Entomology

Comparative effectiveness between entomopathogens and conventional insecticide against *Spodoptera frugiperda* larvae and accompanying alteration in some enzymatic activities

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Abstract

Background: The *Spodoptera frugiperda* (J. E. Smith) fall armyworm (Lepidoptera: Noctuidae) is a dangerous insect pest of an excessive number of crops., with larvae attacking the plants at all growth stages.

Materials: Protecto (*Bacillus thuringiensis*), Biossiana (*Beauveria bassiana*) and BioMeta (*Metarhizium anisopliae*), compared to abamectin (traditional insecticides), were assessed against *S. Frugiperda* in maize field. The enzymatic activities of the larvae were determined 48 hours after exposure to the tested pesticides.

Results: Under laboratory condition, the entomopathogenic bacteria was more effective against fall armyworm (2nd instar) than entomopathogenic fungi, with total mortalities of 96.67 and 93.33% due to Biometa and Biossiana, respectively. Opposite results were obtained in case of 4thinstar larvae, with total mortalities of 53.33 and 50% due to Biossiana and Biometa, respectively. In maize fields, abamectin was the most potent compound in reducing fall armyworm larval population (94.48% reduction) three days post-treatments, followed by Biometa (78.19% reduction), while the least one was Protecto (43.53% reduction). Five and seven days post treatments, the highest reductions (94.66 and 94.84%, respectively) were recorded in abamectin treatment, followed by Biometa (79.38% and 80.56% reduction, respectively). On the other hand, 10 days after treatments, Biossiana treatment induced the highest reduction (77.90%), followed by abamectin (75.41%). Overall average larval reductions, proved that abamectin induced the highest value (89.87%), followed by Biossiana (59.47%) and Biometa (57.91%) while Protecto resulted in the lowest reduction (32.56%) The correspondent total protein ratios were 0.68, 0.06, 0.90, 0.69. In addition, the highest lipid peroxide activity was highest in *S. frugiperda* larvae treated with Protecto (724.40) and abamectin (376.90), but lowest in case of treating the larvae by Biometa (179.52) and Biossiana (158.22). Protecto, Biometa and abamectin applications induced higher acetylcholine esterase activity in the 4th instar larvae compared with larvae treated with Biossiana. The lowest activity of chitinase was detected in case of Biossiana treatment. The chitinase activity was 15.59 in abamectin, and 20.78 in Protecto treatments.

Keywords: entomopathogens, insecticide, fall armyworm, enzymatic activities

Introduction

One of the most common invasive polyphagous pests, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is responsible for damaging around 353 plant species, including cotton, corn, sorghum, sugarcane, turfgrass, and vegetable crops (Montezano 2018; Gamil 2020; Timilsena *et al*. 2022) [25, 13, 40].

Komombo (Aswan Governorate) the *S. frugiperda* was initially discovered in maize fields in 2019 (Dahi *et al*. 2020, Gamil 2020) [9, 13]**.** The larval stage is destructive to the infested crops, as the caterpillars feed on the vegetative and reproductive sections of the host plants (Sarmento *et al*., 2002) [31].

A number of control methods are available to reduce the effects of fall armyworms, such as synthetic insecticides, biopesticides like viruses (e.g., multiple nucleopolyhedrovirus), bacteria, *Bacillus thuringiensis* (*Bt*), botanicals (e.g., neem extracts), genetically modified crops that contain *Bt* toxins, mechanical control methods (e.g., handpicking the caterpillars), or cultural methods (Abrahams *et al*., 2017; Guo *et al*., 2020; Harrison *et al*., 2019; Wan *et al*., 2021) [3, 14, 17, 42].

Entomopathogenic fungi (EPF) include different strains of hyphomycetous fungus, such as *Beauveria bassiana* (Balsamo), *Vuillemin* and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae), which are potentially useful as colonized endophytes belonging to the deuteromycete class. Beauvericin (cyclic hexadepsipeptides), a secondary metabolite mycotoxin that *Beauveria bassiana* releases into its host plants, can poison herbivorous insects with a white muscardine illness (Shah and Pell 2003; Mwamburi 2021) [35, 26]. *B. bassiana* pathogenicity and metabolic activities are primarily a possible source of lipases, which endow the bacterium with a strong virulence factor (Vici *et al*. 2015) [41]. In the insect internal integument, lipases hydrolyze the ester bonds of lipoproteins, lipids, and waxes, which affects cuticle adherence and penetration (Ali *et al*. 2009; Silva *et al*. 2010; Dhawan and Joshi 2017) [4, 37, 11].

According to recent research, entomopathogenic fungi and bacteria are an effective biological control agents against *S. frugiperda* early instars in both lab and field settings (Shahzad *et al*., 2021; Ramos *et al*., 2020) [36, 28].

Therefore, the purpose of the present investigation was to examine the impact of entomopathogenic bacteria (*Bacillus thuringiensis* var. kurstaki), fungi (*Beauveria bassiana* and *Metarhizium anisopliae*), and conventional chemical compound (abamectin) on second and fourth instar larvae of *Spodoptera frugiperda*, as well as the accompanying alteration in some enzymatic activities.

Materials and methods

1. Rearing *S. frugiperda* in the laboratory

Larvae of *S. frugiperda* were collected from infested maize fields in glass jars and transferred to be reared under laboratory conditions (26 ± 2°C and 65 ± 5% RH). The collected larvae were individually placed in cups (2.5 cm diameter and 5 cm height) and fed upon fresh maize leaves until pupation**.** The pupae were sexed, one male and one female were introduced into cages for mating and oviposition. The cages were provided with sugar solution to activate female egg-laying.

2. Tested entomopathogens and insecticide

Evaluated entomopathogens against fall army worm, as well as the conventional insecticide, are listed in Table (1).

Table 1: Common and trade names of compounds evaluated against *Spodoptera frugiperda* larvae

Common name	Trade name	Chemical class	Application rate/100L
Beauveria bassiana (1 X 10^8 colony-forming unit (CFU) mg^{-1})	Biossiana 2,5%WP	Entomopathogenic fungi (biocides)	250 g
Metarhizium anisopliae (1 X 10^8 colony-forming unit (CFU) mg^{-1})	BioMeta 2,5% WP	Entomopathogenic fungi (biocides)	250 g
Bacillus thuringiensis Subsp (Kurstaki)	Protecto (Local) 9.4% WP	Entomopathogenic bacteria (biocides)	250g
Abamectin	Espinosad 1.8% EC	Avermectin	40 ml

3. Pesticide evaluation

To determine the larval mortality percentage, toxicity tests using entomopathogenic bacteria (*Bacillus thuringiensis*) and fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) compared to a traditional insecticide (abamectin) (Table1) were conducted on the second and fourth instar larvae of *S. frugiperda*. Fresh leaves of maize were dipped in each treatment (concentration are shown in Table 1) for 10 seconds, and allow to naturally dry at room temperature for 5 minutes. The treated leaves were introduced into jars having *S. frugiperda* larvae of second and fourth instars (10 larvae per jar, 3 duplicates). The jars were placed in an incubator at 26 ± 2°C and R.H. 65 ± 5°C. Mortalities of larvae were daily noted up to 10 days post-treatments.

4. The effect of entomopathogens and conventional insecticide, against *S. frugiperda* on maize under field condition

An experiment was conducted during 2023 season at maize fields of Kafr-El sheikh Governorate, Egypt, to evaluate the efficacy of entomopathogenic bacteria and fungi compared to the traditional insecticide (abamectin) against *S. frugiperda* (Table1). Four treatments were included in the experiment, which was set up in a randomized full block design [Protecto (*Bacillus thuringiensis*), Biossiana (*Beauveria bassiana*) (1 X 10^8Colony-forming unit (CFU) mg^{-1}) and BioMeta (*Metarhizium anisopliae*) (1 X10^8 Colony-forming unit (CFU) mg^{-1}), abamectin and check (without treatment)]. Every treatment was conducted three times (75 m^2 plot area). A knapsack sprayer provided with one nozzle delivering 200 L water/feddan, was used. Just before spray, 30 plants (10 plants x 3 replicates) were picked up randomly, and numbers of alive *S. frugiperda* larvae were recorded. Three, five, seven, and ten days after treatments, 30 plants were picked up from each plot and numbers of alive larvae were recorded. Percentages of larval reductions were calculated according to the formula of Henderson and Tilton (1955) as follows:

$$\% \text{ population reduction} = \left|1 - \frac{Ta \times Cb}{Tb \times Ca}\right| \times 100$$

Where:

Ta = Number of insects in treated plots after spray
Tb = Number of insects in treated plots before spray
Ca = Number of insects in control plots after spray
Cb = Number of insects in treated plots before spray

5. Enzymatic activity

After 48 hours of exposure to the aforementioned pesticides, crude extract was obtained from *S. frugiperda* larvae in their fourth instar, as compared to the control group. For every treatment, three replicates were allocated. Ten pre-starved larvae were given an acceptable amount of treated maize leaves in each replicate, and the larvae were left for a 24 h. The surviving larvae of each treatment were then prepared for biochemical analysis in the Insect Physiology Laboratory, Plant Protection Research Institute, Dokki, Giza, Egypt.

5.1 Preparation of insect homogenates

Batches of *Spodoptera frugiperda* 4th instar larvae of different treatments as well as the control group was also weighed. A teflon homogenizer with a crushed ice jacket was used to mechanically homogenize each batch in 10 volumes (W/V) of 0.1 M phosphate buffer, pH 7, for a duration of two minutes. The homogenates were then centrifuged using a cooling centrifuge for 30 minutes at 4°C and 4000 rpm. Acetylcholinesterase (A.Ch.E.), chitinase, lipid peroxide (malondialdehyde) activity as well as the soluble protein content were measured in the resulting solution.

5.2. Determination of total protein content

Total proteins were estimated by the method of Bradford (1976) [8] using a standard of bovine serum albumin.

5.3. Determination of lipid peroxide (malondialdehyde)

Lipid peroxide (malondialdehyde) activity was measured according to Satoh (1978) [33] and Satoh *et. al.* (1979) [34].

5.4. Determination of acetylcholinesterase (A. Ch. E.) activity

Acetylcholinesterase (A. Ch. E.) activity was measured according to the method described by Simpson *et.al.* (1964) [38],

using acetylcholine bromide (A. Ch. Br) as asubstrate.

5.5. Determination of chitinase activity

Chitinase was assayed using 3,5-dinitrosalicylic acid reagent to determine the free aldehydic groups of hexosamine liberated on chitin digestion according to Ishaaya and Casida (1974) [19].

6. Statistical analysis

Data was subjected to analysis of variance (ANOVA) and the means were compared with LSD test at 0.05 levels, using the SAS program (SAS Institute, 1988) [32].

Results and discussion

Effectiveness of entomopathogens and conventional insecticide against *Spodoptera frugiperda* under laboratory conditions

Data in Table (1) show the influence of tested entomopathogenic bacteria, fungi, and atraditional insecticide on the mortality percentage of 2nd instar of *Spodoptera frugiperda* larvae under laboratory conditions. No mortality occurred at the second day with all biocides, while complete mortality occurred by the conventional insecticide (abamectin) two days after treatment. Protecto (*Bacillus thuringiensis*) killed all larvae at the 3rd day after treatment.

Thus, the entomopathogenic bacteria was more effective against fall armyworm (2nd instar) than entomopathogenic fungi. Total mortalities were 96.67 and 93.33% due treatments of Biometa (*M. anisopliae*) and Biossiana (*B. bassiana*), respectively.

Table 1: Mortality percentage of 2nd instar *Spodoptera frugiperda* larvae treated with entomopathogens and insecticide under laboratory conditions (26 ± 2 °C,65 ± 5 RH) during 2023 maize season

Days after treatment	Control	BioMeta	Biossiana	Protecto	Abamectin
2nd	0	0.00	0.00	0	100
3rd	0	46.67	46.67	100	0
4th	0	16.67	23.33	0	0
5th	0	23.33	13.33	0	0
6th	0	10.00	10.00	0	0
7th	0	0.00	0.00	0	0
8th	0	0.00	0.00	0	0
9th	0	0.00	0.00	0	0
10th	0	0.00	0.00	0	0
Total	0	96.67	93.33	100	100

Data in table (2) show the influence of tested entomopathogens and conventional insecticides on the mortality percentage of *Spodoptera frugiperda* 4th larval instar under laboratory conditions. No mortality occurred at the second day with all biocides, while the complete mortality occurred by the conventional insecticide (abamectin) two days after treatment, while complete mortality occurred by the conventional insecticide (abamectin) two days after treatment. Protecto (*Bacillus thuringiensis*) killed all larvae by the 5th day from treatment.

Thus, the entomopathogenic bacteria is more effective against fall armyworm (4th instar) than entomopathogenic fungi. Total mortalities were 53.33 and 50% due treatments of biossiana (*B. bassiana*) and biometa (*M. anisopliae*), respectively.

Table 2: Mortality percentage of 4th instar *Spodoptera frugiperda* larvae treated with entomopathogens and insecticide under laboratory conditions (26 ± 2 °C, 65 ± 5 RH) during 2023 maize season

Days after treatment	Control	Biometa	Biossiana	Protecto	Abamectin
2nd	0	0.00	0.00	0	100
3rd	0	0.00	6.67	80	0
4th	0	20.00	3.33	10	0
5th	0	20.00	0.00	10	0
6th	0	10.00	30.00	0	0
7th	0	0.00	13.33	0	0
8th	0	0.00	0.00	0	0
9th	0	0.00	0.00	0	0
10th	0	0.00	0.00	0	0
Total	0	50	53.33	100	100

The present results agree with those of Massochin *et al* (2010) [22] who showed that *B. thuringiensis* resulted in a complete mortality of *S. frugiperda* 2nd instar larvae. Ricardo *et al.* (2000) [30] and Abd El-Salam *et al.* (2018) [1] obtained similar results with mortalities of 80, 40 and 100% as a residual effect of Bt strains against same instar of fall army worm.

The effect of entomopathogens and conventional insecticide, against *S. frugiperda* on maize under field condition

Data presented in Table (3) and Fig. (1) show the influence of tested entomopathogenic bacteria, fungi, and traditional insecticide on the reduction percentage of *Spodoptera frugiperda* population under field condition during 2023 maize season.

Table 3: Potency of entomopathogens and chemical insecticide in reducing *Spodoptera frugiperda* larval population under field conditions at El-Hamol (Kafr-El sheikh Governorate) during 2023 maize season

Treatment	Population reduction % after treatments					
	1 day	3 days	5 days	7 days	10 days	Average ± SE
BioMeta *Metarhizium anisopliae* (1 X 10^8 colony-forming unit (CFU) mg $^{-1}$)	0	78.19	79.38	80.56	51.40	57.91 ± 15.46
Biossiana *Beauveria bassiana* (1 X 10^8 (CFU) mg $^{-1}$)	0	71.91	73.16	74.40	77.90	59.47 ± 14.90
Protecto *Bacillus thuringiensis*	0	43.53	38.25	32.96	48.07	32.56 ± 8.53
Abamectin [*7*mg a.i.l^{-1}]	89.97	94.48	94.66	94.84	75.41	89.87 ± 3.73

Data presented in Table (3) show that abamectin was the most potent compound in reducing the population density of *spodoptera frugiperda* larvae after three days with a value of 94.48% reduction, followed by Biometa (78.19% reduction), while the least one was Protecto (43.53% reduction). Also, after 5 and 7 days, the highest reductions (94.66 and 94.84%) were recorded in abamectin, respectively, followed by Biometa (79.38% and 80.56% reduction respectively). On the other

hand, 10 days after Biossiana treatment, the highest reduction was 77.90%, followed by abamectin (75.41%), of treatments showed that abamectin induced the highest reduction (89.87%) followed by Biossiana (59.47%) and Biometa (57.91%) while protecto induced the lowest reduction (32.56%) in *Spodeptra frugiperda* larval population.

Ali and Ibrahim (2023) [5] obtained more than 60% *S. frugiperda* mortality due to application of two bacterial strains, *Lysinibacillus macroides* and *Brevundimonas olei* in field experiments. El-Hadary *et al.* (2023) showed that using *B. thuringiensis* as a bio-control, succeeded in reducing the larval density of *S. frugiperda* to 64.8% and 75.3% at two governorates (Beni-suef and Al-Qalubia, respectively). These results may be helpful in the IPM programs to bio control of *S. frugiperda* on corn plants. Nashwa Amein (2023) indicated that *B. thuringeinsis* caused a significant prolongation in the larval and pupal duration of *S. frugiperda*, and resulted in a reduction in the percentage of pupation as attributed to the slower metabolic rate of these larvae.

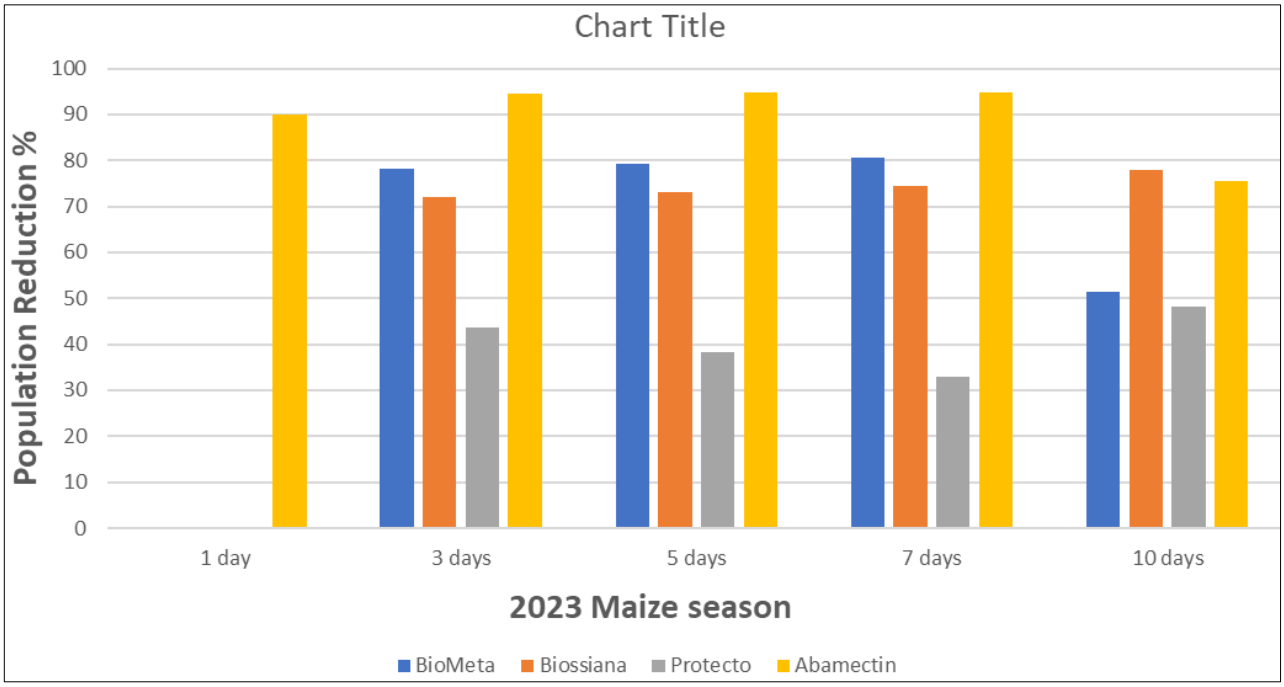

Fig 1: Potency of entomopathogenic bacteria, fungi, and chemical insecticide in reducing *Spodoptera frugiperda* larval population under field conditions at El-Hamol at Kafr-El Sheikh Governorate during 2023 maize season.

Efficiency of the bio-pesticide, *Beauveria bassiana*, *Metarhizium anisopliae* and *Bacillus thuringiensis* on infested maize plants with *S. frugiperda*

Bio-pesticides succeeded in reducing the population of this pest compared with the control. These results are similar to those of Ali and Ibrahim (2023) [5] who obtained a high efficacy of the two bacterial strains, *Lysinibacillus macroides* and *Brevundimonas olei* against *S. frugiperda* with more than 60% reduction in the field.

Total protein content

The results summarized in Table (4) and illustrated in Fig. (2) show the total protein contents in whole homogenates of *Spodoptera frugiperda* 4th larval instar due to infection with entomopathogenic bacteria, fungi, and effect of the chemical compound under laboratory conditions. Untreated (check) had the highest amount of the total protein content (19.48 mg/g. b. wt. /min.) as compared with the other biocides. The lowest protein contents (1.08 mg/g. b. wt. /min.) were assessed in *S. frugiperda* larvae infected with entomopathogenic fungi (biossiana). While the corresponding amounts of protein contents in Protecto and abamectin treatments were 13.36 and 13.15 mg/g. b. wt./min., respectively.

The correspondent total protein ratios due to (Biometa, Protecto, abamectin and Biossiana) treatments as compared with the baseline untreated (control) were 0.90, 0.69, 0.68 and 0.06, respectively.

Table 4: Total protein content in whole homogenates 4th larval instar of *Spodoptera frugiperda* treated with entomopathogens and insecticide under laboratory conditions (26± 2 °C, 65 ± 5 RH) during 2023 maize season

Treatment	Total protein Content (mg/g. b. wt. /min.)	Total protein content ratio
Biometa *Metarhizium anisopliae*	17.61 ± 0.15	0.90
Biossiana *Beauveria bassiana*	1.08 ± 0.31	0.06
Protecto (*Bacillus thuringiensis*)	13.36 ± 0.25	0.69
Abamectin	13.15 ± 0.24	0.68
Control (check)	19.48 ± 0.28	1.00
L.S.D.	21.74	

Total protein content is expressed as: mg/g. b. wt. /min.

$$\text{Activity ratio} = \frac{\text{Enzymatic activity in larvae in different treatments}}{\text{Enzymatic activity in larvae of control (untreated)}}$$

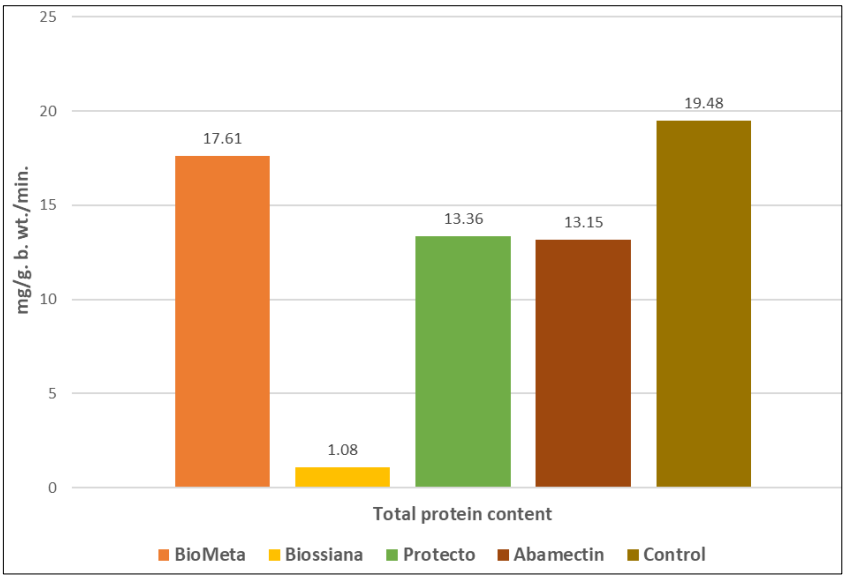

Fig 2: Total protein content in whole homogenates in the 4th larval instar of *Spodoptera frugiperda* treated with entomopathogens and insecticide under laboratory conditions (26 ± 2 °C, 65 ± 5 RH) during 2023 maize season

Lipid peroxide (Malondialdehyde) activity

Data presented in Table (5) and illustrated in Fig (3) reveal that lipid peroxide activity ratios were lower in Biometa and Biossiana compared to the untreated ones. However, Protecto treatment exhibited the highest activity ratio (2.26), followed by abamectin. In the same direction, the highest lipid peroxide activity was highest in *S. frugiperda* larvae treated with Protecto (724.40) and abamectin (376.90), but lowest in case of treating the larvae by Biometa (179.52) and Biossiana (158.22).

Table 5: Lipid peroxide (Malondialdehyde) activity in whole homogenates of *Spodoptera frugiperda* 4th larval instar as influenced by entomopathogens and conventional insecticide under laboratory conditions (26 ±2°C, 65 ± 5RH) during 2023 maize season

Treatment	Lipid Peroxide (Malondialdehyde) activity (Malondialdehyde nmol / g. tissue)	Activity ratio
BioMeta (*Metarhizium anisopliae)*	179.52 ± 1.82	0.56
Biossiana (*Beauveria bassiana)*	158.22 ± 1.00	0.49
Protecto (*Bacillus thuringiensis)*	724.40 ± 3.64	2.26
Abamectin	376.90 ± 19.10	1.18
Control (check)	320.23 ± 10.02	1.00
L.S.D.	17.21	-

Activity is expressed as: Malondialdehyde nmol / g. tissue in sample: Tissue = A Sample/ A Standard X 10/ g. tissue used

$$\text{Activity ratio} = \frac{\text{Enzymatic activity in larvae in different treatments}}{\text{Enzymatic activity in larvae of control (untreated)}}$$

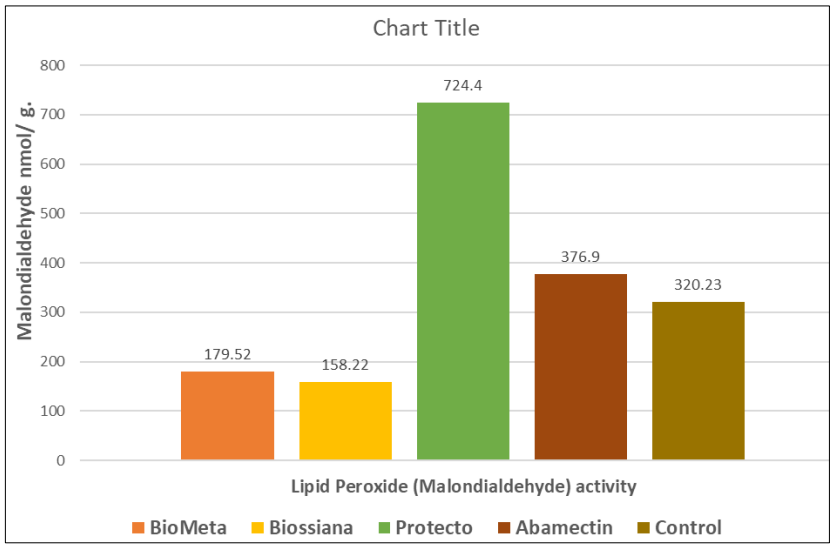

Fig 3: Lipid peroxide (Malondialdehyde) activity in whole homogenates of *Spodoptera frugiperda* 4th larval instar as influenced by entomopathogens and conventional insecticide under laboratory conditions (26 ±2°C, 65 ± 5RH) during 2023 maize season

Acetylcholinesterase (A.Ch.E.) activity

Acetylcholine esterase A.Ch.E. is essential to the preservation of the nerve activity by eliminating acetylcholine released in the passage of an impulse synapses and possible also along axons. Data presented in Table (6) and (Fig. 4) show the inhibitory effect of entomopathogens and conventional insecticide against acetylcholinesterase activity. Entomopathogenic bacteria (Protecto), Biometa and abamectin applications induced higher acetylcholinesterase activity in the 4th instar larvae compared with larvae infected with Biossiana (*Beauveria bassiana*) which was the least. The correspondent activity ratios associated with the four treatments as compared with the baseline control were 1.65, 1.49, 1.48 and 0.27, respectively.

Table 6: Acetylcholinesterase activity in whole homogenates of *Spodoptera frugiperda* 4th larval instar infected with entomopathogens and conventional insecticide under laboratory conditions (26 ± 2 °C, 65 ± 5) during 2023 maize season

Treatment	Acetylcholinesterase activity (µg AchBr release / gm body weight /min.)	Activity ratio
BioMeta *(Metarhizium anisopliae)*	223.24 ± 5.00	1.49
Biossiana *(Beauveria bassiana)*	40.41 ± 0.55	0.27
Protecto *(Bacillus thuringiensis)*	246.01 ± 2.85	1.65
Abamectin	220.67 ± 1.39	1.48
Control	149.47 ± 0.85	1.00
L. S. D	30.71	-

Activity is expressed as: µg AchBr release / gm body weight /min.

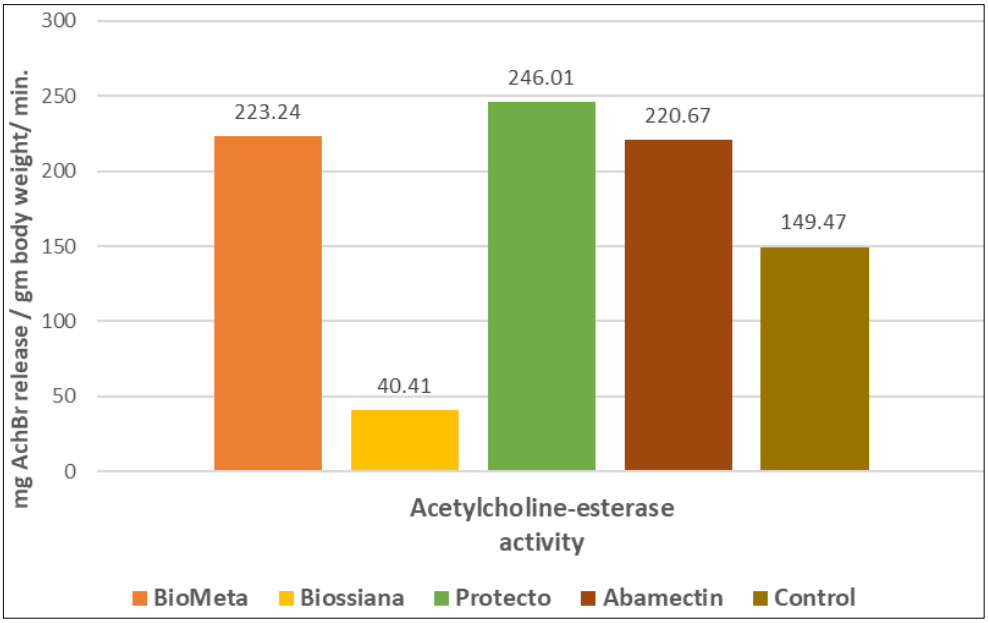

Fig 4: Acetyl cholinesterase activity in whole homogenates of *Spodoptera frugiperda* 4th larval instar infected with entomopathogens and conventional insecticide under laboratory conditions (26 ± 2 °C, 65 ± 5) during 2023 maize season

Chitinase activity

Data in Table (7) and Fig (5) clarifies that chitinase activity was highest (118.67) in the homogenate of *S. frugiperda* larvae (2nd instar) treated with Biometa, followed by the untreated (check) larvae with the activity value of 63.62. However, the lowest activity of chitinase was detected in case of Biossiana treatment. The enzyme activity was 15.59 in abamectin, and 20.78 in Protecto treatments.

The total protein levels and three enzymatic activities (Lipid Peroxide (malondialdehyde), acetylcholinesterase, and chitinase) were disturbed in *S. frugiperda* larval instars treated with entomopathogenic bacteria, fungus, and conventional insecticide.

Table 7: Chitinase activity in whole homogenate larval 4th instar of *Spodoptera frugiperda* treated with entomopathogens and conventional insecticide under laboratory conditions (26 ± 2 °C, 65 ± 5 RH) during 2023 maize season

Treatment	Chitinase activity (µg N-acetylglucosamine released / g. b. wt. / min.)	Activity ratio
BioMeta *(Metarhizium anisopliae)*	118.67 ± 0.16	1.87
Biossiana *(Beauveria bassiana)*	7.06 ± 0.11	0.11
Protecto *(Bacillus thuringiensis)*	20.78 ± 0.38	0.33
Abamectin	15.59 ± 0.06	0.25
Control	63.62 ± 0.65	1.00
L. S. D	3.99	

Each value represents the average of three replicates ± S.E Activity is expressed as: µg N-acetylglucosamine released / g. b. wt. / min.

$$\text{Activity ratio} = \frac{\text{Enzymatic activity in larvae in different treatments}}{\text{Enzymatic activity in larvae of control (non-treated)}}$$

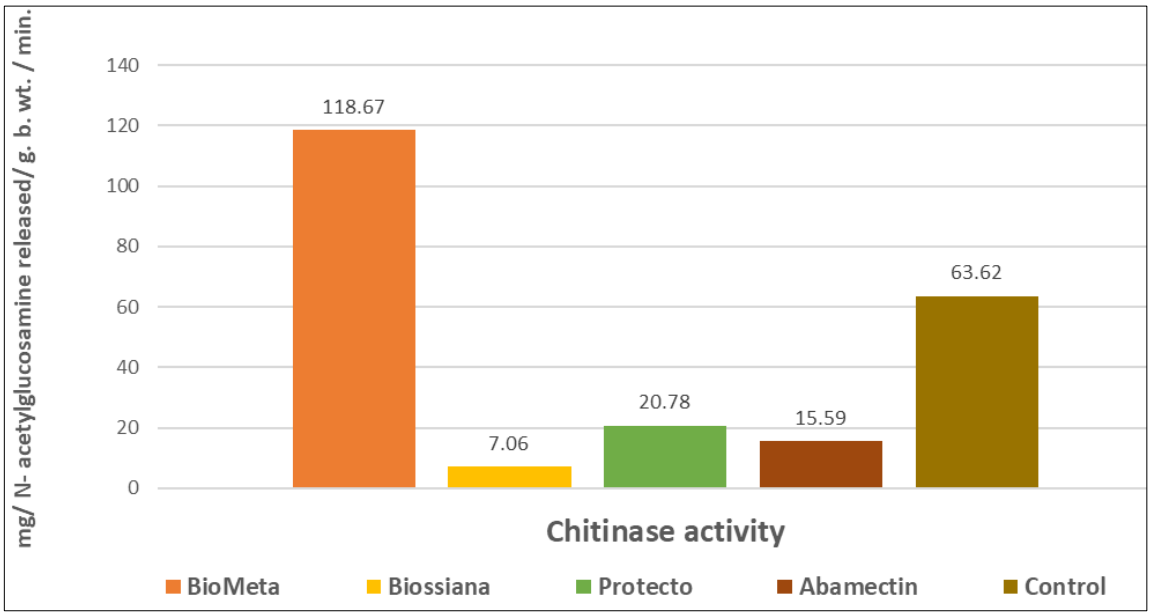

Fig 5: Chitinase activity in whole homogenate larval 4th instar of *Spodoptera frugiperda* treated with entomopathogens and conventional insecticide under laboratory conditions (26 ± 2 °C, 65 ± 5 RH) during 2023 maize season

As shown by Arakane and Muthukrishnan (2010) [6], phylogenetic analysis can separate insect chitinases and chitinase-like proteins into many families. The breakdown of chitin in the old epidermis and the development of new epidermis were impacted when the expression of the *S. frugiperda* chitinase gene was blocked, according to Liu *et al*. (2022) [21]. Additionally, the concentration of chitin increased, preventing the larvae from going through a normal moulting process. According to Merzendorfer and Zimoch (2003) [23], structural remodeling incorporating chitin is necessary for the growth and morphogenesis of insects. For this reason, insects produce chitin synthases and chitinolytic enzymes in several tissues throughout their bodies.

The results of the present study agree with those obtained by Abd-El Wahed *et al.*, (2011) [2] and with Hamama *et al.*, (2015) [16] who reported that changes in enzymatic activities after treatment with bioinsecticides indicated that the changes in the physiological balance of the midgut affect these enzymes. El-Sheikh (2012) [12] studied the effects of *B. thuringiensis* on *S. littoralis* and found that the carbohydrates hydrolyzing enzymes as amylase insignificantly decreased compared to the untreated one, and trehalase significantly decreased.

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