One Health Student Conference USAMV București

Efficacy of the native entomopathogenic nematodes on the survival of *Ceratitis capitata*

Shabalala Carol and Dube Zakheleni

December 3-5, 2025, București

Introduction

- *Citrus sinensis* is widely cultivated globally (Pieterse et al., 2020)
- South Africa is the 2nd-largest citrus exporter worldwide (Chisoro, 2025)
- *Ceratitis capitata* is a major cause of production losses
- Chemical control leads to resistance, residues on fruits, and risks (Cruz et al., 2022, Lengai et al., 2022)
- Entomopathogenic nematodes (EPNs) are a sustainable non-chemical alternative
- No indigenous EPNs registered for use in South Africa (Dunn and Malan, 2025)
- Native EPN use on fruit flies is still limited
- *Heterorhabditis bacteriophora* and *Steinenerma jeffreyense* against *C. capitata*

Materials and Methods

Study area: University of Mpumalanga, laboratory at 20-25°C

Fruit fly eggs were sourced from Citrus Research International

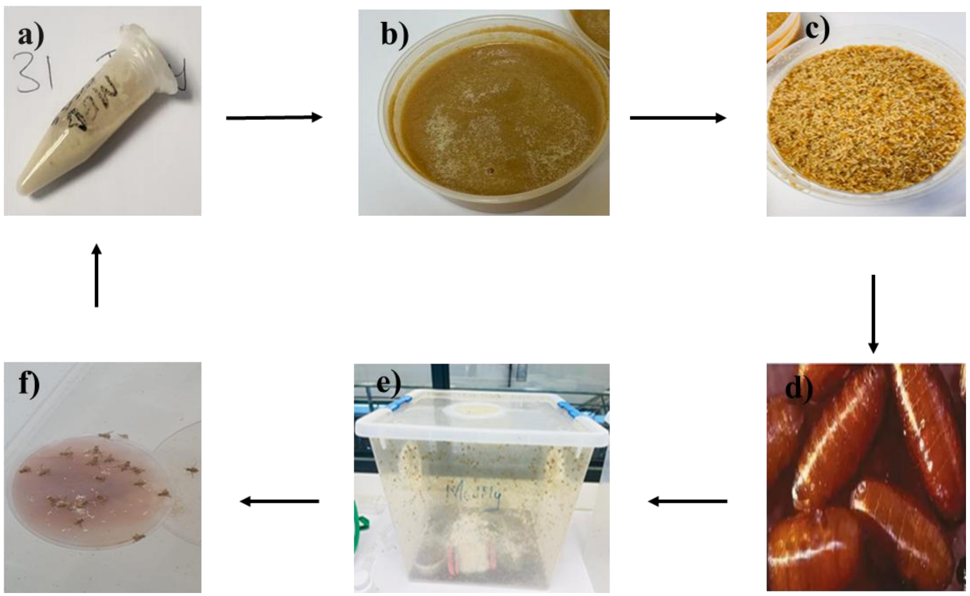

Figure 1: Diagram illustrating the breeding and rearing process of *Ceratitis capitata*. a) eggs, b) carrot-based diet inoculated with eggs, c) larvae feeding on the carrot mixture, d) pupae, e) adults in a rearing cage, and f) females ovipositing on an egg-laying device.

The EPNs were reared using meal worm larvae (*Tenebrio molitor* L.) (Shapiro-Ilan et al., 2016)

Figure 2: Mealworms

Figure 3: Microscopic view of entomopathogenic nematodes

Larval Bioassay

- Five concentrations: 0, 25, 50, 75, and 100 IJs/50 µL
- Completely randomized design with five replications
- Each species was tested in 12 alternating wells of a 24-well plate
- A 50 µL nematode suspension was pipetted into each well
- One *C. capitata* larva was introduced per well
- Data collected: mortality, mortality speed, infectivity, pupation

Figure 4: A 24-well plate

Pupal Bioassay

- Five concentrations: 0, 100, 150, 200, and 250 IJs/50 µL
- One *C. capitata* pupa was introduced per well
- A 50 µL nematode suspension was pipetted into each well
- Each set of 24 wells was placed inside a 2 L container lined with a damp paper towel
- Mortality and infectivity was recorded on the 10th day
- Mortality=No. of pupae introduced−No. of adults emerged
- Data collected: mortality and infectivity

Figure 5: Well plates inside a 2L container

Statistical analysis

- Statistix 10 software (Gomez & Gomez, 1984)
- Normality Check: Shapiro Wilk test (Shapiro & Wilk, 1965)
- Data transformation: Larval and pupal percentage mortality and infectivity were transformed using arcsine $\sqrt{(percentage \div 100)}$
- Mortality speed (larvae) data were transformed using $\log_{10}(x + 1)$
- The whole data were subjected to two-way Analysis of Variance (ANOVA)
- Where significant differences were detected, means were separated using the Least Significant Difference (LSD) test at the 5% probability level

Larval Results and Discussions

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)

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

- No significant interactions between species and concentration
- Concentrations dependent response
- Highest mean mortality and infectivity occurred at 25 IJs/larva
- Reduced mortality at higher concentrations (Puža and Mráček, 2009)
- Competition for limited nutrients and larvae pupated as a self defense

Pupal Results and Discussions

Table 2: Interactive effect of nematode species and concentration on mortality, infectivity, and Relative Impact (RI) of *Ceratitis capitata* pupae

	Mortality (%)				Infectivity (%)	
Concentration	*S. jeffreyense*		*H. bacteriophora*		*S. jeffreyense*	*H. bacteriophora*
	Means	RI (%)	Means	RI (%)	Means	Means
0	0.07^{e}(6.67)	-	0.07^{e}(6.67)	-	0.00^{d}(0)	0.00^{d}(0)
100	0.17^{e}(16.67)	150	0.93^{bc}(80.00)	1099	0.07^{c}(6.67)	0.03^{cd}(3.33)
150	0.08^{e}(8.33)	25	0.81^{cd}(71.67)	975	0.00^{d}(0)	0.13^{b}(13.33)
200	0.22^{e}(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)

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

- Concentration dependent response (Aatif, 2019; Rakubu, 2024)
- Highest mean mortality and infectivity occurred at 250 IJs/pupa
- Different anatomical and physiological constraints (Aatif, 2019)
- Pupae have thicker cuticles and are immobile
- *Heterorhabditis bacteriophora* performed better than *S. jeffreyense*
- *Heterorhabditis bacteriophora*: hermaphroditic and smaller (512-671 µm) (Adams and Nguyen, 2002)
- *Steinenerma jeffreyense*: female and male are larger 784-1043 µm (Malan, 2016)

Conclusions and Recommendations

- *Steinernema jeffreyense* and *Heterorhabditis bacteriophora* reduced the survival of *Ceratitis capitata* under laboratory conditions
- Both EPN species caused mortality in *C. capitata* larvae and pupae
- Larvae: low nematode concentrations are effective
- Pupal: high nematode concentrations are effective
- Future studies should consider factors such as soil type, nematode persistence, and formulation improvements to enhance field performance

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.
- Adams, B.J. and Nguyen, K.B. 2002. Taxonomy and systematics. In Entomopathogenic nematology (pp. 1-33). Wallingford UK: CABI publishing.
- Chisoro, S., 2025. The role of industry associations in export performance: Comparative cases of South Africa's citrus and wine industries (No. 10). Sustainable Global Supply Chains Discussion Papers.
- 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.
- Dongre, P., Doifode, C., Choudhary, S. & Sharma, N. 2023. Botanical description, chemical composition, traditional uses and pharmacology of *Citrus sinensis*: An updated review. Pharmacological Research-Modern Chinese Medicine, 8, 100272.
- Dunn, M.D. and Malan, A.P., 2025. Barriers to the Use of Entomopathogenic Nematodes as Biocontrol Agents: South Africa as a Case Study. South African Journal of Enology and Viticulture, 46, pp.80-92.
- Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Research, 2nd edition. John Wiley and Sons Inc., New York.
- Khan, M.Z., Hidayat, S., Khan, A. and Kazimi, M., 2024. Self-Sufficiency Strategies of Extension Workers to Protect Citrus Fruits from Fruit Flies in Bati Kot District, Nangarhar-Afghanistan. Journal of Natural Science Review, 2(Special. Issue), pp.39-54.
- Lengai, G. M., Fulano, A. M. & Muthomi, J. W. 2022. Improving access to export market for fresh vegetables through reduction of phytosanitary and pesticide residue constraints. Sustainability, 14, 8183.
- 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.
- 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.
- Pieterse, W., Manrakhan, A., Terblanche, J. S. & Addison, P. 2020. Comparative demography of *Bactrocera dorsalis* (Hendel) and *Ceratitis capitata* (Wiedemann)(Diptera: Tephritidae) on deciduous fruit. Bulletin of Entomological Research, 110, 185-194.
- Půža, V., & Mráček, Z. (2009). Mixed infection of *Galleria mellonella* with two entomopathogenic nematode (Nematoda: Rhabditida) species: Steinernema affine benefits from the presence of Steinernema kraussei. Journal of Invertebrate Pathology, 102(1), 40–43.
- 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.
- 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.
- Tsitsips, J.A 1989. Nutrition. In: Robinson, AS. and Hooper, G., ed., Fruit Flies: Their Biology, Natural Enemies and Control, Elsevier, Amsterdam, 3A: 103-119.

Thank you for your attention!

Carol SHABALALA

0648931423

ntokozocaroh@gmail.com

University of Mpumalanga, SA