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Evaluate The Pathogenicity Of Indigenous Entomopathogenic Nematodes Against Tomato Leaf Miner Tuta Absoluta In Wasit Province, Iraq

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Abstract

The study was conducted after collecting the pathogenic nematodes (EPNs) soil samples from Wasit Province, Iraq, using the white trap method using *Galleria mellonella* larvae, then identification as local nematodes (*Heterorhabditis indica, H. megidis*) comparing with commercial nematode *H. bacteriophora*. The commercial and native EPNs were used as biological control agents targeting *Tuta absoluta* larvae and pupae during the tomato growing season in the Suwayra area in Wasit province. A laboratory experiment used one microliter of nematode suspension containing *Heterorhabditis indica* or *H. migidis* and *H. bacteriophora* per larva. All three species of EPNs, both native and commercial, impacted *Tuta absoluta* larvae and resulted in varying mortality rates. The longer exposure time and higher concentration both amplified the effect. After seven days of treatment, the larvae in the current study experienced high mortalities. Among the species utilized in the study, *H. bacteriophora* was the most effective, with the most significant average mortality rate of 94.4% at a 200 Ijs/ml dosage. At the same concentration, *H. megidis* came in second with 90.7%. Additionally, after seven days of mortality, the species *H. bacteriophora*recorded the most significant average mortality rate, at 80.6%, followed by the species *H. indica*, at 77.8%, while the average EPNS species with time in the seven days that showed in *H.bacteriophoria* at 80.6% most effective than the *H.indica* at 77.8% and *H.megidis* at 73.6%, This is mainly significant against the larvae of *T. absoluta*. The examined pupae exhibited sensitivity to infection by all evaluated EPNs, with the highest mortality rate seen at the maximum dose (200 IJs/ml) after 7 days of exposure. which was 100%. The mortality of tested pupae was increased with the increase of EPN concentration and period of exposure in all treatments. The highest average of pupal mortality was recorded at the concentration of (200 Ijs /ml) in the instance of *H. megidis,* which was 94.44%, and the lowest average of pupal mortality was recorded at the concentration of (50 Ijs/ml) in the particular situation of *H. indica,* which was 24.11%. The result also revealed that *H. megidis* was more effective than the other species, recording the highest average of pupal mortality (66.86%), followed by *H. bacteriophora* (58.89%) and the lowest by *H. indica* (54.01%).In the field condition that all entomopathogenic nematodes affect the larvae of *T. absoluta* and the highest percentage of larval fatality was 83.33% in the case of *H. bacteriophora* at the concentration (200 ijs/ml) and the lowest one in the case of *H. migidis* at the concentration (150 ijs/ml). The mortality of larvae was proportional with the concentration, while the p-value in concentration (150 ijs/ml) was 0.720 and in concentration (200 ijs/ml) was 0.609 >0.05. These results were significant, and the L.S.D resulted in 1.762 in concentration (150 ijs/ml) and 1.489 in concentration (200 ijs/ml>0.005); also the findings indicated that the pupae of the leaf miner *Tuta absoluta* exhibited vulnerability to all three examined nematode species. The highest percentage of pupal mortality was 66.67% in the case of *H. bacteriophoraand H. Indica* at a concentration of (200 ijs/ml) and the lowest one in the case of *H. migidis*at the concentration of (150 ijs/ml), which was 33.33%. The mortality of pupae was proportional with the nematode concentration, and the P value in (150 ijs/ml) was 0.903 and in (200 ijs/ml) was 1.186.L.S.D in (150 ijs/ml) was 2.209 and in (200 ijs/ml) was 2.903 this resulted was accepted that showed the affected of entomopathogenic species in the field condition. Our research showed that the pathogenic nematodes of three species (*Heterorhabditis*. ssp.) are effective against the *T. absoluta* stages of life. This biological control is the best and safest way for humans, animals, and plants to eliminate insect pests without collateral damage.

Keywords: *Heterorhabditis indica, Heterorhabditis megidis, Heterorhabditis bacteriophora*, Entomopathogenic nematodes (ENPs), *Tuta absoluta*, biocontrol

Introduction

The most dangerous insect pest to tomatoes is the South American tomato pinworm, *Tuta absoluta* (Meyrick) (Biondi *et al*., 2018; Desneux *et al*., 2010; Mansour *et al.,* 2018; Urbaneja *et al*., 2013). It was swiftly disseminated across Europe, Africa, the Middle East, and Asia, where it promptly attained detrimental levels and evolved into a notable pest of tomatoes cultivated in greenhouses and fields (Han *et al*., 2019; Mansour *et al*., 2018). Abdul Razzak *et al*., (2010) reported that the tomato borer *T. absoluta* was first identified in Iraq in the autumn of 2010 by deploying sex pheromone traps in the Rabia area of Nineveh province. Since then, the insect has rapidly expanded throughout all tomato-growing regions, devastating whole open and sheltered fields. According to Ramirez *et al*., (2010), *T. absoluta* can cause up to 100% damage to crops that are not protected, and it is regarded as a major pest of tomato insects in the region where it is found (Leite *et al,*.2001), The swift assault and its prevalence during the crop maturation phase (Oliveira *et. al.* 2008).The larvae create irregular tunnels and passage ways between the leaf's epidermis in order to feed on all of the plant's aerial components, including the leaves, stems, branches, and fruits. As it consumes the mycelium, it leaves behind tunnels and empty spaces that are hidden by the leaf's outer layer of epidermis (Desneux *et al.,* 2010). The larvae reside within tunnels that shield them from the effects of insecticides, controlling them is quite challenging. In tomato-producing regions worldwide, chemical pesticides are sprayed on this insect at a rate of 8–25 sprays per season. (Retta & Berhe,2015).In addition to the negative impacts of pesticide use on consumers, the environment, and biodiversity, this results in higher production costs and lower economic returns. It also increases the possibility of pesticide-resistant insect strains emerging (Roditakis *et al.,*2015). Previous global studies conducted to assess effectiveness of tomato leaf miners have demonstrated the remarkable capacity of entomopathogenic nematodes to infiltrate tunnels and eliminate tomato leaf miners or their burrowing larvae therein. The percentage of dead larvae varied from 76.3 to 92%, with a(60 infective stage/cm2) rate. (Batalla-Carrera *et al*., 2010). There has been an increasing interest in biological alternative management options for *T. absoluta*, including biological control via the introduction of parasitoids and predators, the application of biopesticides, and the potential utilization of EPNs to reduce reliance on chemical insecticides. (Mansour & Biondi, 2021). Significant biological control methods for various insect pests comprise EPNs. (Grewal *et al*., 2005; Lacey & Georgis, 2012). According to the Heterorhabditidae and Steinernematidae, the members of both families are linked to mutualistic bacteria belonging to the genera *Xenorhabdus* and *Photorhabdus* (Poinar, 1990). By detecting temperature gradients, carbon dioxide levels, movement of the host, and insect excretory secretions, IJs are able to locate their host. IJs then enter the host by spiracles, anus, or natural apertures; moreover, *Heterorhabditis* IJs have teeth that allow them to pierce some insects' cuticles and enter the host. They discharge bacteria into the hemocoel once they've entered, and those bacteria grow and cause septicemia, which kills the host. (Georgis,1992). Using ENPs in integrated pest management programs has a lot of potential. They have been shown to be safer and more effective substitutes for chemical pesticides since they are more targeted. The selectivity and application rates of EPN species affect insect pest susceptibility in different ways. Important variables influencing the activity of EPNs include temperature, moisture, aeration, and soil type. Other variables include the species of EPN, the age of the target insects, and soil fauna. Accordingly(Platt *et al*., 2020) .

The aim of the study

This investigation aimed to ascertain the pathogenicity of EPNS in laboratory and field conditions (*Heterorhabditis indica*, *H. migidis*, and commercial nematode, *H. bacteriophora*) in order to control the larvae and pupa stages of *T. absoluta* using various concentrations of entomopathogenic nematodes.

Material and Methods

Cultivation of *Galleria mellonella* larvae

The larvae of *Galleria mellonella* were maintained on an artificial diet that included 222 g of dry baker's yeast, 890 g of flour, 500 g of glycerin, 500 g of honey, 445 g of milk powder, and 125 g of bee wax. The incubator had 16/8 hours of lighting that was adjusted to 24–25 °C (Mohamed & Coppel 1983). Additionally, bee wax was only utilized to raise and feed greater wax moth larvae.

Sampling soil, capturing it, and keeping EPNs maintained

Several soil samples were taken from various parts of Wasit Province, Iraq (north, south, west, and east) in order to assess the presence of EPNs in Iraqi soil. About 100 samples were collected from these areas, and then they confirmed the presence of entomopathogenic nematodes in 60 areas, meaning the presence of nematodes in three. Areas within the province showed two types of native nematodes (*Heterorhabditis indica, Heterorhabditis megidis* (IRQ.2 (PP869236) Iraq/Wasit), which was found to be the first isolate and diagnosis of *Heterorhabditis*. Sp in Iraq (Nawar,*et al*.,2024) with commercial nematodes (*Heterorhabditis bacteriophora*) this nematode will take from Agricultural Research Directorate \ Ministry of Science and Technology, Baghdad. Which showed the virulence of insect-pathogenic nematodes against *Galleria larvae*. In order to increase the production of the infective stage (IJS) and obtain large numbers of it, waxworm larvae were used for the fourth and fifth stages by using the bioassay method. (Orozco, R, *et al* ,2014).

Obtaining the *Tuta absoluta* from glasshouses that grow tomatoes

T. absoluta was collected from greenhouses that grew tomato plants in Wasit province's Al-Suwayra District, located north of the province. Monitoring was done for *T. absoluta* larvae from the beginning of the tomato planting season. The infestation on the plant leaves was discovered by looking at the tunnels the larvae had created. The infection site was monitored, and the larvae and pupae were collected. In addition, infected tomato leaves were collected, and the field and laboratory experiments were carried out as soon as the larvae and pupae were removed from the infected leaf.

Bioassay

Filter paper bioassay

The three replications were employed in a randomized design for the filter paper experiment. Five to ten larvae are placed in Petri dishes for each replicate. The larvae utilized pupae and were in their third or fourth instar. The larvae were arranged in 9-cm-diameter Petri plates with two sheets of filter paper in between. Then, one milliliter of the nematode suspension containing *Heterorhabditis indica, H. migidis*, and the commercial nematode *H. bacteriophora* was added. The concentrations of the nematode suspension were 0, 50, 100, 150, and 200 IJs (infective juveniles) per microliter, total per microliter. The control treatment was equivalent to one microliter of distilled water, calculated using the equation (c1v1=c2v2) Where C1 is the concentration of IJS in (50)milliliters of nematode suspension, V1 is the volume of tissue flask in(50) milliliters, C2 is the concentration of IJS in 10 microliters, and V2 is the volume of nematode suspension that is withdrawn to calculate the different concentrations, which are (50, 100, 150, & 200 ijs). Note: The equivalent unit of measurement above is larvae/ijs.

The Petri plates were maintained in an incubator at 70% relative humidity, a 12-hour photoperiod, and a temperature of 25 °C. The larvae were assessed three days post-application, and the deceased specimens were examined for indicators of *Heterorhabditis* sp., including a brown hue and decaying insect odor. Following the enumeration of developing pupae, they were maintained in the replications until adulthood (control concentration). Furthermore, the perished larvae were dissected, revealing the presence of the IJS within them.(Rohde *et al*., 2020; Kaya & Stock, 1997).

Soil experiment

Twenty grams of sterilized sandy soil were put in a nine-centimeter-diameter container for the field experiment. To guarantee that the larvae stay in the soil, that the infectious juveniles (IJs) reach the larvae, and that the soil moisture level is approximately 10% using distilled water, ten third and fourth instar larvae were buried 2-3 mm deep in the soil. After that, the larvae were inoculated with a suspension of nematodes (*Heterorhabditis indica*, *H. migidis*, and *Heterorhabditis bacteriophora*) at concentrations of (0 control), 150, and 200 IJs (infectious juveniles/ml). In every concentration, three duplicates were made and buried in the soil. Every four days, the bodies of deceased larvae are observed, and the bodies of the For 10 days, the dead larvae are taken out of the soil. The dead larvae are then put in a Petri dish for each replicate, and a microscope is used to examine the specimens. The exit of the IJs from the dead larvae and pupae is observed, along with a change in the color of the larvae and the presence of a characteristic nematode odor (Sami *et al*., 2023).

Statistical analysis

Every data analysis involved the application of Abbott's formula (Abbott 1925) to account for larval mortality, along with a one-way analysis of variance (ANOVA). The data on the percentage of *Tuta absoluta* (larvae and pupae) still resistant to nematodes were analyzed using the Chi-square test of independence, general analysis of variance, and the statistical program Genstat (fourteenth edition). This data was analyzed by VSN International Ltd. We employed the complete randomized design (CRD) in the lab and the complete randomized block design (CRBD) in the field.

Rustles & Discussion

According to the study's findings, which are shown in Table (1), all three species of EPNs, both native and commercial, impacted *Tuta absoluta* larvae and resulted in varying mortality rates. The longer exposure time and higher concentration both amplified the effect. After seven days of treatment, the larvae in the current study experienced high mortalities. Among the species utilized in the study, *H. bacteriophora* was the most effective, with the most significant average mortality rate of 94.4% at a (200 Ijs/ml) dosage. At the same concentration, *H. megidis* came in second with 90.7%. Additionally, after seven days of mortality, the species *H. bacteriophora* recorded the most significant average mortality rate, at 80.6%, followed by the species *H. indica*, at 77.8%, while the average EPNS species with time in the seven days that showed in *H. bacteriophoria* at 80.6% most effective than the *H.indica* at 77.8% and *H.megidis* at 73.6% this results most significant against larvae of *T.absoluta*. The L.S.D of the Species of entomopathogenic nematodes with time is 46.96 >0.005 and the L.S.D of Species and concentration is 45.71>0.005 this result accepted against larvae of *T.absoluta.*

Table (1) The effect of native nematodes on larvae of *Tuta absoluta* in laboratory conditions.

Nematodes species	Time (day)	Concentration % (Ijs /ml)				Average EPNs with time (%)	Average of EPNs species (%)
		50	100	150	200		
H. bacteriophora*	3	22.2	44.4	55.6	83.3	51.4	69
	5	38.9	66.7	94.4	100	75	
	7	38.9	83.3	100	100	80.6	
Average EPNs with conc.		**33.3**	**64.8**	**83.3**	**94.4**	----------	
H. indica **	3	0.0	11.1	38.9	61.1	27.8	54.3
	5	16.7	44.4	72.2	94.4	56.9	
	7	44.4	72.2	94.4	100	77.8	
Average EPN with conc.		**20.4**	**42.6**	**68.5**	**85.2**	----------	

H. megidis*	3	0	11.1	61.1	77.8	37.5	56
	5	22.2	38.9	72.2	94.4	56/9	
	7	38.9	66.7	88.9	100	73.6	
Average EPNs with Conc**.**		**20.4**	**38.9**	**74.1**	**90.7**	----------	
Average of time	3	7.4	22.2	51.9	74.1	----------	
	5	25.9	50	79.6	96.3	----------	
	7	40.7	74.1	94.4	100	----------	
L.S.D	EPNs Species	**39.91**		Species and time		**46.96**	
	Time	**46.96**		Species and conc.		**45.71**	
	concentrations	**17.13**		Time and conc.		**47.01**	

***L.S.D Least significant differences of means (5% level) >0.005**

Thus, the study's findings demonstrated that *T. absoluta* larvae were vulnerable to every EPN isolates that the effect varied depending on the EPN species, concentration, and time.

Our findings align with those of Fatimah *et al*., (2023), who also observed that the concentration of nematodes and the time of exposure amplified the impact of nematode species on the larval stages of tomato leaf miner. Batalla Carrera *et al*., (2010) investigated the impact of three species of EPNs from the genus *Heterorhabditis* on the life stages of *T. absoluta* in laboratory settings, revealing mortality rates between 78.6% and 100%.The possibility of utilizing entomopathogens against *T. absoluta* was assessed in several studies that were previously acquired in laboratory settings (Ndereyimana *et al*., 2020; Van Damme, 2016). Consistent with our findings, El Aimani *et al*., (2021) conducted a laboratory investigation assessing the virulence and pathogenicity of five distinct local nematode strains, revealing that the *H. bacteriophora* strain (HB-MOR8) induced the highest mortality rate (80–100%) in *T. absoluta* larvae.

In line with the findings of Mutegi *et al*. (2017), who found that the mortality of *Tuta absoluta* larvae by *Heterorhabditis* and *Steinernema* increased with the concentration of EPNs, our study showed that the mortality of larvae increased with the concentration of nematodes .Ben Husin & Port (2021) reported that *Heterorhabditis bacteriophora*, *Steinernema feltiae,* and *Steinernema carpocapsae* were all efficient against *T. absoluta*. The most effective species was *Heterorhabditis bacteriophora*, followed by *Steinernema carpocapsae*. In an in vitro study, El Roby *et al*., (2023) utilized three local nematode isolates *(H. bacteriophora* (EKB20), *Steinernema* sp. (B32), and *Heterorhabditis* sp. (Kasassien isolate)), demonstrating efficacy against larvae with death rates ranging from 70.6% to 94%.It is observed that prolonged exposure time correlates with an elevated mortality rate of *T. absoluta*, as does an increase in EPN concentration. This aligns with Saleh's findings (2023). The peak mortality rate (100%) was achieved with a single entomopathogenic nematode (*S. carpocapsae* E-76) at the greatest application concentration. Others are Ali *et al*., (2023), Husin & Port (2021), and Diego *et al*.,(2023). The application of entomopathogenic nematodes has demonstrated a reduction in pesticide usage, hence fostering sustainable pest control.

The effectiveness against pupae of *T. absoluta* in laboratory conditions

The results of this study presented in Table (3.5) indicated that the tested pupae were susceptible to infection by all tested EPNs, and the highest mortality percentage was recorded at the highest concentration (200 Ijs /ml) after 7 days of exposure, which was 100%. The mortality of tested pupae was increased with the increase of EPN concentration and period of exposure in all treatments. At a concentration of (200 Ijs/ml), *H. megidis* exhibited a mortality rate of 94.44%. In contrast, at a (50 ijs/ml) concentration, *H. indica* demonstrated a mortality rate of 24.11% for *T. absoluta* pupae.The result also revealed that *H. megidis* was more effective than the other species, recording the highest average of pupal mortality (66.86%), followed by *H. bacteriophora* (58.89%) and the lowest by *H. indica* (54.01%). while the average EPNS species with time in the seven days that showed in *H.bacteriophoria* at 86.1% most effective than the *H. megidis* at 79.63% and *H. indica* at 83.33% this results most significant against larvae of *T.absoluta*. The L.S.D of Species and time is 46.96>0.005 and the L.S.D of Species and concentration is 2.802>**0.005** this results more significant against the pupae of *T.absolut*.

Table (2) The effect of native nematodes on pupae of *Tuta absoluta* in laboratory conditions.

***L.S.D Least significant differences of means (5% level) >0.005**

Our findings indicated that increased concentrations of entomopathogenic nematodes enhance their efficacy against

Nematodes species	Time (day)	Mortality (%) Concentration (Ijs /ml)				Average EPNs with time (%)	Average of EPNs species (%)
		50	100	150	200		
*H. bacteriophora**	3	0.00	11.11	61.11	77.78	**37.50**	**58.89**
	5	23.33	38.89	61.11	88.89	**53.06**	
	7	72.22	77.78	94.44	100	**86.11**	
Average EPNs with conc.		**31.85**	**42.59**	**72.22**	**88.90**	----------	
*H. indica ***	3	0.0	11.17	38.83	61.17	**27.79**	**54.01**
	5	11.17	44.50	74.00	88.83	**54.63**	
	7	61.17	66.67	90.67	100	**79.63**	
Average EPN with conc.		**24.11**	**40.78**	**67.83**	**83.33**	----------	
*H. megidis**	3	7.83	38.83	61.17	83.33	**47.79**	**66.86**
	5	44.50	55.50	77.83	100	**69.46**	
	7	61.17	72.17	100	100	**83.33**	
Average EPNs with Conc.		**37.83**	**55.50**	**79.67**	**94.44**	----------	
Average of time	3	9.28	20.37	53.70	74.09	----------	
	5	26.33	46.30	70.98	92.57	----------	
	7	64.85	72.20	95.04	100	----------	
L.S.D	EPNs Species	**40.36**		Species and time		**46.96**	
	Time	**41.54**		Species and conc.		**2.802**	
	concentrations	**14.50**		Time and conc.		**3.829**	

T.absoluta pupae, corroborating the results of Youssef (2015), who also proved the usefulness of several EPNs. *Steirnernema carpocapsae* demonstrated efficacy against pupae in the presence of two fungal species, achieving a mortality rate of 46.7%. The pupal stage has a stronger resistance to nematode infection than the larval stage because of its robust pupal wall; however, empirical evidence demonstrates that the infective juvenile (IJS) of nematodes can breach the pupal wall and establish infection within three days, corroborating the results of Steyn *et al*., (2021) and Vicente *et al*., (2021). Batalla-Carrera *et al*., (2010) concurred with our findings and revealed the effectiveness of *Heterorhabditis* sp. against the larvae and pupae of *T.absoluta* with larvae exhibiting greater susceptibility to infection. Mohamed *et al*., (2023) demonstrated that EPNs from nematode strains *H. indica* NOAC.N1 and NOAC.N2 were able to penetrate the wall of mature pupae and infect them. However, the findings of Garcia-del-Pino *et al*., (2013) are not consistent with our results, who did not observe any mortality in the pupal stage of *T. absoluta* after exposure to three EPNs (*S. carpocapsae, S. feltiae,* and *H. bacteriophora*) and this may be due to the type of species or strain. This is consistent with (Türköz & Kaşkavalcı, 2016; Vicente *et al*., 2021), who explained that pupal mortality after treatment with IJs of *Heterorhabditis* and killing pupae requires a more significant number of IJs than is required for larval control.

The effectiveness of entomopathogenic nematodes against larvae and pupae of *T. absoluta* in field condition

The effectiveness against larvae of *Tuta absoluta* in field conditions

The result of this study that presented in table (3) had been revealed that all entomopathogenic nematode affect the larvae of *T. absoluta* and the highest percentage of larval mortality was 83.33% in the case of *H. bacteriophora* at the concentration 200ijs/ml and the lowest one in the case of *H. migidis* at the concentration 150 ijs/ml. The mortality of larvae was proportional with the concentration.The result also showed that the entomopathogenic nematode differ in their efficiency against larvae of *T. absoluta* and the highest mean of mortality was recorded in the case of *H. Indica* which was 73.61% followed by *H.bacteriophora and H. migidis* which were 69.44% , 63.89% respectively.

Table(3) the effect of native nematodes on *Tuta absoluta* larvae under field conditions

Native samples	Larval mortality rates (%)		Arithmetic means of samples (%)
	150 ijs/ml	200ijs/ml	
H.bacteriophora	55.56	83.33	69.44
H. migidis	50	77.78	63.89
H. Indica	66.67	80.56	73.61
Arithmetic mean of concentration	57.41	80.55	
Pvalue	0.720	0.609	
L.S.D	1.762	1.489	

***L.S.D Least significant differences of means (5% level) >0.005**

***P value Standard errors of means>0.05**

The effectiveness against pupae of *Tuta absoluta* in field conditions

The results had been revealed that the pupae of the leaf miner *Tuta absoluta* were susceptible to all three tested nematode species table(4). The highest percentage of pupal mortality was 66.67% in the case of H. *bacteriophora* and *H. Indica* at the concentration 200ijs/ml and the lowest one in the case of *H. migidis* at the concentration 150 ijs/ml which was 33.33%. The mortality of pupae was proportional with the nematode concentration. The result also showed that the entomopathogenic nematode differ in their efficiency against pupae of *T. absoluta* and the highest mean of mortality was recorded in the case of *H. Indica* and *H.bacteriophora* which was 51.39% while the EPN *H. migidis* recorded the lowest which was 43.05%.

Table (4) Effect of native nematodes on *Tuta absoluta* pupae under field conditions

Native samples	pupal mortality rates (%)		Arithmetic means of samples
	150 ijs /ml	200 ijs /ml	
H.bacteriophora	36.11	66.67	51.39
H. migidis	33.33	52.78	43.05
H. Indica	36.11	66.67	51.39
Arithmetic mean of concentration	35.18	62.04	

Pvalue	0.903	1.186	
L.S.D (0.05)	2.209	2.903	

***L.S.D Least significant differences of means (5% level) >0.005**
***P value Standard errors of means>0.05**

Our findings indicated that *T.absoluta* larvae exhibited greater susceptibility to infection than pupae when exposed to the three nematode species at concentrations of (150 ijs/ml) and (200 ijs/ml). This corroborates the results of Batala Carrera *et al.*, (2010) and Youssef (2015), who reported a higher mortality rate for larvae compared to pupae. Our work established that *H. Indica* exhibits superior efficacy compared to other nematode genera in field applications, corroborating the findings of Mohamed *et al*., (2023), who highlighted the potential of *H. Indica* as an infective juvenile in soil treatment for the control of *T. absoluta* larvae and pupae. Darroch *et al*., (2022) assessed the impact of native isolation of *H. bacteriophora* on *T. absoluta*, revealing a significant mortality rate in the third and fourth larval stages compared to the first and second stages, with mortality rates between 90% and 57.5%. This aligns with Ben Hussein (2017) in assessing the efficacy of locally effective pesticide isolates *H. indica* and *H. bacteriophora* against the larvae and pupae of *Tuta absoluta*, a destructive pest in Syria. The death rate (%) escalated with higher IJ concentration, and EPN isolates of *H. indica* showed greater efficacy than *H. bacteriophora*, corroborating our findings (Ali *et al*., 2023). Patel *et al*., (2024) demonstrated that temperature and soil moisture influenced the infectivity of entomopathogenic nematodes (EPNs), with *H. indica* achieving larval mortality rates of 96% at 25°C and 100% at 28°C. Shamseldean *et al*., (2024) investigated *S. monticolum* and *H. bacteriophora* in both field and laboratory settings to assess their sensitivity to the stages of Tuta about these species, showed that *H. bacteriophora* (HP88) is more virulent than *S. monticolum* versus *T. absoluta*. Tarasco *et al*., (2023) elucidated that acquiring novel species of entomopathogenic nematodes, which hold economic significance due to their potential for biological control of insect pests, is crucial for sustainable management while safeguarding the environment.

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Conclusion

In this study, I showed how to isolate and obtain nematodes from local soil in Wasit province, Iraq, and proved the effectiveness of nematode species (local and commercial) in biological control against tomato leaf miner larvae and pupae. This is considered a safe way to protect the plant from insect pests.

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