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Pathogenicity Study of the Local and Commercial EPNS Isolates Against the Third Instar Larvae of Cotton Leaf Worm *Spodoptera Littoralis* in Baghdad, Iraq

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Abstract: *Spodoptera littoralis* larvae were collected from tomato fields in al-usefiya region , Baghdad, Iraq. Bioassay was done to test the pathogenicity of EPNs isolates against the third instar larvae of cotton leaf worm using three concentrations 100,150 ,200 ijs/ larvae. Results showed that best concentration was 200 ijs/ larvae . At 200 ijs/larvae higher mortality rates were recorded after 5 days of treatment table for native isolates *Oscheius tipulae* ,*Oscheius myriophilis*, *Heterorhabditis bacteriophora* reached 93.00 , 92. 50 , 93.50 % respectively. While Cezar pesticide overcome on all isolates and recorded the highest mortality rate at 99.50% after 5 days of treatment. *Oscheius tipulae* isolate recorded less LC**50** value reached 82.10 ijs/ larvae, While the commercial isolate *Steinernema carpocapsae* had LC**50** value reached 125.77 ijs/larvae .

Keywords: Entomopathogenic nematodes, pathogenicity, bioassay, *Spodoptera littoralis*, Iraq.

INTRODUCTION :

The cotton leaf worm ,*Spodoptera littoralis* (*Lepidoptera : Noctuidae*) is one of the damaging pests in numerous commercial crops causing significant economic losses on a wide range of crops such as tomatoes ,eggplant, pepper in both greenhouses and open fields (Mohamed et al. 2019). *S.littoralis* is difficult to be controlled due to its resistance to synthetic pesticides (Ghulam et al.2017). Beneficial nematodes attack soil borne insect pests, yet are not harmful to humans, animals, plants and safe to the environment, therefore can be used as biological control organisms. Beneficial nematodes that cause disease within an insect are referred to as entomopathogenic and have the ability to kill a wide range of insect pests including cotton leaf worm, caterpillars, cutworms, beetles. (Denno et.al.2008). The present study aimed to evaluate the efficacy of local EPNs isolates *Oscheius tipulae* ,*Oscheius myriophilus*, *Heterorhabditis bacteriophora* and the commercial isolates *Heterorhabditis bacteriophora* , *Steinernema carpocapsae* against the third instar larvae of the cotton leaf worm and effect of time exposure (1,2,3,4,5 days) on mortality rates under laboratory controlled conditions.

MATERIALS AND METHODS:

Rearing of *Spodoptera littoralis*:

Spodoptera littoralis larvae were collected from tomato fields in al-usefiya region , Baghdad, Iraq, transferred to the laboratory and reared at 20 ± 2 ºC. The larvae were fed on an artificial diet .(Chen et .al 2000), and reared in plastic boxes (12cm width ×18 cm length ×8cm

height). Adults were placed in wood cages (40 cm length × 40 cm width) covered with tissue and were fed on 20% sugar solution.

Bioassays:

Laboratory experiments were conducted to test the pathogenicity of EPNs isolates against the third instar larvae of cotton leafworm. Suspension solution of the EPNs was calculated using the dilution method described by (Haitam، 2010) according to the equation :

$$C1 \times V1 = C2 \times V2$$

C1 = concentration of EPNs in the essential suspension (stock)

V1 = total volume of the essential suspension (stock)

C2 = concentration of EPNs in 10 μl

V2 = volume of the suspension solution taken from the essential suspension (10 μl)

For the native and commercial EPNs isolates three concentrations were prepared 100 ,150, 200 ijs/insect. Plastic petri dishes (9 cm) were used for five treatments (3 petri dish for each treatment including control) . Control petri dishes were added only distilled water. EPNs were inoculated using a pipette in each petri dish . Laboratory trials were conducted with 3 larve of S,littoralis for each petri dish , left under laboratory conditions 25 ºC . Mortality rates were calculated at 5 different times (1,2,3,4 ,5)days after inoculation. Insect cadavers were examined in distilled water under an optical microscope . EPNs juveniles were obtained from each infected S. littoralis larvae.

RESULTS AND DISCUSSION:

Bioassay was done to test the Pathogenicity of EPNs on cotton leaf worm under laboratory conditions, overall results showed that there was an interaction between EPNs isolates and mortality of the third instar larvae of cotton leaf worm at all concentrations 100,150,200 ijs/larvae.

1-**Efficacy of nematode isolate at concentration of 100 ijs/larvae on S.littoralis**:

Table(1) Efficacy of nematode isolate at concentration of 100 ijs/larvae on mortality rates third instar of cotton leaf worm at 5 days of exposure time:

Treatments	Mortality rates of Larvae/ Day					Mean
	1	2	3	4	5	
Oscheius tipulae Native	26.67	30.30	43.33	50.00	60.00	42.06
Oscheius myriophilis Native	26.67	31.00	42.50	51.50	60.67	42.46
Heterorhabditis bacteriophora Native	23.33	30.70	43.33	50.00	57.33	40.93
Steinernema carpocapsae Commercial	23.00	30.50	42.00	50.77	57.50	40.75
Heterorhabditis bacteriophora commercial	23.33	30.00	42.60	50.60	58.00	40.90
Cezar L) 100 / (1g Pesticide	60.67	70.91	73.00	90.33	90.33	77.04
LSD(0.05)	6.73**					2.01**
Mean	30.61	37.23	47.79	57.20	63.97	
LSD (0.05)	2.54**					

At 100 ijs/larvae results at table (1) showed that native isolates Oscheius tipulae ,Oscheius Myriophilis achieved mortality rates reached 60.00 ,60.67% respectively after 5 days of treatment. Chemical pesticide Cezar had higher mortality rate on the third instar larvae of cotton leaf worm at 90.33% after 5 days of treatment at 100 ijs/larvae.. We conclude from table(1) that an economic threshold of cotton leaf worm control was considered reliable and practical for scheduling pesticide intervention (EPPO ،2025).Results showed that pathogenicity of native and commercial isolates increases as the exposure time increases. These results disagree with ali ،(2022)which confirmed that the pathogenicity of the local and commercial S. carpocapseae species on the last stage larvae of cucurbit fruit fly had high mortality rates reached 83.33 and 96.66% respectively at 100 ijs/ larvae.

2-**Efficacy of nematode isolate at concentration of 150 ijs/larvae on S.littoralis**:

Table(2) Efficacy of nematode isolate at concentration of 150 ijs/larvae on mortality rates third instar of cotton leafworm at 5 days of exposure time:

	Mortality rates of Larvae/ Day	

Treatments						Mean
	1	2	3	4	5	
Oscheius tipulae Native	43.33	53.30	70.33	73.00	77.00	63.39
Oscheius myriophilis Native	42.50	52.00	71.00	72.50	76.67	62.92
Heterorhabditis bacteriophora Native	41.80	52.70	70.50	72.80	76.00	62.76
Steinernema carpocapsae Commercial	42.00	53.00	60.00	63.77	66.50	57.05
Heterorhabditis bacteriophora commercial	43.00	51.90	59.50	62.60	67.30	56.86
Cezar L) 100 / (1g Pesticide	54.33	69.66	74.33	91.00	93.00	76.46
LSD(0.05)	7.97**					3.56**
Mean	44.49	55.42	67.61	72.61	76.07	
LSD (0.05)	3.01**					

at 150 ijs/larvae results at table(2) showed that native isolates Oscheius tipulae ,Oscheius Myriophilis , Heterorhabditis bacteriophora had significant higher mortality rates reached 77.00, 76.67 ,76.00 % respectively after 5 days of treatment as compared to the commercial isolates *S.carpocapsae H.bacteriophora* which reached 66.50, 67.30 % respectively. While Cezar pesticide was superior in mortality rate over all native and commercial isolates، as he reached 93.00% after 5 days of treatment.These results agree with Abd elazim(2022) when he examined *Heterorhadbitis*.taysearae against *S.littoralis* using different concentrations,data showed that 150 ijs/larvae *H*.taysearae caused 100% mortality rate.The nematode isolates differ in killing abilities due to the virulence of the symbiotically living bacteria that each species of nematodes carry.(Ahmed and Kim , 2018). besides that, EPNs have high efficacy to enter the insect host by avoiding its immune system (Minas etal، 2016).

3-**Efficacy of nematode isolate at concentration of 200 ijs/larvae on S.littoralis**:

At 200 ijs/larvae higher mortality rates were recorded after 5 days of treatment (table 3) for native isolates Oscheius .tipulae ,Oscheius myriophilis, *Heterorhabditis bacteriophora* reached 93.00 , 92.50 , 93.50 % respectively. while Cezar pesticide overcome on all isolates and recorded the highest mortality rate at 99.50% after 5 days of treatment. These results agree with Yagci etal، (2022) find out that nematode isolates *Steinernema .carpocapsae* (Tokat –Baksili05) *Steinernema .feltiae* (Tokat- Emir) had achieved high mortatlity rates against the third instar larvae of *S.littoralis* reached 100%, 97% respectively at 200 ijs/ml when passing 72 ,96 hours after treatment . One of the facters that causes high mortality rates of insect pests is her large natural body openings and lack of tissue hardening(Rohde etal ،2012).Other studies confirmed that Oscheius native nematode species have high efficacy to kill the insect pest and reproduce in it therefore it can be used as biological control agents for a wide range of insect pests. (Abdisa etal ،2024) , besides its high adaptation in tropical and semi tropical climate (Yuksel and Canhilal 2019).

Table(3) Efficacy of nematode isolate at concentration of 200 ijs/larvae on mortality rates third instar of cotton leafworm at 5 days of exposure time:

Treatments	Mortality rates of Larvae/ Day					Mean
	1	2	3	4	5	
Oscheius tipulae Native	70.00	81.00	86.67	89.00	93.00	83.93
Oscheius myriophilis Native	70.50	80.00	85.00	88.50	92.50	83.30
Heterorhabditis bacteriophora Native	71.80	80.70	86.00	89.80	93.50	84.36
Steinernema carpocapsae Commercial	56.67	61.00	62.00	63.50	65.50	61.73
Heterorhabditis bacteriophora commercial	55.00	60.50	61.80	62.60	66.30	61.24
Cesar L) 100 / (1g Pesticide	77.67	84.00	89.33	95.66	99.50	88.43
LSD(0.05)	5.32**					2.38**

Mean	66.94	75.03	79.30	81.51	85.05	
LSD (0.05)	2.01**					

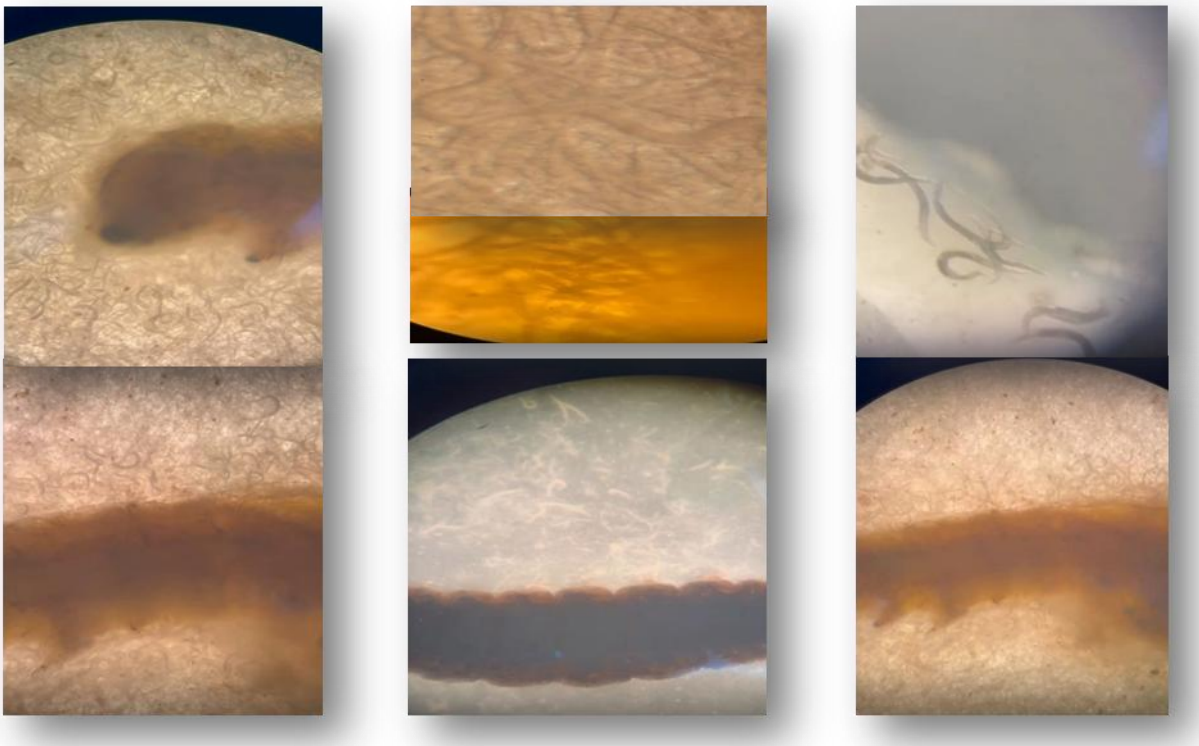
4-**Efficacy of nematode isolates at LC50 (ijs/larvae) on *S.littoralis***:

Results in table (4) showed superiority of *Oscheius tipulae* isolate with less LC**50** value reached 82.10 , followed by LC**50** values for native isolates *Oscheius myriophilis* , *Heterorhabditis bacteriophora* and commercial isolate *Heterorhabditis bacteriophora* reached 88.33 ,102.60, 122.32 ijs/larvae respectively. While the commercial isolate *Steinernema carpocapsae* had LC**50** value reached 125.77 ijs/larvae .

Table (4) LC50 (ijs / insect larvae) for native and commercial EPNs isolates:

Nematode isolates	LC50 Confidence Limits (95%)	*p*	X^2	Slope±SE
Oscheius tipulae Native	82.10 (65.011 - 97.561)	0.35	6.298	0.013 ± 0.002
Oscheius myriophilis Native	88.33 (68.771 - 106.031)	0.18	3.382	0.011±0.002
Heterorhabditis bacteriophora Native	102.60 (85.037 - 120.243)	0.21	3.640	0.011±0.002
Steinernema carpocapsae Commercial	125.77 (102.596 - 153.335)	0.22	3.736	0.008±0.002
Heterorhabditis bacteriophora commercial	122.32 (98.682 – 149.441)	0.29	4.974	0.008±0.002

Figure(1): showing infected *S.littoralis* larvae with entomopathogenic nematodes (ijs) in microscope



CONCLUSIONS:

The native EPNs isolates *Oscheius tipulae, Oscheius myriophilis, Heterorhabditis bacteriophora* had high pathogenicity against the third instar larvae of S.littoralis ,therefore they can be relied on as biological control agents for wide range of insect pests. Besides that, the EPNs isolates are Eco- friendly and have high adaptation under tropical and semi tropical climate. The best concentration for EPNs was 200 ijs/larvae had achieved high mortality rates after 5 days of treatment.

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