Research Article

Field Evaluation of Native Ghanaian Medicinal Botanicals for Management of Plant Root-Feeding Nematodes on Tomato (*Solanum lycopersicum* L.)

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Abstract | This study examined brimstone tree (*Morinda lucida* Benth), neem plant (*Azadirachta indica* A-H de Jussieu) and bitter leaf (*Vernonia amygdalina* Delile) from Ghana against native vertisol-dwelling plant root-feeding nematodes (PRFNs) on tomato plants in the field. The objective was to compare the efficacies among these native Ghanaian medicinal botanicals in suppressing mixture of PRFNs in a field trial. Therefore, treatments were done by introducing 50 mL of 400 g L^{-1} of suspension of smoothly-mashed green leaf extract from each botanical into the rhizosphere of each growing tomato plant in the field. Six weeks later, data for numbers of PRFNs belonging to diverse genera, 'visible root galling severity (VRGS)' and 'root weight gained' in treatments were collected. Using R-software, computed *P*-values of *Shapiro-Wilk* tests for normality in all data were ≤ 0.05. Consequently, non-parametric *Dunn*'s post-hoc test was used for pairwise multiple comparisons of 'mean ± standard error' values between treatments. The result showed that *M. lucida*, *A. indica* and *V. amygdalina* suppressed VRGS by 26 to 33%, relative to control. Moreover, each of the three medicinal botanicals in treatments caused tomato plants to gain similar additional fresh root weights, ranging from 18 to 30%. Notwithstanding, treatments with *M. lucida*, *A. indica* and *V. amygdalina* suppressed overall PRFNs by 67±8, 57±13 and 82±9%, respectively. Also, *V. amygdalina* significantly ($P \leq 0.05$) suppressed root-knot nematodes (*Meloidogyne* spp.) better than *M. lucida* and *A. indica* could achieve. Hence, *V. amygdalina* was more promising. However, considerations for research on more native medicinal botanicals have been discussed.

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Introduction

Soils at any biospheric geographical locations on Earth, on which plants exist or grow, are naturally infested by diverse plant root-feeding nematodes (PRFNs) (Fourie *et al*., 2017; Perry *et al*., 2024). These soil-borne PRFNs are serious problems on crops in the field, thereby threatening global food security and/ or sustainable development goal two (2) of United Nations (Hassan *et al*., 2013; Fourie *et al*., 2017; Devi *et al*., 2024; Kantor *et al*., 2024; Perry *et al*., 2024). This is because mixed infections of growing plant roots by diverse plant root-feeding nematodes (PRFNs) are often observed in the field (Perry *et al*., 2024). Consequently, infected plant roots often suffer from various forms of PRFN-induced damages, which largely include root galling, rots, root lesions, stubby roots and amalgamation of root tips, among others (Heve *et al*., 2015a, b; Fourie *et al*., 2017; Perry *et al*., 2024). Although some plant parasitic nematode groups have been considered to be more damaging than others on crops in the field (Perry *et al*., 2024), parasitism effects of all plant root-feeding nematodes also lead to secondary infections of the attacked plant roots in soil ecosystems (Perry *et al*., 2024). Feeding behaviour of some PRFNs mostly interacts with fungal and bacterial pathogens in the rhizospheres of growing plants in the field, whereas others potentially vector and inject virulent systemic non-cellular akaryotic entities into roots of crops, during feeding on plant roots in a soil environment (Heve *et al*., 2015a; Fourie *et al*., 2017). In effect, dying crops, poorly growing plants and yield losses in cultivated fields are mostly due to the combined damages by phytonematodes themselves and the secondary infections they facilitate in the rhizospheres of crops (Perry *et al*., 2024).

Management of PRFNs using surface applied nematicides in commercially cultivated fields can be very challenging, because soil-borne plant parasitic nematodes are always shielded in complex soil ecosystems. Thus, these unique characteristics of soil-borne PRFNs require integrated management approaches to effectively suppress them in cultivated pieces of farmland in order to meet the rising global demand for food and crop plant commodities (Afzal and Mukhtar, 2024; Perry *et al*., 2024).

Unfortunately, statutory regulatory laws are increasingly banning, restricting and controlling several synthetic agricultural pesticides worldwide (Kwakye *et al*., 2019; Desaeger *et al*., 2020; Sethi *et al*., 2022; Catani *et al*., 2023; FAO, 2024). As a result, research into a search for naturally-available bionematicidal agents as options for management of PRFNs on crops in fields has increasingly received attention across many countries worldwide (Yasmin *et al*., 2003; Dandurand *et al*., 2019; Dutta *et al*., 2019; Okechalu *et al*., 2021; Catani *et al*., 2023; Etim *et al*., 2024; Maher *et al*., 2024; Azeem *et al*., 2025). This is largely because there has absolutely been no serious regulatory pressure on the use of bioagents and/or botanicals for management of plant-damaging nematodes in the field. Thus, many studies have reported nematicidal properties of botanicals, whether they were examined for use as trap crops, biofumigation strategies, or application of their extracts for management of phytonematodes in cultivated fields (Yasmin *et al*., 2003; Dandurand *et al*., 2019; Dutta *et al*., 2019; Okechalu *et al*., 2021; Catani *et al*., 2023; Etim *et al*., 2024; Maher *et al*., 2024).

According to recent reports (Asiedu *et al*., 2019; Nyaku and Brentu, 2022; Heve and Aniapam, 2025; Heve and Otabil, 2025), incidence of diverse PRFNs in Ghanaian soils has been observed in forest areas, grassland environments and agricultural fields. Moreover, from practical standpoint, tomato (*Solanum lycopersicum* L.) plants are generally susceptible to soil-borne PRFNs (Heve *et al*., 2015a). Notwithstanding, we hypothesized that native medicinal plants will offer protection for tomato plant roots against diverse PRFNs in the field. This is because observations in reports by Heve *et al*. (2016, 2017, 2018) showed that field trials reveal the true potential effects of treatments against targeted pests in the field better than *in-vitro* trials under laboratory and glasshouse conditions. Therefore, three locally-available medicinal botanicals, which included brimstone tree (*Morinda lucida* Benth), neem plant (*Azadirachta indica* A-H de Jussieu) and bitter leaf (*Vernonia amygdalina* Delile), among others, in Ghana were tested for their suppressive potential against diverse naturally soil-inhabiting PRFNs on tomato plants in the field. Observations from this field trial were to provide answers to the following questions. If the performances of treatments were compared to those of a control in a field trial, would:

- the selected botanicals significantly suppress naturally occurring diverse PRFNs on tomato plants in an agricultural field?
- suppressive effects of the selected botanicals against diverse PRFNs reduce visible root galling severity (VRGS) of tomato plant roots in treatments?
- suppressive potential of the selected botanicals against diverse PRFNs increase root weight of tomato plants in treatments?

The main objectives of the current study were to use a 'field trial method' to:

- screen the three selected native medicinal plants against any naturally-occurring PRFNs in the field;
- identify the most promising botanical among the three selected ones for management of PRFNs in the field.

Materials and Methods

Study site

The field trial was done in an agricultural field located at (geographical coordinate = 6° 03′02′ N, 0° 00′15′ W; elevation = 70.36 m), in the period from 6th September, 2024 to 8th November, 2024, during which observed precipitation was 329 mm. This site is within an area of the University of Environment and Sustainable Development, which is in the Yilo Krobo Municipal in the Eastern Region of Ghana. Soils in the study site and other areas within Yilo Krobo Municipal are largely calcic vertisols, which have 32 to 41% clay content (Owoade *et al*., 2021; Baidoo *et al*., 2024). The study site was an open-space that had natural resources such as sunlight during the day, irregular rainfall and inherent soil nutrients.

Pre-planting treatments to the study site: The field had been fallowed for more than 10 years. Natural bushy shrubs and grasses present at the study site were weeded close to the ground, using a cutlass to achieve zero-tillage method, which was to maintain structure of topsoil in the field (Lamptey *et al*., 2017; Baidoo *et al*., 2021). On the third day following the day bushy plants were cleared, all the mulches or the plant residues were removed to allow new weeds to regenerate and develop for a month in the study field. After that, zero-tillage by clearing all newly growing weeds was done again, using a cutlass. Again, the use of a cutlass for zero-tillage was to maintain and preserve structure of topsoil, which often houses more diverse soil-borne PRFNs, whose roles were vital in determining the status of tomato plant health in the field for the study.

Confirming incidence of plant root-feeding nematodes (PRFNs) in soil samples from the study site: Asiedu *et al*. (2019), Nyaku and Brentu (2022), Heve and Aniapam (2025) and Heve and Otabil (2025), among others, have sufficiently proved that potential nematodes (PRFNs) could be widespread and endemic in agricultural fields across Ghana. Moreover, Heve and Otabil (2025) have recently reported diverse genera of PRFNs in soil samples from plots within the same study site. Hence, no initial survey was done to establish incidence of any PRFNs at the study site again. Therefore, comparison of PRFNs and their damages on tomato plant roots in replicates of 'control' to those of botanical treatments in the field was more considered to be necessary, similar to the field trial techniques used by Heve *et al*. (2017, 2018).

Culture of tomato seedlings

About 30 kg of the calcic vertisols from an agricultural field located at (geographical coordinate = 6°03′00″ N, 0°00'23″ W; elevation = 77.47 m) was autoclaved at 121 °C for 15 minutes to kill any soil-borne pathogens and phytonematodes. After that, the autoclaved soil sample was allowed to cool overnight.

On 13th August, 2024, about 100 seeds of tomato (variety = ROMA VF) were nursed on 15 kg of the autoclaved soil contained in a plastic basket (60 x 40 x 30 cm^3). After that, the set-up was placed on a garden wooden bench. Developing tomato seedlings were maintained by regular watering copiously until they were transplanted onto the experimental plot, which was simultaneously prepared alongside maintaining tomato seedlings in the nursery. Moreover, the tomato seedlings in the nursery did not receive pesticides and/or fertilizer(s).

Experimental design for treatments before transplanting tomato seedlings

A treatment was from each of the three (3) different medicinal botanicals, which included *M. lucida, A. indica* and *V. amygdalina*. The fourth (4th) treatment was tapwater as the control.

Therefore, the weeded study site had experimental layout designed first before transplanting was done. The experimental plot was divided into 10 rows. Each row had four (4) planting stations, which were pegged. The distance between two successive planting stations in each row was 1 m, whereas the distance between two successive rows was 2.4 m. To achieve a completely randomized block design layout, each of the four (4) treatments was randomly represented in each of the 10 rows. Therefore, each pegged planting station at the study site was labelled for each of the four (4) treatments before transplanting tomato seedlings.

Transplanting tomato seedlings to the pegged planting stations for each of the four (4) treatments

On 6th September, 2024, three-and-half week-old tomato seedlings were transferred from the nursery to each of the pegged planting stations at the study site. Initially, each planting station had two tomato seedlings, but one was later thinned out to maintain a healthy-looking tomato plant for the study.

After transplanting, rainfall was irregular. Therefore, watering was correctly done, when necessary, to sustain the growth of healthy-looking tomato plants in the field. However, after transplanting, none of the growing tomato plants received pesticides and fertilizer(s) in the field.

Preparation of freshly-harvested green leaves of each medicinal botanical for treatments in the field

Phytochemical constituents of leaf extracts from brimstone tree (*M. lucida*), neem plant (*A. indica*) and bitter leaf (*V. amygdalina*) have recently been reported (Okechalu *et al*., 2021; Ujah *et al*., 2021; Okhale *et al*., 2024). Therefore, similar phytochemical analyses of leaf extracts from each of these three (3) medicinal botanicals were not necessary for this study.

About 200 g of freshly-harvested green leaves of each medicinal botanical was smoothly mashed into a watery paste, using a mixer grinder (BOSCH™) at 45,000 rpm (revolutions per minute) for 0.2 hours. About 500 mL of tapwater was added to adjust the concentration of each 'paste suspension' from each medicinal botanical to be 400 g L^{-1}. After that, each suspension was thoroughly stirred, using a hand-held stirrer. Each of the paste suspensions was used for treatments in the field, immediately after freshly preparing it on the same day.

Application of treatments into rhizospheres of tomato plants in the field

According to the report of Okechalu *et al.* (2021), low doses of medicinal botanical extracts poorly suppressed plant-parasitic nematodes. Therefore, on 27th September, 2024, about 50 mL of 400 g L^{-1} of the paste suspension from each medicinal botanical was introduced into the rhizosphere (i.e., root zone) of each growing tomato plant per planting station in the field, using a 10-mL graduated transparent plastic syringe. The treatment for the 'control' was 50 mL of tapwater only, which was used to prepare the leaf extracts from each medicinal plant. Ten (10) replicates were made for each of the treatments, including control, in such a way that a replicate of a treatment was randomly positioned in each of the 10 rows in the experimental plot (see sub-section 2.3). This experimental design was used because distribution of PRFN son crops usually occurs in symptomatic patchy spots across a cultivated field (Heve *et al*., 2015b; Perry *et al*., 2024). After treatments, all the tomato plants were copiously watered when needed in the field, because observed precipitation from 27th September, 2024 to the end of the field trial on 8th November, 2024 was low (i.e., 158 mm). Also, weeds were regularly controlled, using a cutlass. However, after treatments were done, none of the tomato plants received pesticides and fertilizers until data were collected.

Data collection

Visible root galling severity (VRGS) in percentage: On 8th November, 2024, each tomato plant, with a bulk of ample soil around its roots, was carefully uprooted, using a hand-held trowel. The soil sample around the roots of each uprooted tomato plant was gently removed into a 600 mL-polythene bag. The removal of soil from the roots of each uprooted plant made the roots to be visibly clean for visualization and inspection. Therefore, visually-estimated percentage of a tomato plant root system, which was covered by visible galls, was considered to be "VRGS (%)" (Schwarz *et al*., 2024). This gall rating method was also similar to the way Heve *et al.* (2015a) visually estimated the proportion of surface area of a carrot (*Daucus carota* L.), which was covered by *Meloidogyne chitwoodi*-induced galls.

Determination of fresh root weight gained: After estimating VRGS, fresh root weight (FRW) of each tomato plant was measured, similar to the procedures used by Heve *et al.* (2015a). Hence, the percentage of FRW gained by each tomato plant was assessed, using Equation 1 as:

$$R_{wg} = 100\left[\frac{W_r - A_{control}}{A_{control}}\right] \quad \dots(1)$$

Where; R_{wg} was the percentage of fresh tomato plant root weight (FTPRW) gained by each tomato plant in each replicate of each of the treatments with botanicals; W_r was a measured FTPRW for a replicate of a medicinal botanical treatment; $A_{control}$ was the average FRW of all tomato plants from all the replicates of the control.

Extraction of actively-motile live plant root-feeding nematodes (PRFNs) from soil samples and roots: A mixture of different actively-motile live uncharacterized nematodes was extracted from 100 g of each soil sample, removed from roots of uprooted tomato plant in each replicate, using the Baermann funnel method (OEPP/EPPO, 2013; Tintori *et al*., 2022). This same Baermann funnel method, which is similar to the 'mist-chamber system' used by Heve *et al*. (2015a), was used to extract live nematodes from the tomato plant roots, in over one and half weeks.

Identification and quantification of actively-motile live uncharacterized plant root-feeding nematodes PRFNs per tomato plant: Almost all PRFNs from agricultural fields in Ghana are not validly characterized to species level (Nyaku and Brentu, 2022). However, using nematode morphological structures described by Smart and Nguyen (1988), Mekete *et al*. (2012), Heve *et al*. (2015b), Fourie *et al*. (2017), Heve (2021) and Perry *et al*. (2024), the individual live uncharacterized PRFNs in 5 mL of a nematode suspension for each replicate of each treatment were identified to the genus level only, under a microscope. The diagnosed native vertisol-dwelling live uncharacterized PRFNs were counted in a 5 ml of suspension. After that, total number of individual PRFNs belonging to each genus per volume of suspension for each replicate was quantified per tomato plant, using the Equation 2, similar to the procedures used in reports (Heve *et al*., 2015a; Heve and Aniapam, 2025; Heve and Otabil, 2025):

$$N_{gr} = \frac{C \times V}{5} \quad \ldots (2)$$

Where, N_{gr} was the total number of individual nematodes belonging to a genus of PRFNs per tomato plant for a replicate; C was the count of individual nematodes identified for a genus in 5 mL of suspension in a Petri-dish (diameter = 3.5 cm); V was the total volume of a nematode suspension obtained from "100 g of soil sample + tomato roots" in the modified Baermann funnel method for a replicate.

Percentage of plant root-feeding nematodes (PRFNs) suppressed by each medicinal botanical: Numbers of the overall PRFNs were obtained by pooling or summing up the numbers of individuals belonging to each genus of PRFNs, observed in each replicate of each treatment. After that, the percentage of PRFNs suppressed by each medicinal botanical treatment in the field was calculated, using Equation 3 as:

$$D_{sb} = 100\left[\frac{(\bar{y}_{control}) - (P_{rb})}{(\bar{y}_{control})}\right] \quad \ldots (3)$$

Where D_{sb} was the percentage of PRFNs suppressed in each replicate of each of the treatments with medicinal botanicals in the field; $\bar{Y}_{control}$ was the average of numbers of overall plant root-feeding nematodes (OPRFNs) in replicates of the control; P_{rb} was the number of OPRFNs in a replicate of a medicinal botanical treatment.

Data analysis

R software [R.v.4.4.1~/~RStudio.v.SHA-2562c3cf96a] designed by R Core Team (2024) for programming was used for all statistical analyses. The 'mean ± standard errors (SE) of observations were estimated across treatments and then presented in bar graphs.

Statistical diagnostic test for normality in data: Test for normality in data is always recommended so that the correct statistical model can be chosen and used to analyze the data (Gotelli and Ellison, 2013; Dinno, 2015; Mishra *et al*., 2019). In this case, if the *P*-value of *Shapiro-Wilk* test is greater than 0.05, parametric statistical models must correctly be used to analyze the data. However, if the *P*-value of *Shapiro-Wilk* test is less than 0.05 (Gotelli and Ellison, 2013; Dinno, 2015; Mishra *et al*., 2019), non-parametric statistical models must correctly be chosen and applied to the data.

Choice of correct post-hoc test for pairwise comparison of values of 'mean ± standard error' between treatments: *Shapiro-Wilk* test was firstly used to diagnose whether the data collected in this study were normally distributed or not, according to reports by Gotelli and Ellison (2013), Dinno (2015) and Mishra *et al*. (2019). The observed *P*-value of *Shapiro-Wilk* test for normality was ≤ 0.05 for each set of data collected in this study. Therefore, *Dunn*'s test at $P \leq 0.05$ was used for pairwise comparison of 'mean ± SE' values across the categorical groups (i.e., the treatments), similar to the way data were analyzed in recent reports of Heve and Aniapam (2025) and Heve and Otabil (2025).

Results

Response of root lesion nematodes (Pratylenchus spp.) to treatments of tomato plant roots with medicinal botanicals

The 'mean ± SE' value of number of *Pratylenchus* spp. observed in treatments of growing tomato plant roots with either *A. indica* or *V. amygdalina* was significantly ($P \leq 0.05$) the lowest, when compared to that of control (Figure 1A). Thus, the 'mean ± SE' value of number of *Pratylenchus* spp. detected in treatments of roots with *M. lucida* or tapwater (i.e., control) was significantly ($P \leq 0.05$) larger than those in the treatments with *A. indica* and *V. amygdalina* (Figure 1A). However, the 'mean ± SE' value of number of *Pratylenchus* spp. in treatment of roots with *M. lucida* was similar to that of the control (Figure 1A).

Effects of treatments with medicinal botanicals on density of root-knot nematodes (RKN) (Meloidogyne spp.*)*

Each of the three (3) botanicals in treatments significantly ($P \leq 0.05$) had lower 'mean ± SE' values of numbers of RKN (*Meloidogyne* spp.), when compared to that of the control (Figure 1B). The lowest 'mean ± SE' values of numbers of RKN were observed in the treatments of roots with *M. lucida* and *V. amygdalina* (Figure 1B), although the 'mean ± SE' value of number of RKN observed in the treatment with *M. lucida* was similar to that of the treatment with *A. indica* (Figure 1B). Nonetheless, the 'mean ± SE' value of number of RKN observed in the treatment with *A. indica* was significantly ($P \leq 0.05$) larger than that of *V. amygdalina* in treatment (Figure 1B).

Response of dagger nematodes (Xiphinema spp.*) to medicinal botanicals*

Numbers of *Xiphinema* spp. observed in treatments of roots with *M. lucida*, *A. indica* and *V. amygdalina* were not significantly ($P > 0.05$) different from one another (Figure 1C). Moreover, the 'mean ± SE' value of number of *Xiphinema* spp. in the treatment of roots with *V. amygdalina* was similar to that of the control (Figure 1C). However, number of *Xiphinema* spp. in treatment of roots with either *M. lucida* or *A. indica* was significantly ($P \leq 0.05$) lower than that of the control (Figure 1C).

Responses of lance, needle and spiral nematodes to treatments of roots

In Figure 1D, lance nematodes (*Hoplolaimus* spp.) observed across treatments were few. Thus, no significant difference in numbers of *Hoplolaimus* spp. between treatments was observed (Figure 1D).

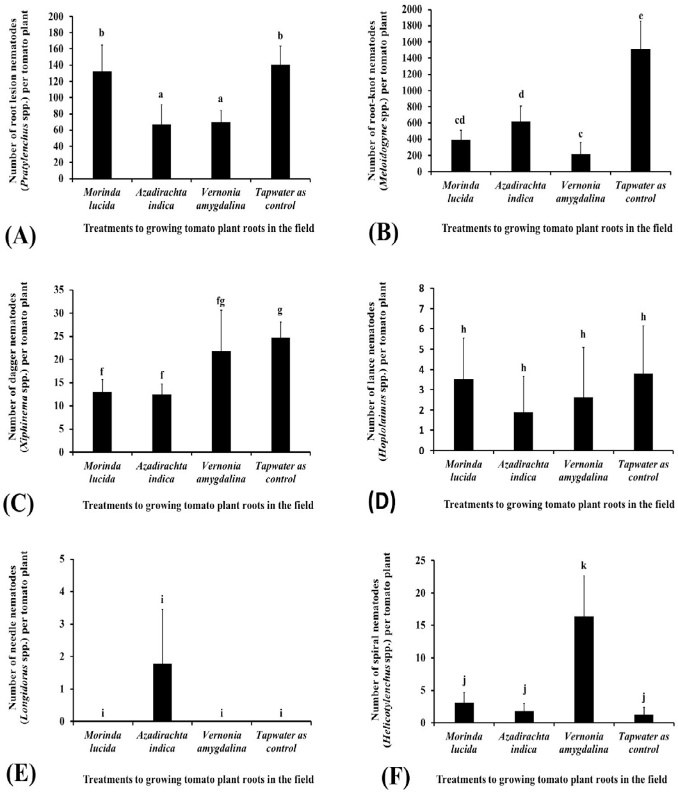

Figure 1: *Number of plant root-feeding nematodes per tomato plant across treatments of roots in the field: (A) root-lesion nematodes (Pratylenchus* spp.*); (B) RKN (Meloidogyne* spp.*); (C) dagger nematodes (Xiphinema* spp.*); (D) lance nematodes (Hoplolaimus* spp.*); (E) needle nematodes (Longidorus* spp.*); (F) spiral nematodes (Helicotylenchus* spp.*). According to Dunn's test at P ≤ 0.05, different lowercase letters on top of bars, in each of the categorical groups A, B, C, D, E and F, denote that 'mean ±SE' values were significantly different between different treatments of roots.*

In Figure 1E, about '2 ± 2' needle nematodes (*Longidorus* spp.) were observed in the treatment of roots with *A. indica*. No *Longidorus* spp. were detected in the three (3) remaining treatments of roots with *M. lucida*, *V. amygdalina* and the control (Figure 1E). The 'mean ± SE' values of numbers of spiral nematodes (*Helicotylenchus* spp.) in the treatments of roots with *M. lucida*, *A. indica* and the control were similar (Figure 1F). However, the 'mean ± SE' value of number of *Helicotylenchus* spp. detected in the treatment of roots with *V. amygdalina* was significantly ($P \leq 0.05$) larger than those of the treatments of roots with *M. lucida*, *A. indica* and tapwater (or the control) (Figure 1F).

Response of overall plant root-feeding nematodes (OPRFNs) to treatments of tomato plant roots

The 'mean ± SE' values of numbers of OPRFNs observed in treatments of roots with *M. lucida*, *A.*

indica and *V. amygdalina* were significantly ($P \leq 0.05$) lower than that of the control (Figure 2A). Although the 'mean ± SE' value of number of OPRFNs in treatment of roots with *V. amygdalina* was the lowest, no significant difference could be observed in numbers of OPRFNs between *V. amygdalina* and *M. lucida* in treatments of roots (Figure 2A). Similarly, the 'mean ± SE' value of number of OPRFNs in treatment of roots with *M. lucida* was not significantly ($P > 0.05$) different from that of the treatment of roots with *A. indica* (Figure 2A). Notwithstanding, the 'mean ± SE' value of number of OPRFNs in treatment of roots with *V. amygdalina* was significantly ($P \leq 0.05$) lower than that of the treatment of roots with *A. indica* (Figure 2A). In effect, the 'mean ± SE' value of percentage of OPRFNs suppressed in treatment of roots with *V. amygdalina* was the highest (i.e., 82 ± 9%) (Figure 2B).

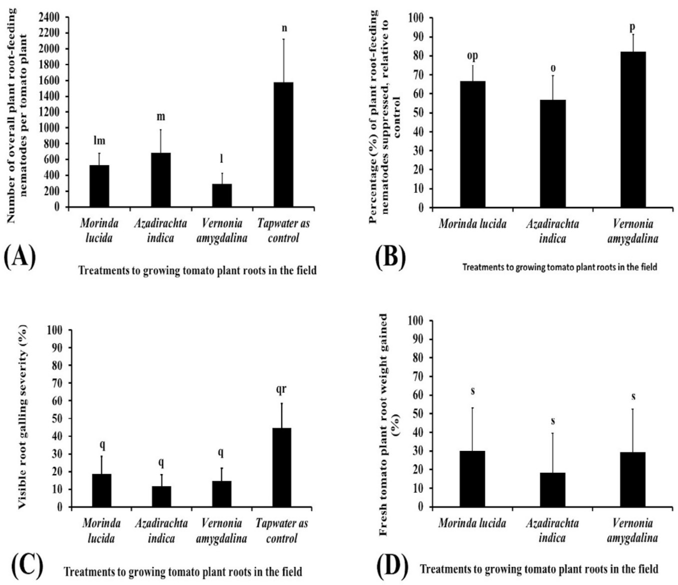

Figure 2: *A summary of detailed observations per tomato plant across treatments of roots in the field: (A) numbers of OPRFNs per tomato plant; (B) percentage (%) of plant root-feeding nematodes suppressed, relative to the control; (C) visible root galling severity (%); (D) fresh tomato plant root weight gained (%), relative to the control. Dunn's test at P ≤ 0.05: different lowercase letters on top of bars, in each of the categorical groups A, B, C and D, indicate that 'mean ±SE' values were significantly different between different treatments of roots.*

Visible root galling severity (VRGS) and fresh root weight gained (FRWG)

The VRGS on tomato plants in treatment with tapwater (or the control) was about 45% (Figure 2C). However, VRGS achieved by the treatment with *M. lucida, A. indica* and *V. amygdalina* were about 19, 12 and 15%, respectively (Figure 2C). Thus, the damage by root-knot nematodes (*Meloidogyne* spp. in Figure 1B) was higher on growing tomato plant roots in the control, when compared to those of each medicinal botanical in treatment (Figure 2C). Nonetheless, the VRGS achieved by each of the botanicals was not significantly ($P > 0.05$) different from that of the control Figure 2V). In effect, the 'mean ± SE' values of FRWG by growing tomato plants in treatments with *M. lucida, A. indica* and *V. amygdalina* were similar (Figure 2D).

Discussion

Medicinal properties of Morinda lucida, Azadirachta indica and Vernonia amygdalina

In Ghana, the three natural botanicals, used in this study, have been effective for treatments of malaria and other numerous diseases caused by microparasitic protozoans (Asante-Kwatia *et al*., 2021). However, *M. lucida* seems to have been explored extensively for its cercaricidal and schistosomicidal activities, whereas *A. indica* and *V. amygdalina* have been used for treatments of schistosomiasis and helminthiasis (Acheampong *et al*., 2020; Asante-Kwatia *et al*., 2021). The effectiveness of crude extracts from these tropical medicinal botanicals against malaria, schistosomiasis and helminthiasis, among others, is largely due to several antimicrobial bioactive organic compounds present in them (Okechalu *et al*., 2021; Ujah *et al*., 2021; Okhale *et al*., 2024). The dominant organic compounds in *M. lucida* include carbohydrates, saponins, terpenes, sterols and flavonoids (Okhale *et al*., 2024). Steroids, saponins, terpenes, tannins, alkaloids, glycosides, flavonoids, oxalic acid and phenols in their varying amounts have been observed in leaf extract of *A. indica* (Ujah *et al*., 2021). Polyphenols, glycosides, saponins, alkaloids, steroid, flavonoids, terpenoids and resins have been reported to be present in leaf extracts of *V. amygdalina* (Okechalu *et al*., 2021). These phytochemicals could possibly depict antiprokaryotic, antiviral, antiprotozoal, antischistosomal, anthelminthic (i.e., nematicidal) and other medicinal properties of these botanicals (Okechalu *et al*., 2021; Ujah *et al*., 2021; Mintah *et al*., 2022; Asafo-Agyei *et al*., 2023; Asase, 2023; Okhale *et al*., 2024).

Variations in suppressive effects of Morinda lucida, Azadirachta indica and Vernonia amygdalina on plant root-feeding nematodes (PRFNs) in the field

Performance of *Solanum sisymbriifolium* (Lamarck) as a trap crop against diverse *Globodera* species in field trials in Idaho, Oregon and Connecticut States of USA was compared to fallowed fields only (Dandurand *et al*., 2019). During a screenhouse trials by Etim *et al.* (2024), no commercially produced nematicide was included among treatments of *Trichoderma* species and neem cake for management of *Meloidogyne incognita* (Kofoid and White) and *Sclerotium rolfsii* (Sacc.) on okra (*Abelmoschus esculentus* L.). Also, based on similar approach used in report of Habriantono *et al.* (2023), the inclusion of commercially-produced nematicide(s) as positive control(s) in the current field trial was largely considered to be unnecessary. This was primarily because observations in treatments could be compared to untreated tomato plants only.

In this study, differential responses of diverse plant root-feeding nematodes (PRFNs) to the three (3) selected medicinal botanicals were observed in an agricultural field, where tomato was cultivated. *Morinda lucida* and *A. indica* were effective in suppressing dagger nematodes (*Xiphinema* spp.), whereas *A. indica* and *V. amygdalina* could be recommended to suppress root-lesion nematodes (*Pratylenchus* spp.) on tomato in the field. Nonetheless, *M. lucida*, *A. indica* and *V. amygdalina* were all effective in reducing densities of uncharacterized root-knot nematodes (RKN) (*Meloidogyne* spp.), which were the most dominant microscopic vertisol-dwelling phytoparasites observed at the study site. Crude extract from seeds of *A. indica* has been effective in suppressing RKN (Yasmin *et al*., 2003). Also, soil treatment with neem (*A. indica*) cake and unknown species of *Trichoderma* significantly suppressed both root galling and reproduction factor of *M. incognita* on okra (*A. esculentus*) (Etim *et al*., 2024). Unfortunately, recent report by Okechalu *et al.* (2021) showed that low doses of powder form of smoothly ground dried leaves of either *V. amygdalina* or *M. oleifera* could not effectively reduce reproduction factor of RKN on carrots in a pot experiment in Nigeria. However, an increase in doses of either *V. amygdalina* or *M. oleifera* suppressed RKN on carrot better (Okechalu *et al*., 2021). In our field trial, a high dose of fresh green leaf extracts from *M. lucida*, *V. amygdalina* or *A. indica* suppressed diverse soil-borne plant root-feeding nematodes (PRFNs) on tomato plants. Several factors may possibly cause the requirement for high doses of crude extracts from medicinal botanicals against PRFNs. Firstly, PRFNs are largely shielded and protected in 'soil medium' in tropical agricultural fields. Secondly, the amounts of antimicrobial bioactive organic compounds retrieved from several medicinal botanicals have extremely been low (Okechalu *et al*., 2021; Ujah *et al*., 2021; Okhale *et al*., 2024).

Unfortunately, the number of *Longidorus* spp. observed in control was similar to that of *A. indica* treatment. Also, the number of *Helicotylenchus* spp. observed in the control was significantly lower than that of *V. amygdalina* treatment. Therefore, we concluded that *A. indica* and *V. amygdalina* could not suppress *Longidorus* spp. and *Helicotylenchus* species in their treatments, respectively. Notwithstanding, *V. amygdalina* was more promising in suppressing the overall phytonematodes observed on growing tomato plants in the field, although it could not manifest effective nematicidal suppressiveness on dagger (*Xiphinema* spp.), lance (*Hoplolaimus* spp.) and spiral (*Helicotylenchus* spp.) nematodes in this study.

According to recent report of Osei-Owusu *et al.* (2023), volatile extracts from native Ghanaian *Ocimum* plant species showed repellency property against malaria parasite-vectoring *Anopheles gambiae* sensu stricto. Azadirachtin in seed and leaf extracts of *A. indica* has been lethal to several insect pests on field crops (Kilani-Morakchi *et al*., 2021). Similarly, extracts from some weed plants show repellency, nematostatic and lethal properties to *M. incognita* (Tariq *et al*., 2022). Crude leaf extracts from *Phyllanthus amarus* (Schumach and Thorn), *Melia azedarach* (L.), *Tridax procumbens* (L.) and *A. indica* cause egg-hatching inhibition and death of second-stage infective juveniles of *M. graminicola* (Dhruw *et al*., 2025). According to Sikder *et al.* (2021), phytohormones can disrupt host-location ability of nematodes (PRFNs). Perhaps, variations in mechanistic nematicidal properties among *M. lucida*, *A. indica* and *V. amygdalina* might have caused differences in their performance against vermiform PRFNs in the field. However, they achieved similar decrease in visible root galling severity (VRGS) of tomato plant. Thus, future research will be required to understand plant root-protecting mechanisms of aqueous leaf extracts from *M. lucida*, *A. indica* and *V. amygdalina* in rhizosphere of crop plants. Of course, less damaged tomato plant roots in treatments of medicinal botanicals could absorb more nutrients and water from soil medium than those in control did. In effect, treatments with leaf extracts of *M. lucida*, *A. indica* and *V. amygdalina* caused tomato plants to gain root weight in the field.

Considerations for research intensification

Numerous medicinal botanicals are in Ghana (Mintah *et al*., 2022; Asafo-Agyei *et al*., 2023; Asase, 2023). Moreover, studies have shown that many of these medicinal plants have effective potential to suppress PRFNs on crops (Yasmin *et al*., 2003; Osei and Sackey-Assante, 2006; Dandurand *et al*., 2019; Dutta *et al*., 2019; Okechalu *et al*., 2021; Ujah *et al*., 2021; Catani *et al*., 2023; Etim *et al*., 2024; Maher *et al*., 2024; Okhale *et al*., 2024; Azeem *et al*., 2025). Notwithstanding, according to Heve and Aniapam (2025), transport of plant commodities from country to country is regulated by statutory laws all over the world. Thus, importing some efficacious varieties of medicinal plants from other countries for field trials in Ghana may be thwarted by strict regulatory laws of countries. This suggests that research must be intensified to screen more local medicinal botanicals in trials towards developing integrated management tactics against uncharacterized diverse PRFNs on crops across Ghana.

In this study, *M. lucida*, *A. indica* and *V. amygdalina* showed similar nematicidal potential, in terms of reducing root galling by root-knot nematodes. Consequently, tomato plants in treatments of these three medicinal botanicals gained similar increase in root weight. In this case, similar performances of these native medicinal botanicals suggest that they may be probably complementary against PRFNs in the field. Therefore, field trial approaches have been adapted in an ongoing project to assess additive, synergistic or antagonistic properties of the three medicinal botanicals against diverse PRFNs in cultivated fields. Possibly, empirical evidence from the ongoing project may provide recommendations to suppress PRFNs on crops in the field for sustainable development.

Conclusions

Morinda lucida, *A. indica* and *V. amygdalina* treatments in rhizosphere of tomato plants suppressed visible root galling severity by 33%. Consequently, tomato plants gained about 25% increase in fresh root weight. Moreover, *M. lucida*, *A. indica* and *V. amygdalina* in treatments reduced population densities of overall PRFNs at varying rates. Among the three native medicinal botanicals, aqueous leaf extract of *V. amygdalina* was more promising for management of diverse crop PRFNs in the field. However, further research is required to examine more native medicinal plants for management of PRFNs.

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Novelty Statement

Our field trial demonstrates that native medicinal botanicals from Ghana could provide effective protection for tomato plant roots against diverse soil-dwelling PRFNs in a cultivated field. Thus, the significance of the current study will reinforce research intensification on more native medicinal botanicals towards development of environmentally-friendly integrated management tactics against PRFNs on crops in the field.

Author's Contribution

Dr. William K. Heve: Conceptualized the study, supervised the collection of data, diagnosed all the nematodes (PRFNs) in suspensions, analyzed the data using R software, wrote the manuscript and carefully edited it for publication.
Abigail M. Norvor: Executed the experiments in the field, collected samples from experimental plot and then used the modified Baermann funnel method to extract diverse PRFNs into suspensions, under the auspices of **Dr. William K. Heve**.
Dr. John Opata: Revised the manuscript and contributed valuable information for publication.

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The authors did not use animal or human bodies as objects or materials for this study.

Conflict of interest
The authors have no conflicting interest to declare.

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