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Toxicological and biochemical parameters of microbial preparations on the cotton leafworm *Spodopter alittoralis* (Lepidoptera :Noctuidae)

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Abstract:

Laboratory experiments were conducted to evaluate the efficacy by sequential treatments of the two entomopathoginc nematode isolates, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* and the entomopathoginc fungus, *Beauveria bassiana* (Biopower1.4%WP) as well as the combined effect of them against the 3rd instar larvae of the cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Data revealed that the nematode, *H. bacteriophora* was more potent than *S. carpocapsae* where their Lc_{50} values after 72h of treatment recorded 53.3 and 81.41 Jv/ml, respectively. On the other hand, the LC_{50} value of the entomopathoginc fungi, *B. bassiana* scored 20.08 gm/l. The combination between the three tested bio-agents using sequential method (*B. bassiana*+ *H. bacteriophora*) and (*B. bassiana* + *S. carpocapsae*) against *S. littoralis* 3rd instar larvae indicated a potantiation effect .The highest larval mortality percentage (93.75%) was recorded by treatment with the combination of the two bioagents. The effect of these pathogens on certain biochemical and physiological aspects of the treated larvae showed that the total protein content and the activity of transaminases were decreased post-infection with each tested bio-agent individually and this reduction was higher when they were used in combination. In contrast, there were an increase in the activity of acid and alkaline phosphatases. The increased activities were higher in treatment with the combined bio-agents than in treatment with each bio-agent only.

Introduction

The Egyptian cotton leaf worm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is considered one of the most destructive phytophagous insect pests in Egypt, not only to cotton plants, but also to other field crops and vegetables (Kandil *et al*., 2003). Intensive use of chemical insecticides for

controlling this pest usually leads to adverse effects on non-target organisms and development of high levels of resistance to organophosphates, carbamates and pyretheroids (Alford, 2000). Therefore, there is always a need for finding out new material having specific modes of action to replace the conventional insecticides. Among the most suitable biological control agents for controlling the cotton leafworm are the entomopathoginc nematodes of the families Steinernematidae and Heterorhabditidae.Those were considered good biocontrol agents because they cause rapid mortality of the insect host without side effects on non-target organisms(Poinar, 1986).The third-stage juvenile of these nematodes is theinfective stage. Those are capable of long-term survival without feeding. These juveniles carry symbiotic bacteria (*Xenorhabdus sp*.) in their intestine to be released into the host`s haemocoel leading to septicemia followed by death of the host insect then the nematodes reproduce within the cadaver (Molyneux *et al*., 1983). Entomopathoginc fungi are similar to most fungal pathogens where they infect their hosts through the external cuticle. The infective units are the conidia which born on conidiophores. The sporulation and germination require high humidity. Fungi gain access to the insect directly through the insect's integument. After germination of the conidia on the insect's cuticle, the fungus penetrates the integument and proliferates throughout the host, ultimately resulting in mortality of the infected host. Host specificity of entomopathoginc fungi varies considerably; some species have a broad host range and others are more restricted (Mudroncekova *et al*., 2013).The present investigation was planned to study the efficacy of the two nematode species, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* as well as the entomopathogenic fungus *Beauveria bassiana* only or in combination using sequential method on toxicological and some biochemical parameters of the cotton leaf worm *S. littoralis* under laboratory conditions.

Materials and methods

1.Tested insect:

A laboratory strain of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) was obtained from Plant Protection Research Institute and it was reared on castor bean leaves under laboratory conditions at 25±2 ^{0}C using the method described by El-Defrawi *et al.* (1964). The 3rd instar larvae were used in all laboratory experiments.

2.Microbial agents:

2.1. Entomopathogenic nematodes:

Two strains *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* Poinar (Rhabditida: Steinernematidae : Heterorhabditidae) were obtained from Pest Physiology Department, Plant Protection Research Institute.

2.2. Entomopathogenic fungus:

Beauveria bassiana as the commercial product Biopower (1.4%WP), produced by/S.T. stares company limit-India**.**

3.Toxicological studies:

Pathogenicity of the nematodes was performed against the freshly moulted 3rdinstar larvae of *S. littoralis*. The inoculums of IJ from *H. bacteriophora* and *S. carrbocabsia* were applied by placing every ten larvae in petri-dishe lined with filter papers. The filter papers were contaminated with 40,80,160 and 200 IJs of each nematode strain. Each concentration was replicated four times. Non-infected larvae were used

as control. Mortality percentages were recorded after three days and corrected using Abbott's formula (Abbott, 1925) whenever necessary.

Median lethal concentration (LC_{50}) of the fungi, Biopower as determined as follow: series of concentrations (4,6,8 and10 gm/100 l.) were prepared by diluting the formulated compound with distilled water. The3rdinstar larvae of *S. littoralis* were placed into plastic cups lined with filter paper and offered to treated leaves (using leaf dipping technique). Each concentration was replicated four times, ten larvae per each replicate. Mortality percentage was recorded after treatment and corrected using Abbott's formula (Abbott, 1925). The data were statistically analyzed using Ldp line to find out the LC_{25} and LC_{50} value.

3.1. Joint action and sequential treatments of fungi, Biopower on larvae infected with nematode, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*:

To examine the interaction between Biopower and the entomopathoginc nematodes, *H. bacteriophora* and *S. carpocapsae* the $LC_{25}$of each nematode (18.15-18.34 Jv/ml) was applied firstly to ten 3rd instar larvae of *S.littoralis*, then after 24h the fungus was applied at the LC_{25} level(6.95gm/l.). In addition, another ten larvae were infected with LC_{25} of the fungus then LC_{25} of each nematode was applied. The experiments were incubated at 25 ± 2^0Cand replicated four times. Ten non-infected larvae were fed on untreated leaves as control. The mortality percentage was recorded and corrected after 72h of infection. The co-toxicity factor was calculated according to (Mansour *et al*., 1966) equation to differentiate the final effect of the combinations were categorized as synergism, antagonism or additive effect. A positive factor of 20 or more was considered potentiation, a negative factor of -20 or less mean antagonism, while a value between -20 and +20 was the additive effect.

3.2. Biochemical studies:

Total body samples were collected from the 3rdinstarlarvae treated with the tested microbial agents, separately or in combination after 72 hours post treatment. Total protein content was measured according to the method described by (Bradford, 1976), acidic and alkaline phosphatases activity was determined according to the method of (Laufer and Schon, 1971). Glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) also were determined as given by (Reitman and Frankel, 1957).

4. Statistical analysis:

Median lethal concentration values (LC_{50}, s) and the regression lines were statistically measured according to Finney (1971) using a software computer program (Ldp-line). Analysis of variance (ANOVA) conducted on all data using SPSS computer program software. And significance between treatment were compared by Duncan's multiple range test (Duncan, 1955).

Results and discussion

Toxicological studies:

1. Toxicity response of *Spodopter alittoralis* 3rd instar larvae to each *Heterorhabditis bacteriophora*, *Steinernema carpocapsae* and *Beauveria bassiana* (Biopower) separately after 72h post treatment:

The LC_{25} values of *H. bacteriophora*, *S. carpocapsae* and *B. bassiana* were 18.15Jv/ml,18.34Jv/mland 6.95g/l, respectively (Table,1). Also, the LC_{50} values of the three pathogens were

53.3Jv/ml,81,4Jv/ml and20.08 g/l, respectively. The current results agree with those mentioned by Reyad (2001) who showed that the tested inoculums's level of *S. carpocapsae* and *H. bacteriophora*was effective against the 3rdlarval instar of *S. littoralis*. Moreover, the level 40 infective juveniles/ml distilled water caused 100% mortality of the host. On the other hand, Anand and Tiwary (2009) observed a high larval morality percentage against the 2nd instar larvae of *S. litura*at the higher fungal spores concentration. Mortality caused by EPF was low at lowerspores' concentrations. It increased with increase of spores' concentration. The (LC_{50}) of *B. bassiana*.value for 3rd instar larvae was (20.08g/l), showing that B. *bassiana* was the least effective agent. This might be attributed to defense mechanisms of target insect. It is well documented that older instars of the cotton leaf worm can tolerate toxic effect of this fungus. EPF species that infect insects have received considerable attention by scientists for their potential use in biological pests control. Some pathogenic fungi have restricted host ranges while others have a wide host range, *e.g., B. bassiana.* Many researchers have focused on the selection of virulent strains for target pests and their development as biological control agents (Godonou *et al*., 2009). Similarly, Anand and Tiwary (2009) observed highest morality rates against 2nd instar larvae of *S. litura*at the highest spores' concentration of fungal isolates. The growth of mycelium was indicated by white spores of *B. bassiana* on the dead *Agrotis ipsilon* larvae treated with the LC_{50} (2 x 10^8spores/ml) after 7, 10, 13 and 16 days. The mycelium started to grow after 7 days from death of infected larvae, and then the insect cadaver was covered by mycelium after 10 days later, the formation and discharge of spores were detected after 13 and 16 days, respectively (Gabarty *et al*., 2014).

Table (1): Toxicity response of *Spodopter alittoralis* 3rdinstar larvae to the tested entomopathogenic nematode and fungi at 72h post treatment.

Treatments	LC25	95% Fiducial limit		LC50	95%Fiducial limit		Slope ±SE
		Lower	Upper		Lower	Upper	
H. bacteriophora	18.15(Jv/ml)	11.70	24.4	53.3(Jv/ml)	43.00	64.29	1.44±0.16
S. carpocapsae	18.34(Jv/ml)	9.46	27.1	81.4(Jv/ml)	63.12	106.79	1.04±0.15
B. bassiana	6.95(g/l.)	5.68	9.0	20.08(g/l.)	13.67	46.24	1.46±0.29

2. Joint action and sequential treatment of the entomopathogenic fungi, *Beauveria bassiana* (Biopower) on larvae infected with each of (*Steinernema carpocapsae* and *Heterorhabditis bacteriophora*):

Data in Table (2) indicated that the combined effect of *B. bassiana* and each entomopathogenic nematode (*H. bacteriophora* and *S.carpocapsae*) as sequential treatment differed from the infection with each pathogen only. In the present study, all combinations showed an increase in the host mortality and gave potentiation effect. The highest effect was observed with the combination of *B. bassiana+H. bacteriophora* (+87.70) followed by *H. bacteriophora+B. bassiana* (+79.16) and *B. bassiana+S.carpocapsae(*+73.90) mixtures, then *S. carpocapsae* + *B. bassiana* (+58.33) mixture. These results agree with those mentioned by Shaira and Noah (2014) who observed that the combination of *B. bassiana*and the nematode*H. Bacteriophora* showed high

larval mortality among *S. littoralis* 3rd instar, which increased with increasing concentrations of fungal spores and/or nematode juveniles the interaction between fungi and nematodes may allow reducing chemical application rates. Additionally, the nematodes may become established and begin to offer a long-term reduction in the larval populations (Klein and Georgis, 1992). This study gives additional support to the importance of combinations between the entomopathogenic fungi and the nematodes for increasing the potentiality control insect pests.

Table (2): Co-toxicity factor and final effect of binary mixtures of entomopathogenic agents against 3rd instar larvae of *Spodoptera littoralis* by using sequential method.

Treatments	Concentration	Observed mortality%	Expected mortality%	Co-toxicity factor	Type of synergistic action
H. bacteriophora+B. bassiana	18.15Jv/ml+6.95g/l.	89.58	50	+79.16	Potentiative
S. carpocapsae+ B. bassiana	18.34Jv/ml+6.95g/l.	79.10	50	+58.33	Potentiative
B. bassiana+H. bacteriophora	6.95g/l.+18.15Jv/ml	93.75	50	+87.70	Potentiative
B. bassiana+S.carpocapsae	6.95g/l.+18.34Jv/ml	86.97	50	+73.90	Potentiative

3. Biochemical influences of *Heterorhabditis bacteriophora, Steinernema carpocapsae* and *Beauveria bassiana* on *Spodoptera littoralis* larvae:

3.1. Total protein content:

3.1.1. Effect of treatment with LC_{50} of each tested bio-agent alone on total protein content:

The obtained results in Table (3) showed a significant reduction in total protein content of *S. littoralis* 3rd instar larvae 72h post-infection with each bio-agent separately compared to control. The highest decrease was recorded in case of infection by *H. bacteriophora*, followed by *S. carpocapsae* and *B. bassiana* with percentages of change - 53.70, - 40.73 and - 30.12%, respectively. The results agreed with Ahmed *et al*. (2014) who found a reduction in total protein of the host larvae post-infection with the nematodes *S. riobrave* and *H. bacteriophora.* This toxic effect of the entomopathogenic nematodes is related to the symbiotic multiply bacteria, rapidly when released into the haemocoel causing a lethal septicemia to the insect host (Dutly, 1959 and Nickle and Welch, 1984). Thus, biochemical changes in the hemolymph composition were expected, since the hemolymph is the main site of action. On the other hand, the present results are in consistence with those obtained by Mazet and Boucias (1995).They found that during the vegetative development of *B.bassiana* in the haemocoel of the beet armyworm,*S.eixuga*; the mycelia tissue invasion phase inhibited host protein synthesis and produced a range of exocellular peptides.

Table (3): Effect of each LC_{50} value of *Heterorhabditis bacteriophora, Steinernema carpocapsae a*nd *Beauveria bassiana* on the total protein content at 72h post-infection of *Spodoptera littoralis* 3rd instar larvae.

Treatments	Total protein (mg/g.b. wt)		
	Mean±SE	Change%	Activity ratio
H.bacteriophora	**8.07±0.11e**	**-53.70**	**0.462**
S.carpocapsae	**10.33±0.29c**	**-40.73**	**0.592**
B. bassiana	**12.18±0.17b**	**-30.12**	**0.698**
Control	**17.43±0.20a**	---------	---------

***means with the same letters are not significantly different at$P \leq 0.05$**

3.1.2. Effect of treatment with combination between the tested bio-agents on total protein content:

Data in Table (4) indicated a significant reduction in the total protein content of *S. littoralis* larvae treated with binary mixtures of the tested bio-agents compared with the control with percentages of change (-91.4, -91.2, -90.1 and -90.9%) for treatment with the binary mixtures of (*H. bacteriophora+B. bassiana*), (*S.carpocapsae+B. bassiana*), (*B. bassiana+H. bacteriophora*) and (*B. bassiana + S. carpocapsae*), respectively. According to lee and Atkinson (1976), this high reduction in protein content could be referred that many nematodes secrete chemicals that facilitate penetration and migration through host tissues, feeding and avoidance of host immunity responses. These chemicals include toxins and digestive enzymes. Such as, proteases which are digestive enzymes that catalyze the cleavage of peptide bonds in proteins. Moreover, some animal parasitic nematodes secrete proteases to assist in skin and tissue penetration (Von Brando, 1973). On the other hand, the cyclic peptide metabolite beauverolide l, cyclosporine a and cyclic were produced by several genera of entomopathogenic fungi. When those were injected into last-instar of *Galleria mellonella*, it activated humoral response and induced a significant release of isozyme and cecopin-like activity into the haemolymph, suggesting stimulatory activity on humoral immune responses. These finding may explain the reduction of protein synthesis after treatment with the entomopathogenic fungi (Vilcinskas *et al.*, 1999).

Table (4): **Total protein content of 3rd instar larvae of *Spodoptera littoralis* treated with of the tested binary combinations of bioagents.**

Treatments	Total protein (mg/g.b. wt)		
	Mean ±SE	Change%	Activity ratio
H. bacteriophora+B. bassiana	2.15±0.08c	-91.4	0.08
S. carpocapsae + B. bassiana	2.20±0.05c	-91.2	0.09
B. bassiana+H. bacteriophora	2.48±0.09b	-90.1	0.09
B. bassiana+Scarpocapsae	2.26±0.11b	-90.9	0.09
Control	25.11±0.07a	--------	--------

***means with the same letters are not significantly different at$P \leq 0.05$**

3.2. Transaminase activities (Glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase**):**

3.2.1. Effect of treatment with LC_{50} of each tested bio-agent only:

Data in Table (5) indicated that infection by *H.bacteriophora, S.carpocapsae*and *B. bassiana* significantly decreased the activity of GOT by percentages of change (-36.89, -29.60 and -14.37%) and GPT (-50.98, -44.48 and -32.60%), respectively as compared to the control. The current data agree with Ahmed *et al.* (2014) who found that the activities of GOT and GPT were highly decreased with infection by *H. bacteriophora* and *S. riobrave* juveniles. In the present study, the significant decline of GOT in *S. carpocapsae* larvae after 72 hr. post-infection by *H. bacteriochlorin* and *B. bassiana*, as compared to control treatment, may be attributed to the significant decline in free amino acids content, as has been pointed out by Kaur *et al.* (1985). Soliman (2002) reported that GOT and GPT activities decreased in *Ceratitis. Capitata* last instar larva infected with *S. riobrave* and *Heterorhabditis* sp. Agreeing with that of the present study. They added that the quantum of free amino acids directly influenced the activity of transaminase at the time of protein synthesis.

Table (5): Glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase activities of *Spodoptera littoralis* 3rd instar larvae treated with the LC_{50} of each bio-agent only.

Treatments	Glutamate-oxaloacetate transaminase (GOT)			Glutamate-pyruvate transaminase (GPT)		
	Mean ±SE	Change %	Activity ratio	Mean ±SE	Chang%	Activity ratio
H. bacteriophora	16.37±0.35d	-36.89	0.631	18.10±0.11d	-50.98	0.490
S. carpocapsae	18.26±0.21c	-29.60	0.703	20.50±0.32c	-44.48	0.555
B. bassiana	22.21±0.24b	-14.37	0.856	24.89±0.28b	-32.60	0.673
Control	25.94±0.78a	---------	----------	36.93±0.24a	---------	----------

*Vertical means with the same letters are not significantly different at P≤ 0.05.

3.2.2. Effect of treatment with the combination between the tested bio-agents on Glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase activities:

As shown in Table (6), there were a significant reductions in GOT and GPT activities of *S. littoralis* 3rd instar larvae treated with binary mixtures of (*H. bacteriophora+B. bassiana*), (*S.carpocapsae+B.bassiana*), (*B. bassiana+H. bacteriophora*) and (*B. bassiana+S.carpocapsae)* compared with the control by percentages of change (-39.04, -15.25, -28.73 and -34.18%) and (-35.43, -25.51, -39.96 and -33.12), respectively. This significant decline in GOT activity that occurred 72h post-infection by *H. bacteriophora* and *B. bassiana*may be attributed to the reduction in free aminoacids content as had been pointed out by Kaur *et al.* (1985).In addition, the current result agreed with that of Soliman (2002) who reported that GOT and GPT activities decreased in *C. capitata* larvae infected with *S. riobrave* and *H.bacteriophora.* They added that the quantum of free amino acids directly influenced the activity of transaminase at the time of protein synthesis.

Table (6): Glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase activities of the 3rd instar larvae of *Spodoptera littoralis* treated with the LC_{50}values of bio-agents in combination.

Treatments	GOT			GPT		
	Mean±SE	Change %	Activity ratio	Mean±SE	Change %	Activity ratio
H. bacteriophora + B. bassiana	11.41±0.16c	-39.04	0.60	25.20±0.03c	-35.43	0.64
S. carpocapsae+ B. bassiana	14.34±0.10b	-15.25	0.76	29.07±0.05a	-25.51	0.74
B. bassiana + H. bacteriophora	13.34±0.05a	-28.73	0.71	23.43±0.09d	-39.96	0.60
B. bassiana + S.carpocapsae	12.32±0.09 d	-34.18	0.65	26.10±0.3b	-33.12	0.66
Control	18.72±0.03b	---------	----------	39.03±0.04a	----------	----------

***Vertical means with the same letters are not significantly different at $P \leq 0.05$.**

3.3. Acidic and alkaline phosphatase activities:

3.3.1. Effect of treatment with LC_{50} of each tested bio-agent only:

From data in Table (7), the activity of alkaline and acid phosphatases, significantly, increased in the treated larvae with *H.bacteriophora, S.carpocapsae* and *B. bassiana* by percentages of change (188.7, 303.9 and 29.3%) and (81.33, 140.80 and 39.41%), respectively as compared to the control. The present results are in accordance with Xia *et al*. (2000) who suggested that acid phosphatase as a lysosomal enzyme, might have a role in autophagy and cell turnover as well as defense. Therefore, it appeared that the enhancement of acid phosphatase activity in *S. littoralis* larvae infected with *S. riobrave, H. bacteriophora* and *B. bassiana* was an attempt by the insect to defend itself against the invasion of the three pathogens. The same authors also added that phagocytosis is known to stimulate the production of lysosomal enzymes of which acid phosphatase is a key component. In addition, acid phosphatase had been found in insect haemocytes and shown to be released into the plasma (Lai-Fook, 1973 and Rowley and Rakcliffe, 1979). Moreover, Cheng (1983) reported that there was a hypersynthesis of acid phosphatase by haemocytes of the mollusk, *Biomphalaria glabrata* during phagocytoses. On the other hand, alkaline phosphatase of secreting products across cell boundaries. The present results agree with Ahmed *et al.* (2014) who reported that the activities of acid and alkaline phosphatase increased because of infection by *Steinernema riobrave* and *H. bacteriophora*.

Table (7): Alkaline and acid phosphatase activities in *Spodoptera littoralis* 3rd instar larvae treated with the LC_{50}value of each bio-agent alone.

Treatments	Alkaline phosphatase			Acid phosphatase		
	Mean±SE	Change%	Activity ratio	Mean±SE	Change%	Activity ratio
H. bacteriophora	5.11±0.073b	188.7	2.88	13.02±0.06b	81.33	1.81
S. carpocapsae	7.15±0.19a	303.9	4.03	17.29±0.12a	140.80	2.40
B. bassiana	2.29±0.16c	29.3	1.29	10.01±0.16c	39.41	1.39
Control	1.77±0.11d	----------	----------	7.18±0.03d	----------	----------

***Vertical means with the same letters are not significantly different at $P \leq 0.05$.**

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.3.2. Effect of treatment with the combination between the tested bio-agents on alkaline and acid phosphatase activities:

Results in Table (8) showed that there was a significant increase in alkaline and acid phosphatases' activity of *S. littoralis* 3rd instar larvae treated with binary mixtures of (*H. bacteriophora+B. bassiana*), (*S.carpocapsae+B. bassiana*), (*B. bassiana+H. bacteriophora*) and (*B. bassiana+S.carpocapsae*) compared with the control by percentages of change (-39.04, -15.25, -28.73 and -34.18%) and (-35.43, -25.51, -39.96 and -33.12), respectively. The present results agree with those obtained by Soliman (2002) who found that acid and alkaline phosphatases activity increased in the larvae of *C. capitata* infected with *S. riobrave* and *H. bacteriophora.*

Table (8): Alkaline and acid phosphatase activities of *Spodoptera littoralis* 3rd instar larvae treated with the LC_{50} value of bio-agents in combination.

Treatments	Alkaline phosphatase			Acid phosphatase		
	Mean±SE	Change%	Activity ratio	Mean±SE	Change%	Activity ratio
H. bacteriophora+B. bassiana	15.14±0.08c	62.97	1.62	14.31±0.1a	72.20	1.72
S. carpocapsae +B. bassiana	10.31±0.10e	10.97	1.10	9.12±0.1d	9.74	1.09
B. bassiana+H. bacteriophora	16.04±0.041a	72.65	1.72	13.07±0.07b	57.28	1.57
B. bassiana+S.carpocapsae	15.65±0.12b	68.46	1.68	12.56±0.04c	51.14	1.51
Control	9.29±0.09d	---------	---------	8.31±0.1e	--------	---------

***Vertical means with the same letters are not significantly different at $P \leq 0.05$.**

In conclusion' using the tested entomopathogenic nematodes and fungi in separate treatments or in combination through using sequential treatment to *S. littoralis* larvae affected some biochemical aspects in the treated larvae. This effect was more potent in treatment with the bio-agents in combination than treatment with each bio-agent alone. Therefore, the current study gives additional support to the importance of combination between the entomopathogenic fungi and nematodes for controlling the cotton leaf worm, *S. littoralis*.

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