Effectiveness of some local and commercial isolates of entomopathogenic nematodes in controlling *Tuta absoluta* larvae and comparing them with the pesticide Oxymatrine in the laboratory

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Abstract

A laboratory study was conducted to evaluate the effectiveness of local isolates *Oscheius onirici*, *Oscheius myriophilus*, commercial isolates *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* of entomopathogenic nematodes (EPNs) to compare them with the plant pesticide Oxymatrine in controlling the tomato leaf miner *Tuta absoluta* on the tomato cultivar Ala in the laboratory. The results showed that the local biological treatment *Oscheius* spp gave the highest mortality rate for the third-stage larvae at concentrations of 100, 300, and 500 Ijs/ml and a cumulative killing rate of 100% during 5 days, respectively, followed by the commercial isolate *H. bacteriophora*, which had a mortality rate of 100%. There were no significant differences between the three isolates; the concentration of 500 Ijs was the highest cumulative killing rate for the biological treatments. At the same time, there was a significant difference between the commercial isolate *S. carpocapsae* and the other isolates, as the larval mortality rate reached 40% compared to the chemical pesticide.The mortality rate of the third-stage larvae *T. absoluta* at concentrations of 1.8, 1.5, and 1 ml during 5 days reached 40%, 36%, and 30%, respectively, as the recommended concentration of 1.8 gave the highest cumulative killing rate of 40% and with a highly significant difference between the three isolates. Still, there was no significant difference between the commercial isolates. The chemical pesticide *S. carpocapsae* reached a 40% mortality rate over 5 consecutive days.

Keywords: *Steinernema carpocapsae*, *Heterorhabditis bacteriophora*, *Oscheius onirici*, *Oscheius myriophilus*, *Tuta absoluta*.

Introduction

Tomato *Solnum lycopersicum* is affected by several key pests, including the tomato fruitworm *Helicoverpa armigera* (Hubner), the vegetable leaf miner *Liriomyza trifolii* (Burgess), the cotton whitefly *Bemisia tabaci* (Gennadius) and the tomato moth *Tuta absoluta*.[1,2], Peru in South America is the original home of the tomato moth *T. absoluta*, and it was later recorded in most of its countries [3]. and entered Iraq in 2010. [4], This insect is the limiting factor in tomato production, as it causes significant economic losses in production because its larvae feed on all parts of the plant, leaves, stems

and fruits, as they make irregular tunnels and passages between the two skins of the leaf, and feed on the spongy layer of the leaf, leaving empty cavities and tunnels covered by the outer skin of the leaves, which later turn into dry spots. The larvae leave their waste at the end of the tunnels. In the case of severe infection, the larva feeds on the entire leaf, and the larva, from the second to the fourth age, spins silky hiding places in the leaves or intertwines the leaves with each other [5, 10].

Losses in tomato production range between 80-100% in open fields and greenhouses if preventive measures are not taken. Controlling this insect is very difficult because its larvae feed inside tunnels that protect them from the effects of pesticides and other conditions. [7], This leads to increased production costs and reduced economic returns in addition to the damage caused by the use of pesticides to the consumer, the environment and biodiversity, and the risk of the emergence of insect strains resistant to pesticides [26, 27].

Biological control of the insect plays an important role in controlling this pest because it is environmentally safe [8], The use of some types of specialized nematodes as pathogens against insects is considered one of the most important of these biological means [17].

Entomopathogenic nematodes are nematodes belonging to the Steinemernatidae and Heterorhabditidae families that have a great ability to destroy a wide range of insect pests that belong to many orders and families, which makes them one of the most important means of biological control. They are characterized by many properties that make them an effective biological control agent due to their close association with symbiotic intestinal bacteria (Enterobacteriaceae, such as the association of *Xenorhabdus* and *Photorhabdus* with the genera *Steinernema* and *Heterohabditis*, respectively [31], and they have several characteristics that facilitate their use due to their wide host range and their symbiotic coexistence with bacteria, which increases their ability to eliminate the host within 48 hours, in addition to the ease of their production and live breeding inside the insect body in vivo using the larvae of the large wax worm *Gelleria mellonella*. [15].

It can also be cultured in vitro in a special medium. [18], Insect-pathogenic nematodes are characterized by remaining active and affecting the host organism for long periods ranging from weeks to months [21]. This study aims to test the effectiveness of some local Iraqi and commercial isolates of insect-pathogenic nematodes and compare them with the pesticide Oxymatrine in controlling a third larval stage of the tomato leaf miner *T. absoluta* in the laboratory in Karbala Governorate.

Materials and Methods

Field survey

The presence of nematodes that are pathogenic to insects was investigated in 30 agricultural soil samples collected through a field survey in some governorates of Iraq during 2023 by following the method of baiting the larvae of the fourth stage of the greater wax worm *G. mellonella* [6]. After (2-5) days, the larvae infected with

nematodes were detected, as the infected larvae were placed in a White trap to extract the third stage Infective Juveniles (Ijs) [33].

The pathogenicity of the EPNs was tested for the five local isolates, including Baghdad Governorate (Al-Rashidiya, Al-Yusufiya, Al-Tajji), Al-Diwaniya (Ghammas area), and Babylon (Hamza Al-Gharbi). The Al-Yusufiya and Al-Diwaniya isolates were the most aggressive and effective in killing the larvae of the greater wax worm within 72 hours. Therefore, these two local isolates belonging to the genus *Oscheius* spp were used to obtain commercial isolates of the nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*, belonging to the Hort Americas Company in controlling the third larval stage of the tomato leaf miner *T. absoluta* in the laboratory.[20, 3, 14, 30], Local isolates of entomopathogenic nematodes were identified to the genus level using the taxonomic key described by Nguyen and Smart [25], and morphometric measurements were taken to classify twenty-third infective instar individuals and twenty adult males and females and the species was distinguished according to [32].

Preparation of Nematode Suspension

EPNs suspension was prepared for the two commercial species *H. bacteriophora* and *S. carpocapsae,* and two local isolates *Oscheius* spp. They were morphologically and molecularly characterized and multiplied in vivo using the last instar larvae of the wax worm *G. mellonella* Lepidoptera: Pyralidae

The greater wax worm was used because of its susceptibility to EPNs. It is a rich source of nutrients available in vivo and has high multiplication potential. It is easily reared on a semi-artificial diet containing 810 g of bulgur, 120 ml of glycerin, 60 g of honey or molasses, and 10 g of yeast. [12, 4]. 10 larvae of *G. mellonella* were placed in a 9 cm diameter Petri dish containing filter paper, and then 100 (Ijs) of the infective stages of the nematode were added. The dishes were kept in the dark at 25 ± 2 °C for 48-72 hours, after which the infected larvae were transferred to a White trap consisting of a 9 cm diameter Petri dish in the middle of an inverted watch glass, on which filter paper was placed, and on top of it the infected larvae emerged from the larval corpse Infective Juveniles after (9-13 days). The suspension was kept in sterile distilled water separately in 50 ml plastic containers at a low temperature of 10-15 °C for two weeks before being used in biological tests.

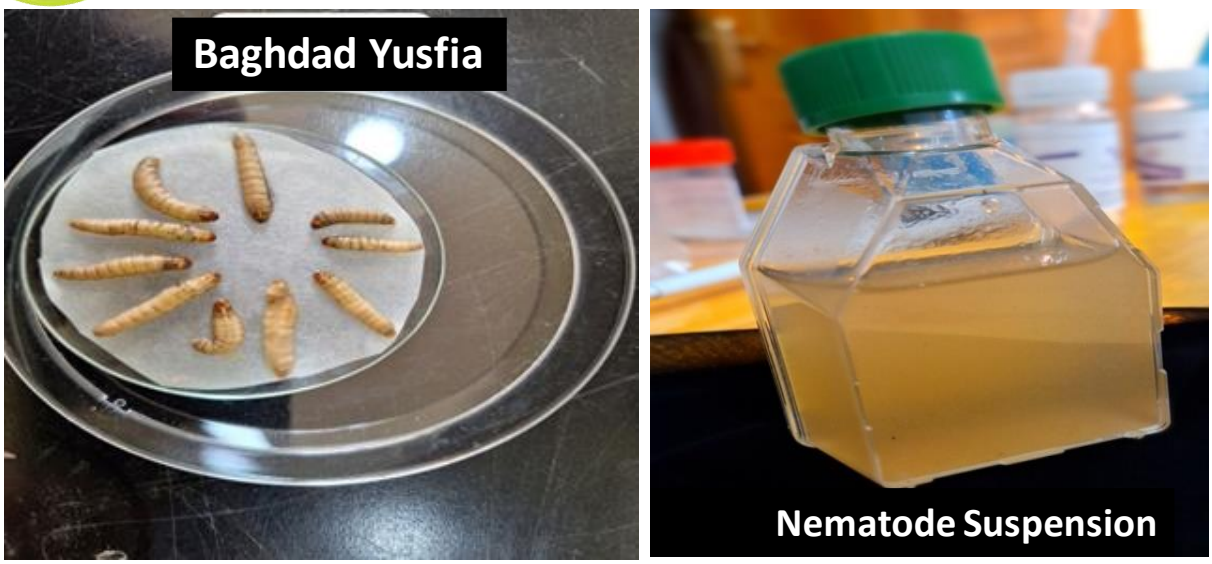

Figure (1): Method of propagating pathogenic nematodes to insects using a White water trap and storing the suspension in a glass beaker [29].

Preparation of concentrations of the pesticide Oxymatrine

The pesticide was obtained from the Department of Crop Protection in the Hur area, Karbala province. Three concentrations of Oxymatrine, 1.8, 1.5 and 1 ml/liter of water, were used for treatments.

Laboratory rearing of tomato leaf miner

Leaves infected with different larval stages were obtained from a plastic house in the Al-Suwaira area, affiliated with Al-Kut Governorate, during the period extending from April to May of 2024. These larval stages were reared on tomato plants at the seedling age, which was previously planted in anvils and placed in wooden rearing cages (50×50×50 cm) covered with soft, transparent, woven cloth in the rearing room at a temperature of 25± degrees Celsius and a relative humidity of % 65± and a lighting period of 16 hours light: 8 hours darkness. The insect was propagated in the laboratory to obtain third-stage larvae to control it [5].

Biological test

This test was conducted for two types of commercial isolates *H. bacteriophora* and *S. carpocapsae*, and two types of local isolates *Oscheius* spp and the pesticide Oxymatrine, according to the recommended concentrations. Petri dishes were prepared with filter paper with a diameter of 9 cm, then a healthy tomato leaf was washed with distilled water, and moistened cotton was placed around the leaf blade to maintain the moisture of the leaf and feed the larvae. Then 10 third-stage larvae of the *T. absoluta* insect were placed in a dish for each treatment. 3 replicates were repeated, and the local and commercial nematode suspension was sprayed separately, which was prepared 100 Ijs 1 ml/liter of water and 300 Ijs and 500 Ijs using a 5 ml hand sprayer.

Concentrations of 1.8, 1.5 and 1 ml/liter of water of the chemical pesticide Oxymatrine were used. *Tuta absoluta* larvae were treated using a 1-liter hand sprayer with 3 replicates for each treatment in a dish. The dishes were tightly covered with adhesive

tape to prevent the larvae from escaping from the dish and were pierced with small holes to ventilate the larvae. The experiment was set up in a completely randomized design with three replicates for the treatment and three for the control factor, which only had water except for Ijs and the pesticide. The percentage of larval mortality was estimated within 24 hours, 48 hours, 72 hours and 5 days, respectively, from the beginning of the treatment according to the equation [2]. The dead larvae were dissected under a magnifying dissecting microscope using forceps and needles by destroying the bodies in Petri dishes to confirm the presence and infection with EPNs. If the insects infected with the genus *Steinernema* sp have light brown *T. absoluta* larvae, the larvae infected with the genus *Heterorhabditis* sp. have blackish-brown or crimson-red larvae [23].

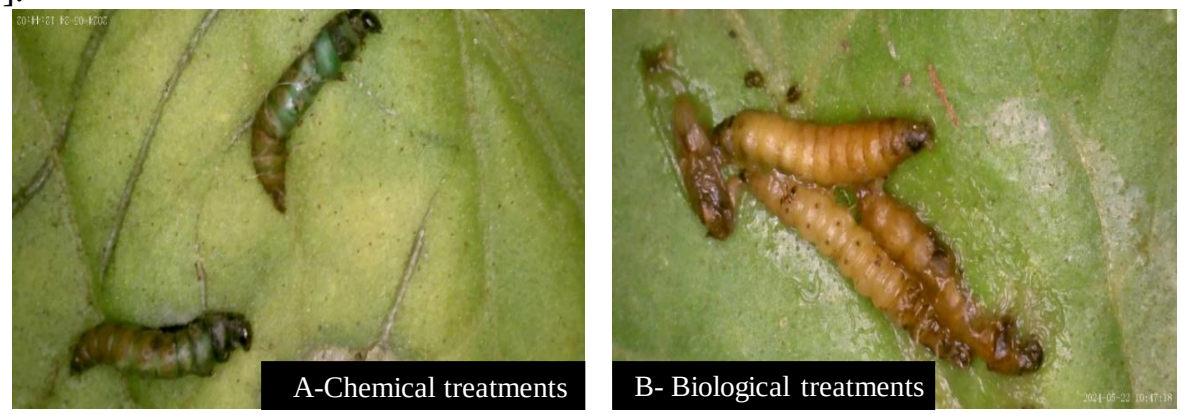

Figure (2): The effect of biological and chemical treatments on the death of third-stage larvae of *Tuta absoluta.*

Statistical analysis

The experiments were designed according to factorial experiments in a Design (C.R.D) using the Statistical Analysis System (SAS) . The significance of the differences between the treatment rates was compared using the Difference (L.S.D) test at a significance level of 0.05 [29] .

Results and Discussion

Effect of local and commercial isolates on the mortality of *T. absoluta* larvae

The results showed (Table 1) testing local isolates and global isolates in the death of third-stage larvae of *T. absoluta* insect at concentrations of 100, 300 and 500 Ijs/m, as the highest mortality rate was for the local isolate 2 of Diwaniyah Governorate *Oscheius* spp and the local isolate of Yusfia Baghdad at 100% within 5 days, followed by the commercial isolate 2 *H. bacteriophora* at 100%. There were no significant differences between the three isolates, while there was a significant considerable difference between the commercial isolate *S. carpocapsae* and the other isolates, as the larval death rate reached 40% compared to the control factor, as the treatment death rate reached 0%.

The effect of the chemical pesticide on the death of tomato leaf miner larvae

The results of (Table 2) showed the effect of the chemical pesticide on the death rate of third-stage larvae *T. absoluta* at concentrations of 1.8, 1.5, 1 ml within 5 days, as the death rate reached 40%, 36%, and 30%, respectively,, as the recommended concentration of 1.8 gave the highest cumulative death rate of 40% with a highly significant difference compared to the control coefficient if it reached 0%.

The effect of biological treatments and their comparison with the chemical factor in the death of tomato leaf miner larvae

It is noted in (Table 3) when comparing the effect of local and commercial isolates compared to the chemical pesticide at the highest concentrations of the four isolates 500 Ijs and the recommended concentration of the pesticide 1.8 ml, the results showed that the local isolates *Oscheius* spp. were significantly superior to the chemical pesticide, as the killing rate reached 40% and the three isolates 100%. There was no significant difference between the commercial isolate *S. carpocapsae* and the chemical pesticide, as the cumulative death rate over 5 consecutive days reached 40%.

Table (1): The effect of biological treatments on the death rate of the third-stage larvae of *T. absoluta* insect after several days of treatment under laboratory conditions.

Treatment	Con.	Mortality / Days				
		1	2	3	5	Average
onirici Oscheius **Baghdad Yusfia 1**	100Ijs	23.33	30.00	46.66	100	50.00
	300 Ijs	20.00	30.00	50.00	100	
	500 Ijs	20.00	23.33	56.66	100	
S. Carpocpsae **Commerciale 1**	100 Ijs	16.66	23.33	30.00	30.00	25.55
	300 Ijs	16.66	20.00	26.66	43.33	
	500 Ijs	16.66	20.00	23.33	40.00	
Oscheius myriophilus **Diwaniyah 2**	100 Ijs	16.66	33.33	50.00	100	50.27
	300 Ijs	20.00	23.33	56.66	100	
	500 Ijs	13.33	30.00	60.00	100	
H. bacteriophora **Commerciale 2**	100 Ijs	26.66	30.00	43.33	100	50.00
	300 Ijs	23.33	30.00	46.66	100	
	500 Ijs	16.66	33.33	50.00	100	
Control		0.00	0.00	0.00	0.00	0.00
Average of time		15.33	21.77	36.00	67.55	
Average of concentrations	100	300		500		
	35.00	35.33		35.16		
L.S.D. 0.05	**Treatment**	**Concentrations**		**Time**		**Interaction**
	3.5812	2.774		3.2031		12.406

Table (2): The percentage of effectiveness of the chemical pesticide Oxymatrine against third-stage larvae of the tomato leaf miner *Tuta absoluta* under laboratory conditions.

Treatment	Con.	Mortality / Days				
		1	2	3	5	Average
Oxymatrine	1.8 m/L	13.33	23.33	23.33	40.00	25.00
	1.5 m/L	16.66	20.00	26.66	36.66	25.00
	1 m/L	20.00	23.33	26.66	30.00	25.00
	Control	0.00	0.00	0.00	0.00	0.00
Average of time		12.50	16.66	19.16	26.66	
L.S.D. 0.05	Treatment			Time		Interaction
	3.5812			6.7898		13.58

Table (3): Effect of the highest concentrations of biological treatments and their comparison with the chemical pesticide on the mortality rate of third-stage larvae of *Tuta absoluta* after several days of treatment under laboratory conditions.

Treatment	Con.	Mortality / Days				
		1	2	3	5	Average
***onirici Oscheius* Baghdad Yusfia 1**	500 Ijs/ml	20.00	23.33	56.66	100.00	49.99
***S. Carpocpsae* Commerciale 1**	500 Ijs/ml	16.66	20.00	23.33	40.00	24.99
***Oscheius myriophilus* Diwaniyah 2**	500 Ijs/ml	13.33	30.00	60.00	100.00	50.83
***H. bacteriophora* Commerciale 2**	500 Ijs/ml	16.66	33.33	50.00	100.00	49.99
Oxymatrine	1.8 m/L	13.33	23.33	23.33	40.00	25.00
Control		0.00	0.00	0.00	0.00	0.00
Average of time		13.33	21.66	35.55	63.33	
L.S.D. 0.05		Concentrations		Time		Interaction
		1.5869		2.5578		4.1447

In this study, it is evident that *H. bacteriophora*, *S. carpocpsae* and *Oscheius* spp. Effective against *T. absoluta* larvae, which reached mortality at 100, 300 and 500 Ijs/ml concentrations. The mortality rate of *T. absoluta* larvae increased with increasing concentrations. In this case, the highest concentration tested (500 Ijs/ml) achieved the highest mortality rate under laboratory conditions. This increase in mortality with increasing concentration can be attributed to the many symbiotic bacteria released by EPNs when they penetrate the larvae, [13]. Steinernematida Ijs retains *Xenorhabdus* symbionts within its intestinal sac. At the same time, *Photorhabdus* cells stick together in the anterior part of the intestine of *Heterorhabditids* and release them upon invasion of an insect host [11]. Once the nematodes enter the host, they release bacteria that kill the larvae by producing toxins and hydrolytic enzymes, digesting the internal contents of the host and thus providing food for the EPNs [19]. This is consistent with the study

by [24], who reported that increasing the dose of EPNs usually increases larval mortality in managing banana weevil *Cosmopolites sordidus*. Exposure periods of 24, 48, 72 hours and 5 days affected *T. absoluta* larval mortality. The highest larval mortality was recorded after 72 h of exposure when the highest concentration of infective events was used (500 Ijs/ml).

It is likely that the longer the time and the greater the amount of Ijs available, the more EPNs could search for and infect the larvae and subsequently release the bacteria that caused the toxicity that led to death.

Previous research conducted worldwide using EPNs to control the effectiveness of tomato leaf miners [8], demonstrated the high efficiency of EPNs to penetrate the tunnel and kill the larvae of tomato leaf miners inside the tunnels, with a percentage mortality2 of larvae ranging between 76.3 and 92% at a rate of 60 infective stages/cm2. In another study, the mortality rate of tomato leaf miner larvae inside tunnels at temperatures of 10, 15, 20 and 25 °C ranged from 60-100% in laboratory tests using the nematode *Heterorhabditis bacteriophora*, while the mortality rate of tomato leaf miner pupae was relatively low 16.6-20.83% [10]. In a test conducted in Egypt [34]. the mortality rate of the fourth instar larvae of the tomato leaf miner reached 93.3% in the laboratory after 3 days of treatment with a dose of 1000 infective stages 1 ml of the nematode *S. carpocapsae* in Turkey, the results indicated the effectiveness of some local isolates of some types of nematodes that are pathogenic to insects [16]. in controlling the tomato leaf miner.

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