Entomology

Evaluation of *Beauveria bassiana* and *Heterorhabditis bacteriophora* as biocontrol agents for *Spodoptera frugiperda* [J.E. Smith] (Lepidoptera: Noctuidae) and their effect on its biochemical parameters

Entesar N Haron[1*], Nevien M Gaber[1] and Soheir F Abd EL-Rahman[1]

[1] Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

Correspondence Author: Entesar N. Haron

Received 2 Sep 2023; Accepted 17 Oct 2023; Published 30 Oct 2023

Abstract

The fall armyworm, *Spodoptera frugiperda* [J.E. Smith] (Lepidoptera: Noctuidae), is widely distributed in the world and is a multi-feeding insect that attacks many economic field crops and almost all vegetables, causing significant economic losses. The present study was carried out to evaluate the effects of three *Beauveria bassiana* fungal isolates, (B1, B2, and B3) and the nematode (*Heterorhabditis bacteriophora*) in combating third-instar larvae of the *S. frugiperda* in the laboratory. Five tested concentrations and the deep-leaf approach were applied. In addition, the effect of the three fungal isolates and nematodes on the total protein and activities of some enzymes (transaminases, phenoloxidases, and alkaline phosphatase (ALP)) in the blood lymph of third instar *S. frugiperda* larvae was studied after treatment with LC_{50} values of B1 and *H. bacteriophora*. The results showed that the mortality percentages in B1 were higher compared to B2 and B3. According to LC_{50} values, B1 was more toxic followed by B2 than B3 (LC_{50} = 5.52E+07 conidia/ml, 1.26E+08 conidia/ml and 3.38E+09 conidia/ml, respectively). Moreover, the mortality rate was higher in *H. bacteriophora* than the three fungal isolates. The LC_{50} of nematodes was 23.67 IJs/ml. Additionally, the time-response efficacy (LT50) of nematodes on *S. frugiperda* larval mortality was lower compared to that of *B. bassiana* treatments. It was 0.71 and 9.5 days for *H. bacteriophora* and *B. bassiana* (B), respectively at high concentrations for each one. The tested compounds significantly decreased the total protein and GOT, GPT levels. While, phenoloxidase, and ALP were significantly increased with compared to the control group.

Keywords: Entomopathogenic fungi, Nematodes, Armyworms, Protein and Enzyme activities

Introduction

The fall armyworm, *Spodoptera frugiperda* [J.E. Smith] (Lepidoptera: Noctuidae), is widely regarded as a significant threat to global food security due to its destructive impact. (FAO, 2018) [17]. *S. frugiperda* impacts a range of commercially significant crops; infestation rates in maize fields Have been reported to vary between 33% and 100%. (Sisay *et al*., 2019) [45]. In Egypt, the management of harmful insect populations primarily depends on conventional insecticide applications. (Vattikonda and Sangam, 2017; Ismail, 2021) [48, 29], while these insecticides often pose significant toxicological risks to the environment (Tiryaki and Temur, 2010; Chowański *et al*., 2014) [47, 11]. This situation has prompted the exploration of alternative, environmentally friendly, selective, and effective pest control strategies. One promising approach involves the utilization of entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPNs).

EPF and EPN serve as essential components of integrated pest management programs (IPM) and are appreciated for their effectiveness, specificity, and low environmental damage compared to conventional insecticides. Prominent genera of entomopathogenic fungi, such as *Beauveria*, *Metarhizium*, and *Isaria*, are widely recognized for their broad application in managing diverse insect pests. (Feng *et al*., 1990) [19]. There are many fungal-derived bioinsecticides and the most commonly used fungal species are those of the Beauveria genus, Cordyceps, Isaria, Lecanicillium, Metrarhizium and Nomuraea (Feng *et al*., 1990; Zimmermann, 2007) [19, 51]. *B. bassiana*, for instance, has demonstrated significant virulence against pests like whiteflies and aphids, positioning it as a favored choice in sustainable agricultural methods (Javed *et al.,* 2019 [30]. Additional studies revealed that bioassays using *M. anisopliae* and *B. bassiana* on newly hatched *S. frugiperda* larvae achieved a 100% mortality rate with strains Ma41, Ma22, Mr8, and Bb9, while strain Bb39 showed a lower mortality rate of 74%. (Cruz-Avalos *et al.,* 2019) [12]. Also, isolates of *M. anisopliae* and *B. bassiana* caused mortality in the 2nd larvae of *S. frugiperda* by 67.8% and 64.3%, respectively (Ramanujam *et al*., 2020) [41].

EPNs have attracted significant global interest due to their contribution to in IPM programs, owing to their safety for non-target organisms, human health, and the environment, as well as their distribution, virulence, and practical applications. (Çağlayan *et al*., 2021; Ali *et al*., 2022, Kumar *et al*., 2022; Peçen and Kepenekci, 2022) [9, 3, 34, 37]. *Heterorhabditis* and *Steinernema* are types of entomopathogenic nematodes (EPNs) that induce sepsis in insects. This occurs within 24 to 48 hours when the nematodes release symbiotic bacteria from the genera *Xenorhabdus* or *Photorhabdus*. These bacteria proliferate and secrete cytotoxic, exoenzymes, and antibiotics (Kaya *et al*., 2006 and Koppenhöfer 2007) [31, 32]. *Heterorhabditis megidis* SOz01 has also been used as a powerful insecticide on the pine saw beetle, *Monochamus alternatus*, reaching mortality rates of 86 to 100% under laboratory conditions (Ozawa *et al.*, 2023) [36].

Interactions between host insects and both fungi and nematodes that cause insect diseases not only result in the death of the insect host but also lead to major changes in the host's immune and defense systems. Additionally, *B. bassiana*, *M. anisopliae*, and various *Isaria* species can penetrate the insect cuticle and grow within the host. In response to fungal invasion, insects trigger an immune reaction that includes activating several defense mechanisms, such as the production of antimicrobial peptides and a pigmentation response, both of which are regulated by phenol oxidase enzymes. (Kunc *et al*., 2017) [33]. Entomopathogenic nematodes (EPNs), particularly those from the genera Steinernema and Heterorhabditis, also introduce symbiotic bacteria from the genera Xenorhabdus or Photorhabdus into their host organisms.These bacteria compromise the host's immune system by interfering with hemocyte function, thereby diminishing the insect's capacity to initiate a robust immune response (Kaya *et al*., 2006) [31]. However, these immune reactions may not always be sufficient to thwart infection, as fungi and nematodes have developed strategies to counteract the immune defenses of the host. (Zimmermann, 2007 and Kunc *et al*., 2017) [51, 33].

To develop integrated control programs for FAW, it is necessary to use alternative methods that reduce environmental damage compared to chemical insecticides. Therefore, the aims of the current research use the *Beauveria bassiana* isolates of the fungus and a *H. bacteriophora* nematode species to control FAW in controlled laboratory settings.

Materials and Methods

Insect culture

S. frugiperda larvae were collected from infested maize fields in the El Ayat area, Giza Governorate (29°17′09.5″N, 30°48′54″E). These larvae were bred for ten generations in the laboratory under controlled conditions (27 ± 2 ˚C and 65 ± 2% RH) and fed fresh castor plant leaves to establish a susceptible strain, following the methodology developed by (El-Defrawi *et al*., 1964) [18].

Entomopathogenic fungi (EPF)

The entomopathogenic fungi utilized in this study included *Beauveria bassiana* (Bals.), specifically isolates B1, B2, and B3. Isolate B1 and B2 were obtained from soil samples (Ali *et al*., 2020) [2], Meanwhile, strain B3 was obtained from the Plant Protection Research Institute (PPRI). The fungal spores were cultured on Sabouraud dextrose yeast agar (SDAY) medium at a temperature of 25.0 ± 1.0 °C for 14 days in a dark environment.

Entomopathogenic nematode (EPN)

EPN selected for this study is *Heterorhabditis bacteriophora*, belonging to the family Heterorhabditidae. This specimen was obtained from the Pests Physiology Department at the Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza.

Toxicological studies

Pathogenicity of *B. bassiana* on *S. frugiperda*

To prepare the stock spore suspension, mature spores were rinsed with distilled water containing 0.01% Tween 80. The spores were collected by washing with distilled water mixed with 0.01% (v/v) Tween 80 and then filtered through cheesecloth to prevent mycelial clumping. The spore concentration was measured using a hemocytometer (Neubauer improved HBG, Germany). Serial dilutions (10^6, 5×10^6, 10^7, 5×10^7, and 10^8 conidia/ml) were made in distilled water with 0.01% Tween 80 for isolates (B1, B2, B3). The pathogenicity of the fungi at five concentrations was assessed against the 3rd larval instar of *S. frugiperda* using the leaf dipping technique. Castor leaf discs (10 × 8 cm) were dipped in each concentration for 10 seconds and allowed to dry completely. After an 8-hour fasting period, the larvae were placed on the treated discs to feed. Control leaf discs were prepared by dipping in distilled water with 0.01% Tween 80. Each concentration included five replicates (10 larvae/ replicate), with individual larvae exposed in each replicate, and maintained under controlled laboratory conditions. Mortality data were recorded every two days for 12 days. Abbott's formula (Abbott, 1925) [1] was applied to adjust the mortality percentage and to calculate the LC50 and LC90 values.

Pathogenicity of the *H. bacteriophora* on *S. frugiperda*

Freshly molted third larval instar of *S. frugiperda* were utilized for the bioassay experiments. Serial concentrations of *H. bacteriophora* in distilled water were prepared at 10, 50, 100, 200, and 300 IJs/ml. The leaf dipping method, as outlined earlier, was used for the experiment. Larvae were exposed to the various concentrations, while a control group was treated with distilled water. Each dish received 1 ml of water to facilitate nematode movement. The percentage of mortality was recorded daily for three days following the treatment. Abbott's formula (Abbott, 1925) [1] was applied to adjust the mortality rates and to calculate the LC50 and LC90 values.

Quantification enzymes activity

Third instar larvae were treated with the LC50 values of the compounds being tested. After a 24-hour exposure period, 0.5 g samples of both treated and untreated live larvae were frozen. The frozen samples were homogenized in distilled water using a Teflon homogenizer and then centrifuged at 8000 rpm for 15 minutes at 2°C in a refrigerated centrifuge. (Amin, 1998) [5]. The resulting pellets, which included the homogenates and upper sediments, were stored in a deep freezer at -20°C until needed for biochemical assays to determine:

- Total proteins, as determined by the Bradford (I976) [8] method.
- Transaminases (GOT, GPT) using Reitman and Frankle's (1957) [42] methodology.
- Phenoloxidase activity was measured using a modified method described by Ishaaya (1971) [28].
- Alkaline phosphatases were measured using Powell and Smith's (1954) [39] methodology.

Statistical analysis

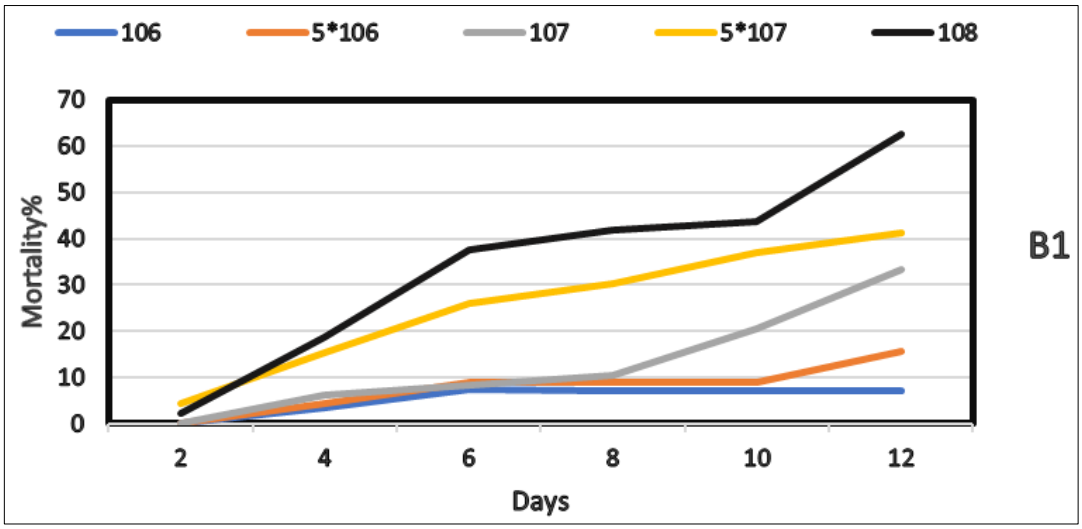
Statistical analysis to estimate the LC50 and LT50 values was conducted using the methodology described by Finney (1971) [15] with the assistance of the "LdPLine®" software [http://embakr.tripod.com/ldpline/ldpline.htm]. The Chi-square value was calculated for additional data analysis. A one-way ANOVA was conducted on the complete dataset using SPSS software, followed by Duncan's multiple range tests to evaluate the significance of differences between treatments.

Results

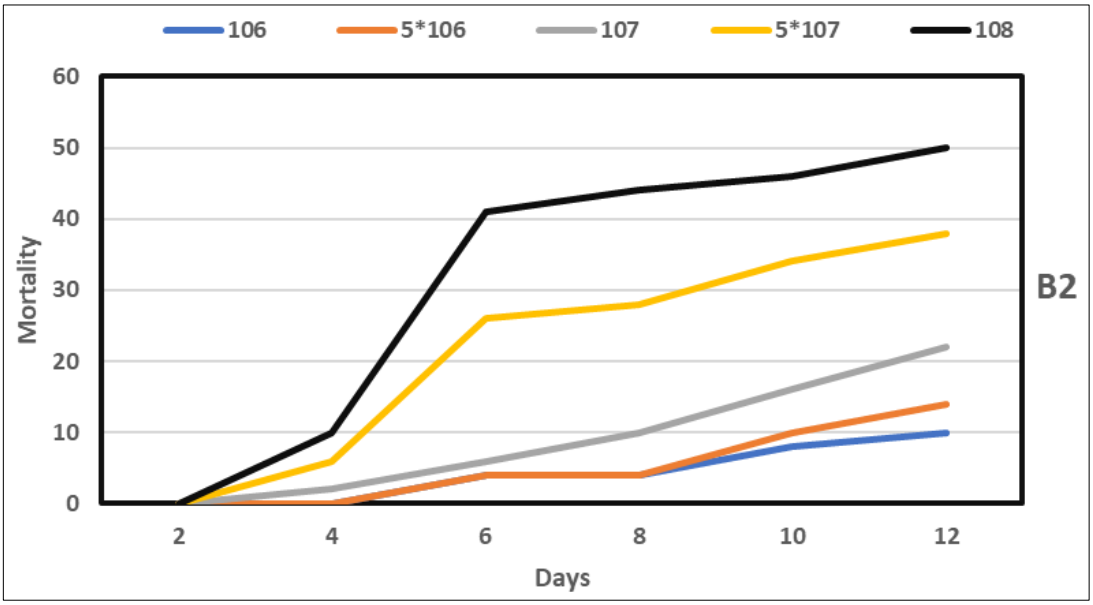
Pathogenicity of the tested *Beauveria bassiana* to *Spodoptera frugiperda*

Mortality was assessed for *S. frugiperda* larvae fed on castor leaves contaminated with spore suspensions at concentrations of 10^6, 5 X 10^6, 10^7, 5 X 10^7, and 10^8 conidia/ml. of *B. bassiana* isolates (B1, B2, and B3).

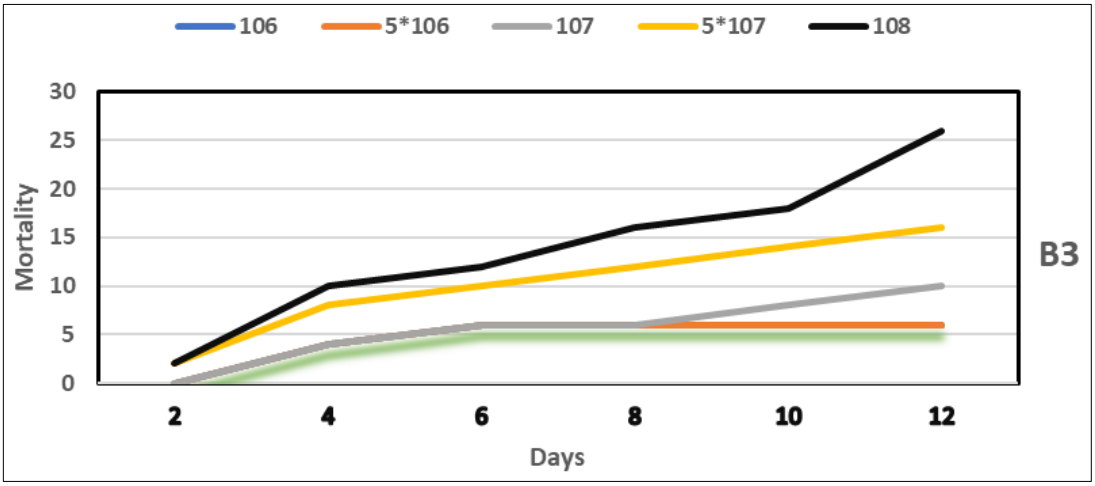
The results in Figure (1: a, b, c) show an increase in the total mortality percentages of *S. frugiperda* larvae treated with different concentrations of the tested *B. bassiana* isolates**.** Figure (2) also shows *S. frugiperda* larvae covered with spores of *B. bassiana*. The fungal pathogens increased with increasing time and concentration of *B. bassiana*.



(a)



(b)



(c)

Fig 1: Total mortality percentages of *S. frugiperda* larvae treated with different concentrations of the tested *B. bassiana isolates.*

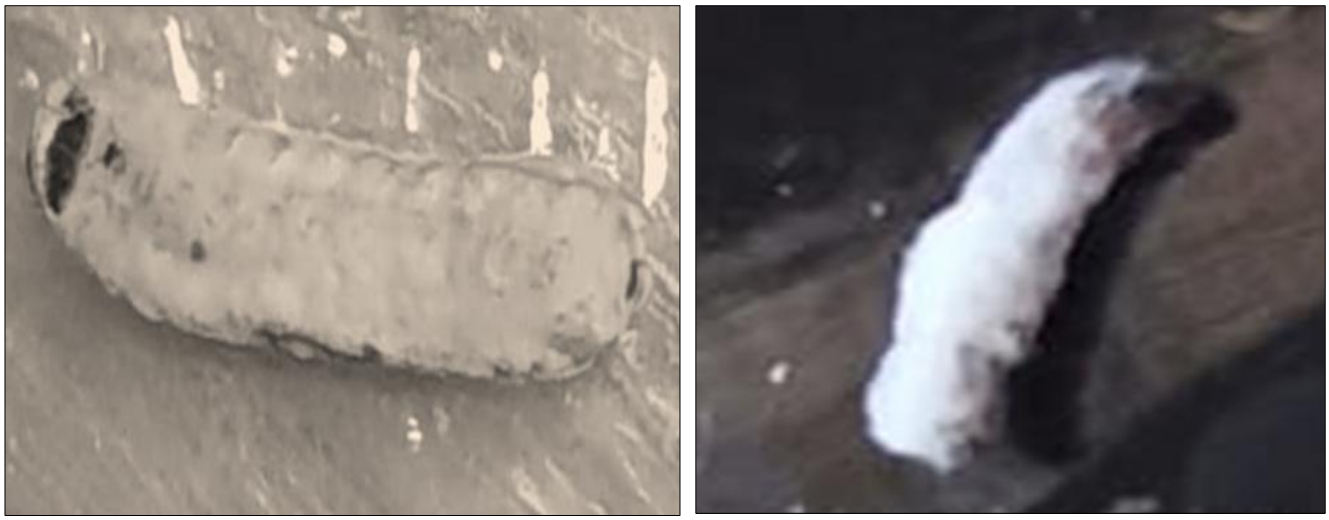


Fig 2: Mycosed larvae of covered with abundant external white fungal growth *S. frugiperda*

As shown in Table (1), isolate B1 was more effective against the third instar of *S. frugiperda* compared to B2 and B3. The LC50 values were 5.52E + 07, 1.26E + 08, and 3.38E + 09 conidia/ml for B1, B2, and B3, respectively.

Table 1: Lethal concentrations of *B. bassiana* isolates on *S. frugiperda*

Isolates	LC_{50} (conidia/ml)	Lower limit	Upper limit	Index	RR	Slope	LC90 (conidia/ml)	Chi-square (X2	LT_{50} (day)
B1	5.52E+07	3.80E+07	9.01E+07	100	1	0.84	1.82E+09	5.81	9.56
B2	1.26E+08	7.16E+07	3.06E+08	43.73	2.28	0.68	9.89E+09	1.80	10.16
B3	3.38E+09	6.32E+08	2.27E+11	1.63	61.31	0.49	1.38E+12	2.64	34.44

B1, B2, B3 = *B. bassiana* Index compared with B1 Resistance Ratio (RR) compared with B1 LC_{50} and LT_{50} refer to median lethal concentration and median lethal time, respectively.

There appear to be significant differences in their effectiveness at a concentration of 1 x 10^8 conidia/ml (Table 2). The order of the most effective isolates was B1, B2, and B3, respectively. At day 12, the mortality percentage of the isolates ranged from 28% for B3, 50% for B2, and 62.68% for B1 at a concentration of 10^8 spores/ml.

Table 2: Mortality % of *S. frugiperda* larvae after treated with *B. bassiana* at 10^8 conidia/ml

Isolates	Day after treatment					
	2	4	6	8	10	12
B1	10.7±3.5a	18.52±8a	38.08±7.55a	42.3±9.74a	44.3±10.8a	62.68±13.22a
B2	0±0b	10±7.07a	40±7.07a	44±7.48a	46±8.71a	50±14.14a
B3	0±0b	8±38.36a	10±4.47b	14±4b	18±5.83b	28±8.36 b
F	9.17**	2.55	6.67*	5.10*	3.62*	10.38**
P	0.00	0.12	0.01	0.02	0.07	0.00

Mean mortality % + SE, Means in a Column followed with the same letter(s) are not significantly different at 5% level of probability. B1, B2, B3= *B. bassiana*, *= significant, **= Highly significant

Pathogenicity of the tested *Heterorhabditis bacteriophora* on *S. frugiperda*

The effectiveness of five nematode concentrations was assessed on the 3rd larval instar of *S. frugiperda*. The data in Table (3) and Figure (3) indicate that larval mortality significantly increased (p<0.01) with higher concentrations. The pathogenicity of the nematodes also increased with both time and concentration. On the first day post-treatment, mortality rates were 18%, 34%, 36%, 50%, and 62% at concentrations of 10, 50, 100, 200, and 300 IJs/ml, respectively. After 72 hours, mortality rates were 34%, 60%, 70%, 88%, and 90% at the same concentrations.

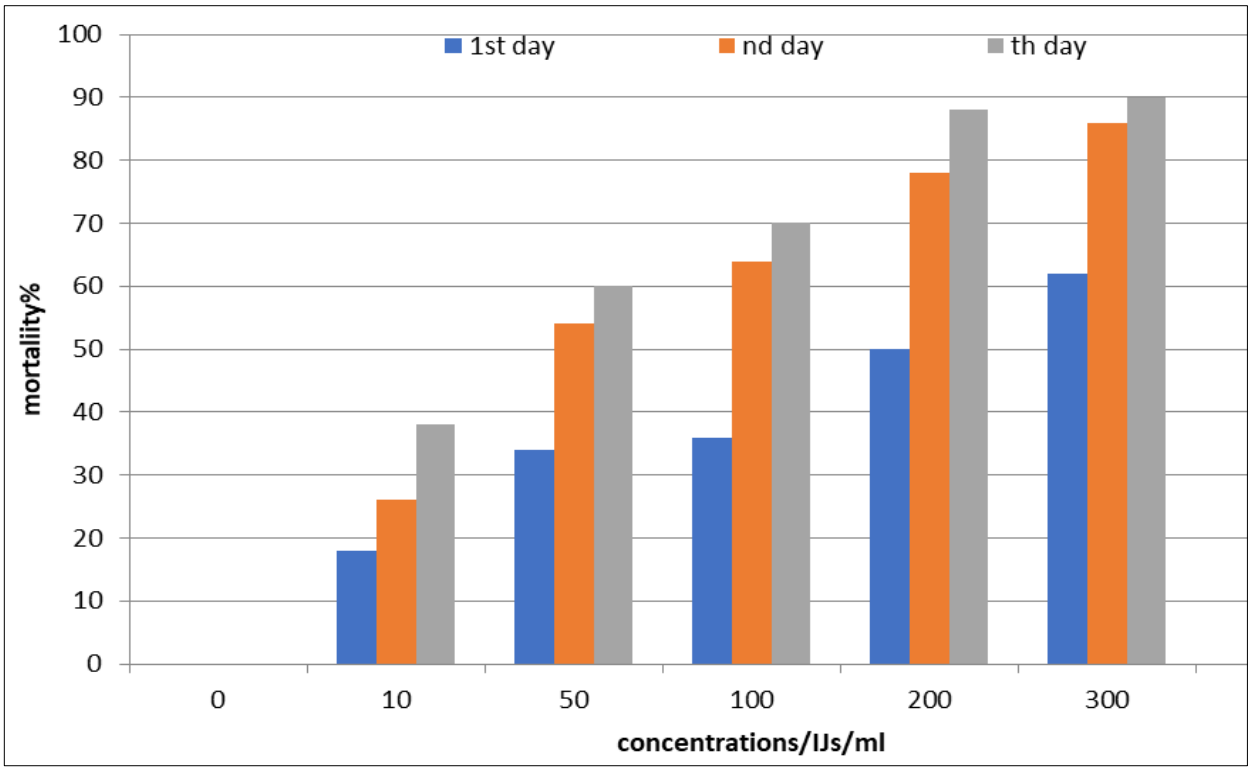


Fig 3: Mortality percentages of the 3rd larvae *S. frugiperda* during the period of treatment with *H.bacteriophora*

Table 3: Accumulative Mortality %+SE of *H. bacteriophora* on *S. frugiperda*

Concentrations (IJs/ml)	Day after treatment		
	1	2	3
10	18±6.6c	26±6.78d	34±6.78a
50	34±2.5bc	54±5.09c	60±3.16b
100	36±2.5b	64±5.09bc	70±3.16b
200	50±8.36ab	78±6.63ab	88±5.83a
300	62±4.9a	86±6.00a	90±4.47a
F	9.33**	15.42**	21.95**
P	0.0	0.0	0.0

Means in a Column followed with the same letter(s) are not significantly different at 5% level of probability, *= significant, **= Highly significant

The LC_{50} and LC_{90} values of third instar larvae of *S. frugiperda* exposed to nematodes were calculated in Table (4). Nematode exhibited mortality of 50% at a concentration of 2.37E+01 IJs/ml the LC_{90} values was 3.00E+02 IJs/ml. Data showed there are significant differences in the efficacy at all concentrations along the period of the experiment. Also, LT50 was 0.71day at 300 IJs/ml.

Table 4: Lethal concentrations of *H. bacteriophora* isolates on *S. frugiperda*

LC	Con.	Lower limit	Upper limit	Slope	Chi-square (X2
	(IJs/ml)	(IJs/ml)	(IJs/ml)		
25	6.22E+00	2.36E+00	1.13E+01		
50	2.37E+01	1.35E+01	3.49E+01		
75	9.00E+01	6.45E+01	1.29E+02	1.16	3.38
90	3.00E+02	1.99E+02	5.52E+02		
95	6.16E+02	3.65E+02	1.41E+03		
99	2.37E+03	1.10E+03	8.45E+03		

Quantification enzymes activity

The impact of entomopathogenic fungi and nematodes on the total protein content, transaminase, phenoloxidase, and alkaline phosphatase (ALP) enzyme activity of 3rd instar *S. frugiperda* treated with the LC50 of these pathogens was evaluated after 24 hours of treatment (Table 5). The results revealed a highly significant difference in total protein activity between the treatments and the control. Treatment with the LC50 of both pathogens resulted in a significant decrease in total protein content. The total protein content decreased by 40.5% and 14.6% for insects treated with EPF and EPN, respectively, compared to the control.

Furthermore, there was a considerable inhibition of glutamic oxaloacetic transaminase (GOT) in the control and treatment groups, with inhibition rates of -78.7% and -44.5% for B1 and nematodes, respectively. Also, GPT activity increased in the hemolymph of uninfected (control) instar larvae more than in infected larvae, activity reduction by *B. bassiana* and *H. bacteriophora* at 24 were (-1.32E+01%) and (-2.28E+01%), respectively (Table, 5).

The LC50 effect of EPF and EPN on the defense enzymes of treated *S. frugiperda* was shown by the data in Table (5). Defensive enzymes such phenoloxidase were activated when *S. frugiperda* was treated with LC50 of EPF and EPN, according to the results. Phenoloxidase levels were generally greater after EPF treatment than after EPN treatment. After 24 hours of treatment with EPF and EPN, the phenoloxidase activity were 1020.3, 870.0 (mO.D. units/min/mg protein), respectively. While it was 819.0 (mO.D. units/min/mg protein) for control.

However, there was a significant difference in alkaline phosphatase (ALP) between the treatments and the control, with a 41.4% rise in treated nematodes relative to the control treatment. While entomopathogenic fungi treatment showed enzyme was increased, recording (28.4%) compared with control (Table, 5).

Table 5: Changes in some biochemical activity in *S. frugiperda* after 24 h of treatment with the LC_{50} of B1 (*B. bassiana*) and *H. bacteriophora* under laboratory condition

Treatments	Total protein (mg/ g.b.wt)	Change %	GOT (mU/ mg protein	Change %	GPT *(mU/ mg protein*	Change %	Phenoloxidase *(mO.D. units/min/ mg protein*	Change %	ALP *(mU/ mg protein*	Change %
B1	24.5±0.7c	-40.5	305.3±7.9c	-78.7	1906.7±37.1b	-1.32E+01	1020.3±13.2a	24.58	206.7±5.7b	28.4
Nematodes	35±0.6b	-14.6	794.3±27.9b	-44.5	1695.7±17.2c	-2.28E+01	870.0±5.8b	6.23	227.7±5.4a	41.4
Control	41.1±0.6a	_____	1430.3±41.3a	____	2197.3±55.4a	_____	819.0±9.5c	_____	161.0±6.1c	____
F	163.7**	_____	374.5**	___	40.1**	_____	110.38**	_____	35.4**	____
P	0.0	_____	0.0	___	0.0	_____	__0.0____	_____	0.0	____

Means in a Column followed with the same letter(s) are not significantly different at 5% level of probability. **= Highly significant, Alkaline phosphates=ALP

Discussion

S. frugiperda, the fall armyworm, is a significant invasive pest that can lead to substantial reductions in crop yields. The data indicate that the effectiveness of EPF and EPN in causing mortality in third instar larvae of **S. frugiperda** significantly improved with higher concentrations and prolonged exposure times. For EPF, mortality results from extensive tissue damage, toxicosis, cellular dehydration, and the failure to absorb vital nutrients. Eventually, the fungal hyphae emerge from the host insect's body, releasing spores and starting a new infection cycle. (Perez *et al*., 2014). These findings are consistent with those reported by several researchers, including Rajula *et al*. (2021) [40]. Six isolates of *B. bassiana* were assessed for their effectiveness against the fall armyworm, seeing mortality rates that varied, with a maximum of 91.67% after twelve days post-inoculation. While some earlier studies have reported mortality rates reaching 100%, which is remarkable, rates of 60% and above are generally considered effective for managing insect populations (Hassan *et al*., 2019) [25]. Moreover, it has been confirmed that the lethal effect of B. bassiana is directly correlated with its concentration, with higher concentrations demonstrating increased efficacy against the pest (Yasin *et al*., 2019). Our findings reinforce this observation, as the highest mortality rates for all isolates were observed at a concentration of 1 × 10^8 conidia/ml.

Fergani and Refaei (2021) [20] reported that *B. bassiana* showed significant efficacy against *S. littoralis.* Additionally, Hu *et al.* (2021) [26] evaluated the pathogenic effects of various *B. bassiana* strains on *Hyphantria cunea*, finding that the LC50 values for strains Bb10331 and Bb7725 were 4.72 × 10^6 and 3.28 × 10^6 conidia/ml, respectively, after 120 hours of treatment. The corresponding LT50 values at a concentration of 1 × 10^8 conidia/ml were 71.13 hours and 74.54 hours. Moreover, the results for controlling *S. frugiperda* using *B. bassiana* are consistent with those from similar studies. El Husseini (2019) [14], who evaluated a local isolate of the fungus against *S. exigua*, noting that larval mortality rates increased with higher concentrations of conidiospores.

The findings confirmed that *Heterorhabditis bacteriophora* exhibited a strong insecticidal effect on *S. frugiperda* larvae. Research by Ozawa *et al*., (2023) [36] indicated that *H. megidis* significantly impacted *Monochamus alternatus* larvae, with inoculation of over 20 infective juveniles leading to 86–100% larval mortality. They observed that various nematode stages could reproduce within the insect host in approximately one week, as most larvae succumbed within 3–4 days post-inoculation. Andaló *et al*. (2010) [4] evaluated seventeen nematode populations at concentrations of 100, 250, and 500 infective juveniles per larva. Laboratory tests showed that at a concentration of 200 infective juveniles per larva, both *Steinernema arenarium* and *Heterorhabditis sp.* RSC02 achieved mortality rates of 100% and 97.6%, respectively. Garcia *et al*., (2008) [10] An assessment was conducted on the application methods for the entomopathogenic nematodes *Heterorhabditis indica* and *Steinernema* sp. to manage *S. frugiperda* infestations in corn crops. Insects possess a variety of defense mechanisms to combat invading pathogens, which encompass both morphological and immunological strategies aimed at protecting against these threats (Kunc *et al*., 2017; Berger and Jurcova, 2012; Irving *et al*., 2005) [33, 6, 27]. The analysis of enzyme activity showed a highly significant difference in the effectiveness of all treatments compared to the control group. Proteins, which are essential constituents of all living organisms, encompass a wide range of substances, including enzymes and hormones (Fagan *et al*., 2002) [16]. Our findings indicated that both *B. bassiana* and *H. bacteriophora* exerted a suppressive influence on the proteins of *S. frugiperda*, with *B. bassiana* demonstrating a more pronounced depressant effect compared to *H. bacteriophora*. It is anticipated that there may be a subsequent reduction in protein levels, attributed to the significant impact of entomopathogenic fungi on transaminases. These variations in results between entomopathogenic fungi and *H. bacteriophora* could be linked to the characteristics of the fungal spores. The influence of entomopathogenic fungi on the primary metabolites of the infected insects may stem from their utilization and consumption during the germination and penetration processes of the fungal conidia, which require these metabolites for nutritional purposes (Sewify and Moursy, 1993) [44]. Therefore, these results are consistent with those of other researchers. Sandhu *et al*. (2012) [43]. It was determined that entomopathogenic fungi infect their hosts through a process that involves penetrating the host's cuticle. For these fungi to thrive and reproduce, they must breach the cuticle and access the insect's internal environment to extract necessary nutrients. Once the fungus penetrates, it proliferates and spreads into the hemolymph by producing blastospores. Total protein content in the locusts treated with *M. anisopliae* showed a significant decline across all treatments. The results indicated notable biochemical changes in the locusts infected with the fungus.

Recently, Ghoneim *et al*. (2023) [23] identified that the entomopathogenic nematodes *S. carpocapsae* and *H. bacteriophora* exhibited significant pathogenicity towards the larvae of *Agrotis ipsilon*, he demonstrated that *H. bacteriophora* displayed a more significant reducing effect on the activity of GPT when interacting with *A. ipsilon*. This

disruption of phosphatase and transaminase activities resulted from the interactions between this nematode and its symbiotic bacteria, As a result, the host insect is led to its death. Moreover, these two species of EPNs significantly impacted the key body metabolites present in the hemolymph of the mentioned insect (Ghoneim *et al*., 2022) [22]. The findings highlight the potential of *S. carpocapsae* and H. bacteriophora as effective biological control agents against *A. ipsilon* larvae. The observed decline in total protein and the reduction in soluble protein in the host's hemolymph during parasitic interactions could be attributed to the secretion of proteolytic enzymes into the insect's hemocoel, leading to the breakdown of host proteins (Gillespie *et al*., 2000) [24].

Our findings indicated that both *B. bassiana* and *H. bacteriophora* had a significant impact on the activity of defensive enzymes, particularly phenoloxidases and alkaline phosphatase (ALP). Phenoloxidase (PO) is integral to the biosynthesis of melanin, which is essential for the immune response in insects. This enzyme not only plays a vital role in defense mechanisms but also supports various physiological processes, including the sclerotization of the cuticle, which is crucial for insect survival. Furthermore, during the process of wound healing, PO helps to reduce the loss of hemolymph by promoting the swift deposition of melanin at the injury site (Becker *et al*., 2001; Zibaee *et al*., 2011) [7, 50]. Importantly, phenoloxidases showed marked activation 24 hours after treatment, aligning with the penetration phase (St-leger *et al*., 1987) [46]. Additionally, Gabarty *et al*., (2013) [21] proved that the protein and phenoloxidase concentration of treated insect by entomopathogenic fungi, *B. bassiana* and *M. anisopliae*, substantially increase after one day.

Numerous studies have demonstrated a synergistic effect between entomopathogenic nematodes (EPN) and their symbiotic bacteria, which enables them to effectively bypass the immune defenses of insects, leading to the host's death within a period of 24 to 48 hours (Lu *et al*., 2017) [35]. This phenomenon has been experimentally validated in the case of *Spodoptera exigua* larvae (Darsouei *et al*., 2017) [13]. Furthermore, it is important to emphasize the significant role of symbiotic bacteria in overcoming the immune responses of their hosts. Ghoneim *et al*. (2023) [23] reported an increase in alkaline phosphatase (ALP) activity in the hemolymph of larvae that were six hours old. In contrast, a significant decrease in ALP activity was observed in the hemolymph of larvae treated with EPN, particularly at 24 and 48 hours post-treatment.

Conclusion

Entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPN) can be effectively integrated into a pest management strategy aimed at *S. frugiperda* due to their lethal impact on this pest. They also have a role in influencing changes in enzymes activity and the protein content of the insect, which leads to insect susceptibility. Nevertheless, nematodes demonstrate greater speed and efficacy compared to fungi in their pest control capabilities.

References

1. Abbott WW. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 1925;18:265-267.
2. Ali SS, Haron ENS, Ahmed MA, Abas AA, Elshaier ME. Isolation of entomopathogenic fungi and efficacy as a biological control agent on red spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae). Journal of Plant Protection Research Institute. 2020;3(2):761-770.
3. Ali M, Allouf N, Ahmad M. First report of entomopathogenic nematode *Steinernema affine* (Nematoda: Steinernematidae) in Syria and its virulence against Galleria mellonella L. (Lepidoptera: Pyralidae). Egyptian Journal of Biological Pest Control. 2022;32:101-114. https://doi.org/10.1186/ s41938-022-00602-x.
4. Andaló V, Santos V, Moreira GF, Moreira CC, Moino Junior A. Evaluation of entomopathogenic nematodes under laboratory and greenhouse conditions for the control of *Spodoptera frugiperda*. Ciência Rural. 2010;40(9):1862-1869. https://doi.org/10.1590/S0103-84782010005000151.
5. Amin TR. Biochemical and physiological studies of some insect growth regulators on the cotton leaf worm, *Spodoptera littoralis* (Boisd.). Ph.D. thesis, Faculty of science, Cairo Univ, 1998, 164p.
6. Berger J, Jurèová M. Phagocytosis of insect haemocytes as a new alternative model. Journal of Applied Biomedicine. 2012;10:35-40. Doi: https://doi.org/ 10.2478/v10136-012-0003-1.
7. Becker G, Sugumaran M, Cooper EL (Eds.). *Phylogenetic perspectives on the vertebrate immune system* (Vol. 484). Springer Science & Business Media. 2001;404:169-183.
8. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal. Biochem. I976;72:248-254.
9. Çağlayan A, Atay T, Kepenekci İ. Efficacy of some native Entomopathogenic nematodes against the alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae), and the lucerne beetle, *Gonioctena fornicate* (Brüggemann) (Coleoptera: Chrysomelidae), adults under laboratory conditions. Egyptian Journal of Biological Pest Control. 2021;31:89-95. https://doi.org/10. 1186/s41938-021-00436-z
10. Garcia LC, Raetano CG, Leite LG. Application technology for the entomopathogenic nematodes *Heterorhabditis indica* and *Steinernema* sp. (Rhabditida: Heterorhabditidae and Steinernematidae) to control *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) in corn. Neotropical Entomology. 2008;37:305-311. https://doi.org/10.1590/S1519-566X2008000300010
11. Chowański S, Kudlewska M, Marciniak P, Rosiński G. Synthetic Insecticides- is There an Alternative? Polish Journal of Environmental Studies. 2014;23(2):291-302.
12. Cruz-Avalos AM, Bivián-Hernández MA, Ibarra JE, Rincón-Castro MCD. High Virulence of Mexican Entomopathogenic Fungi Against Fall Armyworm, (Lepidoptera: Noctuidae). J Econ Entomol. 2019;112:99–107.
13. Darsouei R, Karimi J, Hosseini M, Ghadamyari M. Immune defense components of *Spodoptera exigua* larvae against entomopathogenic nematodes and symbiotic bacteria. Biocontrol Science and Technology. 2017;27(7):867-885. DOI: https://doi.org/10.1080/ 09583157.2017.1350632

14. El Husseini MM. Effect of the fungus, *Beauveria bassiana* (Balsamo) Vuillemin, on the beet armyworm, *Spodoptera exigua* (Hübner) larvae (Lepidoptera: Noctuidae), under laboratory and open field conditions. Egyptian Journal of Biological Pest Control. 2019;29(1):52-56. https://doi.org/10.1186/s41938-019-0158-0
15. Finney DJ. Probit analysis: A statistical treatment of the sigmoid response curve; Cambridge university press. London, New York: Melbourne, 1971, p333.
16. Fagan WF, Siemann E, Mitter C, Denno RF, Huberty AF, Woods HA, *et al*. Nitrogen in insects: implications for trophic complexity and species diversification. The American Naturalist. 2002;160(6):784-802. https://doi.org/10.1086/343879
17. FAO. Integrated management of the fall armyworm on maize a guide for farmer field schools in Africa, 2018. http://www.fao.org/faostat/en/.
18. El-Defrawi ME, Toppozada A, Mansour N, Zeid M. Toxicological studies on the Egyptian cotton leafworm, Prodenia litura I. Susceptibility of different larval instars to insecticides. J. Econ. Entomol. 1964;57:591-593.
19. Feng MG, Johnson JB, Kish LP. Survey of entomopathogenic fungi naturally infecting cereal aphids in irrigated cereal crops in southwestern Idaho. Environ Entomol. 1990;19:1534–1542.
20. Fergani YA, Refaei EAE. Pathogenicity induced by indigenous *Beauveria bassiana* isolate in different life stages of the cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) under laboratory conditions. Egyptian Journal of Biological Pest Control. 2021;31:1-7. https://doi.org/10.1186/s41938-021-00411-8
21. Gabarty A, El-Sonbaty SM, Ibrahim AA. Synergistic effect of gamma radiation and entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* on the humoral immune enzyme response in cotton leaf worm *Spodoptera littolaris* (Boisd). Egypt. Acad. J. Biol. Sci. 2013;6(3):1-10. DOI: 10.21608/eajbsa.2013.13221
22. Ghoneim K, Tanani M, Hassan HA, Bakr NA. Comparative Efficiency of The Entomopathogenic Nematodes, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*, against the Main Body Metabolites of *Agrotis ipsilon* (Lepidoptera: Noctuidae). Egyptian Academic Journal of Biological Sciences, C Physiology & Molecular Biology. 2022;14(2):57-66. Doi.10.21608/EAJBSC.2022.260471
23. Ghoneim K, Hassan HA, Tanani M, Bakr NA. Enzymatic Disturbance in Larvae of The Black Cut Worm, *Agrotis ipsilon* (Lepidoptera: Noctuidae), by Infection with The Entomopathogenic Nematodes, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*. Egyptian Academic Journal of Biological Sciences, C Physiology & Molecular Biology. 2023;15(1):121-142. DOI: 10.21608/EAJBSC.2023.285633
24. Gillespie JP, Burnett C, Charnley AK. The immune response of the desert locust *Schistocerca gregaria* during mycosis of the entomopathogenic fungus, *Metarhizium anisopliae* var acridum. Journal of Insect Physiology. 2000;46(4):429-437. https://doi.org/10.1016/S0022-1910(99)00128-6
25. Hassan FR, Abdullah SK, Assaf LH. Pathogenicity of the entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. endophytic and a soil isolate against the squash beetle, *Epilachna chrysomelina* (F.) (Coleoptera: Coccinellidae). Egypt. J. Biol. Pest Control. 2019;29:1–7.
26. Hu R, Bai P, Liu B, Yu J. On the virulence of two *Beauveria bassiana* strains against the fall webworm, *Hyphantria cunea* (Durry) (Lepidoptera: Erebidae), larvae and their biological properties in relation to different abiotic factors. Egyptian Journal of Biological Pest Control. 2021;31:1-7. https://doi.org/10.1186/s41938-021-00452-z
27. Irving P, Ubeda J, Doucet D, Troxler L, Lagueux M, Zachary D, *et al*. New insights into Drosophila larval haemocyte functions through genome wide analysis. Cell Microbiology. 2005;7:335-350. doi:10.1111/j.1462-5822.2004.00462.x.
28. Ishaaya I. Observation on the phenoloxidase system In the armored scale *Aonidiella aurantii* and *Chrysomphalus aonidum*. Comp. Biochem. Physiol. 1971;39 B:935-943.
29. Ismail SM. Field persistence of certain new insecticides and their efficacy against black cutworm, *Agrotis ipsilon* (Hufnagel). Bulletin of the National Research Centre. 2021;45:17-24. https://doi.org/10.1186/s42269-020-00481-y
30. Javed K, Javed H, Mukhtar T, Qiu D. Efficacy of *Beauveria bassiana* and *Verticillium lecanii* for the management of whitefly and aphid. Pakistan Journal of Agricultural Sciences. 2019;56(3):669-674.
31. Kaya HK, Aguillera M, Alumai A, Choo HY, De la Torre M, Fodor A, *et al*. Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world. Biological control. 2006;38:134-155.
32. Koppenhöfer HS. Bacterial Symbionts of Steinernema and Heterorhabditis. Nematology Monographs & Perspectives. 2007;5:735-808.
33. Kunc M, Badrul A, Pavel H, Ulrich T. Monitoring the effect of pathogenic nematodes on locomotion of Drosophila larvae. Fly. 2017;3:1–10. DOI: 10.1080/19336934.2017.1297350
34. Kumar D, Kumari P, Kamboj R, Kumar A, Banakar P, Kumar V. Entomopathogenic nematodes as potential and effective biocontrol agents against cutworms, Agrotis spp.: present and future scenario. Egyptian Journal of Biological Pest Control. 2022;32:42-51. https://doi.org/10.1186/s41938-022- 00543-5
35. Lu D, Macchietto M, Chang D, Barros MM, Baldwin J, Mortazavi A, *et al*. Activated entomopathogenic nematode infective juveniles release lethal venom proteins. PLoS Pathogen. 2017;13:e1006302. DOI: 10.1371/1006302.
36. Ozawa S, Maehara N, Takatsuka J, Aikawa T, Nakamura K. Insecticidal effect of the entomopathogenic nematode *Heterorhabditis megidis* (Nematoda: Heterorhabditidae) baited from the soil on the larvae of *Monochamus alternatus* (Coleoptera: Cerambycidae). Applied Entomology and Zoology. 2023;58(2):197-203. https://doi.org/10.1007/s13355-023-00820-1
37. Peçen A, Kepenekci I. Efficacy of entomopathogenic nematode isolates from Turkey against wheat stink bug, Aelia rostrata Boheman (Hemiptera: Pentatomidae) adults under laboratory conditions. Egyptian Journal of Biological Pest Control. 2022;32:91-97. https://doi.org/10.1186/s41938-022-00590-y

38. Perez LS, Florido JEB, Navarro SR, Mayagoitia JFC, Lopez MAR. Enzymes of Entomopathogenic Fungi Advances and Insights. Advances in Enzyme Research. 2014;2:65-76.
39. Powell MEA, Smith MJH. The determination of serum acid and alkaline phosphatase activity with 4-aminoantipyrine. J. Clin. Pathol. 1954;7:245-248.
40. Rajula J, Pittarate S, Suwannarach N, Kumla J, Ptaszynska AA, Thungrabeab M, *et al*. Evaluation of native entomopathogenic fungi for the control of fall armyworm (Spodoptera frugiperda) in Thailand: A sustainable way for eco-friendly agriculture. Journal of Fungi. 2021;7(12):1073-1086. https://doi.org/10.3390/jof 7121073.
41. Ramanujam B, Poornesha B, Shylesha AN. Effect of entomopathogenic fungi against invasive pest *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in maize. Egyptian Journal of Biological Pest Control. 2020;30:100-105. https://doi.org/10.1186/s41938-020-00291-4
42. Reitman S, Frankel S. Colourimetric method for aspartate and alanine transaminases. Amer. J. Clin. Pathol. 1957;28:56.
43. Sandhu SS, Sharma AK, Beniwal V, Goel G, Batra P, Kumar A, *et al*. Myco-biocontrol of insect pests: factors involved mechanism, and regulation. Journal of Pathogens. 2012;1:10. https://doi.org/10.1155/2012/126819
44. Sewify GH, Moursy EB. Impact of the entomopathogenic fungus, Verticillium lecanii on lipids and free fatty acids of cabbage aphid, *Brevicoryne brassicae.* Bulleten Entomology Society of Egypt. 1993;71:153-162.
45. Sisay B, Simiyu J, Mendesil E, Likhayo P, Ayalew G, Mohamed S, *et al*. Fall Armyworm, *Spodoptera frugiperda* Infestations in East Africa: Assessment of Damage and Parasitism. Insects. 2019;10(7):195-204. https://doi.org/10.3390/insects10070195
46. St-leger RJ, Cooper RM, Charnley AK. Production of cuticle-degrading enzymes by the entomopathogen Metarhizium anisopliae during infection of cuticles from *Calliphora vomitoria* and *Manduca sexta*. Microbiology. 1987;133(5):1371-1382. https://doi.org/10.1099/00221287-133-5-1371
47. Tiryaki D, Temur C. The fate of pesticide in the environment. Journal of Biological and Environmental Sciences. 2010;4(10):29-32.
48. Vattikonda SR, Sangam SR. Effect of forskolin on the growth and differentiation of the ovary of *Papilio demole*us L. (Lepidoptera: Papilionidae). International Research Journal of Environmental Science. 2017;6:13-17.
49. Yasin M, Wakil W, Ghazanfar MU, Qayyum MA, Tahir M, Bedford GO. Virulence of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against red palm weevil, *Rhynchophorus ferrugineus* (Olivier). Entomol. Res. 2019;49:3-12.
50. Zibaee A, Bandani AR, Talaei-Hassanlouei R, Malagoli D. Cellular immune reactions of the sunn pest, *Eurygaster integriceps*, to the entomopathogenic fungus, *Beauveria bassiana* and its secondary metabolites. Journal of Insect Science. 2011;11:1-16. doi: 10.1673/031.011.13801.
51. Zimmermann G. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Science and Technology. 2007;17(6):553–596. https://doi.org/10.1080/09583150701309006.