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Efficacy of Entomopathogenic Nematodes Against *Pieris brassicae* (L.) (Lepidoptera: Noctuidae)

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Abstract
For the control of different insect pests, entomopathogenic nematodes (EPNs) have the ability to replace larvicidal activity. The pathogenicity of two EPN species, *Steinernema monticolum* and *Heterorhabditis bacteriophora*, was tested in laboratory trials against the cabbage butterfly, *Pieris brassicae* (L.). The two nematode species used in this study were *H. bacteriophora* and the Korean species *S. monticolum*, at concentrations of 0, 10, 20, 40, 80, and 160 IJs/cm2. Only 2 samples effectively displayed larvicidal activity according to the strains' pathogenicity. The treatments with *H. bacteriophora* and *S. monticolum* at 160 IJs/cm2 produced the greatest mortality percentage (87.50 and 85 %) at the 2nd instar larval, respectively. At the 4th instar, *H. bacteriophora* and *S. monticolum* treatments at 160 IJs/cm2 resulted in corresponding larval mortality rates of 97.50 and 87.50 %. Pupae had mortality rates of (90 %) for *H. bacteriophora* and *S. monticolum*, respectively, at 160 IJs/cm2; (77.50 and 72.50%) for both the evaluated EPNs, respectively, at 80 IJs/cm2. The percentage of *P. brassicae* larval mortality reduced by the tested EPN isolates was substantially higher than the untreated control. The percentage of larval mortality significantly rose with the lengthening of time periods, reaching its peak at 72 hours, according to the results. Even after 48 and 72 hours of exposure, the strains of *S. monticolum* and *H. bacteriophor*a demonstrated powerful larvicidal action at low concentration. This research showed that *P. brassicae* was successfully controlled by the EPN strains *S. monticolum* and *H. bacteriophora* HR2.

Keywords: biocontrol, *heterorhabditis bacteriophora*, *steinernema monticolum*, pathogenicity, *pieris brassicae*

Introduction
One of the primary reasons limiting the output of cabbage is the cabbage butterfly, *P. brassicae* (L.) (Lepidoptera: Pieridae), which severely damages the crop by feeding as a larva on leaves. (Mazurkiewicz *et al*. 2017) [16]. In order to control *P. brassicae*, chemical insecticides have been a common practise among cabbage growers. However, the negative effects of chemicals on the environment, such as groundwater contamination, pesticide resistance, toxicity risks, and the loss of biodiversity of beneficial natural enemies, call for an effective alternative method of crop insect pest management that is eco-friendly and safe for organisms other than the target pests. Steinernematidae and Heterorhabditidae-related entomopathogenic nematodes (EPNs) are deadly parasites of insect pests that are safe for humans, other vertebrates, and non-target organisms, simple to use, and have no harmful effects on the environment (Abbas *et al*. 2021) [1]. Additionally, they work well with a variety of chemical pesticides (Laznik and Trdan 2014) [13].
Heterorhabditidae and Steinernematidae-family entomopathogenic nematodes (EPNs), which live in soil, are naturally obligate insect parasites (Kaya and Gaugler 1993) [10]. These nematodes have developed a mutualistic relationship with the genera of bacteria. According to Arthurs *et al*. (2004) [3], Photorhabdus, which is connected to Heterorhabditis spp., is transported in the intestine of infected juveniles (IJs). Xenorhabdus is related to Steinernema species and is associated with a particular vesicle in the IJs' gut. Nematodes search for possible hosts by observing insect cues. (Lewis *et al*. 2006) [14]. After finding a host, IJs infect it by penetrating the epidermis (especially in Heterorhabditis spp.) or through an opening like the mouth, anus, or spiracles. IJs penetrate the host, shed their outer cuticle (Sicard *et al*. 2004) [20], beginning to consume hemolymph, and the expulsion of symbionts happens either through faeces in Steinernema species or regurgitation in Heterorhabditis species (Grewal *et al*. 2005) [7]. The host is killed by the nematode-bacteria combination within 24 to 48 hours by septicemia or toxaemia (Forst and Clarke 2002). The nematodes are recolonized by bacteria as they emerge as IJs from the dead insect in quest of new hosts (Poinar 1990) [17].
Approximately 80% of the more than 100 EPN species that have been identified worldwide are steinernematid, and 13 percent of these species have been commercialised (Abbas *et al*. 2021) [1]. EPNs have been widely utilised in the biological control of numerous commercially significant pests found in a variety of habitats (Grewal *et al*. 2005) [7]. However, the use of EPNs against foliar pests has been improved by the incorporation of adjuvants or the formulation of EPNs to delay desiccation or increase leaf coverage and IJ persistence (Head *et al*., 2004) [8].
The purpose of the current research was to offer the fundamental knowledge required for the use of locally isolated EPNs as a biological control agent. The research examined the pathogenicity of two nematode species, *S. monticolum* and *H. bacteriophora* (Poinar 1990) [17] (strain HP88), against *P. brassicae* in a laboratory setting.

Materials and Methods
Rearing of Pieris brassicae
Larvae of the cabbage butterfly, *P. brassicae*, were collected from the cabbage growing fields at Giza Governorate, Egypt, was transferred to the laboratory of Vegetables Pests Research Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt. The larvae were

brought to the lab, kept at room temperature, and put in rearing cages (20 cm×12 cm). Unsprayed cabbage leaves were gathered from the field to use as larval food. Every 24 hours, the feed was replaced, and the cages were carefully cleaned at the same time to avoid any contamination. The neonate larvae were given the opportunity to grow by consuming the cabbage leaves until they reached complete maturity. *P. brassicae* was harvested at the proper instar and prepared for the bioassay by being put in sterilised plastic containers with a mesh for ventilation. Every day, dead *P. brassicae* larvae were taken out of the rearing box, and wet cotton wool was used to clean the faecal pellets.

Entomopathogenic nematodes

Heterorhabditis bacteriophora (Poinar 1990) [17] (strain HP88) and the Korean species *Steinernema monticolum* (Stock *et al*. 1997) [17] were the two species of nematodes used in the current research, according to Ibraheem (2015) [7]. Following Woodring and Kaya (1998) [22] approach, both nematode species were reared at 26 °C on late-instar larvae of the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Galleridae). Using modified White traps, Kaya and Stock (1997) [11] were able to collect the nematode infective juveniles (IJs) that emerged from insect cadavers. They were kept at 10 °C for a week, then permitted to acclimatise at room temperature for 45–60 minutes. The viability of the samples was then determined by observing movement with a zoom stereomicroscope. (Ibraheem 2015) [7].

Effect of nematode concentrations

Petri plates (9 cm) were used for bioassays. There were 20 g of sand soil in each unit. (Table 1). 7% (w/w) of the soil's moisture was modified. IJs were evenly applied to the soil surface in 1 ml of distilled water at rates of 0, 5, 10, 20, 40, 80, and 160 IJs/cm2. 10% (w/w) was the final soil moisture value. Ten *P. brassicae* larvae per container were then placed on the soil surface after the containers were maintained at room temperature for one hour. For every concentration, there were four replicates. The containers were housed in a growth chamber for 72 hours under carefully monitored conditions. Then, the larvae were gently separated away from the substrate and kept separately under controlled circumstances until adult emergence. In order to confirm nematode infection, 25% of the dead larvae were arbitrarily chosen and dissected using stereomicroscope image analysis software (Olympus Soft Imaging Solutions) three days later. The experiment was conducted twice.

Larvicidal activity

Each nematode species was added to the 9 centimetre Petri dish in triplicate along with 10 larvae of the tested *P. brassicae* strains at various concentrations (1.00, 1.30, 1.60, 1.90, and 2.20 IJs/cm2). Young (vegetative stage) cabbage leaves supplied the 2nd and 4th larval instars. As a control, one Petri dish was used without the suspension of EPNs. Calculations were made of the quantity of dead larvae at 24, 48, and 72 hours. Pathogenic strains were those that destroyed more than 50% of the larvae. Using different EPN suspension concentrations, the quantitative larvicidal activity of both nematode strains against *P. brassicae* was tested. For each concentration, the infected larvae were examined under a stereo zoom microscope after a 72-hour exposure period.

Table 1: Mortality percentage of *P. brassicae* stages after entomopathogenic nematode treatments

Treatments	Stages	*Pieris brassicae* mortality (%) after treatments					
		Steinernema monticolum			*Heterorhabditis bacteriophora*		
		24h	48h	72h	24h	48h	72h
Control	2nd instar	0.00	0.00	0.00	0.00	0.00	0.00
	4th instar	0.00	0.00	0.00	0.00	0.00	0.00
	Pupa	0.00	0.00	0.00	0.00	0.00	0.00
10 IJs/cm2	2nd instar	17.5	32.50	42.50	20.00	30.00	42.50
	4th instar	22.50	32.50	40.00	32.50	52.50	65.66
	Pupa	20.00	32.50	45.00	22.50	35.00	45.00
20 IJs/cm	2nd instar	25.00	37.50	50.00	27.50	40.00	50.00
	4th instar	35.00	45.00	55.00	45.00	67.50	77.50
	Pupa	27.50	37.50	52.50	35.00	47.50	57.50
40 IJs/cm2	2nd instar	32.50	47.50	62.50	35.00	47.50	57.50
	4th instar	47.50	57.50	67.50	55.00	72.50	85.00
	Pupa	40.00	50.00	60.00	45.00	57.50	70.00
80 IJs/cm2	2nd instar	37.50	55.00	70.00	42.50	60.00	70.00
	4th instar	55.00	67.50	77.50	67.50	80.00	90.00
	Pupa	47.50	62.50	72.50	52.50	65.00	77.50
160 IJs/cm2	2nd instar	52.50	67.50	85.00	50.00	72.50	87.50
	4th instar	65.00	80.00	87.50	77.50	90.00	97.50
	Pupa	55.00	75.00	90.00	60.00	77.50	90.00
LSD ($p<0.05$)	2nd instar	4.64	4.17	6.44	5.80	7.24	10.03
	4th instar	5.35	6.03	6.05	4.37	8.46	9.80
	Pupa	5.80	5.69	8.78	4.95	4.39	8.54

Statistical analysis

In order to satisfy the assumptions of normality and homogeneity of variances, the mortality of insects was control-corrected (Abbott 1925) [2] and Arcsine transformed when necessary. Control-corrected mortality was analysed using a one-factor of variance (ANOVA) in every experiment.

The corrected percent mortality data that were thus obtained for various concentrations of *P. brassicae* (L.) were then submitted to probit analysis using the method described by

Finney in 1971. Data on the concentration-mortality relationship were collected. LSD ($P < 0.05$) values were also computed to differentiate between treatment averages.

Results

Bioassay of *P. brassicae*

Table 1 provides data on the effectiveness of EPNs for controlling *P. brassicae* larvae. In comparison to the untreated control, the studied EPN isolates significantly reduced *P. brassicae* larval mortality. Results showed that as time periods increased the percentage of larval mortality significantly increased, reaching its maximum at 72 hours, then 48, then 24 hours. After 72 h, the highest percentage of larval mortality was observed at the 2nd instar larvae treated with *H. bacteriophora* (87.50 %) and *S. monticolum* (85 %) at 160 IJs/cm2, followed by those at 80 IJs/cm2 (70%), producing identical outcomes for both EPNs. At 40 IJs/cm2, *S. monticolum* and *H. bacteriophora* had larvae mortality rates of (62 %) and (57 %), respectively (Fig. 1). The 4th instar larvae mortality with *H. bacteriophora* (97.50 %) and *S. monticolum* (87.50 %) at 160 IJs/cm2, followed by those at 80 IJs/cm2, *H. bacteriophora* (90 %) and *S. monticolum* (77.50 %). At 40 IJs/cm2, *H. bacteriophora* had larvae mortality rates of (85 %) and *S. monticolum* (67.50 %), respectively (fig.2). In pupae at 160 IJs/cm2, *H. bacteriophora* and *S. monticolum* both caused (90 %) pupa death. However, at 80 IJs/cm2, *H. bacteriophora* and *S. monticolum*, respectively, had larval death rates of (77.50 %) and (72.50 %), followed by those at 40 IJs/cm2, *H. bacteriophora* (70 %) and *S. monticolum* (60 %) respectively (Fig. 3). The untreated comparison showed no signs of larval mortality.

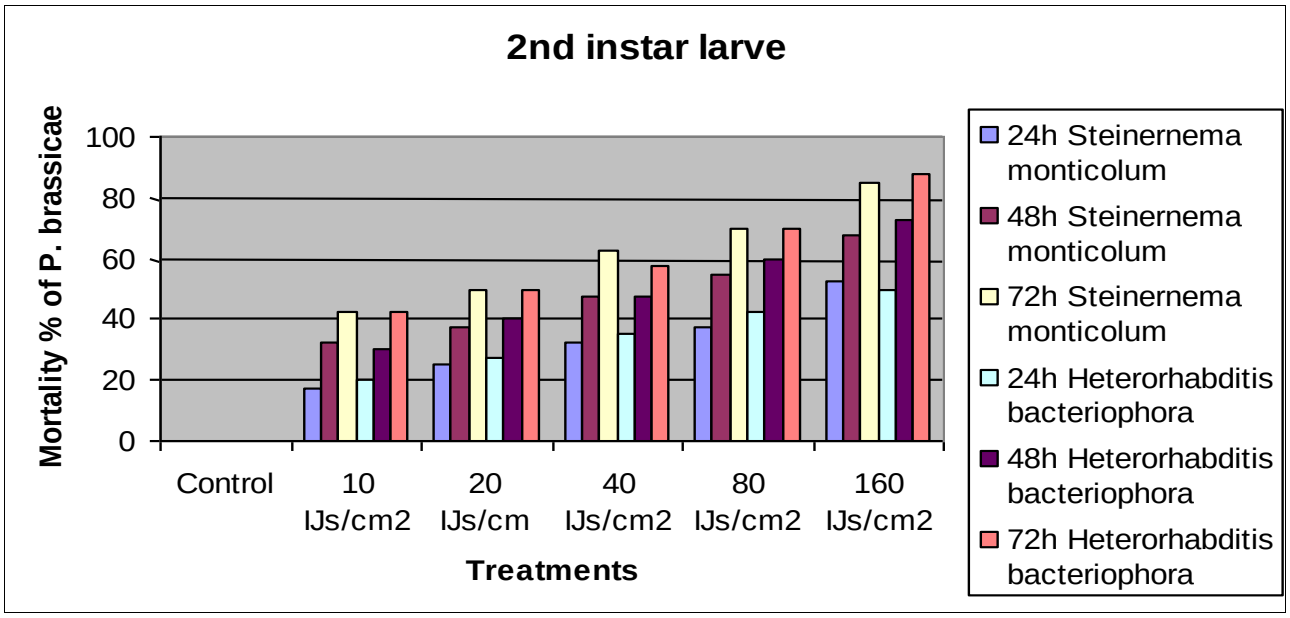

Fig 1: Efficacy of *S. monticolum* and *H. bacteriophora* against 2nd instar *P. brassicae* larvae

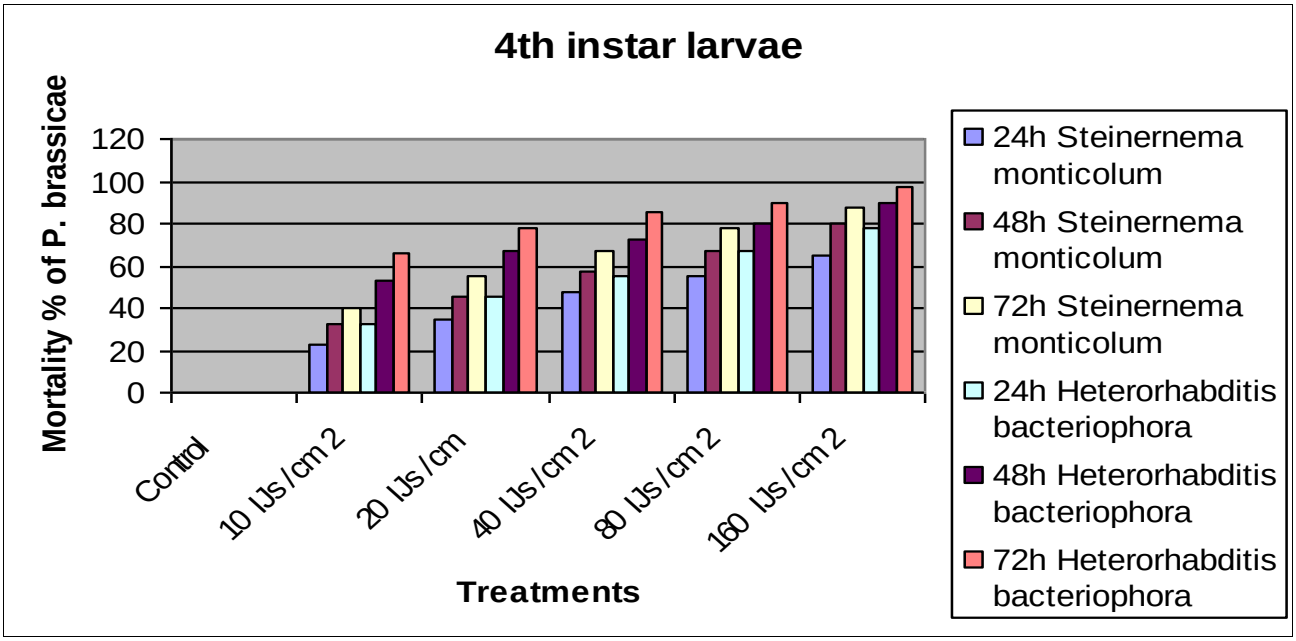

Fig 2: Efficacy of *S. monticolum* and *H. bacteriophora* against 4th instar *P. brassicae* larvae

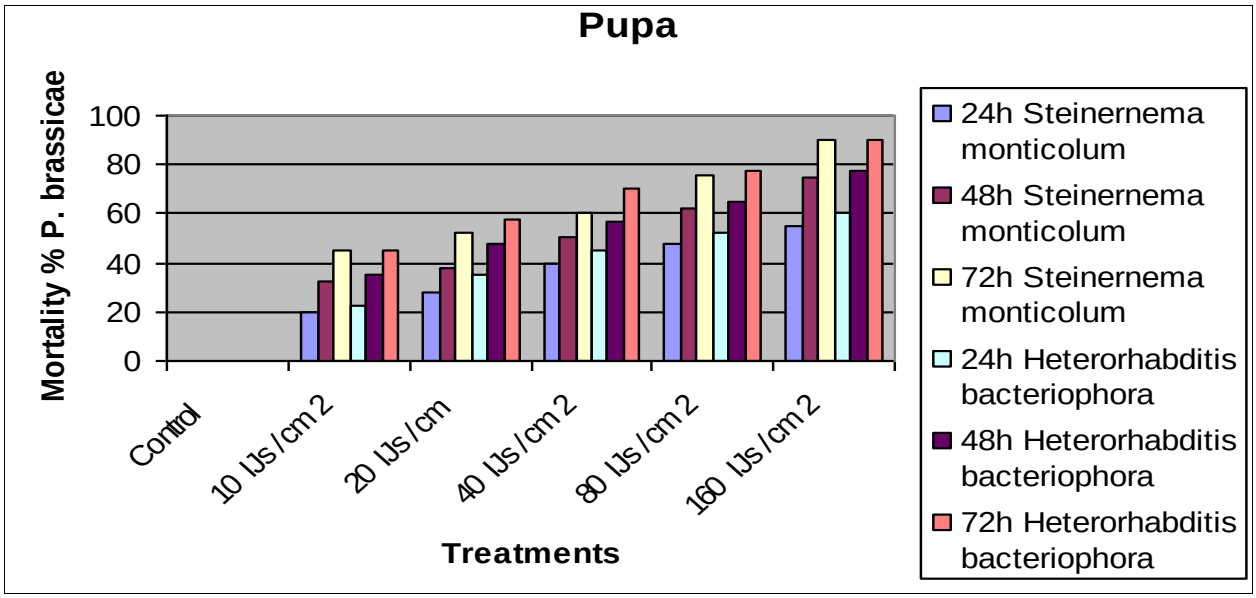

Fig 3: Efficacy of *S. monticolum* and *H. bacteriophora against P. brassicae* pupae

After 48 hours at the 2nd instar larvae, the treatment demonstrated the highest percentage of larval mortality for *H. bacteriophora* and *S. monticolum* at 160 IJs/cm2 (72.50 % and 67.50 %), respectively, followed by *H. bacteriophora* and *S. monticolum* at 80 IJs/cm2 (60 % and 55 %). At 40 IJs/cm2, *H. bacteriophora* and *S. monticolum* resulted in a death rate of (47.50%) for each (Fig.1). Treatments for the 4th instar larvae mortality included *H. bacteriophora* (90 %) and *S. monticolum* (80 %) at 160 IJs/cm2. At 80 IJs/cm2, *H. bacteriophora* and *S. monticolum*, respectively, were responsible for 80 % and 67.50% of the larval deaths. In the untreated control, there was no evidence of larval death (Fig.2). Pupal mortality for *H. bacteriophora* and *S. monticolum* at 80 IJs/cm2 was 65.50 % and 62.50 %, respectively. The effectiveness of EPNs on pupal mortality was observed for *H. bacteriophora* (77.50 %) and *S. monticolum* (75.50 %) at 160 IJs/cm2. (Fig. 3). The results after 24 hours showed that the highest mortality rates of the 2nd instar larvae were recorded in treatments with *S. monticolum* and, *H. bacteriophora*, respectively, at 160 IJs/cm2 (52 % and 50 %), followed by those at 80 IJs/cm2 *H. bacteriophora* (42.50 %) and *S. monticolum* (37.50 %), and then at 40 IJs/cm2 *H. bacteriophora* (35 %) and *S. monticolum* (32.50 %). The next-best outcome was (65 %) for *S. monticolum* at the same dosage, followed by (77.50 %) for the mortality of 4th instar larvae by *H. bacteriophora* at 160 IJs/cm2. *H. bacteriophora* and *S. monticolum* caused pupal mortality rates of 60 % and 55%, respectively, at 160 IJs/cm2, but EPNs caused mortality rates of 52.50 and 47.50 % at 80 IJs/cm2. In the untreated comparison, there was no larval mortality seen. The remaining treatments differed from one another, but not significantly.

Bioassay of Log probit analysis larvicidal activity

To determine the lethal amounts of EPNs for the various *P. brassicae* larval instars, the virulence test was performed. The information regarding the effectiveness of the two strains of *S. monticolum and H. bacteriophora* (HR2) against the second, fourth, and pupal stages of *P. brassicae* is summarised in (Table 2). EPN was used at 10, 20, 40, 80, and 160 IJs/cm2, and *P. brassicae* were recorded when EPNs (at LC50 level) were used 48 and 72 hours after EPN treatment or LC90 level (Table 2). When EPNs were applied at LC90, an effect was seen in that the best larvicidal activity was achieved during the 72-hour exposure period. Even at 48 and 72 hours after exposure, *S. monticolum* and *H. bacteriophora* EPN strains demonstrated powerful larvicidal action at low concentrations (Table 2).

Table 2: Log probit analysis of the larval and pupal activity of *P. brassicae*, a type of cabbage butterfly, after treatment with entomopathogenic nematodes

Nematode species	Stages	Exposure time (hrs)	LC50 (IJs/cm2) 95% LCl-UCL	LC90 (IJs/cm2) 95% LCl-UCL	Intercept	Slope±SE	χ2 value	P value
Steinernema monticolum	2nd instar larvae	48	46.96 (22.88–82.03	69.06 (39.53–88.92)	−1.25	1.3±0.22	0.57	0.005**
		72	23.02 (14.74–35.12)	51.62 (33.06–80.10) −	−1.21	1.5±0.22	1.54	0.006**
	4th instar larvae	48	35.47 (23.60–53.32)	79.91 (49.98–94.25)	−1.50	01±0.23	0.16	0.003**
		72	22.02 (14.94–32.46)	59.58 (40.42–87.82) −	−1.37	1.3±0.23	0.11	0.004**
	Pupae	48	36.18 (23.28–56.24)	80.22 (51.61–97.08)	−1.49	1.5±0.22	0.99	0.003**
		72	20.86 (13.76–29.93)	73.54 (48.51–94.49)	−1.28	01±0.23	4.92	0.005**
Heterorhabditis bacteriophora	2nd instar larvae	48	39.94 (25.15–63.44)	75.72 (47.67–89.12)	−1.46	1.5±0.22	0.47	0.003**
		72	20.38 (13.36–31.08)	48.82 (32.01–74.46)	−1.29	02±0.23	2.87	0.005**
	4th instar larvae	48	29.66 (18.16–48.44)	78.70 (48.19–97.92)	−0.83	1.5±0.23	1.15	0.024*
		72	22.11 (14.10–34.67)	49.84 (31.78–78.16)	−0.76	01±0.26	1.25	0.046*
	Pupae	48	32.99 (20.63–56.76)	73.12 (45.72–95.20)	−1.27	1.5±0.22	0.40	0.005**
		72	21.69 (14.46–32.52)	55.26 (36.85–82.86)	−1.24	1.5±0.23	0.83	0.006**

Discussion

Overall findings on the efficacy of the tested EPNs showed that *H. bacteriophora* treatment of 4th instar larvae at 160 IJs/cm2 for 72 h (97 %) was superior to other treatments. However, it was discovered that *S. monticolum* treatment at 160 IJs/cm2 was the following effective treatment (87.50 %). In light of this, the findings of the current research support Sharma *et al*.'s (2018) [19] findings that *P. brassicae* caused larval net mortality of 86.2 and 66.5% under laboratory conditions, respectively. The same outcomes were also documented by (Askary and Ahmad, 2020) [4]. Mantoo and Zaki (2014) [15] obtained results that corroborate the findings of an increase in IJs inoculum levels. The time required for larval mortality decreased, but when the larval size increased, the time required for larval mortality also increased. It was also demonstrated that EPNs may be a successful alternative to insecticides. Foliar applications are becoming more and more popular for controlling insect pests, according to Laznik *et al*. (2010).

The mortality was calculated using various amounts following 48 and 72 hours of exposure. The concentration and exposure duration affect the mortality rate. However, after 48 and 72 hours, the treatment with *H. bacteriophora* at very low concentrations showed the greatest mortality range. Despite having a slow mortality rate in 48 and 72 hours of exposure, *S. monticolum* restrained the larval growth in the early pupal stage. The findings are consistent with those of Sabry *et al*. (2016) [16] study of *H. bacteriophora*'s pathogenicity against vine mealy bug in South African farms. The data showed that as time intervals, such as 24, 48, and 72 hours, increased, the mortality rate also increased.

The obtained results support the findings of Mantoo and Zaki (2014) [15], who found that while the time required for larval mortality reduced with an increase in the inoculum level of IJs, it increased when the size of the larva increased. Other researchers Askary and Ahmad (2020) [4] and Abbas *et al*. (2021) [1] reported similar findings.

In comparison to *S. monticolum*, the efficacy of *H. bacteriophora* EPN isolates was vastly more effectively. Future field experiments are justified in order to thoroughly assess the potential of local EPN isolates against *P. brassicae* because high EPN efficacy obtained under laboratory conditions cannot be readily extrapolated to field efficacy.

Abbreviations

EPNs: Entomopathogenic nematodes; IJs: Infective juveniles; cm2: Centimeter square; h: Hour; LSD: Least signifcant diference

References

1. Abbas W, Javed N, Haq IU, Ahmed S. Pathogenicity of entomopathogenic nematodes against cabbage butterfy (*Pieris brassicae*) Linnaeus (Lepidoptera: Pieridae) in laboratory conditions. Int J Trop Insect Sci,2014:41:525-531. https://doi.org/10.1007/s42690-020-00236-2
2. Abbott WS. A method of computing the efectiveness of an insecticide. J Econ Entomol,1925:18:265-267.
3. Arthurs S, Heinz KM, Prasifka JR. An analysis of using entomopathogenic nematodes against above-ground pests. Bull Entomol Res,2004:94:297-306. https://doi.org/10.1079/BER2003309
4. Askary TH, Ahmad MJ. Efcacy of entomopathogenic nematodes against the cabbage butterfy *Pieris brassicae* (L.) (Lepidoptera: Pieridae) infesting cabbage under feld conditions. Egypt J Biol Pest Control,2020:30:39. https://doi.org/10.1186/s41938-020-00243-y
5. Finney DJ. Probit analysis. 3rd edition. Cambridge university press, 1971, 318.
6. Forst S, Clarke D. Bacteria–nematode symbiosis. In: Gaugler R (ed) Entomopathogenic nematology. CABI Publishing, 2022, 57-77. ISBN 0851995675
7. Grewal PS, Ehlers RU, Shapiro-Ilan DI. Nematodes as biocontrol agents. CABI Publishing. ISBN 0851990177, 2005.
8. Head J, Lawrence AJ, Walters KFA. Efcacy of the entomopathogenic nematode, *Steinernema feltiae*, against *Bemisia tabaci* in relation to plant species. J Appl Entomol,2004:128:543-547. https://doi.org/10.1111/j.1439- 0418.2004.00882.x
9. Ibraheem HIH. Entomopathogenic nematodes as biocontrol agents of *Tuta absoluta* on tomato plants M.Sc. Thesis Fac. Agric., Cairo Univ, 2015, 80.
10. Kaya HK, Gaugler R. Entomopathogenic nematodes. Annu Rev Entomol,1993:38:181-206. https://doi.org/10.1146/annurev.en.38.010193.001145
11. Kaya HK, Stock SP. Techniques in insect nematology. In: Lacey LA (ed) Manual of techniques in insect pathology. Academic Press, San Diego, 1997, 281-324.
12. Laznik Z, Toth T, Lakatos T, Vidrih M, Trdan S. Control of the Colorado potato beetle (Leptinotarsa decemlineata [Say]) on potato under feld conditions: a comparison of the efficacy of foliar application of two strains of *Steinernema feltiae* (Filipjev) and spraying with thiametoxam. J Plant Dis Prot, 2010:117:129-135.
13. Laznik Z, Trdan S. The infuence of insecticides on the viability of entomopathogenic nematodes (Rhabditida: *Steinernematidae and Heterorhabditidae*) under laboratory conditions. Pest Manag Sci,2014:70:784-789.
14. Lewis EE, Campbell JC, Grifn C, Kaya HK, Peters A. Behavioural ecology of entomopathogenic nematodes. Biol Control,2006:38:66-79. https://doi.org/ 10.1016/j.biocontrol.2005.11.007
15. Mantoo MA, Zaki FA. Biological control of cabbage butterfy, *Pieris brassicae* by a locally isolated entomopathogenic nematode, Heterorhabditis bacteriophora SKUAST-EPN-Hr-1 in Kashmir. SKUAST J Res,2014:16:66-70.
16. Mazurkiewicz A, Tumialis D, Pezowicz E, Skrzecz I, Błażejczyk G. Sensitivity of *Pieris brassicae*, *P. napi* and *P. rapae* (Lepidoptera: Pieridae) larvae to native strains of Steinernema feltiae (Filipjev, 1934). J Plant Dis Prot,2017:124:521-524. https://doi.org/10.1007/s41348-017-0118-4
17. Poinar GO. Biology and taxonomy of Steinernematidae and Heterorhabditidae. In: Gaugler R, Kaya HK (eds) Entomopathogenic nematodes in biological control. CRC Press, Boca Raton, 1990, 23-58.
18. Sabry AH, Metwallya HM, Abolmaatyb SM. Compatibility and efcacy of entomopathogenic nematode, *Steinernema carpocapsae* all alone and in combination with some insecticides against Tuta absoluta. Der Pharm Let,2016:8:311-315.
19. Sharma R, Singha B, Choudhury SR, Sharma G. Isolation and microscopic investigation of

Entomopathogenic nematodes (EPNs) occurring in Barak Valley, Assam, India. Int J Curr Microbiol App Sci,2018:7:1835-1839.
20. Sicard M, Brugirard-Ricaud K, Pages S, Lanois A, Boemare NE, Brehelin M, *et al*. Stages of infection during the tripartite interaction between *Xenorhabdus nematophila*, its nematode vector, and insect hosts. Appl Environ Microbiol,2004:70:6473-6480. https://doi.org/10.1128/ AEM.70.11.6473-6480.2004
21. StocK SP, Choo HY, Kaya HK. An entomopathogenic nematode, *Steinernema monticolum* (Rhabditida: Steinernematidae) from Korea with a key to other species. Nematologica,1997:43:15-29.
22. Woodring JL, Kaya HK. *Steinernematid* and *heterorhabditid* nematodes: a handbook of techniques. Arkansas Agricultural Experiment Station, Fayetteville, Arkansas. South Cooper Bull,1998:331:1-30.