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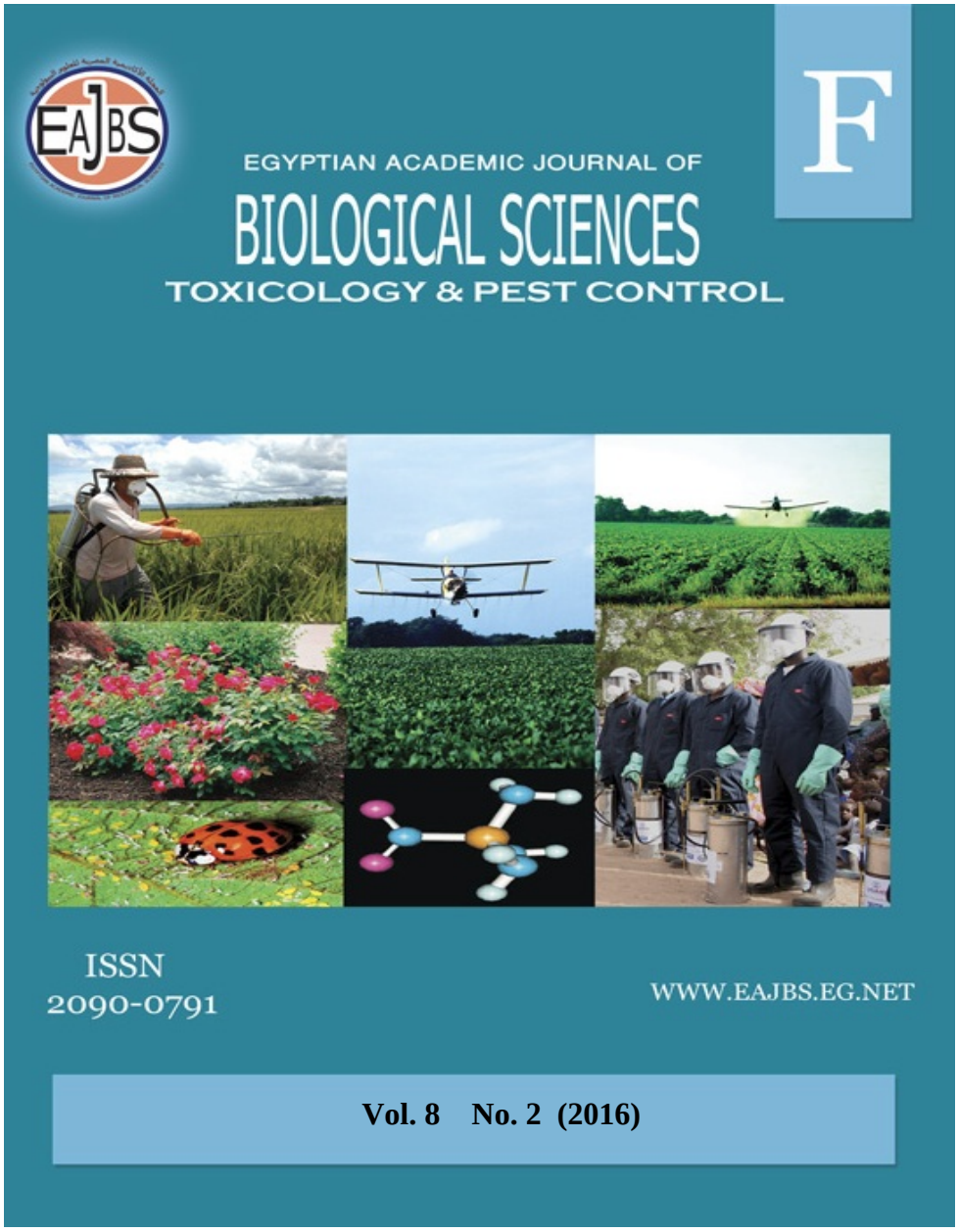

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The Efficacy of Entomopathogenic Nematodes on the Pink Bollworm, *Pectinophora gossypiella*

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ABSTRACT

The development of indigenously isolated entomopathogenic nematodes as biological control agents was investigated. The study involved two nematode species (*Steinernema riobrave* and *Heterorhabditis bacteriophora* Poinar (HP_{88} strain) and their pathogenicity against larvae of pink bollworm, *Pectinophora gossypiella*, under laboratory conditions. The observed mortality caused by the both tested nematodes at different time intervals was recorded. The establishment of nematodes in a host depends greatly on its ability to manage host defenses so, total larval body contents (total protein and free fatty acids) were recorded as well as activities of detoxification enzymes (peroxidase and phenoloxidase) were screened. Results indicated that, the two nematodes had variable significantly effects. *S. riobrave* recorded a highly significantly affect than *H. bacteriophora.* In addition, there are relationship between mortality rate and time exposure of both nematodes against *P. gossypiella* larvae, the susceptibility increased with the time exposure increased. Total proteins content significantly increased after treatment with *S. riobrave* (24.58 mg protein/gbwt).Whilst *H. bacteriophora* caused a non-significant increase in the proteins content (22.95 mg protein/gbwt). In addition, it is could be noted that, female showed a slight significant increase in total proteins content (23.81 mg protein/gbwt) than male (23.10 mg protein/gbwt). Both nematode species increased the free fatty acids than control and the contents of free fatty acids were higher in female (1355.78 ug triolein/gbwt) than male (469.33 ug triolein/gbwt). Nematodes, *H. bacteriophora* and *S. riobrave* showed the lowest enzyme activities of phenoloxidase (17.87 and 17.72 OD units/min/gbwt, respectively). In addition, values recorded for male and female did not differed significantly. Data demonstrated that peroxidase activity of the pink bollworm larvae was higher in female than male. The enzyme activity increased relative to control by 13.55 and 74.92% in the case of *H. bacteriophora* male and female with values of 3.10 and 5.79 Δ OD 430/min/gbwt, respectively. In conclusion, we can say that the all parameters determined were higher in female than male, except in the case of phenoloxidase. Entomopathogenic nematodes, *S. riobrave* and *H. bacteriophora* can play an important role in defeating the host immune system and can control larvae of *P. gossypiella.*

INTRODUCTION

Pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), is one of the most serious pests of cotton. It bore into the squares or bolls within short time of hatching. Also, final instar larvae overwinter as diapausing and damage the following season. Late instar PBW cut out of the boll and drop to the ground to enter diapause in the top few centimeters of soil, or remain in the bolls and diapause near or within the seeds.

Some pathogens like entomopathogenic nematodes located near the soil surface, thus making them a potential candidate for PBW control. Entomopathogenic nematodes (EPNs) that belong to the families; Steinernematidae and Heterorhabditidae, associated with their symbiotic bacteria *Xenorhabdus*, and *Photorhabdus*, respectively (Gaugler, 2002). These bacteria when released from the nematode intestine, multiply rapidly in the host haemolymph, causing a lethal bacteremia within 24-48 hours. Also, EPNs could secrete insecticidal active substances, including toxins, proteases, and so on, contributing to the lethal effect on infected host insects (Toubarro *et al*., 2009). The lethal processes caused by insecticidal active substances are often related to the activity changes of some enzymes in the host insects (Grewal *et al*., 2005). The present study will enhance the understanding of the physiological and biochemical virulence determinants of the EPN towards pink bollworm. The specific objectives as follow:

Evaluate the efficacy of two different nematode species, *S. riobrave* and *H. bacteriophora* Poinar (HP_{88} strain), on first and full-grown larvae of PBW.

Evaluate the effects of LC_{50} of these nematodes species on the PBW total larval body contents (total protein & free fatty acids) as well as activities of detoxification enzymes (peroxidase & phenoloxidase).

MATERIALS AND METHODS

Insects: Newly hatched larvae of *P. gossypiella* were obtained from a colony maintained in the Bollworms Department Laboratory, Plant Protection Research Institute, ARC, for several generations at 27 ± 1°C and 75 ± 5% relative humidity (RH). Larvae reared on a modified artificial diet as described previously by Abd El-Hafez *et al.* (1982).

Nematodes: Imported nematode species, *S. riobrave* and *H. bacteriophora* Poinar (HP_{88} strain) were supplied by Dr. El-Sadawy, NRC- Egypt. For nematodes mass culturing, the last instar larvae of the greater wax moth, *Galleria mellonella* were used as hosts according to Shamseldean *et al*. (2008).

Susceptibility of *P. gossypiella* first instar larvae infected with the nematodes, *S. riobrave* and *H. bacteriophora*.

All bioassays were doing on the newly hatched larvae of (PBW). Nematodes (*S. riobrave* or *H. bacteriophora*) stock solutions containing about 160 IJs/1 ml distilled water. Four dilutions in water from each nematode were prepared in half-descending order then homogenized mixed with 100.0 g of artificial diet to obtain the tested concentrations, i.e.; 0.0625, 0.1250, 0.2500 and 0.5 ppm for each nematode species. Each concentration was fold into three Petri dishes (9 cm in diameter) as replicates. Ten newly hatched larvae were place on the surface of the diet using a soft brush. Another group of 3 Petri dishes was prepared containing the same diet but mixed with equal volume of distilled water (DW) used as control. Larvae were allowed to feed on the tested diets for three hours. Afterwards all alive larvae were transferred individually to glass vials (2 X 7 cm) containing a small piece of normal diet. Vials were plugged with absorbent cotton and incubated at 27 ± 1°C and 75 ± 5% RH. The accumulative mortality percentage was recorded at 1, 2 & 3 hrs. also, 1, 2& 7 days post-infections, respectively, to estimate the LC_{50} values.

Susceptibility of *P. gossypiella* full-grown larvae infected with the nematodes, *S. riobrave* and *H. bacteriophora*.

Thirty of full-grown larvae of (PBW) were placed in three plastic cups. Each cup (one replicate) contain 10

larvae of PBW placed on tissue paper and infected with nematodes, *S. riobrave* or *H. bacteriophora* at concentrations (20, 40, 80 and 160 IJs/1 ml distilled water). Then incubated at 27 ± 1°C and 75 ± 5% RH and mortalities were recorded.

Percentages of mortalities (first & full-grown larvae) were corrected according to Abbott's formula (Abbott, 1925). The data were subject to probity analysis (Finney, 1971) through software computer program to obtain the LC_{50} and slope values. In addition, the efficiency of different compounds was measured by comparing the tested nematode with the most effective nematode using toxicity index (Ti) which calculated using the equation of Sun (Sun, 1950) as follows:

$$Ti = \frac{LC_{50} \text{ of A}}{LC_{50} \text{ of B}} \times 100$$

Where; A: the most effective nematode
B: the other tested nematode

The potential biochemical effects for LC_{50} values of nematodes, *S. riobrave* and *H. bacteriophora*:

This part of study was conducted in order to determine total body contents (proteins & free fatty acids) and some vital enzyme activities (phenoloxidase & peroxidase) in the full-grown larvae of PBW after treatment with tested EPNs. Full-grown larvae (≈30 larvae) placed on plastic cups and treated with LC_{50} of the two tested EPNs. One day (24 h) after treatment, the survived full-grown larvae were collected, weighted and frozen (at –20ºC) for analysis. The total larval bodies were homogenized in distilled water using a chilled glass-teflon tissue grinder for 3 min. Homogenates were centrifuged at 3500 rpm for 10 min at 5ºC and the supernatants were kept in deep-freezer till the biochemical determinations. The optical densities (OD) were read spectrophotometrically by spectronic 1201 (Milton Roy Co., USA) and centrifugation was carried out by a refrigerated centrifuge (Gs-6r, Beckman, USA).

Total proteins were determined by the method of Bradford (1976). Free fatty acids are estimated using the method described by Sadasivam & Manickam (1991), and estimated by titrating it against KOH in the presence of phenolphthalein indicator. Phenoloxidase activity (EC 1.14.18.1) was determined according to a modification of Ishaaya (I971). Peroxidases (EC 1.11.1.7) activity was determined with modifications of the method described by Vetter *et al.* (1958).

Statistical analysis

Toxicological data were statistically calculated through a Proban program, software computer program (Jedrychowski, 1991). The variability in response to the tested materials was determined based on LC_{50} and slope value. Analysis of variance (ANOVA) was conducted on all data using Costat computer program software. Means were compared by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Cumulative mortality and LC_{50} values of *P. gossypiella* larvae after different intervals exposure time of treatment with *S. riobrave* and *H. bacteriophora.*

Data in (Table 1 & Fig. 1) indicated that, the EPNs, *S. riobrave* and *H. bacteriophora* have great efficacy against 1st instar larvae of PBW at nematode rates of 0.0625 to 0.5000 ppm. Mortality percentages had a positive relationship with both the concentration and post-treatment period. Larvae started death on first hour in all treatments were 23.17 & 7.26 % at 0.0625 ppm (lowest concentration) of two nematodes, respectively. Whereas the higher dosage (0.5000 ppm) gives ≈100% mortality on day 7. The low concentration (0.1250 ppm) gives ≈50% mortality after 1 h with *S. riobrave* comparable to 3 h with *H. bacteriophora* post treatments. These percentages reached to 86.45 & 76.43% after 7 days with both nematodes, respectively.

Table 1: Corrected mortality of *S. riobrave* and *H. bacteriophora* against the newly hatched larvae of *P. gossypiella* after different intervals.

Nematode conc. (ppm)	*S. riobrave*	*H. bacteriophora*
1- hour after treatment		
0.0625	23.17	7.26
0.1250	44.26	21.87
0.2500	67.17	46.17
0.5000	84.93	72.05
2- hours after treatment		
0.0625	26.58	19.09
0.1250	57.29	37.43
0.2500	83.97	59.24
0.5000	96.43	78.46
3- hours after treatment		
0.0625	43.45	24.84
0.1250	71.69	45.40
0.2500	90.53	67.30
0.5000	97.99	84.42
1- day after treatment		
0.0625	53.61	29.88
0.1250	76.27	54.27
0.2500	92.10	79.00
0.5000	98.39	93.71
2- days after treatment		
0.0625	60.25	39.96
0.1250	79.50	61.55
0.2500	92.45	80.91
0.5000	98.39	93.71
7- days after treatment		
0.0625	73.53	43.55
0.1250	86.45	76.43
0.2500	94.71	97.41
0.5000	98.46	99.95

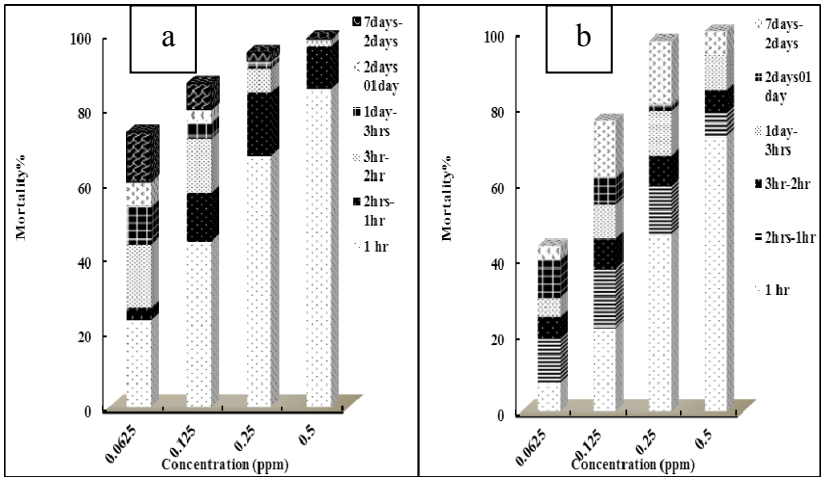

Fig. 1: Cumulative mortality of *P. gossypiella* larvae after different intervals of treatment with *S. riobrave* (a) and *H. bacteriophora* (b)

This indicates that *S. riobrave* requires the least exposure time to the PBW larvae to cause 50% mortality and kill larvae more rapidly than with *H. bacteriophora*. The concentration-mortality relationship of PBW larvae to the EPNs was typically expressed as LC_{50} values and compared as Ti (Table

2). Based on the LC_{50} values and toxicity indices, *S. riobrave* was the most potent nematode for controlling PBW (Ti= 100.0%). LC_{50} values ranged from 0.1481 to 0.0504 ppm after 1 hour and 7 days of treatment with *S. riobrave*, respectively. While it ranged from 0.2757 and 1.033 ppm for *H. bacteriophora*, respectively (Table 2).

Table 2: LC_{50} (ppm) and slope values of *S. riobrave* and *H. bacteriophora* tested against the newly hatched larvae of *P. gossypiella* after different intervals.

Treatment	LC_{50} (95% confidence limits)	Slope± SE	Ti
1- hour after treatment			
S. riobrave	0.1481(0.1252-0.1733)	1.9562± 0.2131	100
H. bacteriophora	0.2757(0.2387-0.3259)	2.2604± 0.2308	53.72
2- hours after treatment			
S. riobrave	0.1068(0.0648 – 0.1492)	2.6888± 0.4191	100
H. bacteriophora	0.1866(0.1580 – 0.2215)	1.8406± 0.2090	57.23
3- hours after treatment			
S. riobrave	0.0730(0.0588 – 0.0859)	2.4539± 0.2768	100
H. bacteriophora	0.1441(0.0648 – 0.2527)	1.8731± 0.3918	50.66
1- day after treatment			
S. riobrave	0.0804(0.0666 – 0.0933)	2.5568± 0.2742	100
H. bacteriophora	0.1274(0.1104-0.1451)	2.4868± 0.2362	63.19

Full grown larvae of *P. gossypiella* were also susceptible to *S. riobrave* than to *H. bacteriophora* (Tables 3 & 4). Infectivity tests with *S. riobrave* nematodes showed that, the highest concentration (160 IJ/ml) killed 73.87% male and 87.15% female larvae, respectively .While *H. bacteriophora* killed 49.63 and 56.09% of male & female larvae, respectively. So, it could be said that female was more susceptible than male to both nematodes species. Toxicity indices calculated for male and female full-grown larvae infected with *H. bacteriophora* (44.69 for male & 45.69 for female) were one-half that calculated for *S. riobrave* infected larvae (100 for male and female, Table 4). These findings are in agreement with those of early workers who have also reported the efficacy of different Steinernema spp. and Heterorhabditis spp. against PBW (Henneberry, 1997 and Henneberry *et al.*, 1996 & 1998); and other Lepidopterous species (Nyasani *et al*., 2007; Shairra & Nouh, 2014 and Hassan *et al*., 2016).

A positive increase in percentage mortality of PBW 1st instar with increase in concentration and exposure time was observed (Table 2). These results agreed with Mahar *et al*. (2004) for diamondback moth larvae (DBM). In addition, the present results confirm that the larval mortality started on first hour. This is in agreement with Lindegren *et al.* (1993) whereas, they found that the time required for nematode infection of PBW larvae was 24 h or less. The results demonstrated that *S. riobrave* can readily attack PBW larvae and cause mortality within a short time than with *H. bacteriophora* (Table 2). Under ideal conditions, host death occurs within 24-48 hrs after infection. The exposure time has an implication on the efficacy of the entomopathogenic nematode strain to control PBW in the field as field effectiveness of entomopathogenic nematodes is limited by desiccation, extreme temperatures, UV radiation and relative humidity in the microclimate (Mason & Wright, 1997). Therefore, a nematode isolate that is able to cause mortality in the PBW larvae in the shortest time possible is desired to overcome the above-mentioned limitations in the field and shortest time

of 1st instar larvae exposure to nematode before interring the bolls.

In addition, Table 2, showed that both on the basis of mortality and LC_{50} values *S. riobrave* emerged as the most potent species (LC_{50}= ranged from 0.1481 to 0.0504 ppm after 1 hour and 7 days of treatment, respectively). This research finding is in agreement with, Correa-Cuadros *et al.* (2014) and Shairra *et al.* (2016). This finding agreed with other report that the efficacy of various EPN species or strains for controlling a particular insect pest may differ significantly (Kondo & Ishibashi, 1988). Also, Shairra & Nouh (2014) found that pathogenicity of both nematodes, *H. bacteriophora* Poinar (HP_{88} strain) and *S. riobrave* against 3rd larval instars of cotton leaf worm, *Spodoptera litraltois.*

Table 3: Corrected mortality of *S. riobrave* and *H. bacteriophora* against the full-grown larvae of *P. gossypiella* after different intervals.

Concentration	*S. riobrave*	*H. bacteriophora*
Male		
20	14.95	0.10
Female		
20	14.71	18.04

The LC_{50} values of *S. riobrave* was the most potent nematodes after 48 & 72 hours of treatment on the contrary, *H. bacteriophora.* The defense reactions against nematodes and their associated bacteria may play an important role of nematode dose, infective juvenile age, exposure period, host species, host size, larval diet and starvation, were tested as factors affecting penetration of nematodes isolates. Also herein, both nematode species were effective against full-grown larvae (Table 4). Full-grown larvae of PBW were more susceptible to *S. riobrave* than to *H. bacteriophora*. Female was more susceptible than male to both nematodes species. Differences in the susceptibility among insect life-cycle stages are have been observed by Shannag & Capinera (1995). However, most success has been achieved in insect pests that spend some stages in the soil where infective juveniles (IJs) are protected from environmental extremes. So, EPNs can effectively control PBW whereas it drill into the ground as diapause larvae. So, it could be contribute in decreasing the damage and loses in the following season. EPNs could applied in spring, summer or fall.

Table 4: LC_{50} (IJs) and slope values of *S. riobrave* and *H. bacteriophora* tested against the full grown larvae of *P. gossypiella* after different intervals.

Treatment	LC_{50}	Slope± SE	Ti
Male			
S. riobrave	72.44	1.627	100
H. bacteriophora	162.12	1.627	44.69
Female			
S. riobrave	54.33	2.416	100
H. bacteriophora	118.70	1.182	45.77

A spring application, prior to pupation, may reduce the susceptible overwintering PBW larval population. Timing would be less critical in a fall application after plowdown because the PBW population would be limited to susceptible diapausing larvae. Summer applications may be effective since the

cotton canopy has reached a size where it is able to shade the soil and maintain a high humidity beneath the plants. Time of application of nematodes, as well as cost of application, need to be determined under field conditions.

Biochemical studies:

The present study was carried out to clarify the effects of the median lethal concentrations (LC_{50}) of the EPNs recorded for full-grown larvae on some biochemical parameters (total protein and free fatty acid contents) and some enzyme activities (phenoloxidase and peroxidase) of pink bollworm. The changes in larval protein contents are given in Table (5). Indicated that, total proteins content significantly increased after treatment with *S. riobrave* (24.58 mg protein/gbwt). Whilst *H. bacteriophora* caused a non-significant increase in the protein content (22.95 mg protein/gbwt). Also, it could be noted that female showed a slight significant increase in total protein contents (23.81 mg protein/gbwt) than male (23.10 mg protein/gbwt).

Table 5: Changes in total body contents of proteins (mg/gbwt) in 4th instar larvae of *P. gossypiella* treated with LC_{50} concentrations of nematodes, *S. riobrave* and *H. bacteriophora*.

Treatment	Male		Female		LSD (5%)	Total
	Mean ± SD	Change%*	Mean ± SD	Change%*		
Control	21.72^{bB} ± 0.30	—	23.93^{abA} ± 0.51	—	0.96	22.83^{b} ± 1.27
H. bacteriophora	22.73^{bA} ± 0.31	4.65	23.17^{bA} ± 0.57	-3.18	1.03	22.95^{b} ± 0.47
S. riobrave	24.83^{aA} ± 1.32	14.31	24.33^{aA} ± 0.49	1.67	2.26	24.58^{a} ± 0.93
LSD (5%)	**1.60**		**1.05**			**0.85**
Total	23.10^{B} ± 1.54		23.81^{A} ± 0.69		**0.70**	23.45 ± 1.21

Means within columns with same letter (s) are not significantly different from each other at 5% level of probability. Capital letter genius -Small letter treatment.

* $\%\text{Change} = \frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$

Data from the current study is consistent with that obtained by (Hassan *et al*., 2016); (El-Sadawy *et al*., 2009) in *Agrotis ipsilon* 6th larval instar; Parasarcophaga aegyptiaca and Argas (persicargas) persicus when infected with *H. bacteriophora* and *S. riobrave*. In addition, (El-Bishry, 1989) reported that haemolymph protein of *S. littoralis* was markedly reduced 30 hrs. post-nematode infection. Author attributed this reduction to the proteolytic activity detected in the haemolymph of infected larvae, this activity was believed to be the main cause of the host quick death.

Results presented in Table (6) showed that EPNs caused an increase in total free fatty acids content in the male. The mean values recorded were 406.33 and 605.67 ug triolein/gbwt for *H. bacteriophora* and *S. riobrave*, respectively. It was increased relative to control by 2.61 and 52.95, respectively. In female, *H. bacteriophora* caused a significant increase in free fatty acids content by 75.41%. In contrast, *S. riobrave* caused insignificant decrease by 1.23%.

In conclusion, two nematode species increased the free fatty acids (1156.5 and 839.67 ug triolein/gbwt) than control (741.5 ug triolein/gbwt). And the contents were higher in female (1355.78 ug triolein/gbwt) than male (469.33 ug triolein/gbwt).

Table 6: Changes in total body contents of free fatty acids (ug triolein/gbwt) in 4th instar larvae of *P. gossypiella* treated with LC_{50} concentrations of nematodes, *S. riobrave* and *H. bacteriophora*.

Treatment	Male		Female		LSD (5%)	Total
	Mean ± SD	Change%*	Mean ± SD	Change%*		
Control	396^{bB} ± 5.29	—	1087.0^{bA} ± 60.22	—	**96.91**	741.5^{c} ± 380.40
H. bacteriophora	406.33^{bB} ± 5.69	2.61	1906.67^{aA} ± 100.66	75.41	**161.62**	1156.5^{a} ± 824.23
S. riobrave	605.67^{aB} ± 15.82	52.95	1073.67^{bA} ± 39.15	-1.23	**67.68**	839.67^{b} ± 839.67
LSD (5%)	**20.33**		**142.64**			**64.15**
Total	469.33^{B} ± 102.73		1355.78^{A} ± 417.81		**52.38**	912.56 ± 543.24

Means within columns with same letter (s) are not significantly different from each other at 5% level of probability. Capital letter genius - Small letter treatment

$$* \ \%\text{Change} = \frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$$

Data in Table (7), showed the effect of EPNs on the activity of phenoloxidase. *H. bacteriophora* and a *S. riobrave* had the lowest enzyme activities (17.87 and 17.72 OD units/min/gbwt, respectively) in comparable with control (21.00 OD units/min/gbwt). In addition, no significantly values differed between male and female of PBW.

Table 7: Changes in the Phenoloxidase activity (OD units/min/gbwt) in 4th instar larvae of *P. gossypiella* treated with LC_{50} concentrations of nematodes, *S. riobrave* and *H. bacteriophora*.

Treatment	Male		Female		LSD (5%)	Total
	Mean ± SD	Change%*	Mean ± SD	Change%*		
Control	20.27^{aA} ± 0.70	—	21.73^{aA} ±1.41	—	**2.53**	21.00^{a} ± 1.28
H. bacteriophora	18.67^{bA} ± 0.25	-7.89	17.07^{bB} ± 0.38	-21.45	**0.73**	17.87^{b} ± 0.92
S. riobrave	17.9^{bA} ± 0.36	-11.69	17.53^{bA} ± 0.59	-19.33	**1.10**	17.72^{b} ±0.48
LSD (5%)	**0.95**		**1.82**			**0.91**
Total	18.95^{A} ± 1.13		18.78^{A} ±2.36		**0.75**	18.86±1.80

Means within columns with same letter (s) are not significantly different from each other at 5% level of probability. Capital letter genius - Small letter treatment

$$* \ \%\text{Change} = \frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$$

Data in Table (8), in total demonstrated that peroxidase activity of PBW larvae was higher in female than male. In details, the enzyme activity increased relative to control by 13.55 and 74.92% in the case of *H. bacteriophora* male and female with values of 3.10 and 5.79 Δ OD 430/min/gbwt, respectively.

On the other hand, *S. riobrave* (1.31 and 3.03 Δ OD 430/min/gbwt) caused a decrease in the enzyme activity by 0.52 and 0.08%, respectively.

In conclusion, we can say that the all parameters determined were higher in female than male, except in the case of phenoloxidase. Also, it could be noted that both nematodes caused an increase in the total protein and free fatty acids contents. In contrast, they caused a decrease in phenoloxidase activity. On the other hand, peroxidase activity increased following treatment with *H. bacteriophora* only.

The bacterial symbionts create a favorable environment for the nematode to propagate by suppressing the immune protein of the insect (Gotz *et al*., 1981) and provide nutrition for the nematode to develop and reproduce (Poinar, 1983).

Table 8: Changes in the peroxidase activity (ΔOD430/min/gbwt) in 4th instar larvae of *P. gossypiella* treated with LC_{50} concentrations of nematodes, *S. riobrave* and *H. bacteriophora*.

Treatment	Male		Female		LSD (5%)	Total
	Mean ± SD	Change%*	Mean ± SD	Change%*		
Control	2.73^{bB}± 0.12	—	3.31^{bA}± 0.16	—	**0.32**	3.02^{b}±0.34
H. bacteriophora	3.10^{aB}± 0.06	13.55	5.79^{aA}± 0.25	74.92	**0.41**	4.45^{a}±1.49
S. riobrave	1.31^{cB}± 0.12	- 0.52	3.03^{bA}± 3.03	- 0.08	**0.22**	2.17^{c}± 0.95
LSD (5%)	**0.20**		**0.34**			**1.18**
Total	2.38^{B}±0.82		4.05^{A}±1.32		**1.15**	

Means within columns with same letter (s) are not significantly different from each other at 5% level of probability.

Capital letter genius - Small letter treatment * $\%\text{Change} = \frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$

The lethal processes caused by insecticidal active substances are often related to the activity changes of some enzymes in the host insects (Grewal *et al*., 2005). The molecular interactions between nematodes, bacteria, and insect hosts that lead to the impairment of the insects' defense responses have not been fully recognized**.** Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide (H_2O_2), and lipid peroxides, arising from aerobic metabolism and dietary prooxidants, can damage proteins, lipids, and other important macromolecules. Fatty acids play numerous roles in living organisms. They are a source of energy (Manteiga *et al*., 2013), and the building blocks of cell membranes in living organisms (Calderone *&* Lin, 2001 and Pernal *et al*., 2005). Living organisms must therefore remove or scavenge ROS before cell damage occurs (Ahmad, 1992 and Feig *et al*., 1994).

Phenoloxidase (PO), the enzyme responsible for the biosynthesis of melanin, is considered as an important component of insects' immune system. The enzyme is not only involved in defense reactions but also in other physiologically important processes, such as sclerotization of the cuticle, an essential step for the survival of all insects (Sugumaran *et al*., 2000).

This study is a clear demonstration that entomopathogenic nematodes (i.e., *S. riobrave* and *H. bacteriophora*) have great potential that should be exploited in pink bollworm management.

A series of field trials using these entomopathogenic nematodes would be appropriate to verify the laboratory results and to see if the laboratory results can be extrapolated to the field.

More work also needs to be done on the economics of using entomopathogenic nematodes in the management of PBW.

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