Bulletin of Faculty of Science, Zagazig University (BFSZU)
e-ISSN: 1110-1555
Volume-2023, Issue-1, pp-181-191
https://bfszu.journals.ekb.eg/journal
Research Paper DOI: **10.21608/bfszu.2022.155722.1167**

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Evaluation of Beauvaria bassiana metabolite pretreated with silica nanoparticles against the cotton leafworm, Spodoptera littoralis (Boisd.)

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ABSTRACT : **Plant herbivorous insects are danger to the agricultural production of crops. Insecticides resulted in habitat destruction due to high toxicity and resistance. Hence, the development of alternatives to such insecticides is a sustainable approach to supreme crop production with the least damage is a crucially prerequisite. As a result, the current study was carried out to evaluate the potential effect of arbuscular mycorrhizal (AM) fungi along with *Beauvaria bassiana* silica nanoparticles (Si NPs) as a new approach to protect cotton (*Gossypium hirsutum* L.) against *Spodoptera littoralis*. AM and non-AM inoculated cotton plants were infested with *S. littoralis* and then treated with metabolites of *B. bassiana* Si NPs or Chlorpyrifos**.

Date of Submission: 11-08-2022 Date of acceptance: 22-09-2022

I. INTRODUCTION

White gold, cotton (*Gossypium hirsutum* L.) is an important natural fiber and economic crop that provides substantial benefits to humans **(Gao *et al.*, 2020)**. Cotton has a diversity of applications, principally medicinal and many other usages such as pigments, derivatives for cattle feed and different uses of the oil extracts **(Hesam *et al.*, 2020**) and it is not only a natural fiber resource but also a food and feed for billions of humans and livestock **(Noreen *et al.*, 2020)**. Meanwhile; cotton is facing enormous biotic and abiotic stresses with insect pests being most prominent. Massive destruction caused by insects needs to be controlled for maintaining fruitful cotton crop production **(Aslam *et al.*, 2020)**.

In the last decades, the production of cotton suffers from increased incidence of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), which is one of the most destructive pests as well does serious damage to many important agricultural crops in Egypt such as groundnut, soybean, tomato, sweet potato and tobacco **(Ragaei and Sabry, 2011; Xu *et al.*, 2020).**

Development of effective control methods against the cotton leafworm, *S. littoralis* is urgently needed. One of these effective methods is the chemical pesticides application. These pesticides are the most common tool used to control pests and diseases. The pesticide manufacturing companies endorsed pesticides at a definite dose, however, pesticides dealers often propose overdosing to the farmers **(Metwally and Abdelhameed, 2019)**; these higher doses can probably harm the host crops and their associated soil beneficial microbes in agro-ecosystems **(Hage-Ahmed *et al.*, 2018)**. Also, there are other reports of the development of resistance in *Spodoptera* sp against a wide range of insecticides **(Xu *et al.*, 2020)**. The increasing demands for reduction of chemical inputs in agriculture and increased resistance to insecticides have given a considerable stimulus to the production of alternative forms of insect-pest control.

Biological control is another attractive alternative method to chemical pesticides due to its non-toxicity for human and other organisms and being less environmentally harmful **(Ak, 2019)**. Additionally, they neither leave toxic chemical residues in the environment nor induce resistance in their insect hosts **(Patocka, 2016; Poveda *et al.*, 2020)**. Interestingly, entomopathogenic fungus, *Beauvaria bassiana* with broad host range is known as an effective organism to control agricultural pests **(Inglis *et al.*, 2001)**. Its mode of action includes the production of a large array of biologically active metabolites **(Ak, 2019)** such as toxic proteins, enzymes, and bioactive secondary metabolites to overawed the insect immune system and modify the host performance **(Elbanhawy *et al.*, 2019)**.

Recently, silica nanoparticles (Si NPs) have cleared promising potential in such field. The application of Si NPs in crops provides an effective component of integrated management of insect pests because it leaves no pesticide residues, can be easily integrated with other pest management practices and has induced resistance in many plant species against insects **(Divya, 2014).** Interestingly, recent studies have used nanotechnology as an emerging science that possibly significantly revolutionize the food and agricultural industry by using nanoparticles for pest control **(Xu *et al.*, 2020)**

Additionally, the symbiosis of arbuscular mycorrhizal fungi (AMF) with the plant roots can provide several benefits to the host such as facilitated the nutrient uptake from the soil **(Rizzo *et al.*, 2020; Metwally, 2020)**, augmented its growth and help the plants better cope with abiotic and biotic stress **(Metwally and Abdelhameed 2018; Sanmartín *et al.*, 2020)**.

The application of nanotechnology in combination with AM fungi can be a promising biological alternative to chemical fertilizers where, nanoparticles have been lately tested as fertilizers for effective protection to different plants **(Abdelhameed *et al.*, 2021)**. Therefore, to overcome the harmful effects of chemical residues to human health, several studies have been explored as we see in the present study which deals with the using of eco-friendly alternatives such as AM fungi and *B. bassiana* Si NPs as bio controls against *S. littoralis* on cotton plant. Nevertheless, reports dealing with their combined bio control activity against insect infection are limited. As a result, the study presented herein is accompanied by two hypotheses; the first was that AM fungal inoculation and/or Si NPs application enhances the growth and acts as a stimulator to increase the health efficiency of cotton plants against the cotton leafworm, *S. littoralis*. The second was that *B. bassiana* Si NPs along with AM fungi increases the effectiveness of cotton plants as a bio control against this dangerous Egyptian pest to lessen the harmful effects of chemical pesticides.

. II. Materials and Methods

- Materials:

1-Spodoptera littoralis (Boisd.) insect culture

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) was originally obtained from a well-established culture reared at the Department of Cotton Leafworm, Plant Protection Research Institute, EL-Sharkia Branch. The egg masses were kept in glass jars covered with muslin and fastened with rubber band under laboratory conditions of 26± 1ºC, 70±5 % relative humidity and 12 h light/ 12 h dark cycle of photoperiod till hatching **(Gamil, 2004)**. Egg masses were placed in glass jars on castor bean leaves, *Ricinus communis*. The newly hatched larvae were provided daily with castor bean leaves in mating cages for further studies **(El- Defrawi *et a.*, 1964)**.

1.1- *Beauvaria bassiana* (Balsamo)

The fungal isolate of *B. bassiana* was obtained from Assiut University, Mycology Center, Egypt and was cultured on potato dextrose agar, PDA medium for 2 weeks at 28 ±1ºC and 50-60 relative humidity. Metabolites of *B. bassiana* applied with Si NPs were prepared by inoculating 1mL of *B. bassiana* spore suspension and 1mL of Si NPs solution in Erlenmeyer flask (250 mL) containing 100 mL sterile potato dextrose broth (PDB) medium and incubated at 28 ± 1°C for 15 days. Cell filtrate and mycelial mat were separated by using Whatman filter paper No.1. Therefore, metabolites became ready for foliar application.

1.3- **AM fungal inoculum**

Spores of AM fungi were isolated from rhizospheric soil from Sharkia Governorate, Egypt via wet sieving and decanting technique **(Gerdmann and Nicolson, 1963)**. Approximately 200 g of air-dried soil was distributed in 2 L of water in a large jar and the suspension was left intact for 10–15 min. The suspension was then decanted 2–3 times through the stack of sieves of 400, 250, 180 and 38µm in diameter. The residue from each sieve was collected into a small flask and the morphology of AM fungal spores and sporocarps were observed and identified by using Manual for identification. The mixture of identified spores of *Funneliformis mosseae*, *Funneliformis constrictum*, *Gigaspora margarita* and *Rhizophagus irregularis* together in pots filled with sterilized sandy clay soil were propagated on Sudan grass (*Sorghum sudanenses* Pers.) roots as an appropriate trap plant for inoculum production. AM inoculum consisted of AM spores, hyphae and colonized root fragments.

2-tested compounds

2-1- Chemical pesticide, Chlorpyrifos

It was supplied by Dow Agro Sciences and was used at the recommended dose of 5ml /L of water.

2-2- Silica nanoparticles (Si NPs)

Hydrophilic Si NPs was amorphous (50 nm) with about 99.9% purity produced from Egypt Nanotech Company Limited, 6 th October, Cairo, Egypt and was used at a concentration of 500 ppm.

3- Soil sample collection

The soil sample was collected from Minia AL-Qamh, EL-Sharkia Governorate, Egypt. The soil was sterilized to remove native AMF by 2% formaldehyde, covered for 7 days and left for 25 days for aeration **(Thakur and Sharma, 2018)**.

4- Experimental design

A 2-year pot experiment was conducted in an environmental growth chamber under controlled conditions of 16 h light/8 h dark cycle (day/night) at 30±4 ºC and 70-80 % humidity in the Botany and Microbiology greenhouse Department, Faculty of Science, Zagazig University, Egypt. Seeds of a variety Giza 86 of cotton, *Gossypium hirsutum* L. Merr. obtained from Agriculture Research Center, Giza, Egypt, were surface sterilized in 7% sodium hypochlorite for 10 min, subsequently rinsed with sterilized water and sown in sterilized plastic pots with 30 cm diameter top, 25 cm diameter base and 40 cm depth, filled with 10 kg sterilized soil. Two different treatments were administered to the potted plants as follow:

1- Non-AM treatments: cotton seeds were planted in pots received 100 g sterilized soil / pot besides filtered washings of an equal amount of AM soil inoculum.

1- AM treatments: cotton seeds were planted in pots received 100 g Sudan grass root fragments / pot at sowing date (approx. 80 spores/g trap soil).

Each treatment was divided into two groups according to Si NPs application as follows:

1- Control (-NPs) treatments: each pot received 150 mL of tap water at a fixed time (10 days before the artificial infestation) at a rate of 50 mL/ 3 days.

2- (+NPs) treatments: each pot received 150 mL of its treatment of Si NPs solution at a fixed time (10 days before the artificial infestation) at a rate of 50 mL/ 3 days.

Fifty days after sowing (DAS), plants were artificially infested using one egg mass of the cotton leafworm/ plant. When the 1st instar larvae emerged from eggs on leaves, they were allowed to feed on plants until reaching the 2nd instar larvae then, the tested compounds, *B. bassiana* Si NPs and Chlorpyrifos were sprayed separately.

5- **Measurements**:

To initiate the choice experiments, survived larvae were collected after 3, 5 and 7 days post spraying tested compounds from 3 pots of each AM and Non-AM plants (sprayed with Chlorpyrifos or *B. bassiana* Si NPs or previously supplemented with Si NPs) and control ones for biochemical assay.

-Preparation of samples for biochemical assay:

Larval samples used for biochemical assays were collected after 3, 5 and 7 days post treatment. Untreated larvae were used as control. Larvae were weighed and homogenized in distilled water using a Teflon homogenizer surrounded with a jacket of crushed ice. The homogenates were centrifuged at 5000 rpm for 10 min at 5 ºC.

1- Determination of total soluble protein:

Total soluble protein in homogenate of larvae was carried out as described by **Gornall *et al.* (1949)**. A known volume of homogenate (0.2 mL) was added to 5mL of Biuret reagent and incubated for 30 min at 20–25 ºC. The absorbance of the sample against a blank was measured at 546 nm using a spectrophotometer (APEL Japan, Model: PD-303). Total soluble protein was expressed as mg protein/ g.b.w.

2-Determination of GOT and GPT

The reaction mixture contains 1 mL of a mixture of phosphate buffer (pH 7.4), 0.2 mμ α- keto glutaric and 200 mμ D, L- alanine or L-aspartate was added to 0.2 mL of larval homogenate and the mixture was incubated for 30 min. Then, 10 mL of (0.4 N) NaOH was added. The optical density of the produced brown color is measured after 5 min at 520 nm. The enzyme activity was expressed as μg pyruvate/ mL/ min for GPT and μg oxaloacetate/ g.b.w/ min for GOT

Statistical Analysis

Each result was presented as the mean of three replicates. The significant differences between treatments were statistically evaluated by two-way analysis of variance (ANOVA) using SPSS 15.0 (Statistical Package for the Social Science, SPSS, Richmond, VA, USA). $p < 0.05$ was considered to be significant.

III.RESLUTS And DISCUSSIONS

- **Total soluble protein (TSP) content**

Data in **Table (1)** showed the changes in TSP in the homogenate of *S. littoralis* larvae fed on cotton plants after 50 DAS and at different time intervals post treatment with fungal metabolites or Chlorpyrifos. Also, there were high significant differences between metabolites and Chlorpyrifos treatments where, under non-AM (+NPs) conditions, changes % in larvae fed on cotton plants treated with metabolites and Chlorpyrifos was higher by (274.735% and 332.716%), respectively over the control after the 3rd day post treatment. However, throughout the 5th and the 7th day after treatment, in spite of increasing TSP content in untreated larvae with increasing the age of larvae, higher reduction in TSP content was observed in treated larvae compared to untreated ones, whereas

changes % were (-34.307 and -50.581%) for metabolites and (-8.201 and -56.317%) for Chlorpyrifos at the 5th and the 7th day post treatment, respectively.

Meanwhile; under non- AM (-NPs) conditions data cleared that both of metabolites and Chlorpyrifos caused reduction in TSP content in *S. littoralis* fed on treated cotton plants at different times intervals in comparison with the control. Additionally, there were high significant differences in TSP content between different treatments, whereas the change % ranged between (-86.837 and -45.705%) for metabolites at the 7th day and Chlorpyrifos at the 3rd day post treatment, respectively.

On the other hand, data cleared that both metabolites and Chlorpyrifos high significantly affected TSP content in larvae fed on AM (+NPs) cotton plants where; at the 5th and the 7th day after application, both of Chlorpyrifos and metabolites gave high change % in TSP content ranged between (-68.313%) for metabolites at the 7th day and (-30.779%) for Chlorpyrifos at the 5th post application. Only at the 3rd day, Chlorpyrifos caused an increase in TSP content of (39.671%) and metabolites slightly reduced TSP content by (-1.587%) as compared to the control **(Table 1)**.

Regarding TSP in *S. littoralis* fed on AM (-NPs) cotton plants, data in **Table (1)** revealed that there were high significant differences between TSP contents in treated larvae during time intervals after spraying the cotton plants with metabolites or Chlorpyrifos. The highest Change % of (-90.807%) was recorded for metabolites at the 7th day while; both tested insecticides caused nearly close change % of (-80.608 and -81.2895) at the 3rd day for metabolites and Chlorpyrifos, respectively.

Table 1: Total soluble protein (TSP) content of *S. littoralis* infested mycorrhizal (AM) and non-mycorrhizal (non-AM) cotton plants under different treatments after 50 DAS.

AMF status	Si NPs Status	Treatments	TSP	The 3rd day	The 5th day	The 7th day
Non-AM	+ NPs	Control	Conc.	12.3733^{g}	25.9333^{e}	39.177^{d}
		Metabolites	Conc.	46.367^{d}	17.0366^{gh}	19.36^{e}
			C%	274.735	-34.307	-50.581
		Chlorpyrifos	Conc.	53.54^{c}	23.8066^{e}	17.113^{f}
			C%	332.716	-8.201	-56.317
	- NPs	Control	Conc.	99.2367^{b}	100.36^{b}	120.977^{b}
		Metabolites	Conc.	33.0433^{e}	19.0766^{fg}	15.923^{f}
			C%	-66.702	-80.992	-86.837
		Chlorpyrifos	Conc.	53.88^{c}	38.4233^{c}	43.19^{c}
			C%	- 45.705	-61.714	-64.298
AM	+NPs	Control	Conc.	36.5433^{e}	29.0033^{d}	41.583^{c}
		Metabolites	Conc.	35.9633^{e}	15.96^{h}	13.176^{g}
			C%	-1.587	-44.971	-68.313
		Chlorpyrifos	Conc.	51.04^{c}	20.0766^{f}	16.563^{f}
			C%	39.671	-30.779	-60.168
	-NPs	Control	Conc.	124.33^{a}	130.0733^{a}	135.367^{a}
		Metabolites	Conc.	24.11^{f}	16.19^{h}	12.443^{g}
			C%	-80.608	-87.553	-90.807
		Chlorpyrifos	Conc.	23.2633^{f}	20.4933^{f}	21.033^{e}
			C%	-81.289	-84.245	-84.462
F				***	***	***
P				0.0000	0.0000	0.0000

* C = Concentration expressed as (mg/g.b.w.) * C % (Change %) = $\frac{\text{treatment}-\text{control}}{\text{control}}$ X 100

- **Glutamic pyruvic transaminase (GPT)**

Data illustrated in **Table (2)** showed that there were high significant differences between different treatments under non-AM (-NPs) conditions. Generally, both of metabolites and Chlorpyrifos gradually decreased GPT activity as compared to control throughout the period from the 3rd to the 7th day. Metabolites gave the highest RA% of (-87.722%) at the 7th day while; at the same period Chlorpyrifos reduced GPT activity to approximately half value by (-54.822%) as compared to control. Additionally, fungal metabolites decreased SA during all the exposure periods from (28.276 to 12.696 µg pyruvate/ g.b.w/ min) at the 3rd and the 7th day, respectively. In the same manner, Chlorpyrifos decreased SA from (61.533 to 46.716 µg pyruvate/ g.b.w/ min) at the 3rd to the 7th day, respectively.

Furthermore, GPT activity was decreased in *S. littoralis* infested non-AM (+NPs) cotton plants under metabolites and Chlorpyrifos treatments. The highest RA % of (-83.443%) was recorded for metabolites at the 7th day while; the lowest RA% of (-26.576%) was recorded for Chlorpyrifos at the 3rd day **(Table 2)**.

Moreover, GPT activity was high significantly reduced in *S. littoralis* at different time intervals post treatment of AM (-NPs) cotton plants with metabolites or Chlorpyrifos. The highest RA% of (-88.367%) was recorded for metabolites at the 7th day while; the lowest RA% of (-37.359%) was recorded for Chlorpyrifos at the 3rd day. Also, Chlorpyrifos slightly reduced SA from (35.363 to 35.37 µg pyruvate/ ml/ min) at the 5th and the 7th day, respectively.

On the other hand, in untreated larvae, GPT activity in *S. littoralis* fed on AM (+NPs) cotton plants gradually increased from (74.543 to 81.39 µg pyruvate/ g.b.w/ min) while; metabolites decreased it from (23.66 to 11.66 µg pyruvate/ g.b.w/ min) from the 3rd to the 7th day, respectively. Additionally, Chlorpyrifos decreased GPT activity from (37.64 to 31.836 µg pyruvate/ g.b.w/ min) at the 3rd and the 5th day, respectively and then increased it again to (35.13 µg pyruvate/ g.b.w/ min) at the 7th day.

Table 2: GPT of *S. littoralis* infested mycorrhizal (AM) and non-mycorrhizal (non-AM) cotton plant under different treatments after 50 DAS

AMF status	Si NPs Status	Treatments	GPT	The 3rd day	The 5th day	The 7th day
Non-AM	- NPs	Control	SA.	93.48^{b}	96.856^{a}	103.406^{b}
		Metabolites	SA.	28.276^{i}	26.656^{h}	12.696^{gh}
			RA%	-69.751	-72.478	-87.722
		Chlorpyrifos	SA	61.533^{e}	53.796^{d}	46.716^{d}
			RA%	-35.323	-44.457	-54.822
	+ NPs	Control	SA.	76.676^{c}	79.64^{b}	81.963^{c}
		Metabolites	SA.	24.22^{k}	23.083^{i}	13.57^{g}
			RA%	-68.447	-71.015	-83.443
		Chlorpyrifos	SA.	56.36^{g}	42.73^{e}	44.49^{e}
			RA%	-26.576	-44.568	-45.719
AM	- NPs	Control	SA.	95.29^{a}	97.183^{a}	107.283^{a}
		Metabolites	SA.	26.63^{j}	17.3^{k}	12.48^{gh}
			RA%	-72.053	-82.198	-88.367
		Chlorpyrifos	SA.	59.69^{f}	35.363^{f}	35.37^{f}
			RA%	-37.359	-63.611	-67.031
	+ NPs	Control	SA.	74.543^{d}	77.42^{c}	81.39^{c}
		Metabolites	SA.	23.66^{k}	19.293^{j}	11.66^{h}
			RA%	-68.217	-74.972	-85.673
		Chlorpyrifos	SA.	37.64^{h}	31.836^{g}	35.13^{f}
			RA%	-49.886	-58.700	-56.837
F				***	***	***
P				0.0000	0.0000	0.0000

SA(Specific Activity) expressed as µg pyruvate/g.b.w.

RA% (Relative Activity %) = $\frac{\text{treatment}-\text{control}}{\text{control}}$x 100.

Glutamic oxaloacetic transaminase (GOT)

Data illustrated in **Table (3)** showed that there were high significant differences between different treatments under non-AM (-NPs) condition. Generally, both of metabolites and Chlorpyrifos gradually decreased GOT activity in *S. littoralis* infested cotton plants as compared to control throughout the time intervals post treatment. Metabolites gave the highest RA% of (-49.972%) at the 5th day while; Chlorpyrifos gave the lowest RA% of (-24.146%) at the 3rd day post treatment. Also, Chlorpyrifos decreased SA all over the periods of application from (150.376 to 142.436 µg oxaloacetate/ g.b.w/ min) at the 3rd and the 7th day, respectively. Meanwhile; metabolites reduced SA from (116.606 to 100.176 µg oxaloacetate/ ml/ min) at the 3rd and the 5th day, respectively and increased it again to (141.633 µg oxaloacetate/ g.b.w/ min) at the 7th day.

Furthermore, GOT activity generally decreased in *S. littoralis* infested non- AM (+NPs) cotton plants following application of metabolites and Chlorpyrifos as compared to control at time intervals post treatment. The highest RA% value of (-62.233%) was recorded for metabolites at the 7th day while; the lowest RA% of (-31.517) was recorded for Chlorpyrifos at the 3rd day.

Taking into account the effect of fungal metabolites and Chlorpyrifos on GOT activity of *S. littoralis* infested AM (-NPs**)** cotton plants, data illustrated in **Table (3)** cleared that there were high significant differences between different treatments. Generally, both of metabolites and Chlorpyrifos generally reduced GOT activity in treated larvae as compared to control. Metabolites gave the highest RA% of (-61.921%) at the 5th day while; Chlorpyrifos recorded the lowest RA % of (-22.239%) at the 3rd day. Additionally, Chlorpyrifos gradually decreased GOT activity throughout the periods of application from (149.32 to 103.42 µg oxaloacetate/ g.b.w/ min) at the 3rd and the 7th day, respectively. Meanwhile; metabolites decreased GOT activity from (99.443 to 74.34 µg oxaloacetate/ g.b.w/ min) at the 3rd to the 5th day, respectively and then increased it again to (77.276 µg oxaloacetate/ g.b.w/ min) at the 7th day.

On the other hand, GOT activity gradually decreased in larvae infested AM (+NPs) cotton plants either treated with Chlorpyrifos or metabolites as compared to control as it recorded the highest reduction of (-55.562%) for metabolites at the 7th day and the lowest reduction of (-33.451%) for Chlorpyrifos at the 3rd day.

Table 3: GOT of *S. littoralis* infested mycorrhizal (AM) and non-mycorrhizal (non-AM) cotton plant under different treatments after 50 DAS
SA (Specific Activity) expressed as µg oxaloacetate/ g..b.w./ min

AMF status	Si NPs status	Treatments	GOT	The 3rd day	The 5th day	The 7th day
Non-AM	- NPs	Control	SA.	198.246[a]	200.24[a]	217.193[a]
		Metabolites	SA.	116.606[g]	100.176[g]	141.633[e]
			RA%	-41.181	-49.972	-34.78
		Chlorpyrifos	SA	150.376[d]	146.973[d]	142.436[e]
			RA%	-24.146	-26.601	-34.419
	+ NPs	Control	SA.	190.263[c]	196.27[b]	199.733[b]
		Metabolites	SA.	86.52[i]	87.133[i]	75.423[j]
			RA%	-54.526	-55.605	-62.233
		Chlorpyrifos	SA.	130.296[f]	129.08[e]	127.356[f]
			RA%	-31.517	-34.233	-36.236
AM	- NPs	Control	SA.	192.026[b]	195.23[b]	198.063[c]
		Metabolites	SA.	99.443[h]	74.34[j]	77.276[i]
			RA%	-48.213	-61.921	-60.984
		Chlorpyrifos	SA.	149.32[e]	114.893[f]	103.42[j]
			RA%	-22.239	-41.149	-47.784
	+ NPs	Control	SA.	149.463[e]	152.24[c]	149.93[d]
		Metabolites	SA.	84.806[j]	88.396[i]	66.51[k]
			RA%	-43.259	-41.680	-55.562
		Chlorpyrifos	SA.	99.466[h]	96.283[h]	89.97[h]
			RA%	-33.451	-36.477	-39.887
F			***	***	***	***
P			0.0000	0.0000	0.0000	0.0000

RA% (Relative Activity %) = $\frac{\text{treatment}-\text{control}}{\text{control}}$ x 100

IV.Discussion

Recent study has highlighted the importance of AMF, Si NPs, *B. bassiana* Si NPs and Chlorpyrifos in protecting cotton plants against *S. littoralis* attack where, AMF help plants to tolerate stress. In addition, nanoparticles were previously used in the field of plant protection and pest control **(Xu *et al.*, 2020)**. Finally, the entomopathogenic fungi had proved their good role as biological control agents through the production of a large array of biologically active metabolites **(Ak, 2019)**. On the other hand, our results confirmed the effective biocontrol activity of root mycorrhizal colonization against infestation of cotton plants with *S. littoralis*, which resulted in a considerable variation in the tested insect biochemical parameters as represented herein.

Proteins are essential constituents of the general animal cells and in the maintenance of different activities. Because protein is essential to chitin synthesis, the depletion of these metabolic macromolecules indicates that chitin production must be inhibited. In addition, proteins are essential for energy production. The insect body contains thousands of different types of proteins, each with a very specific purpose **(Tunaz and Uygun, 2004)**. A protein may be merely structural giving form and strength to the exoskeleton or binding cells together into biochemical reaction, the storage and transport of a nutrient or waste product of the movement of a specific molecule across cell membranes **(Salgado, 1997)**.

Total proteins are major components necessary for an organism to develop, grow and perform its vital activities. In our study, a significant decrease in TSP content was detected in larvae fed on AM or non-AM cotton plants treated with either Chlorpyrifos or fungal metabolites at 50 DAS in most treatments as compared to control while; this reduction was more pronounced in Chlorpyrifos treatments than metabolites ones. A similar observation was reported in *S. littoralis* larvae exposed to Avermactin **(Dahi *et al.*, 2009).** This result was also in the same line with **Abd El-Rahman *et al.* (2019)** who conducted that Dimilin 48%SC caused the highest change

percentage of 33.07% in TSP of the 4th instar larvae of *S. littoralis*. As well as, the total protein content was decreased by 26 % in the 4th instar larvae of *S. littoralis* treated with Chlorpyrifos insecticide **(Fetoh and Asiry, 2013)**. Additionally, **Abd El-Rahman *et al*. (2019)** found that Owner 5%EC, Dimilin 48%SC, Strong 30% SC and Emafel 45% significantly decreased TSP content of the 4th instar larvae of *S. littoralis* and such decline in TSP content could suggest mobilization of amino acids to meet energy demands in detoxification of chlorpyrifos **(EL-Khayat *et al*, 2011)**. Moreover, exposure of the 5th instar *Bombyx mori* larvae to Fenitrothion and Ethion caused depletion in TSP content that was followed by an increase in free amino acids **(Nath *et al*., 1997).** Also, decreased TSP content was detected in *Tribolium castaneum* treated with Spinosad **(Hussain *et al*., 2009)**. This reduction in TSP content may be due to inhibition of DNA and RNA synthesis (**El-barky *et al*., 2008**) or to elevating protease enzyme that hydrolyzes protein as reported in the 4th instar larvae of *S. littoralis* treated with Tebufenozide, Teflubenzuron, Flufenoxuron and Methoxyfenozide **(Sabry and Khedr, 2014).** The reduction of TSP in our study may reflect the decrease in the enzymatic activities of various enzymes (**Abdel-Aziz *et al*., 2007)**. Additionally, protein was very efficiently utilized by insects and most species derived the main part of their nourishments from this nutrient. The significant decrease of TSP content was also reported in studies on *Musca domestica* treated with Tebufenozide (**Assar *et al*., 2010**); on the 6th instar larvae of *S.littoralis* treated with Spinosad compound **(El-Sheikh, 2012**) and on *Sesamia cretica* larvae treated with the same compound (**Osman *et al*., 2014)**. On the other hand, our results about the decline in TSP content was also supported by the explanation of (**Mosleh *et al*., 2003)** that the decrease in TSP content might be due to a mechanical lipoprotein formation which will be used to repair damaged cells, tissues, and organs or might be referred to mobilization of amino acids during insecticide stress to meet the energy. Also, the reduction of TSP might be due to the destructive effect on some of the cerebral neurosecretory cells of the brain responsible for secretion of the protein of the treated *S. littoralis* as reported by (**Hamouda and Dahi, 2008**) who proved that, Spintoram has a neurotoxic effect leading to production of hormones that utilize protein. Furthermore, Methoxyfenozoid compound binds to the ecdysteroid receptors and thereby disrupting the insect hormonal balance (**Palli and Retnakaran, 2001**).

Moreover, the decline in TSP content observed under Si NPs conditions may be due to the insecticidal effect of Si NPs which resulted from interaction of free radicals released within the host, with DNA leading to modification of sugar moieties of DNA and thus affecting protein production **(Patil, 2009)**. Furthermore, this result could be explained by the indirect effect of NPs on insect metabolites by altering plant quality for herbivores **(Dimkpa and Bindraban, 2017**).

In this work, TSP content was also affected in *S. littoralis* when cotton plants treated with fungal metabolites as compared to the control. Similarly, the crude extracts of *Metarhizium anisopliae* decreased TSP of the 6th **(El-Sonbaty *et al*., 2016)** and the 2nd **(Resquin-Romero, 2016)** instars larvae of *S. littoralis*. Also, **AL-Shannaf and Khedr (2013)** revealed that entomopathogenic fungi, *Metarhizium anisopliae* (Bioranza) Sorok, affected *S. littoralis* in Egyptian clover fields than control and in a similar work, **Al-Shannaf *et al*., (2006)** reported that two bioinsecticides, Spinosad and Viroset also affected *S. littoralis*. These result may be due to the high insecticidal and virulence activities of crude extracts of *B. bassiana* against *S. littoralis* **(Resquin-Romero *et al*., 2016)** or due to the low molecular weight insecticidal toxins produced by *B. bassiana* which may have a role in the demise of the insect by inducing a primary response and phenoloxidase activity **(Elsevier, 2018)**. On the other hand, **Sahar *et al*. (2016)** revealed that *B. bassiana*, *Metarhizium anisopliae*, *Paecilomyces lilacinus* and *Lecanicillium attenuatum* affected TSP in haemolymph of the 5th instar larvae of *S. littoralis*. Moreover, **Abdullah (2019)** indicated that ethyl acetate extracts of both *B. bassiana* and *Trichoderma harzianum* affected *S. littoralis* through the action of compounds found in their metabolites.

Interestingly, GPT and GOT help in the production of energy and serve as a strategic link between the carbohydrates and protein metabolism and are known to be decreased during various physiological and pathological conditions and suggested that this may be attributed to the occurrence of reversible binding between insecticides and enzymatic site of action on the enzyme surface **(Azmi *et al*, 1998)**. This is may be due to the fact that the relationships between protein synthesis and transaminase levels were affected by the hormonal control of protein synthesis and neurosecretory hormones which involved in the regulation of transaminase levels **(Etebari *et al*., 2007**).

In the present study, both of fungal metabolites and Chlorpyrifos generally decreased GOT and GPT enzymes activities as compared to the control in AM and non-AM cotton plants under –NPs and +NPs conditions while; this reduction was more pronounced in AM plants than non-AM ones in most treatments. Meanwhile; Chlorpyrifos was more effective in reducing transaminases activities than metabolites. Similarly, Chlorpyrifos and Spinosad insecticides were reported to cause similar effect on *S. litura* treated larvae (**Singh and Sohi, 2008**).These changes are confirmed by **Abd EL-Aziz (2014)** who reported that decreased transaminases levels had been correlated with protein anabolism in some instances and with protein catabolism in some others. Also,

El-Shershaby *et al*. (2008) reported that throughout 120 h post treatment of the 4th instar larvae of *S. littoralis* with Dipel-2x insecticide, fluctuated changes in GOT and GPT activities were observed. Besides, Coumarin, Neemix and Lannate insecticides decreased the activity of GOT and GPT in the 4th instar larvae of *S. littoralis* **(Gaaboub *et al*., 2012**) and the same result was also observed when larvae treated with Indoxacarbd and Methoxyfenozoid **(Khaled and Farag, 2015)**. On the other hand, **Abd-EL-Aziz (2014)** indicated that Indoxacarb, Emamectin benzoate and Pyridalyl insecticides inhibited GPT and GOT activities of the 4th instar larvae of *S. littoralis*.

In this work, our observation about the effect of fungal metabolites applied with Si NPs in reducing GPT and GOT activities may be due to the ability of *B. bassiana* to reduce consumption of plants by their herbivores **(María *et al*., 2019)** through changes in volatile emissions and some biochemical parameters of the plant and hence affecting insect metabolic processes as transaminases **(Moloinyane and Felix, 2019)**. Another explanation for the decline of transaminases in our study is that, it may be related to the toxic action of Si NPs, either supplemented to the soil or to the fungal growth medium, which was previously recorded by **EL-Helaly *et al*. (2016)** who studied the effect of Si NPs in comparison with Si and Diazinon insecticide on *S. littoralis* by applying 4 different concentrations of 200,300,400, and 500 ppm of the three tested compounds. Their results showed that Si NPs caused higher toxic action values than the other treatments. In the same manner, **Shaker *et al*. (2016)** tested six different concentrations (1000, 500, 250, 125, 62.5 and 31.25 mg/l) of CuO NPs against *S. littorals* and they observed that NPs have a significant effect on the insect as compared to Methomyl compound. Our results about the toxic action of Si NPs, either supplemented to the soil or to the fungal growth medium, could be explained by other workers that represented different explanations and evidences about the toxic effect of NPs on insects where **Gojova *et al*. (2007)** found that the toxic effects of NPs can be attributed to interaction of free radicals released within the host as a result of toxic effect of NPs with host DNA.

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