Short Communication

Management of *Meloidogyne incognita* associated with okra (*Abelmoschus esculentus*) using moringa (*Moringa oleifera*)

Mehrnoush Aminisarteshnizi

Aquaculture Research Unit, School of Agricultural and Environmental Sciences, University of Limpopo, Sovenga, South Africa

Corresponding author email: mehrnoush.aminisarteshnizi@ul.ac.za

Okra is one of the important vegetable crops grown in tropical and sub-tropical areas worldwide. Different parts of the crop, including fresh leaves, buds, flowers, pods, stems, and seeds, are usable. Okra contains various benefits for humans, including fibre and vitamins. One of the critical pests for okra is root-knot nematode which causes crop yield loss. Therefore, an investigation was conducted in 2022 at the Aquaculture Research Unit laboratory at the University of Limpopo to evaluate the effectiveness of moringa (*Moringa oleifera*) application in controlling root-knot nematode in okra. During a survey of an okra field, *Meloidogyne incognita* was identified from the root samples. Leaf and fruit powder of moringa, along with positive control (fenamiphos) and a negative control (tap water), were used to study their efficacy against *M. incognita*. In pot trials, seedlings of okra were inoculated with 3000 eggs and second-stage juveniles (J2) of *M. incognita*; with treatments comprising leaf and fruit powder of moringa; 56 days after inoculation, the moringa leaf and fruit powder treatments compared to the negative control had, respectively, 69.2 and 56.4% fewer eggs and J2 in soil; 84.2 and 74.5% lower gall number; and 69.6 and 36.6% lower gall index. by 56.4 – 69.2%. The percentage improvements for the positive control were not significantly different from those for moringa leaf powder (P > 0.05). These results showed that moringa leaf powder is promising for controlling root-knot nematode in okra.

Keywords: *Moringa oleifera*, biological control, okra, *Meloidogyne incognita*, moringa, root-knot nematode

Okra (*Abelmoschus esculentus*) is a flowering plant in the mallow family. Okra originated in West Africa, Ethiopia, Southeast Asia, and South Asia and is an economically important vegetable crop grown worldwide in tropical and sub-tropical areas (Gemede et al. 2014). Fresh leaves, buds, flowers, pods, stems, and seeds of okra are usable. The crop contains water, protein, carbohydrates, dietary fiber, vitamin C, and vitamin K (Mihretu et al. 2014). Free-living (Shokoohi 2023; Shokoohi and Eisenback 2023) and plant-parasitic nematodes are the primary organisms in the soil that interact with each other. Plant-parasitic nematodes (*Meloidogyne spp)* are a main threat to agricultural crops (Shokoohi et al. 2023). Several *Meloidogyne* species are associated with okra (Perry et al. 2009). The genus *Meloidogyne* represents over 100 species (Karssen et al. 2013); *M. incognita* is the most dangerous plant-parasitic nematode, which causes yield loss in crops. Therefore, control of the nematode is very important in reducing plant diseases.

Although chemical nematicides have successfully managed root-knot nematodes in most crops, the products have often adversely affected animal, human, and environmental health systems (Aminisarteshnizi 2021a). Since plant extracts could be excellent alternatives for managing nematode population densities, many plant species have been examined for their nematicidal or nematostatic properties (Akpheokhai et al. 2012).

Moringa oleifera is the most widely cultivated species of the genus *Moringa*. Farmers use the moringa tree parts as a control measure due to its pesticidal, antifungal, and antifeedant properties (Sowley et al. 2014). The potential benefits of *M. oleifera* to effect biological control of root-knot nematodes in maize were demonstrated as moringa leaves and seeds have been found to possess

0041–3216/2024/010115–119

pesticidal properties (Ammer et al. 2016). Páez-León et al. (2022) demonstrated that the ethyl acetate present in moringa leaf extract has great potential for combating agricultural nematodes. Therefore, this study attempts to investigate the effects of leaf and seed powder from *M. oleifera* for its efficacy in suppressing *M. incognita* under greenhouse conditions.

Materials and methods

An experimental trial was conducted in 2022 at the Aquaculture Research Unit laboratory, University of Limpopo, using moringa leaf and fruit powder as a control measure for *M. incognita.*

Rearing of Meloidogyne species

The *Meloidogyne* species' population was reared on 3-week-old susceptible okra in plastic pots from a single egg mass collected from infected okra roots; 56 days after rearing, infected roots were removed from pots and rinsed with running tap water to remove soil particles and debris. Roots were cut into 2 - 3 cm long pieces and placed in a kitchen blender with 500 ml 1% sodium hypochlorite (NaOCl) solution to extract eggs and second-stage juveniles (J2) (Perry et al. 2009). Eggs and J2 were then collected on a 38-µm sieve after passing the aliquot through a 125-µm sieve to remove the root debris. Eggs and J2 on a 38-µm sieve were gently washed to remove excess NaOCl solution and collected in clear water in a beaker. Eggs and J2 were then placed on filter paper in a petri dish and kept in a temperature-regulated incubator at 25 ± 2ºC for 4 days until J2 was hatched (Perry et al. 2009).

Identification of the nematode

Meloidogyne species isolated were identified as *M. incognita* using morphological and morphometrical characteristics. The identified species were verified with molecular sequencing of 28S rDNA of the monoculture females, which were previously examined with a stereomicroscope (Olympus CH-2) to confirm conspecificity (Aminisarteshnizi 2021b).

Preparation of plant powder

Fresh leaves and fruits of moringa were collected from the University of Limpopo, South Africa. The leaves and fruits were separately shade-dried at room temperature and ground using an electrical steel grinder.

Greenhouse experiment

Plastic pots, 20 cm diameter, were filled with steam-pasteurised 3 kg clay and sand mixed at 2:1 (v/v) ratio, with pH = 7 and EC = 2 dS/m. The four leaf-stage of the okra seedlings were transplanted in the newly filled plastic pots. Due to heterogeneous conditions in the glasshouse, treatments were arranged in a randomised complete block design with five replications and four treatments: moringa leaf powder, moringa fruit powder, control (tap water) and fenamiphos 0.06. Plants were maintained under greenhouse conditions at temperatures ranging between 26 - 28ºC and watered as needed. Each seedling was fertilised using 5 g 2:3:2 (26) NPK fertiliser 2 days after inoculation to provide essential macro-and micro-nutrients. The experiment was repeated twice.

Inoculation with *M. incognita* second-stage juveniles (J2)

One hole (2 cm diameter) was made in each seedling rhizosphere, with 60 mL water containing 5,000 J2 *M. incognita* added to the bottom of the hole. After 24 h, moringa leaf and fruit powder were inoculated into the soil at the rate of 5 g per pot (Doaa et al. 2021). The powders were combined with the soil around the rhizosphere, where the nematodes were inoculated. Fenamiphos was applied at 0.06 mg/mL tap water: 1ul per pot (Jansson and

Rabatin 1997). Tap water was the untreated control (100 mL per pot). Plants were removed from the pots 56 days after inoculation and roots were excised from shoots. Each plant's root system was rinsed under running tap water and blotted dry on a paper towel. Recorded plant variables were root length, dry shoot mass, shoot length, and dry root mass (g). The number of galls per root system and gall index, J2 per 100 mL soil sample (Costa et al. 2020) were recorded.

Statistical analysis

Analyses of variance (ANOVA) were calculated using SPSS (ver. 2021) software. The significant differences among treatments were determined according to the least significant differences (LSD) at $P < 0.05$ level of probability.

Results

Nematode variables

According to morphological (e.g., J2, females and perineal pattern) and morphometrical characters and 28S rDNA sequence of *M. incognita* used in the current study had 99% identity with the molecularly identified populations for *M. incognita*.

The nematicide treatment (fenamiphos) produced the lowest numbers of J2 /100 mL in soil (45.3), gall number (7.87), and gall index (1.1), but there were no significant differences from the results obtained using moringa leaf powder ($P > 0.05$). Untreated soil had more nematodes than soils treated with fenamiphios and with both moringa leaf and fruit powder (Table 1).

Table 1: The effect of control of *M. incognita* associated with okra using moringa leaf and fruit powders

Treatment	J2 in soil/100 g soil	R.I%	Gall number	R.I%	Gall index	R.I%
Moringa leaf powder	50.5^{a}	-69.2	13.0^{a}	-84.2	1.8^{a}	-69.6
Moringa fruit powder	71.5^{b}	-56.4	21.0^{b}	-74.5	3.8^{b}	-36.6
Control	164.0^{c}	0.0	82.5^{c}	0.0	6.0^{c}	0.0
Fenamiphos 0.06	45.3^{a}	-72.3	7.8^{a}	-90.4	1.1^{a}	-81.6
SE	0.74		0.67		0.42	

R. I: relative impact (%) = [(treatment/control) - 1] × 100. Different letters in superscript signify statistically significant differences from one another ($P \leq 0.05$)

The moringa powders reduced the number of eggs and J2 in soil by 56.4 - 69.2%, gall number by 74.5 - 84.2%, and gall index by 36.6 - 69.6%. The best moringa treatment was leaf powder which resulted in significantly fewer nematodes and galls than the control ($P \leq 0.05$), and this was not significantly different from fenamiphos ($P > 0.05$). There was also a significant difference between moringa fruit powder and control ($P \leq 0.05$)

Plant growth parameters

The result indicated dry root mass (g), shoot length (cm), root length (cm), and dry shoot mass (g) were increased due to the use of fenamiphos 0.06, however, there was no significant difference among moringa leaf powder, moringa fruit powder and fenamiphos 0.06 ($P > 0.05$) (Table 2)

Table 2: Plant-parameter values using moringa leaf and fruit powder

Treatment	Dry root mass (g)	Shoot length (cm)	Root length (cm)	Dry shoot mass (g)
Moringa leaf powder	0.3[a]	28.7[a]	59.5[a]	1.4[a]
Moringa fruit powder	0.3[a]	26.9[a]	48.2[a]	1.3[a]
Control	0.2[b]	18.8[b]	27.8[b]	1.0[b]
Fenamiphos 0.06	0.4[a]	29.2[a]	60.3[a]	1.4[a]
SE	0.047	2.22	1.38	0.10

Different letters in superscript signify statistically significant differences from one another ($P \leq 0.05$)

Discussion

The study showed nematicidal effects against *M. incognita* by leaf powder of moringa significantly reducing the gall index and gall root number of this nematode pest. These effects were pronounced for moringa plant powder, in particular, which was highly effective against *M. incognita*. These results agree with those obtained by Páez-León et al. (2022) with aqueous extracts of leaves of moringa (*M. oleifera*), against *Haemonchus contortus* and *Nacobbus aberrans*. Another study by Sowley et al. (2014) showed moringa leaf powder could control root-knot nematode. Several studies have reported 70 - 100% root-knot nematode mortality using different aqueous moringa formulations extracts. El-Ansary and Al-Saman (2018) showed moringa could control root-knot nematode *M. incognita* in bananas. In the present study, the increase in the percentage of immobility and mortality caused by the higher concentrations of moringa leaf powder was observed. This is in agreement with Sowley et al. 2014.

Conclusion

There is a great need for eco-friendly nematode control. The leaf and fruit powder of moringa possess nematicidal characteristics and can be used to manage root-knot nematodes. Although this plant is commonly available in most parts of South Africa, it should be more surveyed to control root-knot nematodes in okra orchards. Therefore, because this study was a greenhouse pot test, it is more realistic to infer that the findings indicate the potential for moringa to be used in an integrated pest management programme.

References

Akpheokhai, I.L., A.O. Claudius-Cole, and B. Fawole. 2012. "Evaluation of Some Plant Extracts for the Management of *Meloidogyne incognita* on Soybean (*Glycine max*)." *World Journal of Agricultural Sciences* **8**:429–435.

Aminisarteshnizi, M. 2021a. "Efficacy of Neem (*Azadirachta indica*) Formulations on Suppression of Root-Knot Nematodes (*Meloidogyne javanica*) and Growth of Eggplant (*Solanum melongena*)." *Research on Crops* **22 (1)**: 158–162.

Aminisarteshnizi, M. 2021b. "Lipid Content in the Juvenile, Female and Male of *Acrobeles complexus* Nematode." *Research on Crops* **22 (1)**: 167–170.

Ammer M.R., S. Zaman, M. Khalid, M. Bilal, S. Erum, D. Huang, and S. 2016. "Optimization of Antibacterial Activity of *Eucalyptus tereticornis* Leaf Extracts Against *Escherichia coli* Through Response Surface Methodology." *Journal of Radiation Research and Applied Sciences* **9 (4)**: 376–385.

Costa S.R., S. Chin, and U. Mathesius. 2020. "Infection of *Medicago truncatula* by the Root-Knot Nematode *Meloidogyne javanica* Does not Require Early Nodulation Genes." *Frontiers in Plant Science* **11**. Doi: 10.3389/fpls.2020.01050.

Doaa, K, A.R. Refaei, and F.A.M. Mostafa. 2021. "Management of Meloidogyne incognita Infecting Eggplant Using Moringa Extracts, Vermicompost, and Two Commercial Bio-Products. Egypt J Agronematol **20 (1):** 1–16. 1. https://doi.org/10.21608/ejaj.2021.134910.

El-Ansary, M.S., and M.A. Al-Saman. 2018. "Appraisal of *Moringa oleifera* Crude Proteins for the Control of Root-Knot Nematode, *Meloidogyne incognita* in Banana. *Rendiconti Lincei Sci. Fisich. Nat.*, 29: 631-637. https://doi.org/10.1007/s12210-018-0692-9.

Gemede H.F., N. Ratta, G.D. Haki, and A.Z.W.F. Beyene. 2014. "Nutritional Quality and Health Benefits of Okra (*Abelmoschus esculentus*): A Review." *Global Journal of Medical Research: K Interdisciplinary* **14 (5)**: 28–37.

Jansson, R., and S. Rabatin. 1997. "Curative and Residual Efficacy of Injection Applications of Avermectins for Control of Plant-Parasitic Nematodes on Banana." *Journal of Nematology*. **29 (4S)**: 695–702.

Karssen, G., W. Wesemael, and M. Moens. 2013. "Root-Knot Nematodes." In *Plant Nematology*, 2nd ed., edited by R.N. Perry and M. Moens, 73–108. *CAB International, Wallingford, UK.*

Mihretu, Y., G. Wayessa, and D. Adugna. 2014. "Multivariate Analysis among "Okra" (Abelmoschus esculentus (L.) Moench) Collection in South Western Ethiopia." *Journal of Plant Sciences* **9 (2)**: 43–50.

Páez-León, S.Y., M. Carrillo-Morales, O. Gómez-Rodríguez, G. López-Guillén, G. Castañeda-Ramírez, E. Hernández-Núñez, A. Wong-Villareal, and L. Aguilar-Marcelino. 2022. "Nematicidal Activity of Leaf Extract of *Moringa oleifera* Lam. Against *Haemonchus contortus* and *Nacobbus aberrans*." *Journal of Helminthology* **96**: E13.

Perry, R.N., M. Moens, and J.L. Starr. 2009. "Root-Knot Nematodes." *CAB International, Wallingford, UK.*

Shokoohi, E. 2023. "Impact of Agricultural Land Use on Nematode Diversity and Soil Quality in Dalmada, South Africa." *Horticulturae* **9 (7)**:749.

Shokoohi, E., and J. Eisenback. 2023. "Description of *Anaplectus deconincki* n. sp. from South Africa." *Journal of Helminthology* **97**: e52. doi: 10.1017/S0022149X23000330.

Shokoohi, E, N. Moyo, and F. Gouveia. 2023. "Relationship of Nematodes in Natural and Disturbed Land with Physicochemical Properties in Magoebaskloof, Limpopo Province, South Africa." *Biologia* **18**:3223–3233.

Sowley E., F. Kankam, and J. Adomako. 2014. "Management of Root-Knot Nematode (*Meloidogyne spp.*) on Sweet Pepper (*Capsicum annuum* L.) with Moringa (*Moringa oleifera* Lam.) Leaf Powder. *Archives of Phytopathology and Plant Protection* **47 (13)**: 1531–1538.