Scientific Papers. Series B, Horticulture. Vol. LXX, No. 1, 2026
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFICACY OF THE NATIVE ENTOMOPATHOGENIC NEMATODES (EPN) ON THE SURVIVAL OF *CERATITIS CAPITATA*

Ntokozo C. SHABALALA, Zakheleni P. DUBE, Ludzula MUKWEVHO

University of Mpumalanga, Private Bag X11283, Mbombela 1200, South Africa

Corresponding author email: 222010541@ump.ac.za

Abstract

Ceratitis capitata (Wiedemann, 1824) (Mediterranean fruit fly) is one of the most economically damaging pests of fruits globally, causing significant yield losses and trade restrictions. Despite the presence of both native and non-native entomopathogenic nematodes (EPNs) in South African soils, indigenous species remain underexplored for use in pest management programs. This study objective was to evaluate the efficacy of two indigenous isolates; Steinernema jeffreyense and Heterorhabditis bacteriophora against the larval and pupal stages of C. capitata under laboratory conditions. To achieve the objective, a 2 × 5 factorial experiment with five replications in a completely randomised design was set up. Variables measured were larval and pupal mortality, mortality speed, EPN infectivity, and the number of pupae after larval exposure. There were no significant interactions between EPN and concentration for the larval trial, while all variables in the pupal trial had highly significant interactions. Generally, both species caused larval and pupal mortality across treatments, with H. bacteriophora performing better than S. jeffreyense. Larval and pupal mortalities were concentration dependent, with the highest larval mortality at 25 IJs and pupal mortality significant only at 250 IJs. These findings suggest potential for biological control of C. capitata.

Key words*: biological control, Ceratitis capitata, entomopathogenic nematodes, Heterorhabditis bacteriophora, Steinernema jeffreyense*

INTRODUCTION

Phytosanitary pests, such as *Ceratitis capitata,* are widely distributed polyphagous pests of commercial fruits in South and North America, Southeast Asia, Europe, and Africa (Authority et al., 2019, Dong et al., 2022, Jaffar and Lu, 2022, Papadogiorgou et al., 2024, Authority et al., 2025). *Ceratitis capitata* attacks over 300 plant species, particularly in tropical and subtropical regions, including South Africa (Abd-Elgawad, 2021; Sookar et al., 2021; Dong et al., 2022). The infestation by *Bactrocera dorsalis* (Hendel, 1912) and *C. capitata* is associated with estimated yearly financial losses of US$1 billion worldwide and US$42 million in Africa (Motswagole et al., 2019, López et al., 2024).

Management of *C. capitata* in orchards includes behavioural, chemical, biological, cultural, and mechanical tactics, with synthetic chemicals being the most used (Candia, 2019, Reddy et al., 2020, Ganie et al., 2022, Rodriguez et al., 2024, Wang et al., 2024). However, the use of synthetic chemicals poses ecotoxicological risks, which may include water and soil pollution, insect resistance, and chemical residues on fruits, affecting the export quality of the crop (Mokrini et al., 2020, Cruz et al., 2022, Lengai et al., 2022, Ali et al., 2023). Therefore, there is a clear demand for eco-friendly alternatives that are also highly effective. Biological control has attracted research and developmental attention as a reliable alternative to reducing reliance on synthetic chemicals, because it is very effective, with minimal environmental contamination and insect resistance (Baker et al., 2020, Lengai et al., 2022, Maurya et al., 2022, Costa et al., 2023, Kasana, 2024).

Entomopathogenic nematodes (EPNs) are one biological agent that has demonstrated effectiveness in the management of many insect pests, including *Thaumatotibia leucotreta* and many tephritid fruit flies (Belien, 2019; Coleman, 2020; Mkiga, 2020). The use of entomopathogenic nematodes could help alleviate fruit fly densities in many cropping systems (Fouad et al., 2020, Sallam et al., 2024). Unlike chemicals, entomopathogenic nematodes actively seek out their hosts by detecting chemical cues released by the larvae or pupae in the soil (Zhang et al., 2021, Kallali et

al., 2024). Currently, there is limited information on the use of locally isolated EPNs in the management of fruit flies. Therefore, the current study objective is to evaluate the efficacy of two local EPN isolates: Steinernema *jeffreyense* and *Heterorhabditis bacteriophora*, in the management of *C. capitata*.

MATERIALS AND METHODS

Study sites

The study was conducted at the University of Mpumalanga, Mbombela Campus, in South Africa under laboratory conditions.

Sampling and rearing of insects

Sourcing and rearing of mealworm (Tenebrio molitor) (Linnaeus, 1758) larvae

Mealworm larvae were sourced from Feeders Africa and reared in containers filled with wheat bran, which served as the primary substrate and food source (Figure 1). Freshly grated carrots were provided as a food source for the adults and a moisture source for the larvae. The containers had holes to ensure adequate ventilation, yet small enough not to allow the insect to escape (Figure 1). Egg cardboard crates were placed inside the containers to provide shelter and ovipositing surfaces for the adult beetles (Figure 2).



Figure 1. Rearing container of mealworms with wheat bran, ventilation holes, and grated carrot provided as a food and moisture source. Photo by NC Shabalala (2025)

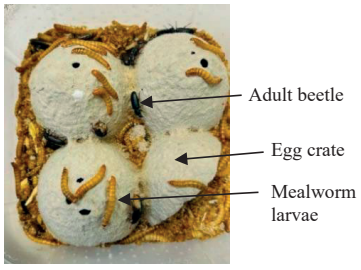

Figure 2. Rearing container of mealworms showing egg cardboard crate used as shelter and ovipositing surfaces for adult beetles. Photo by NC Shabalala (2025)

Sourcing and rearing of Ceratitis capitata

Five hundred eggs of *Ceratitis capitata* were sourced from Citrus Research International, Mbombela, South Africa. The eggs were placed in a circular container filled with a carrot-based medium (Tsitsipis, 1989) prepared by mixing carrot powder (BioNutricia extract) with warm water to achieve a moist consistency (Figure 3). The mixture served as the primary food source for the larval stage. The container was then placed inside a 27 L clear plastic container (Figure 4). Distilled water was sprayed regularly on the carrot mixture to maintain a high-humidity environment. The eggs were allowed to hatch and larvae to develop through the first three instar stages before being moved out, mimicking their natural behavior of exiting fruit to pupate. Unhatched eggs were left in the original circular container until they hatched; those that did not hatch were disposed of. The third instar larvae were collected using forceps and used for the larval bioassay. Some of the third instar larvae were left in the circular container inside the 27 L container until they reached the pupal stage. The pupae were then collected using forceps for use in the pupal bioassay; the rest were transferred into a Petri dish and subsequently placed back into a new 27 L container, which had a net sleeve through a side opening, to ensure adequate ventilation and prevent adult flies from moving out. Once adults emerged, they were provided with a sugar-yeast mixture (3:1) in a Petri dish, along with water supplied in a circular container lined with a paper towel that was lightly moistened (Figure 5). For egg collection, a custom-designed artificial oviposition device was used. This consisted of a plastic container with damp paper towel and rotten *Citrus sinensis* on either side of the device, sealed with perforated lids (Figure 6). The holes allow adult females to insert their ovipositors and lay eggs into the medium, ensuring a continuous supply of eggs.

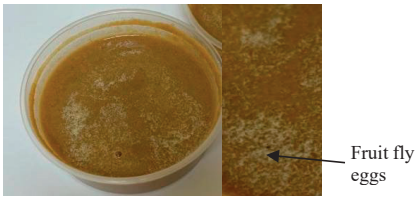

Figure 3. Carrot-based medium in circular containers with fruit fly eggs. Photo by NC Shabalala (2025)



Figure 4. Circular container with carrot-based medium placed inside a 27L clear plastic container used for rearing larvae. Photo by NC Shabalala (2025)

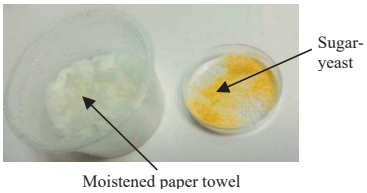

Figure 5. Sugar-yeast mixture (3:1) in a Petri dish, with water provided in a circular container lined with moistened paper towel. Photo by NC Shabalala (2025)

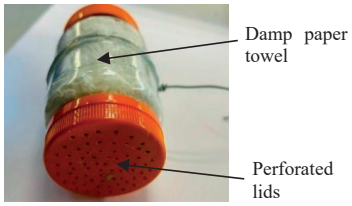

Figure 6. Custom-designed artificial oviposition device consisting of a plastic container with damp paper towel and rotten *Citrus sinensis*, sealed with perforated lids. Photo by NC Shabalala (2025)

Sourcing and rearing of entomopathogenic nematodes

Entomopathogenic nematodes (EPNs) were obtained from the University of Stellenbosch, South Africa. The *in vivo* approach was used for rearing the EPNs as previously described (Shapiro-Ilan et al., 2016). Briefly, four Petri dishes (9 cm in diameter) were filled with circular filter paper (Whatman No. 1). About 800 µL suspension containing the EPNs were evenly distributed onto the filter paper in each Petri dish. Ten mealworm (*Tenebrio molitor*) last instar larvae were then introduced into each Petri dish. The Petri dishes were covered with their lids, labelled with the date and EPN species, and placed inside a 5L plastic container to maintain humidity. The containers were stored at room temperature (20-25°C). Petri dishes were examined daily for signs of infection and larval death, as determined by the discoloration or decay of the mealworm larvae. After two days, cadavers were carefully removed and rinsed with distilled water on a 25 µm sieve, then transferred to sterile 9 cm Petri dishes. Ten days later, the 9 cm Petri dish containing wet filter paper and cadavers was placed inside a 15 cm-wide glass Petri dish, leaving the small Petri dish open. The 15 cm Petri dish was filled with water to half the level of the 9 cm Petri dish. The large Petri dish was closed, labelled accordingly, and left undisturbed at room temperature until the infective juveniles began to emerge and move into the water contained in a 15 cm Petri dish. Once EPN concentrations were high enough to become visible in the water, the 9 cm Petri dish was removed. The water containing EPNs was carefully poured into a beaker and allowed to settle for approximately 30 minutes. The infective juveniles (IJs) suspension was then transferred into culture flasks. The flasks were stored horizontally to ensure even distribution of nematodes. The nematode suspension was kept at 12.8°C. The flasks were shaken weekly to improve oxygen levels in water and prevent EPNs from climbing out of water, the lid was slightly loosened to allow air exchange.

Experimental design and bioassays

Larval bioassay

A 5 × 2 factorial experiment in a completely randomised design was used to test effect of EPNs on *C. capitata*. The first factor consisted of five EPN concentrations of 0, 25, 50, 75 and 100 IJs per 50 µL and the second factor were the two EPNs; *Steinernema jeffreyense* or *Heterorhabditis bacteriophora.* The EPN inocula were prepared from the culture flask by diluting the nematode suspensions with distilled water. Prior to inoculation, a 13 mm diameter filter paper disc was placed in each well of a 24-well bioassay plate. Each EPN was tested in 12 alternating wells of a 24-well plate to minimize potential IJ migration between wells. For each treatment, 50 µL of the nematode suspension was pipetted into the well, and a single third-instar fruit fly larva of *C. capitata* was placed on the filter paper. Control wells received only 50 µL of distilled water and fruit fly larvae. A completely randomized design with five

replicate plates per treatment was employed, resulting in 60 larvae per treatment per nematode. The plates were covered with a paper towel and a glass plate, secured with a rubber band. Each respective treatment plate was then placed inside a 2-L plastic container lined with damp paper towels to maintain humidity. All plates were incubated at 25 ± 2 °C. Larval survival was recorded daily for a period of three days. Mortality was determined at each observation by the difference between the total number of larvae introduced and those surviving. Dead larvae were dissected under a stereomicroscope to confirm the presence of nematode infection.

Pupal bioassays

To ascertain the survival of pupae, the same procedure described for larvae was followed, except that the EPN concentrations tested were 0, 100, 150, 200, and 250 IJs per 50 µL. Prior to inoculation, a 13 mm diameter filter paper disc was placed in each well of a 24-well bioassay plate. Each concentration was applied by pipetting 50 µL of the nematode suspension into the well. A single one-day-old pupa of *Ceratitis capitata* was then placed into each treated well. Control wells received only 50 µL of distilled water and pupa. Each species was tested in 12 alternating wells of a 24-well plate. A completely randomized design with five replicate plates per treatment was used. The plates were covered with a paper towel and a glass plate, secured with a rubber band, and placed inside 2-L plastic containers lined with damp paper towels to maintain humidity. All plates were incubated at 25 ± 2°C. The number of adults emerging was recorded daily for both nematodes. The experiment was terminated on the 10th day. Pupae that failed to turn into adults or showed visible signs of infestation (e.g., discoloration, decay, or disintegration) were dissected, and if nematodes were found inside, then they were recorded as dead. Nematode infection was confirmed by the presence of EPNs inside a dissected pupa. Mortality was calculated as the difference between the number of pupae introduced and the number of adults that successfully emerged.

Statistical analysis

Data were tested for normality using the Shapiro-Wilk test (Shapiro & Wilk, 1965). The data found to deviate from normality were transformed to meet the assumptions of ANOVA using $\log_{10}$ (x + 1) or arcsine, depending on the data type. The entire data set were subjected to analysis of variance (ANOVA) through Statistix 10 software (Gomez & Gomez, 1984). Where significant differences were detected, means were separated using the Least Significant Difference (LSD) test at the 5% probability level ($P \leq 0.05$).

RESULTS AND DISCUSSIONS

Shapiro Wilk test showed that all variables (mortality, mortality speed, pupation, and infected) were not normally distributed ($P \leq 0.05$) and were transformed accordingly.

Larval bioassay

There were no significant interactions between EPN and concentration on all measured variables. Concentrations had significant effects on all measured variables, whereas EPN were only significant for larval mortality speed. Concentration effects were concentration dependent, with concentrations of 25, 50, 75, and 100 IJs/larva not differing from each other for mortality, mortality speed, pupation, and infectivity, but all differed from the control (Table 1). The control was significantly different from all other concentrations, except 75 IJs for mortality (Table 1). The highest mean mortality, mortality speed, and infectivity of 27.4, 4.0, and 30.9% occurred at 25 IJs/50 µl, followed by 50 IJs/50 µl at 19.90, 2.8, and 26.6 %, respectively, while the control recorded the least at 1.6, 0.1, and 0%, respectively. The highest pupation of 91.8% occurred at the control.

Table 1. Effect of entomopathogenic nematode concentration on *Ceratitis capitata* larval mortality (M), mortality speed (MS), number of pupae (P), infectivity (I), and Relative Impact (RI)

Concentrations	M (%)	RI (%)	MS	RI	P (%)	RI (%)	I (%)
0	0.02^{b}(1.6)	-	0.03^{b}(0.1)	-	0.13^{a}(91.8)	-	0.00^{b}(0)
25	0.28^{a}(27.4)	1612	0.56^{a}(4.0)	3900	0.80^{b}(69.3)	-24	0.32^{a}(30.9)
50	0.20^{a}(19.9)	1143	0.48^{a}(2.8)	2700	0.94^{b}(77.6)	-15	0.27^{a}(26.6)
75	0.17^{ab}(15.7)	881	0.38^{a}(2.7)	2600	1.02^{b}(84.3)	-8	0.24^{a}(22.4)
100	0.19^{a}(19.2)	1100	0.57^{a}(3.2)	3100	0.87^{b}(75.8)	-17	0.17^{a}(16.6)
F value	3.07		5.78		4.74		4.84
P value	0.028^{*}		0.001^{**}		0.003^{**}		0.003^{**}
$LSD_{0.05}$	0.078		0.131		0.117		0.080

*Significant at $P \leq 0.05$ **Highly significant at $P \leq 0.001$; Values in brackets are untransformed means; All means followed by the same letter are not statistically different from each other. RI (%) = $\left(\frac{Treatment}{Control} - 1\right) \times 100$

Heterorhabditis bacteriophora (3.92) exhibited a higher mortality speed compared to *Steinernema jeffreyense* (1.20) (Table 2). Relative to *S. jeffreyense*, *H. bacteriophora* had 3.27 times faster mortality (Table 2).

Table 2: Effect of *Steinernema jeffreyense* and *Heterorhabditis bacteriophora* on *Ceratitis capitata* larvae mortality speed across all concentrations

Species	Mortality speed
S. jeffreyense	0.27^{b}(1.20)
H. bacteriophora	0.54^{a}(3.92)
F value	10.19
P value	0.002^{**}
$LSD_{0.05}$	0.082

**Highly significant at $P \leq 0.001$; Values in brackets are untransformed means

Both entomopathogenic nematodes infected fruit fly larvae, with *H. bacteriophora* achieving the highest infectivity among treatments. A nonlinear relationship between concentration and infection was observed, where infectivity increased at lower concentrations but decreased at higher ones. This could be due to density-dependent interactions inside the host (Selvan, 1993). At lower concentrations, mortality increased, probably because the number of nematodes was adequate for effective searching, locating, and penetrating the host. However, when too many nematodes invade a single host, they begin to compete for limited nutrients, reducing their survival and successful development. This could explain the observed reduction in infectivity at higher concentrations relative to lower concentrations.

Mortality speed, defined as how quickly nematodes killed the host, was highest at 25 IJs/larva. This indicates that from the first day after inoculation, larvae began dying more rapidly compared with other treatments. More larvae pupated in the control, as expected of the fly life cycle, which is approximately three days to pupate (Weldon, 2022). At higher concentrations, increased pupation rates were observed, particularly at 75 IJs/larva. This may suggest that larvae accelerated pupation to escape infection pressure. However, no direct studies have shown that *Ceratitis capitata* pupate faster when exposed to EPNs; however, a similar phenomenon has been reported in other insect–parasite systems. *Lobesia botrana* larvae exposed to parasitoids developed faster and reached maturity earlier, possibly as a strategy to reduce the duration of exposure to parasitism (Vogelweith et al., 2013). Infectivity values were generally higher than mortality at most concentrations, however, the opposite trend occurred at 100 IJs/larva, where mortality exceeded infectivity. This was likely because some larvae died early from physical damage caused by excessive nematode penetration attempts, before the symbiotic bacteria could establish and produce metabolites that suppress the host immune system or outcompete other microbes (Grewal et al., 1994). Since the nematodes rely on their symbiotic bacteria to create a suitable environment and provide nutrition the larvae need to survive and proliferate. As a result, when confirming the cause of death, no nematodes were found even though the larvae were dead.

Thus, the plateau in mortality above 25 IJs/larva in this study aligns with findings where further increases in nematode density result in diminishing returns.

Previous reports have indicated a linear concentration-dependent mortality, in which

mortality increases consistently with increasing concentration, as observed in *S. carpocapsae*, *H. bacteriophora*, and *S. feltiae* (Elhadidy, 2021; Yağcı, 2021; Sayed, 2023). This pattern often occurs when host-searching behaviors favor nematode movement and infection success and also where food supply is non-limiting.

Both EPNs used in the current study are considered cruising foragers that actively search for hosts (Rakubu, 2024; Langford, 2014). However, in this study, *H. bacteriophora* showed faster killing ability, with mortality observed from the first day. It exhibited higher mortality relative to *S. jeffreyense*, likely due to key differences in reproductive strategy (Cassells, 2024). *Heterorhabditis bacteriophora* produces hermaphrodites in its first generation inside the host cadaver, allowing reproduction even when few infective juveniles are present, whereas *S. jeffreyense* requires male–female mating in the first generation, which may slow early propagation. Another contributing factor could be the size of infective juveniles, *H. bacteriophora* is smaller (512-671 µm) (Adams and Nguyen, 2002) compared to *S. jeffreyense* (784-1043 µm) (Malan, 2016), which gives *H. bacteriophora* an advantage in penetrating host openings. Additionally, the presence of a dorsal tooth in *Heterorhabditis* enables active cuticle penetration (Griffin et al., 2005). These traits, combined with efficient host searching and rapid early reproduction, likely contributed to the superior performance of *H. bacteriophora* in this study.

Pupal bioassay

There were significant interactions between EPN and concentration on all measured variables. Concentration effects varied with changes in nematode concentration, with 100 IJs/pupa not differing significantly from 150 and 200 IJs/pupa in mortality, and 100 and 150 IJs/pupa not differing significantly in infectivity. However, all other concentrations (0, 100, 150, 200, and 250 IJs/pupa) differed significantly from each other (Table 3).

Table 3. Combined effect of *Steinernema jeffreyense* and *Heterorhabditis bacteriophora* concentrations on mortality and infectivity of *Ceratitis capitata*

Concentration	Mortality (%)	Infectivity (%)
0	0.07^{d}(6.6)	0.00^{d}(0)
100	0.55^{bc}(48.3)	0.05^{c}(4.9)
150	0.45^{c}(40.0)	0.07^{c}(6.6)
200	0.64^{b}(54.1)	0.16^{b}(15.8)
250	1.00^{a}(80.0)	0.27^{a}(26.6)
F value	62.52	48.18
P value	0.000^{**}	0.000^{**}
$LSD_{0.05}$	0.060	0.021

**Highly significant at $P \leq 0.001$; Values in brackets are untransformed means

The highest mean mortality and infectivity of 80.0 % and 26.6 %, respectively, both occurred at 250 IJs/pupa. The next concentration was 200 IJs/pupa with mortality and infectivity at 54.1 % and 15.8 %, respectively, while the control (0 IJs/pupa) recorded the least mortality and infectivity of 6.6 % and 0 %, respectively.

For *Steinernema jeffreyense*, concentrations of 0, 100, 150, and 200 IJs/pupa did not differ significantly in mortality, while 250 IJs/pupa caused a significantly higher mortality (68.3%) than all other concentrations (Table 4).

Table 4: Interactive effect of entomopathogenic nematode and concentration on mortality and infectivity of *Ceratitis capitata* pupae

	Mortality				Infectivity	
Concentration	*S. jeffreyense*		*H. bacteriophora*		*S. jeffreyense*	*H. bacteriophora*
	Means	RI	Means	RI	Means	Means
0	0.07^{c}(6.67)	-	0.07^{c}(6.67)	-	0.00^{d}(0)	0.00^{d}(0)
100	0.17^{c}(16.67)	150	0.93^{bc}(80.00)	1099	0.07^{c}(6.67)	0.03^{cd}(3.33)
150	0.08^{c}(8.33)	25	0.81^{cd}(71.67)	975	0.00^{d}(0)	0.13^{b}(13.33)
200	0.22^{c}(21.67)	225	1.06^{b}(86.67)	1199	0.08^{bc}(8.33)	0.24^{a}(23.33)
250	0.75^{d}(68.33)	924	1.25^{a}(91.67)	1274	0.25^{a}(25.00)	0.29^{a}(28.33)
F value	15.56				7.10	
P value	0.000**				0.000**	

**Highly significant at $P \leq 0.001$; Values in brackets are untransformed means; All means followed by the same letter are not statistically different from each other. RI (%) = $\left(\frac{Treatment}{Control} - 1\right) \times 100$

Infectivity for *S. jeffreyense* differed significantly across concentrations, except between 100 and 200 IJs/pupa. For *Heterorhabditis bacteriophora*, mortality differed significantly across all concentrations, except that 100 IJs/pupa did not differ from 150 and 200 IJs/pupa (Table 4). The 250 IJs/pupa treatment of *S. jeffreyense* did not differ significantly from the 150 IJs/pupa treatment of *H. bacteriophora* in mortality. Infectivity of *H. bacteriophora* differed significantly among concentrations; however, 200 and 250 IJs/pupa did not differ significantly, while 100 IJs/pupa did not differ from the control.

Heterorhabditis bacteriophora exhibited a higher mortality (67%) and infectivity (14%) when compared with *Steinernema jeffreyense* with 24 % and 8 %, respectively, (Table 5). Relative to *S. jeffreyense*, *H. bacteriophora* had 2.8 times faster mortality and 1.75 times faster infectivity (Table 5).

Table 5. Effect of *Steinernema jeffreyense* and *Heterorhabditis bacteriophora* on mortality and infectivity of *Ceratitis capitata* pupae

Species	Mortality (%)	Infectivity (%)
Steinernema jeffreyense	0.26^{b}(24.33)	0.08^{b}(8.00)
Heterorhabditis bacteriophora	0.82^{a}(67.33)	0.14^{a}(13.67)
F value	218.60	17.44
P value	0.000^{**}	0.000^{**}
$LSD_{0.05}$	0.038	0.013

**Highly significant at $P \leq 0.001$; Values in brackets are untransformed means

In the pupal bioassays, mortality increased with concentration. For example, *S. jeffreyense* caused 67% mortality at 250 IJs/pupa, which was higher than at other concentrations, while *H. bacteriophora* caused 92% mortality at 250 IJs/pupa. These observations are in full agreement with other reports that observed concentration-dependent mortality in pupal bioassays (Aatif, 2019; Rakubu, 2024). Such outcomes illustrate that while larval-stage responses may plateau due to density dependence at the currently used concentrations, the pupal stage continues to increase with increasing doses. This could be due to differences in anatomical and physiological constraints between larvae and pupae. Pupae, have a thicker cuticle that reduces mobility and also pose a greater barrier to infection. Higher IJ concentrations increase the chances of penetration through sheer greater numbers or opportunities for infection. Mortality was higher than infectivity across all concentrations because not all observed deaths are due to nematode infestation. Infectivity, however, was confirmed only when nematodes were found inside pupae that failed to emerge. Some pupae did not emerge, but dissection revealed fully formed adults inside; these were recorded as alive. Pupae that failed to emerge and showed no signs of adult development were recorded as dead.

CONCLUSIONS

Both entomopathogenic nematodes caused significant mortality against *Ceratitis capitata* larvae and pupae, with *Heterorhabditis bacteriophora* performing better across all concentrations. The larval stage exhibited density-dependent mortality effects, whereas the pupal stage showed concentration-dependent mortality.

Based on these findings, both nematodes are promising candidates for the biological control of *C. capitata,* but *H. Bacteriophora* appears to be particularly more efficient at higher concentrations when targeting less mobile pupal stages, while lower concentrations were still effective for the larval stage.

Further field experiments are needed to validate these laboratory results under variable environmental conditions. Future studies should also consider factors such as soil type, nematode persistence, and formulation improvements to enhance field performance and ensure the effective integration of EPNs into integrated pest management (IPM) programs.

ACKNOWLEDGEMENTS

The authors acknowledge the University of Mpumalanga for providing the academic environment and resources necessary for this research. My gratitude also goes to the organizations that provided essential materials and training. The Agricultural Research Council (ARC) and Citrus Research International (CRI) are thanked for their support, and the National Research Foundation (NRF) for financial

assistance. I also thank the University of Stellenbosch for the opportunity to attend their EPN short course, which greatly enhanced my understanding, and for providing the EPN cultures used in this study.

REFERENCES

Aatif, H., M., Hanif, M., S., Ferhan, M., Raheel, M., Shakeel, Q., Ashraf, W., Ullah, M. I. & Ali, S., 2019. Assessment of the entomopathogenic nematodes against maggots and pupae of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), under laboratory conditions. *Egyptian Journal of Biological Pest Control,* 29**,** 1-5.

Abd-Elgawad, M. M., 2021. The Mediterranean fruit fly (Diptera: Tephritidae), a key pest of citrus in Egypt. *Journal of Integrated Pest Management,* 12**,** 28.

Adams, B.J. and Nguyen, K.B., 2002. Taxonomy and systematics. *In Entomopathogenic nematology* (pp. 1-33). Wallingford UK: CABI publishing.

Ali, S., Ahmad, N., Dar, M., A., Manan, S., Rani, A., Alghanem, S., M. S., Khan, K., A., Sethupathy, S., Elboughdiri, N. & Mostafa, Y. S., 2023. Nano-agrochemicals as substitutes for pesticides: prospects and risks. *Plants,* 13**,** 109.

Authority, E. F. S., Loomans, A., Diakaki, M., Kinkar, M., Schenk, M. & Vos, S., 2019. Pest survey card on *Bactrocera dorsalis*. *EFSA Supporting Publications,* 16**,** 1714E.

Authority, E. F. S., Tramontini, S., Gilioli, G., De La Peña, E., Rzepecka, D., Scala, M., Sánchez, B., Nougadère, A., Vos, S., Research, C. F. A. & Economics, 2025. *Bactrocera dorsalis* Pest Report to support the ranking of EU candidate priority pests. Wiley Online Library.

Baker, B. P., Green, T. A. & Loker, A. J., 2020. Biological control and integrated pest management in organic and conventional systems. *Biological Control,* 140**,** 104095.

Belien, T., 2019. Entomopathogenic nematodes as biocontrol agents of insect pests in orchards. *CABI Reviews***,** 1-11.

Candia, I. A. F., 2019. Ethological control of the Mediterranean fruit fly *Ceratitis capitata* (Wied.).

Cassells, M., 2024. Resource Defence by the Entomopathogenic Nematodes Steinernematidae and Heterorhabditidae. National University of Ireland Maynooth.

Coleman, O., 2020. Efficacy of Entomopathogenic Nematodes for Control of *Tuta absoluta* in South Africa. North-West University (South Africa).

Costa, C. A., Guiné, R. P., Costa, D. V., Correia, H. E. & Nave, A., 2023. Pest control in organic farming. *Organic Farming.* Elsevier.

Cruz, R. M., Krauter, V., Krauter, S., Agriopoulou, S., Weinrich, R., Herbes, C., Scholten, P. B., Uysal-Unalan, I., Sogut, E. & Kopacic, S., 2022. Bioplastics for food packaging: environmental impact, trends and regulatory aspects. *Foods,* 11**,** 3087.

Dong, Z., He, Y., Ren, Y. Wang, G., & Chu, D., 2022. Seasonal and year-round distributions of *Bactrocera dorsalis* (Hendel) and its risk to temperate fruits under climate change. *Insects,* 13**,** 550.

Elhadidy, N.M., Badr, F.A. and Azzazy, A.M., 2021. Potential and bio-chemical effects of *Steinernema carpocapsae* (Rhabditida: Steinernematidae) an entomopathogenic nematode against *Bactrocera zonata* and *Ceratitis capitata* (Diptera: Tephritidae). *Journal of Plant Protection and Pathology*, *12*(10), pp. 725-732.

Fouad, M., Salah, L., Youssef, B., Abdelali, B., Hicham, L., Sbaghi, M., Imren, M., Ozer, G., Paultiz, T., & Lahlali, R., 2020. Potential of native entomopathogenic nematodes for the control of the Mediterranean fruit fly (*Ceratitis capitata*) (Wiedemann) (Diptera: Tephritidae).

Ganie, S., A., Rehman, S., A., Nisar, T., Paray, M., Bano, P. & Khurshid, R., 2022. Fruit Fly Management and Control Strategies: A Review. *Biopesticides International,* 18.

Gomez, K.A. and A.A. Gomez, 1984. *Statistical Procedures for Agricultural Research*, 2nd edition. John Wiley and Sons Inc., New York.

Grewal, P.S., Gaugler, R. and Lewis, E.E., 1993. Host recognition behavior by entomopathogenic nematodes during contact with insect gut contents. *The Journal of Parasitology*, pp. 495-503.

Griffin, C.T., Boemare, N.E. and Lewis, E.E., 2005. Biology and behaviour. In *Nematodes as biocontrol agents* (pp. 47-64). Wallingford UK: CABI Publishing.

Jaffar, S. & Lu, Y., 2022. Toxicity of some essential oil constituents against oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Insects,* 13**,** 954.

Kallali, N., S., Ouijja, A., Goura, K., Laasli, S.-E., Kenfaoui, J., Benseddik, Y., Blenzar, A., Joutei, A., B., El Jarroudi, M. & Mokrini, F., 2024. From soil to host: Discovering the tripartite interactions between entomopathogenic nematodes, symbiotic bacteria and insect pests and related challenges. *Journal of Natural Pesticide Research,* 7**,** 100065.

Kasana, B., 2024. The role of biocontrol in integrated pest management systems. *Ukrainian Journal of Ecology,* 14**,** 60-62.

Kung, S.P., Gaugler, R. and Kaya, H.K., 1990. Soil type and entomopathogenic nematode persistence. *Journal of Invertebrate Pathology*, *55*(3), pp. 401-406.

Langford, E.A., Nielsen, U.N., Johnson, S.N., and Riegler, M., 2014. Susceptibility of Queensland fruit fly, *Bactrocera tryoni* (Froggatt)(Diptera: Tephritidae), to entomopathogenic nematodes. *Biological Control*, *69*, pp. 34-39.

Lengai, G., M., Fulano, A., M. & Muthomi, J. W., 2022. Improving access to export markets for fresh vegetables through reduction of phytosanitary and pesticide residue constraints. *Sustainability,* 14**,** 8183.

López, S., Acín, P., Gómez-Zubiaur, A., Corbella-Martorell, C., & Quero, C., 2024. A shift in the paradigm? A male-specific lactone increases the response of both sexes of the olive fruit fly *Bactrocera*

oleae to the food lure ammonium bicarbonate. *Journal of Pest Science,* 97**,** 965-978.

Malan, A.P., Knoetze, R., and Tiedt, L.R., 2016. *Steinernema jeffreyense* n. sp.(Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. *Journal of Helminthology*, *90*(3), pp. 262-278.

Maurya, R., P., Koranga, R., Samal, I., Chaudhary, D., Paschapur, A., U., Sreedhar, M., & Manimala, R. N., 2022. Biological control: A global perspective. *International Journal of Tropical Insect Science,* 42**,** 3203-3220.

Mkiga, A. M., 2020. Damage to Citrus and Vegetables by *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) and Prospects for Control with Entomopathogenic Fungi. North-West University (South Africa).

Mokrini, F., Laasli, S.-E., Benseddik, Y., Joutei, A., B., Blenzar, A., Lakhal, H., Sbaghi, M., Imren, M., Özer, G. & Paulitz, T., 2020. Potential of Moroccan entomopathogenic nematodes for the control of the Mediterranean fruit fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). *Scientific Reports,* 10**,** 19204.

Motswagole, R., Gotcha, N. & Nyamukondiwa, C., 2019. Thermal biology and seasonal population abundance of *Bactrocera dorsalis* Hendel (Diptera: Tephritidae): implications for pest management. *International Journal of Insect Science,* 11**,** 1179543319863417.

Papadogiorgou, G., D., Papadopoulos, A., G., Moraiti, C., A., Verykouki, E. & Papadopoulos, N. T., 2024. Latitudinal variation in survival and immature development of *Ceratitis capitata* populations reared in two key overwintering hosts. *Scientific Reports,* 14**,** 467.

Rakubu, I.L., Katumanyane, A. and Hurley, B.P., 2024. Host-foraging strategies of five local entomopathogenic nematode species in South Africa. *Crop Protection*, *176*, p.106525.

Reddy, K., V., Devi, Y. K. & Komala, G., 2020. Management strategies for fruit flies in fruit crops - A review. *JETIR,* 7**,** 1472-1480.

Rodriguez, C., C., Barud, F., J., Gómez, M., P., Sayra, J. & López, M. L., 2024. Host influence on life history traits of *Ceratitis capitata* Wiedemann in an arid region of Argentina. *Bulletin of Entomological Research,* 114**,** 729-736.

Sallam, R., F., Shalaby, F., F., Hafez, A., A., Abdel-Hameid, N. F. & Shairra, S. A., 2024. Efficacy of different entomopathogenic nematode isolates against the peach fruit fly, *Bactrocera zonata* (Saund.) (Diptera: Tephritidae). *Egyptian Journal of Biological Pest Control,* 34**,** 12.

Sayed, R.M., El Sayed, T.S. and Rizk, S.A., 2023. Assessing the efficacy of un and gamma-irradiated entomopathogenic nematode, *Steinernema carpocapsae,* as an eco-friendly approach to control *Agrotis ipsilon* larvae. *International Journal of Tropical Insect Science*, 43(2), pp.609-616.

Selvan, S., Campbell, J.F. and Gaugler, R., 1993. Density-dependent effects on entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) within an insect host. *Journal of Invertebrate Pathology*, *62*(3), pp.278-284.

Shapiro, S.S. and Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). *Biometrika*, *52*(3-4), pp.591-611.

Shapiro-Ilan, D., I., Morales-Ramos, J. A. & Rojas, M., G., 2016. *In vivo* production of entomopathogenic nematodes. *Microbial-based biopesticides: methods and protocols***,** 137-158.

Sookar, P., Patel, N. & Ramkalawon, P., 2021. *Bactrocera dorsalis*, an invasive fruit fly species in Mauritius. *Fruits,* 76**,** 269-275.

Toepfer, S., Kurtz, B. and Kuhlmann, U., 2010. Influence of soil on the efficacy of entomopathogenic nematodes in reducing *Diabrotica virgifera virgifera* in maize. *Journal of pest science*, *83*(3), pp.257-264.

Tsitsipis, J.A., 1989. Nutrition. In: Robinson, AS. and Hooper, G., ed., *Fruit flies*: *Their Biology*, *Natural Enemies and Control*, Elsevier, Amsterdam, 3A: 103-119.

Vogelweith, F., Moret, Y., Thiery, D. and Moreau, J., 2013. *Lobesia botrana* larvae develop faster in the presence of parasitoids. *PLoS One*, *8*(8), p.e72568.

Wang, Y.-H., Wee, S.-L., De Faveri, S., G., Gagic, V., Hossain, S., Cheng, D., -F., Chouangthavy, B., Han, P., Jiang, H.-B. & Krutmuang, P., 2024. Advancements in Integrated Pest Management strategies for *Bactrocera dorsalis* in Asia: Current status, insights, and future prospects. *Entomologia Generalis,* 44**,** 1091-1116.

Weldon, C., 2020. *Ceratitis capitata* (Mediterranean fruit fly). *CABI Compend*, *12367*.

Yağcı, M., Akdeniz Fırat, T., Erdoğuş, F.D. and Şahin, M., 2021. Virulence of four entomopathogenic nematodes against different stages of the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Dipteryesa: Tephritidae). *Egyptian Journal of Biological Pest Control*, *31*(1), p.126.

Zhang, X., Li, L., Kesner, L. & Robert, C., A. M., 2021. Chemical host-seeking cues of entomopathogenic nematodes. *Current Opinion in Insect Science,* 44**,** 72-81.