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Insecticidal effect and biochemical studies of entomopathogenic nematode strains against fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae).

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Abstract:

The fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is a significant invasive pest that has recently expanded to many countries worldwide. It is a polyphagous pest, destroying almost all vegetables and numerous commercial agricultural crops worldwide. The purpose of the current study was to compare between the efficacy of a newly isolated local strain of entomopathogenic nematodes (EPNs), *Steinernema* coded as (15) and the foreign strain *S. carpocapae* (All) against 3rd larval instar of *S. frugiperda* under laboratory conditions with four concentrations (5, 10, 20, and 40 infective juveniles IJs/ml/larvae). Our results revealed that the newly local strain recorded the maximum larval mortality, reaching 100%, while the foreign strain caused 91.67% larval mortality at concentration (40 IJs/ml) after 72 hr. of treatment. Our results showed that the relative potency of the newly local strain was 1.98 more potent than the foreign strain. By studying the effect of LC_{50} (4.4778 IJs/ml) of the newly isolated local strain of EPN on biochemical aspects of larval haemolymph such as total protein and some enzymes activity. Results showed that the treated larvae had lower protein content in compared to control. In addation, the activity of enzymes (phenoloxidase (PO) and carboxylesterase (CarE)) was affected by newly local strain treatment. In other way, (PO) and (CarE) enzymes activities were increased significantly in treated larvae in compared to control.

Keywords: *Spodoptera frugiperda*, entomopathogenic nematodes, total protein, carboxylesterase (CarE), phenoloxidase (PO).

1. Introduction

Fall armyworms (FAWs), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), are a major danger to the food security and income of millions of smallholder farmers and consumers in recent years [(1)].

The primary difficulties with this devastating pest were its great capacity for dispersal, higher fertility rate (>2000 eggs), long-distance migration exceeding 500 km, continuous yearly generation rate, tolerance to climatic circumstances, and intense fire up to 100 kilometres every night [(2)].

Despite the application of various control strategies, farmers continue to rely on both new and conventional chemical insecticides to eradicate this pest. High resistance to the main classes has been acquired as a result of excessive and continual use of these compounds [(3)].

Consequently, the necessity to create alternative, non-chemical equipments and efficient, eco-friendly techniques to control the FAWs infestation are growing. Natural enemies like parasitoids, pathogens and predators are highly suggested for biological control when it comes to eco-friendly management practices. According to this concept, entomopathogenic nematodes (EPNs) from the families Heterorhabditidae (Rhabditida) and Steinernematidae have been employed extensively as likely biocontrol agents on a variety of economically significant insect pests during the past few decades [(4)].

Furthermore, EPNs are mutualistically associated with two symbiotic pathogenic bacteria, *Xenorhabdu*s for *Steinernema* and *Photorhabdus* for *Heterorhabditis* [(5)].

Insect host can be penetrated by EPNs through a natural opening like a cuticle, anus, mouth, or spiracles to reach the hemocoel and release their bacteria, which then multiply quickly into haemolymph, breaking down the insect tissue and generating a variety of immune-suppressive factors such as haemolysins, hydrolytic enzymes, antimicrobial compounds, and complexes of toxins against the target host; as a result, the insects died between 24 and 72 hr. after application [(6)].

Enzymes involved in detoxification are crucial for the survival of insects exposed to both endogenous and exogenous xenobiotics [(7)].

Carboxylesterase (CarE) is a crucial detoxifying enzyme that has been linked to pesticide resistance through its metabolic roles, including catalyzing hydrolysis of amide, sulphate, and ester [(8)].

Phenoloxidase (PO) activity formed Melanin coating around the invading

pathogen. Cuticular (PO) is a melanizing enzyme which is produced in response to infection by activated haemocytes [9].

Therefore, this study aimed to assess the bio-efficacy of two steinernematid EPNs species, foreign strain, *Steinernema carpocapsae* (All) and newly isolated local strain *Steinernema* coded as (15) against 3rd larval instar of *S. frugiperda* and to study the biochemical aspect and changes in enzymes activity of treated larvae with LC_{50} of newly isolated strain.

2. Material and Methods:

2.1. Culture of *Spodoptera frugiperda*:

Initial culture of *S. frugiperda* larvae was collected from infested cabbage fields in villages of Shebin Al-Qanater Centre, Qalyubiya Governorate, Egypt. The insect rearing technique was carried out in accordance with standards provided by [2]. The pest was reared under laboratory conditions without any contamination with insecticides for more than six generations at 27 ±2°C and 65- 70% R.H before the beginning of the experiments. Larvae were reared in small groups in a 20-ml plastic container to avoid cannibalism and fed on fresh castor bean leaves *Ricinis communis* (L.), until development of pupae which transferred to another jar inside a rearing cage (30×30×30 cm) until development of adults. When moths emerged, they supplied with 10% honey solution for feeding and oviposition.

2.2. Entomopathogenic nematodes (EPNs):

Two steinernematid species, *Steinernema carpocapsae* (All) (Rhabditida: Steinernematidae), received from Ramon Georgis, Biosys, Palo Alto California, USA, and a newly isolated EPN, *Steinernema* coded as (15), which was obtained from soil samples from orange horticulture, Domiatte Governorate, in 2023 and identified molecularly, then submitted their sequence to the NCBI-GenBank database. According to [10]., nematodes were cultured in vitro on the last larval instar of *Galleria mellonella;* following harvesting, IJs were kept in 500 ml vented culture flasks arranged horizontally and filled with 150 ml of distilled water at 14 °C. The flasks had been shaken once a week to enhance IJ survival and aeration. IJs were used over the first three weeks following its emergence and harvest from White's traps [11]. IJs had been stored at 22°C for the previous 24 hr for use in experiments.

2.3. Pathogenicity bioassays:

For bioassay against 3rd larval instar of *S. frugiperda,* different concentrations of tested steinernematid EPN (5, 10, 20, 40 IJs/ml/larvae) were used. Twelve larvae per each concentration/species were placed

singly into 24-Cell culture plate (Greiner, Germany) lined with a double circle of Whatman filter paper and received one ml of the aforementioned concentration. Castor bean leaves was provided as source of food. Control received only distilled water. All treatments were incubated under laboratory conditions at 25±2 °C; 16:8 hrs photoperiod; and 65 R.H for 72 hr. Experiments were repeated three times and larval mortality was recorded daily. The LC_{50} (median lethal concentration) and slope were also determined.

Larval bioassay for determination of the biochemical aspects as total protein and some enzymes.

Thirty larvae of third instar were treated with LC_{50} of the local strain *Steinernema* coded as (15) also used thirty larvae treated only with the same volume of water as a control for investigation the influenced contents of total protein in treated larvae and untreated ones (control) at three-time intervals 24, 48 & 72 hr. after treatment.

2.4. Preparation of insects for analysis:

The treated larvae were homogenized in distilled water (50 mg /1 ml). Cooling centrifuge (ST – 2 Mechanic-Preczyina, Poland) was used for centrifugal homogenates for 15 min at 8000 r.p.m. at 2 ºC. Supernatants, which are referred as enzyme extract can be kept for at least one week at temperature below 0ºC without noticeable loss in activity when stored. The deposits were discarded according to [12].

Protein Estimation:

Total proteins were determined by [13].

Phenoloxidase (PO) assay:

PO activity was determined according to a modification [14].

Carboxylesterase (CarE) assay:

CarE activity was measured according to the method described by [15].

3. Statistical analysis:

Data obtained were analyzed using one- way analysis of variance (ANOVA) followed by LSD post-Hoc multiple comparisons [16]. When ANOVA statistics were significant ($P< 0.05$), the means were compared by Duncan's multiple range test and also, independent- samples T- test at ($P< 0.05$) were used to compare between two groups with SPSS software package version 20 Program. The values of percentage mortality and their standard error (SE) were calculated. Moreover, the LC_{50}, LC_{95} and slope were calculated using probit analysis [17].

4. Results and discussion

4.1. Entomopathogenic nematodes against 3rd larval instar of fall armyworm (FAW).

Based on the obtained results in **Table (1)**, the mortality of larvae treated

by nematodes after 24 hr of treating appeared to be significant **(P< 0.05)**, where the treatment with *Steinernema carpocapae* (All) against 3rd larval instars caused (33.33%) larval mortality after 24 and 48 hr post-treatment at the lowest concentration (5 IJs/ml). While at the highest concentration (40 IJs/ml), the mortality percentage was increased up to 58.33 and 75% after 24 & 48 hr., respectively. The mortality reached the maximum rate of 91.67% larval mortality at the highest concentration after 72 hr. post-treatment of *S. carpocapae* (All). In the application of the newly isolated local strain, *Steinernema* coded as (15), results showed that after 24 & 48 hr. of treatment, the larval mortality percentage was (41.67%) recorded at the lowest concentration (5 IJs/ml). There was a sharp increment in mortality with increasing concentrations, whereas the mean number of dead larvae reached 100% at concentrations of (20 and 40 IJs/ml) after 72 hr. of exposure. There was a significant increase in percentage of larval mortality with increasing the concentrations as well as time in comparing to that of control (0%). Our optical observations showed that, following nematode exposure, the larval feeding behaviour was extremely weak in every treatment compared to the control.

By comparing the newly isolated local strain, *Steinernema* coded as (15) with the foreign strain *S. carpocapae* (All), the percentage of larval mortality after 72 hr. of exposure with the strain *Steinernema* coded as 15 and *S. carpocapae* (All) at the highest concentration (40 IJs/ml) was 100 and 91.67%, respectively, but at the lowest concentration (5 IJs/ml), the newly isolated local strain appears to have no significant increase in larval mortality (58.33%) compared to that recorded by the foreign strain (41.67%).

The present results are in agreement with **(18)** who observed that *S. carpocapsae* strain was effective against 4th larval instar and pupa of *Pieris rapae* L. Additionally, several studies were carried out against other insects; the diamondback moth, *Plutella xylostella* **(19)** evaluated *S. carpocapsae* and *H. bacteriophora* against *P. xylostella* and observed that *S. carpocapsae* was more effective, and by application of *Steinernema feltiae, S. carpocapsae,* and *H. bacteriophora* against the same previous larvae, *S. carpocapsae* revealed the highest virulence as reported by **(20)**. Further investigation was carried out by **(21)** who revealed that utilizing various *Steinernema diaprepesi* concentrations led to increased larval mortality in the last larval instar of FAW after 144 hr. Several studies have examined the effectiveness of various *nematode*

strains against *Spodoptera frugiperda* globally [22] who concluded that *S. carpocapsae* (Weiser) and *H. indica* (Poinar) exhibited a high rate of ovicidal, larvicidal, and pupicidal actions of *S. frugiperda*. Also, according to [23] was found that *S. carpocapsae* was particularly potent against younger larvae (first-third) exclusively; in an additional way, [24] observed that both indigenous isolates, *S. siamkayai* (APL 12.3) and *H. indica* (AUT 13.2), were effective against the second and fifth larval instars of FAW. Also [25] noted that 100% mortality of the 3rd and 4th larval instars of *S. frugiperda* died as a result of *H. indica* (NBAIIH38) and *S. carpocapsae* (NBAIRS59).

According to [26] who found that the Rwandan strain of *S. carpocapsae* (RW14-G-R3a-2) caused 100% mortality in the second and third instars very quickly, although the rate of mortality dropped to 75% in the sixth instar. In addition, [27] evaluated that *S. carpocapsae* and *H. indica* against all larval instar of *S. frugiperda* and they clarified that *S. carpocapsae* treatment was more virulent and effective against all larval instats, showing 100% mortality at various nematode concentrations 48-72 hr. after exposure, on the other hand, *H. indica* only killed 100% of early instars after 96 hrs, but late instars needed more time. On the contrary, *S. riobrave* proved ineffective against FAW larvae even at high concentrations [28].

The larval mortality may be due to generating more effective immunological suppressor against to the insect's antimicrobial peptides [29]; also [30] showed that entrance of IJs into the host's hemocoel, releasing their symbiotic bacteria that cause septicemia and eventually kill the host.

Table (1) Mean and Percentage of mortality of 3rd larval instar of *S. frugiperda* treated with two isolates, *S. carpocapae* (All) and *Steinernema* coded as (15) nematodes at 25 ±2 ºC after 24, 48 &.72hr.

Hours	Nematode strain	Mean no. of dead larvae ± SE (% of mortality)					F-Value	P-Value
		40 (IJs/ml)	20 (IJs/ml)	10 (IJs/ml)	5 (IJs/ml)	control		
24	*Steinernema carpocapae* (All)	0.58 ± 0.1^{be} (58.33%)	0.42 ± 0.15^{bd} (41.67%)	0.42 ± 0.15^{bc} (41.67%)	0.33 ± 0.14^{acde} (33.33%)	0.00 ± 0.00^{a} (0%)	2.690	0.04***
	Steinernema coded as 15	0.67 ± 0.1^{b} (66.67%)	0.50 ± 0.15^{b} (50%)	0.42 ± 0.15^{b} (41.67%)	0.42 ± 0.15^{b} (41.67%)	0.00 ± 0.00^{a} (0%)	3.467	0.013***
	T-Value	0.689^{NS}	0.689^{NS}	1.000^{NS}	0.689^{NS}			
48	*Steinernema carpocapae* (All)	0.75 ± 0.1^{b} (75%)	0.50 ± 0.15^{bd} (50%)	0.50 ± 0.15^{bc} (50%)	0.33 ± 0.14^{acd} (33.33%)	0.00 ± 0.00^{a} (0%)	4.618	0.003***
	Steinernema coded as 15	1.00 ± 0.00^{c} (100%)	0.92 ± 0.08^{c} (91.67%)	0.75 ± 0.13^{c} (75%)	0.42 ± 0.15^{b} (41.67%)	0.00 ± 0.00^{a} (0%)	18.31	0.000***
	T-Value	0.082^{Ns}	0.027**	0.223^{NS}	0.689^{NS}			
72	*Steinernema carpocapae* (All)	0.92 ± 0.08^{cd} (91.67%)	0.58 ± 0.15^{bd} (58.33%)	0.50 ± 0.15^{b} (50%)	0.42 ± 0.15^{b} (41.67%)	0.00 ± 0.00^{a} (0%)	7.380	0.000***
	Steinernema coded as 15	1.00 ± 0.00^{c} (100%)	1.00 ± 0.00^{c} (100%)	0.83 ± 0.11^{c} (83.33%)	0.58 ± 0.15^{b} (58.33%)	0.00 ± 0.00^{a} (0%)	25.200	0.00***
	T-Value	0.339^{NS}	0.017**	0.091^{NS}	0.436^{NS}			

Each value represents mean of 12 replicates ± SE, "each replicate contain 1 larva".
ANOVA P-Value: ***= Significantly different at P<0.05. Mean ± SE followed by same letters are not significantly different in the same row (P > 0.05) (F-test).
Student's (t) test: **=Significant difference between *S. carpocapae* (ALL) and *Steinernema* coded as (15) at (P<0.05.) (T- test). N.S= non-significantly different.

Data represented in **Table (2)** probit analysis showed that LC_{50} values at 72 hr. after treatment were (8.8484 and 4.4778 IJs/ml) for *S. carpocapae* (All) and *Steinernema* coded as (15), respectively. Data also revealed that the LC_{95} of both selected strains against 3rd larval instar of *S. frugiperda* was (112.9275 IJs/ml) for *S. carpocapae* (All) and (14.3926 IJs/ml) for *Steinernema* coded as (15). The toxicity index based on LC_{50} of *S. carpocapae* (All) was 50.60% and 100% for *Steinernema* coded as (15). The relative potency of *Steinernema* coded as (15) was nearly 1.98 times more potent than *S. carpocapae* (All) against 3rd larval instar of *S. frugiperda*. From previous results, probit analysis showed that the LC_{50} of *Steinernema* coded as (15) was lower in value than the foreign strain, which confirmed the *Steinernema* coded as (15) was the more virulent. Our results are similar to **(31)** who investigated the effectiveness of EPN *S. carpocapsae*

and *H. bacteriophora* against last instar larvae of *Osphranteria coerulescens* and observed that *S. carpocapsae* LC_{50} (2.7 IJ larva−1) was more effective than *H. bacteriophora* (9.0 IJ larva^{-1}). [20] found that *S. carpocapsae*, *S. feltiae* and *H. bacteriophora* had virulence effect against larvae of *P. xylostella*, LC_{50} values were 8.95, 11.57 and 28.46 IJ L^{-1}, respectively.

So, it is commonly recognized that one of the most crucial aspects of a regression line is its slope value; the slopes in regression equations show how strongly the EPN virulence affects the different DBM maturation phases. Every regression relationship demonstrates a negative slope, suggesting that EPN virulence is time-dependent on the host and decreases with exposure., [18] who showed that LC_{50} values of *S. carpocapsae* were 18.148 for larvae (IJs/ larva) of *Pieris rapae* L.; [32] who observed that the median lethal concentration (LC_{50}) for *S. feltiae* (Filipjev), *S.carpocapsae* (Weiser),a nd *H. bacteriophora* (Poinar) were 35.97, 44.0 and 104.5 IJ/ml, respectively against last larval instar of clearwing moth *Paranthrene diaphana*., Beside[33] who evaluated the efficacy of EPN (*S. feltiae*, *S. carpocapsae* and *H. bacteriophora*) against 2nd larval instar of *Tuta absoluta* and observed that the LC_{50} values were 156.01, 225.13, and 317.66 IJs/ml, respectively. Also, [34] who assessed the lethal values of EPN stains *S. carpocapsae* and *H. indica* against the 4th larval instar of *Spodoptera littoralis* and they observed that *S. carpocapsae* had higher toxicities than *H. indica* at LC_{35}, LC_{50}, and LC_{90}.

Table (2) Effect of two isolates, *S. carpocapae* (All) and *Steinernema* coded as (15) nematodes (LC_{50}) and (LC_{95}) against 3rd larval instar of *S. frugiperda* at 25 ±2 ºC after 24, 48 &.72hr.

Nematode strain	LC_{50} (IJs)	Toxicity index	Relative potency	LC_{95} (IJs)	Slope	Chi-Square
***Steinernema carpocapae* (All)**	8.8484	50.60	1	112.9275	1.4873± 0.5904	1.6126
***Steinernema* coded as 15 (identified)**	4.4778	100	1.98	14.3926	3.2439± 1.2722	0.3611

Table (3) Effect of LC_{50} of *Steinernema* coded as 15 on total protein content of 3rd larval instar of *S. frugiperda* after 24, 48 &72hr.

Exposure time/hours	(mg protein/g.b.wt)		F-value	P-value
	Control	*Steinernema* coded as (15)		
24	49.07±0.74[a]	42.97±1.29[a]	0.977	0.023
48	44.60±0.95[b]	32.37±0.88[b]	0.035	0.001*
72	37.60±1.45[c]	24.90±0.62[c]	1.318	0.006*
F-value	28.328	31.185		
P-value	0.001	0.000		

Number: Three replicates for each treatment, protein content in the same column followed by the different superscript are significantly different (ANOVA) followed by Duncans test, $p<0.05$.

* Values (Mean ± SE) were significantly different than control in various time intervals in same rows ($P<0.05$, Independent- Samples T-test). Control= untreated larvae with nematode.

4.2. Effect of LC_{50} of *Steinernema* coded as 15 on phenoloxidase (PO) of 3rd larval instar of *S. frugiperda* after 24, 48 &72hr.

The results, as shown in **Table (4)** indicated that there was a significant difference in phenoloxidase activity between treated larvae with *Steinernema* coded as 15 and control larvae after exposure time 24 hr. PO activity was significantly (**P< 0.05**) dropped from 121.00±2.08 to 78.43±1.55 O.D. units/min/g.b.wt in treated larvae. In the same way, in untreated larvae, PO activity significantly decreased from 92.23±1.24 to 74.87±1.33 O.D. units/min/g.b.wt.

Generally, the activity of PO enzymes in treated larvae was more than those in control The obtained results agreed with **(36)** who found that dramatically increased in PO activity in 4th larval instar of the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* after treated with LC_{20} and LC_{80} of *S. carpocapsae*., also the present findings confirmed with results obtained by **(39)** who noticed significant increasing in PO activity in treated nymphs of *Locusta migratoria* L with LC_{50} of *S. carpocapsae* after both 24& 48hr. in compared to control. In contrast to our results, **(40)** observed that the infection caused by *S. carpocapsae* against hispid beetle**,** *Octodonta nipae* was seen causing a slight reduction of the host's phenoloxidase activity; **(34)** observed that PO enzyme activity increased in treated 4th larval instar of *S. littoralis* with *S. carpocapsae, H.*

indica and a binary combination of EPNs in compared to control.

Table (4) Effect of LC_{50} of *Steinernema* coded as 15 on phenoloxidase of 3rd larval instar of *S. frugiperda* after 24, 48 &72hr.

Exposure time/hours	(O.D.units/min/g.b.wt)		F-value	P-value
	Control	*Steinernema* coded as (15)		
24	92.23±1.24[a]	121.00±2.08[a]	1.140	0.001[*]
48	75.87±1.21[b]	75.70±2.22[b]	2.177	0.951
72	74.87±1.33[b]	78.43±1.55[b]	0.102	0.157
F-value	59.631	166.237		
P-value	0.000	0.000		

Number: Three replicates for each treatment, phenoloxidase in the same column followed by the same superscript are not significantly different (ANOVA) followed by Duncans test, p>0.05.

[*] Values (Mean ± SE) were significantly different than control in various time intervals in same rows (P<0.05, Independent- Samples T-test). Control= untreated larvae with nematode.

4.3. Effect of LC_{50} of *Steinernema* coded as (15) on carboxylesterases (CarE) of 3rd larval instar of *S. frugiperda* after 24, 48 &72hr.

The results displayed in **Table (5)** demonstrate a significant rise in CarE activity in the treated larvae, 80.90±1.93 ug Meb/min/g.b.wt, in contrast to the untreated larvae that showed 38.00±1.79 ug Meb/min/g.b.wt after 24hr. of treatment. The activity of CarE then abruptly reduced in both treated and untreated larvae after 48 hours, but it then significantly increased **(P< 0.05)** again afte72 hr., reaching 96.37±2.30 and 48.47±2.03 ug Meb/min/g.b.wt in treated and control larvae, respectively. This result is in harmony with the results obtained by **(7)** who observed that the activity of CarE increased during the first 8 hr. after treating the last larval instar of *G. mellonella* by a new EPN species, *H. beicherriana* n. Sp, then the activity of CarE enzyme dropped, but it again rose after 32 and 40 hr., Also **(41)** noticed the activity of CarE enzyme was higher in treated larvae of *A. ipsilon* by *S. Abbasi* than those treated by *H. zealandica*.; **(42)** who observed that after treatment of larvae of *Tenebrio molitor* by different concentrations of EPN, *H. beicherriana*, there was negative relationship between concentrations revealed and the activity of CarE enzyme; where at lower concentrations, increased but decreased at the high concentrations.

Table (5) Effect of LC_{50} of *Steinernema* coded as (15) on carboxylesterases of 3rd larval instar of *S. frugiperda* after 24, 48 &72hr.

Exposure time/hours	(ug Meb/min/g.b.wt)		F-value	P-value
	Control	*Steinernema* coded as (15)		
24	38.00±1.79[a]	80.90±1.93[a]	0.069	0.000*
48	31.00±1.31[b]	45.03±1.99[b]	0.220	0.006*
72	48.47±2.03[c]	96.37±2.30[c]	0.042	0.000*
F-value	25.556	160.125		
P-value	0.001	0.000		

Number: Three replicates for each treatment, carboxylesterases in the same column followed by the same superscript are not significantly different (ANOVA) followed by Duncans test, p>0.05.

* Values (Mean ± SE) were significantly different than control in various time intervals in same rows (P<0.05, Independent- Samples T-test). Control= untreated larvae with nematode.

5. Conclusion

Pathogenicity bioassays of the two entomopathogenic nematode species: the first was a foreign strain, *S. carpocapsae* (All), and the second strain was a newly isolated local strain, *Steinernema* coded as (15), against 3rd larval instar of FAW. In actuality, the newly isolated local strain *Steinernema* coded as (15) was more virulent and effective than *S. carpocapsae* (All). *Steinernema* coded as (15) demonstrated a considerable reduction in the total protein content due to the nematode's presence inside the hemolymph; it also caused significant alterations of enzyme activities like phenoloxidase (PO) and carboxylesterase (CarE). Based on the obtained results, the newly isolated local strain, *Steinernema* coded as (15), should be strongly suggested as promising biocontrol agent against this invasive FAW for environmentally friendly farming.

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