
Control	238.6 ± 1.8 a	282.3 ± 1.5 a	123.3 ± 2.4 a
F Value	**631*** **	**508*** **	**97*** **
L.S. D	**1.99789516029**	**2.40117114781**	**1.997895160**

Numbers of the same letters have no significant difference

CONCLUSION

The use of entomopathogenic fungi, such as Bio-Power® and Bio-Magic®, has the potential to be an effective and environmentally friendly alternative to traditional pesticides for controlling *Spodoptera frugiperda*. However, further research is necessary to comprehensively evaluate the efficacy, safety, and long-term environmental impacts of these Bio-pesticides.

Declarations:

Ethical Approval: Not applicable.

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