Research Article

Combining Freshly-Harvested Green Leaves of Medicinal *Morinda lucida*, *Azadirachta indica* and *Vernonia amygdalina* for Effective Management of Diverse Invasive Soil-Borne Crop-Damaging Phytoparasitic Nematodes Reduced Okra (*Abelmoschus esculentus*) Plant Biomass

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Abstract | This study examined whether multiple combinations of freshly-harvested green leaves of medicinal brimstone, neem and bitter leaf trees against phytoparasitic nematodes (PPNs) could be complementary to improve okra plant biomass (OPB). Therefore, field evaluation technique was methodically used to collect data of visual root-galling severity (VR-GS), overall PPNs suppressed (OPPNss) and OPB for analysis. The VR-GS was positively correlated, (correlation coefficient (*r*) = 0.32; *P*-value = 0.07569), with number of botanicals combined (NBC) in treatments. Percentage of OPPNss in treatments that had single, two-combined and three-combined botanicals was 84±6, 85±7 and 91±5%, respectively. However, relative biomass of an okra plant was negatively correlated, (*r* =-0.34; *P*-value = 0.05615), with NBC in treatments. Consequently, all single botanicals in treatments triggered an increase in OPB, whereas two-combined or three-combined botanicals in treatments largely caused a decrease in OPB. Freshly-harvested green leaves of *A. indica*, *M. lucida* and *V. amygdalina* in single botanical treatments achieved 7.32, 18.22 and 43.4% increase in OPB, respectively. Therefore, *V. amygdalina* will most likely increase okra yield better, while suppressing PPNs.

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Introduction

Okra (*Abelmoschus esculentus* L.) is a widely cherished vegetable for having ample nutrients. It is often used to be a major component of health-focused dietary medicinal formulations (Elkhalifa *et al*., 2021). After mangoes, okra is the second largest income-generating crop for livelihood in Yilo Krobo Municipal (YKM) of Eastern Region in Ghana (first author's personal communication with YKM

Agricultural Extension Agents). However, okra plant root system (OPRS) is highly susceptible to diverse phytoparasitic nematodes (PPNs), although root-knot nematodes (*Meloidogyne* spp.) are more problematic on this crop (Srivastava *et al*., 2012; Mukhtar *et al*., 2014, 2017; Patil *et al*., 2020; Prajapati *et al*., 2021; Danso and Abugri, 2022; Baah *et al*., 2024; Perry *et al*., 2024; Gawade *et al*., 2025). These PPNs on OPRS can cause okra yield losses up to 40% if the right management techniques are not adopted against them (Shendge *et al*., 2010; Mukhtar *et al*., 2014, 2017; Prajapati *et al*., 2021; Gawade *et al*., 2025).

Nematicide and non-nematicide options have been assessed and suggested to vegetable crop growers to use against PPNs in the field (Noling, 2002; Shendge *et al*., 2010; Bhosle *et al*., 2012; Mukhtar *et al*., 2017; Patil *et al*., 2020; Prajapati *et al*., 2021; Baah *et al*., 2024; Gawade *et al*., 2025; Heve *et al*., 2025). Unfortunately, synthetic nematicides, which are more effective in suppressing PPNs in field conditions, are seriously regulated across the world (Kwakye *et al*., 2019; Desaeger *et al*., 2020; Sethi *et al*., 2022; Catani *et al*., 2023; FAO, 2024). Thus, the high global demand for food and plant commodities, coupled with increasing severity of PPNs on crops in the field, require research considerations to upgrade non-nematicide approaches (Afzal and Mukhtar, 2024; Abd-Elgawad, 2024). Some of the upgrading efforts for effective non-nematicide strategies include research and advancement into the use of beneficial microbial entities and botanicals for management of PPNs on crop plants (Dandurand *et al*., 2019; Dutta *et al*., 2019; Okechalu *et al*., 2021; Catani *et al*., 2023; Abd-Elgawad, 2024; Baah *et al*., 2024; Etim *et al*., 2024; Maher *et al*., 2024; Azeem *et al*., 2025; Heve *et al*., 2025).

In a recent field trial, Heve *et al*. (2025) examined and compared efficacies among neem plant (*Azadirachta indica* Jussieu), brimstone tree (*Morinda lucida* Benth) and bitter leaf (*Vernonia amygdalina* Delile) for management of PPNs on tomato (*Solanum lycopersicum* L.). The three botanicals achieved similar percentage increase in tomato plant root (TPR) weight. Although *V. amygdalina* could be recommended for management of PPNs, the three botanicals again had similar percentage decrease in TPR galling severity (Heve *et al*., 2025). Therefore, we hypothesized, in the current study, that combining the botanicals in treatments would be complementary against PPNs in the field. Our main objective was to examine whether performances of multiple combinations of these three botanicals against PPNs could be additive, synergistic or antagonistic. Therefore, similar field trial procedures in report of Heve *et al*. (2025) were used to study the effects of multiple combinations of these three botanicals on both PPNs and okra plant biomass.

Materials and Methods

The study site and field preparation

The study site (SS) was at the coordinate (6° 3'0.66" N, 0° 0'4.64" W, with elevation = 69 m). The site had undulating topographical landscape. Soils in Yilo Krobo Municipal, including the SS, had been characterised to be predominantly calcic vertisols that have high clay content, ranging between 32 and 41% (Owoade *et al*., 2021; Baidoo *et al*., 2024).

Bushes at SS were cleared close to the ground, using a cutlass. This manual weed clearing was adopted to achieve zero-tillage that was to conserve topsoil structure, any beneficial soil-borne microorganisms, phytopathogens (i.e., bacteria and fungi), microscopic phytoparasitic nematodes (PPNs), microinvertebrates and earthworms, among others (Zheng *et al*., 2020; Długosz *et al*., 2024).

Pre-treatment organization in experimental plot

The experimental plot was divided into seven rows, similar to the approach used by Heve *et al*. (2025). Along each row, eight planting stations were pegged. The distance between two successive pegged stations within a row was one (1) m, whereas the distance between two successive rows was 2 m. After pegging the planting stations, a hole (depth = 20 cm; width = 15 cm; length = 20 cm) was created at the position of every peg in each row, using a hoe. After that, the holes were labelled again for each of intended treatments in such a way that all the treatments could be arranged in a randomized complete block design layout (RCBDL) in the field. Hence, each treatment unit was randomly positioned in each of the seven rows, similar to the way it was done in recent report of Heve *et al*. (2025).

Pre-planting treatments with green leaves of medicinal botanicals in holes created in the experimental plot

On March 23 and 24, 2025, fresh green leaves of *M. lucida*, *A. indica* and *V. amygdalina* were separately harvested at the geographical coordinates (6°3'3.10" N, 0°0'16.06" W), (6°3'2.07" N, 0°0'13.86" W) and (6°1'39.82" N, 0°27'23.42" W), respectively. On March 25, 2025, about 50 g of green leaves of *M. lucida* alone was added to a randomly-selected hole in each row and then covered with soil. On the same day, similar procedures used for *M. lucida* were repeated for *A. indica* alone, *V. amygdalina* alone, two-combined and three-combined botanicals. However, for two botanicals combined in a treatment, 50 g of their green leaves was maintained in ratio 1:1, similar to the technique used in report of Heve *et al.* (2018). Also, for three medicinal botanicals combined in a treatment, 50 g of combined green leaves was from *M. lucida*, *A. indica* and *V. amygdalina* in a ratio 1:1:1 (Heve *et al.*, 2018). An "empty hole" that did not receive green leaves of the botanicals was the control. In all, there were eight (8) treatments. A replicate of each treatment was randomly positioned in each of the seven (7) rows. Thus, each of the eight (8) treatments was replicated seven times and then arranged in RCBDL on the plot at the study site, similar to the experimental design used in reports of Heve *et al.* (2018, 2025).

The 50 g of green leaves, incorporated into soil at each planting station, decomposed from March 25, 2025 until planting was done on April 9, 2025. Precipitation between these two dates was 42 mm.

Planting at pegged stations after incorporating 50 g of green leaves of medicinal botanicals into soil

On April 9, 2025, four (4) viable okra (*A. esculentus* L.; variety = Ladies' fingers) seeds were sown at each labelled planting station, which had already received treatments for 'control', single, two or three botanicals combined. After germination, the okra seedlings were thinned out to maintain one at each pegged planting spot.

Maintenance of growing okra plants against weeds, leaf-damaging pests and climate change

Using a hoe for soil tillage, weeds were controlled in the experimental plot to allow healthy-looking okra seedlings develop well. According to recent reports (Rinaldi *et al.*, 2019; Choo *et al.*, 2020), suspensions of smoothly-mashed garlic (*Allium sativum* L.) protect crop plants against insect pests and okra-damaging snails, among others. Therefore, 7.5 g of garlic cloves was smoothly mashed in a mixer grinder (BOSCH™) at speed of 45,000 rpm (revolutions (minute)$^{-1}$) for 5 minutes. After that, a litre of tapwater was added to the smoothly-mashed garlic paste (SMGP), thereby adjusting the concentration of the paste to be 7.5 g L^{-1}. On April 16, 2025, about 10 mL of the suspension of SMGP was sprinkled over leaves of a growing okra seedling at each planting spot, using a 20-mL measuring cylinder. Again, on April 30, 2025, about 10 mL of similar concentration of freshly prepared SMGP in suspension was sprinkled over the leafy parts of each developing okra plant at each planting station. The two consecutive applications of SMGP suspensions were to effectively repel leaf-chewing insects (i.e., coleopterans) from all delicate growing okra plants at their early stage. Hence, influence of applied mashed-garlic suspensions on okra plants would be similar in all replicates of treatments and control.

No fertilizers were applied. Also, none of the okra plants was irrigated. Precipitation from April 9, 2025 (the date of sowing) to June 23, 2025 (the date of harvesting) was 281 mm, whereas daily temperatures varied from 23 to 34 °C. Moreover, okra plants were exposed to sufficient daily sunshine hours, which varied between six (6) and 10.

Data collection

Assessment of visual root-galling severity (VR-GS) and okra plant biomass (OPB): When the okra plants were 11-week-old from the date of planting, each was carefully uprooted on June 23, 2025. The amount of soil around the root system was gently removed into a 600-mL polythene bag, thereby making the roots of each okra plant appear visibly clean (Heve *et al.*, 2025). Each clean root system of each okra plant was then examined for incidence of root galls. Hence, VR-GS was visually estimated using the procedures in reports (Heve *et al.*, 2015a, 2025; Schwarz *et al.*, 2024). After that, OPB was determined by weighing each whole uprooted fresh okra plant for each replicate of a treatment.

Extraction, identification and quantification of actively-motile phytoparasitic nematodes (PPNs)

The modified Baermann funnel method (MBFM) (OEPP/EPPO, 2013; Tintori *et al.*, 2022), recently recommended by Bui *et al.* (2025) and Heve *et al.* (2025), was used to extract live PPNs from both '200 mL of fresh rhizosphere soil sample' and 'fresh okra plant roots' into suspensions for each replicate of a treatment over a week. Using a microscope, all the

PPNs in 5 mL of a suspension for each replicate of a treatment were identified to genus level only, according to their morphological features in reports (Smart and Nguyen, 1988; Mekete *et al*., 2012; Heve *et al*., 2015b; Fourie *et al*., 2017; Ghaderi *et al*., 2018; Heve, 2021; Chen *et al*., 2023; Nimnoi *et al*., 2024; Perry *et al*., 2024; Sroisai *et al*., 2024). After that, total number of identified individual nematodes belonging to each genus of PPNs in a volume of nematode suspension collected from MBFM was quantified using Equation 1, similar to the approach used by Heve *et al*. (2025), as:

$$N_g = \frac{(C_g)V}{5} \quad \dots(1)$$

Where N_g was the total number of individuals belonging to a genus of PPNs; C_g was the number of individual nematodes belonging to a genus of PPNs in a 5 mL of nematode suspension from MBFM; V was total volume of nematode suspension collected from MBFM for each replicate of each treatment.

Assessment of overall phytoparasitic nematodes (PPNs) suppressed in each replicate of a treatment

Similar to the methods used by Heve *et al*. (2025), overall phytoparasitic nematodes (OPPNs) for each replicate of a treatment was obtained by summing N_g (in Equation 1) for each of all the genera of PPNs. Therefore, the percentage of OPPNs suppressed (OPPNss) in each treatment was assessed using Equation 2, similar to the procedure used by Heve *et al*. (2025), as:

$$P_t = 100\left[\frac{T_{control} - T_{treatment}}{T_{control}}\right] \quad \dots(2)$$

Where P_t was the percentage of overall phytoparasitic nematodes suppressed (OPPNss) in treatment; $T_{control}$ was the overall phytoparasitic nematodes (OPPNs) observed in control; $T_{treatment}$ was OPPNss in treatment.

Data analysis

R software [R.v.4.5.1 and RStudio.v.SHA-2562c3cf96a] by R Core Team (2025) for computing was used to analyze data. *Shapiro-Wilk* test was first used to examine normality in data; the *P*-values of the test were < 0.05, thereby proving that data lacked normality, according to reports by Gotelli and Ellison (2013), Dinno (2015), Mishra *et al*. (2019) and Heve *et al*. (2025). Nonetheless, parametric two-way (or multi-factor) analysis of variance (ANOVA) was fitted to unbalanced data better than its analogous non-parametric Scheirer-Ray-Hare test could be (Scheirer *et al*., 1976; Gotelli and Ellison, 2013; Noskov *et al*., 2019; Klasson, 2020; Sourisse *et al*., 2024; Najafi *et al*., 2025; Sinha, 2025). This observation was similar to the first author's previous experience in his recent report (Osei-Owusu *et al*., 2023). This is because the *P*-values of the parametric two-way ANOVA model were lower than those of the analogous non-parametric Scheirer-Ray-Hare model. Therefore, "two-way" ANOVA was used to determine whether the categorical factors could be the significant sources of variation in the data for VR-GS, OPPNs, P_t or OPB. The factors considered to be the likely sources of variation included (1) "incidence of no, single, two-combined and three-combined botanicals in treatments (IBT)", (2) "treatments in rows (TR)" and (3) "interaction between IBT and TR". After that, the non-parametric Dunn's test was applied to data to compare values of "mean ± standard errors (SE)" between treatments, according to suggestions in reports (Gotelli and Ellison, 2013; Dinno, 2015; Mishra *et al*., 2019; Heve *et al*., 2025). Pearson correlation coefficient was used to examine linear relationship between two variables. The "mean ± SE" values were presented in either bar graphs or Tables.

Results

Multiple combinations of fresh green leaves of the medicinal botanicals in treatments against visual root-galling severity (VR-GS)

Incidence of botanicals in treatments (IBT) and "interaction between IBT and treatments in rows (TR)" significantly (P < 0.05) caused variation in VR-GS (Table 1). Thus, VR-GS values varied between the individual treatments (Figure 1A). The VR-GS values in treatments of *V. amygdalina* alone, *M. lucida* alone and '*A. indica* + *V. amygdalina*' were significantly (P < 0.05) lower than those in control, '*V. amygdalina* + *M. lucida*', '*A. indica* + *M. lucida*' or '*V. amygdalina* + *M. lucida* + *A. indica*' treatment (Figure 1A). Also, the VR-GS values in *A. indica* treatment were significantly (P < 0.05) less than those in control (Figure 1A). However, the mean±SE values of VR-GS in treatments of *A. indica* alone, '*V. amygdalina* + *M. lucida*', '*A. indica* + *M. lucida*' and '*V. amygdalina* + *M. lucida* + *A. indica*' were not significantly (P > 0.05) different from one another (Figure 1A).

Values of the VR-GS in treatments of single botanicals were significantly (P < 0.05) lower than those in

Table 1: *Multi-functional "two-way" analysis of variance (ANOVA), predicting categorical sources of variation in each set of data collected from the field trial.*

Categorical source of variation	Sets of data measured and collected from the field trial											
	Visual root-galling severity (VR-GS)			Number of overall phytoparasitic nematodes (OPPNs)			Percentage of OPPNs suppressed (P_t in equation 2)			Okra plant biomass (OPB)		
Categorical source of variation	DF_1	MS	*P*-value	DF_1	MS	*P*-value	DF_2	MS	*P*-value	DF_1	MS	*P*-value
Incidence of botanicals in treatment (IBT) ¶	3	2495.50	0.00015 ***	3	599069.6	< 0.00001 *****	2	3149.84	0.3286 NS	3	0.063462	0.19039 NS
Treatment in rows (TR)	6	422.56	0.2753 NS	6	65724.53	0.0254 *	6	598.65	0.9682 NS	6	0.01618	0.8682 NS
Interaction between IBT and TR	18	856.03	0.00183 **	18	141681.4	<0.000003 *****	12	936.58	0.9764 NS	18	0.018234	0.966 NS
Residuals	84	330.59	NA	84	25728.57	NA	42	2755.82	NA	84	0.039128	NA

¶: Only incidence of no, single, two-combined and three-combined botanicals in treatments (IBT), which had overall replicates to be 7, 21, 21 and 7, respectively, was considered; ***DF_1*** *was the degree of freedom, when data for replicates of control were included;* ***DF_2*** *was the degree of freedom, when data for replicates of control were excluded;* ***MS*** *was mean squares, whereas P-value was probability statistic for F statistic of parametric two-way ANOVA test;* ***"Two-way" ANOVA* test:** **, **, *** and ***** indicate significance at P < 0.05, P < 0.01, P < 0.001 and P < 0.00001, respectively, whereas "NS" denotes no significance;* **NA** *was "not applicable".*

Table 2: *Numbers of phytoparasitic nematodes (PPNs) per okra plant in treatments that had green leaves of medicinal botanicals.*

Diverse invasive soil-borne phytoparasitic nematodes (PPNs)	Medicinal botanical treatments, with control, to okra plant roots							
	Control (i.e., no green leaves)	*A. indica* (AZ)	*M. lucida* (ML)	*V. amygdalina* (VA)	AZ+ML	AZ+VA	ML+VA	AZ+ML+VA
Meloidogyne spp.	464±55 A	13±2 C	7±7 C	126±77 B	3±3 C	0±0 C	130±92 B	15±10 C
Scutellonema spp.	13±11 D	0±0 D	8±7 D	0±0 D	6±5 D	0±0 D	0±0 D	0±0 D
Xiphinema spp.	27±5 E	0±0 F	30±7 E	24±4 E	0±0 F	2±2 F	0±0 F	0±0 F
Trichodorus spp.	28±3 G	17±8 GH	0±0 H	16±11 GH	12±7 GH	0±0 H	5±3 H	25±7 G
Pratylenchus spp.	44±8 I	5±4 K	4±3 K	7±4 JK	8±7 JK	9±5 JK	31±14 IJ	10±7 JK
Radopholus spp.	3±2 L	0±0 L	0±0 L	0±0 L	0±0 L	0±0 L	0±0 L	0±0 L
Trichotylenchus spp.	16±10 M	0±0 M	0±0 M	0±0 M	8±7 M	0±0 M	1±1 M	0±0 M
Belonolaimus spp.	18±10 N	0±0 N	22±10 N	2±2 N	0±0 N	5±5 N	0±0 N	8±6 N
Amplimerlinius spp.	7±5 O	0±0 O	4±3 O	0±0 O	0±0 O	5±5 O	9±6 O	5±3 O
Hoplolaimus spp.	26±15 P	0±0 P	0±0 P	0±0 P	17±14 P	0±0 P	0±0 P	0±0 P
Longidorus spp.	15±4 Q	0±0 R	0±0 R	4±3 R	0±0 R	17±5 Q	3±3 R	0±0 R
Helicotylenchus spp.	13±11 S	0±0 S	4±3 S	0±0 S	8±7 S	0±0 S	0±0 S	0±0 S
Rotylenchus spp.	3±2 T	0±0 T	4±4 T	0±0 T	0±0 T	0±0 T	0±0 T	0±0 T
Tylenchorhynchus spp.	15±6 U	0±0 U	9±5 UV	0±0 U	0±0 U	0±0 U	0±0 U	0±0 U
Rotylenchulus spp.	17±2 W	0±0 X	0±0 X	0±0 X	0±0 X	0±0 X	6±5 X	0±0 X

According to Dunn's test at P < 0.05, different uppercase letters against 'mean±SE-values' in each row denote significant difference between treatments , with control.

either control or '*V. amygdalina* + *M. lucida* + *A. indica*' treatment (Figure 1B). Also, significant difference was observed in VR-GS values between 'single botanical' and 'two combined botanicals' treatments (Figure 1B). However, the values of VR-GS in '*V. amygdalina* + *M. lucida* + *A. indica*' treatment were similar to those in control (Figure 1A, B). Excluding all observations in control, an increase in values of VR-GS in Figure 1B was positively correlated, (r = 0.32; *P*-value = 0.07569), with an increase in number of botanicals combined in treatments.

Multiple combinations of fresh green leaves of the medicinal botanicals in treatments against diverse phytoparasitic nematodes (PPNs) on okra plant roots

The significant categorical sources of variation in numbers of overall phytoparasitic nematodes (OPPNs) included IBT, TR and "interaction between

IBT and TR" (Table 1). However, in Table 2, numbers of uncharacterized PPN species of *Scutellonema*, *Radopholus*, *Trichotylenchus*, *Belonolaimus*, *Amplimerlinius*, *Hoplolaimus*, *Helicotylenchus*, *Rotylenchus* and *Tylenchorhynchus* in each of the medicinal botanical treatments were not significantly ($P > 0.05$) different from those observed in control. In contrast, number of either *Meloidogyne* spp. or *Rotylenchulus* spp. in all the medicinal botanical treatments was significantly ($P < 0.05$) lower than that in control (Table 2). Number of *Xiphinema* spp. in treatment of *A. indica*, '*A. indica* + *M. lucida*', '*A. indica* + *V. amygdalina*', '*M. lucida* + *V. amygdalina*' or '*A. indica* + *M. lucida* + *V. amygdalina*' was significantly ($P < 0.05$) less than that of control (Table 2).

Similarly, number of *Trichodorus* spp. in treatment of *M. lucida*, '*A. indica* + *V. amygdalina*' and '*M. lucida* + *V. amygdalina*' were significantly ($P < 0.05$) lower than that of control (Table 2). Numbers of *Pratylenchus* spp. in all treatments, except the trial involving '*M. lucida* + *V. amygdalina*', were significantly ($P < 0.05$) less than that of control (Table 2). Also, numbers of *Longidorus* spp. in all treatments, except the trial involving '*A. indica* + *V. amygdalina*', were significantly ($P < 0.05$) lower than that of control (Table 2).

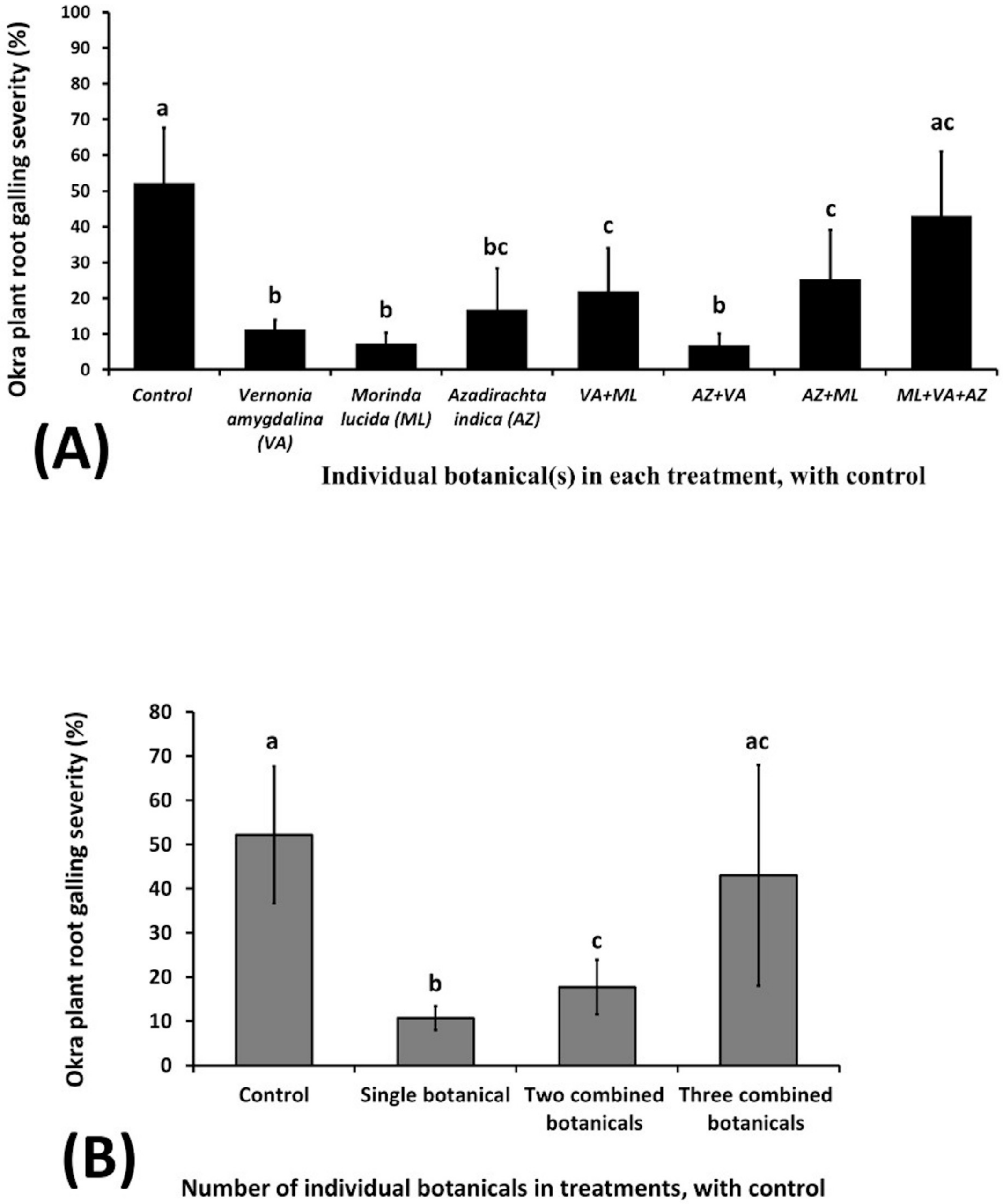


Figure 1: *Observations revealing: (A) the performance of individual treatments against visual root-galling severity (VR-GS); (B) antagonistic effects of combining botanicals in treatments on VR-GS. According to Dunn's test at* $P \leq 0.05$*, different lowercase letters on top of bars denote significant differences in VR-GS between treatments.*

The numbers of OPPNs observed in each of the individual treatments of single, two-combined or three-combined medicinal botanicals were significantly ($P < 0.05$) lower than that of control (Figure 2A). Numbers of OPPNs achieved in treatments of *A. indica*, *M. lucida*, '*A. indica* + *M. lucida*', '*A. indica* + *V. amygdalina*' and '*A. indica* + *M. lucida* + *V. amygdalina*' were not significantly ($P > 0.05$) different from one another (Figure 2A). Moreover, numbers of OPPNs observed in treatments of *M. lucida*, '*A. indica* + *M. lucida*' and '*A. indica* + *M. lucida* + *V. amygdalina*' were not significantly ($P > 0.05$) different from those achieved in treatments of *V. amygdalina* and '*M. lucida* + *V. amygdalina*' (Figure 2A). However, the number of OPPNs obtained in treatment of either *A. indica* alone or '*A. indica* + *V. amygdalina*' was significantly ($P < 0.01$) lower than that observed in treatment of *V. amygdalina* alone or '*M. lucida* + *V. amygdalina*' (Figure 2A).

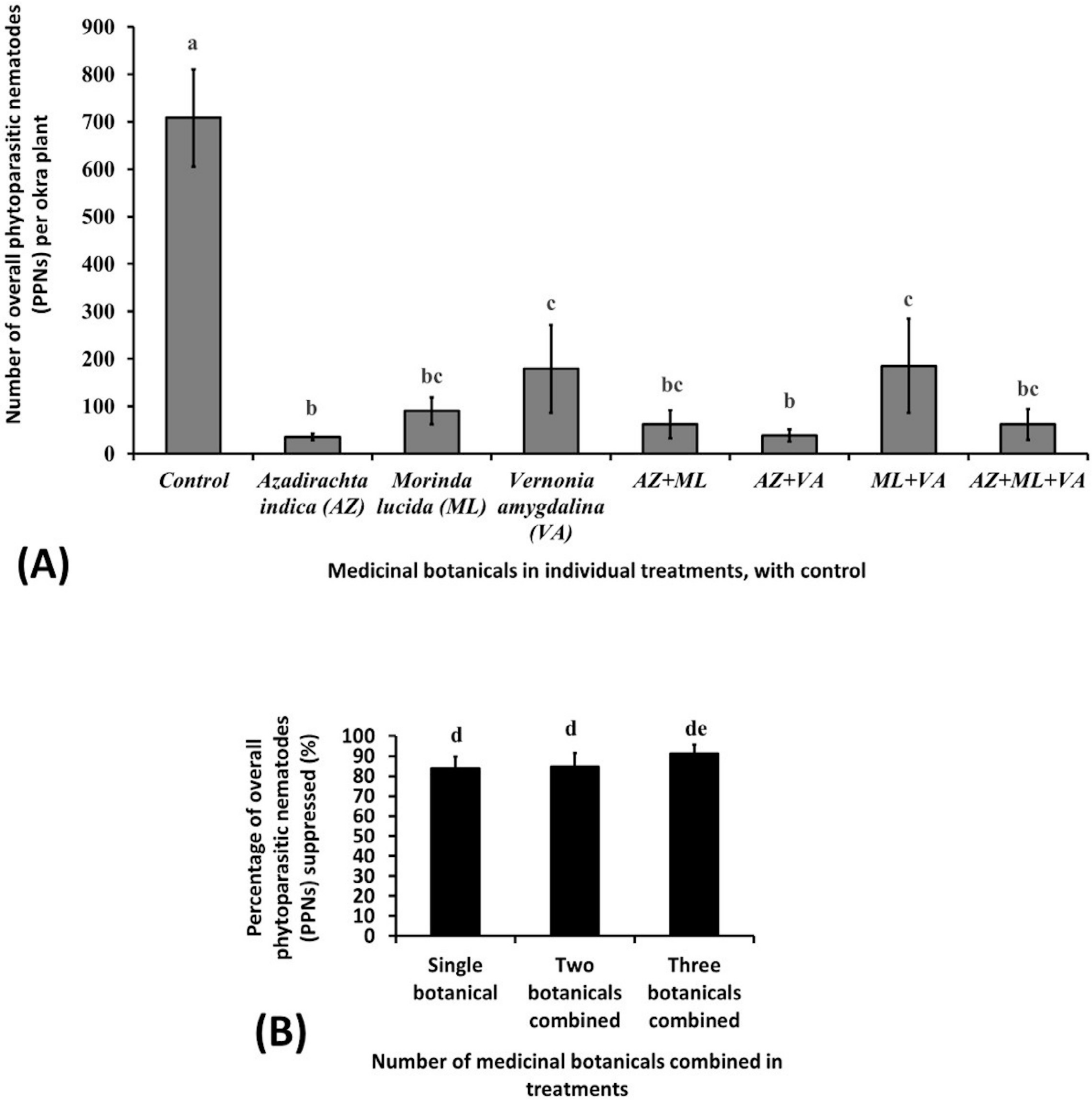
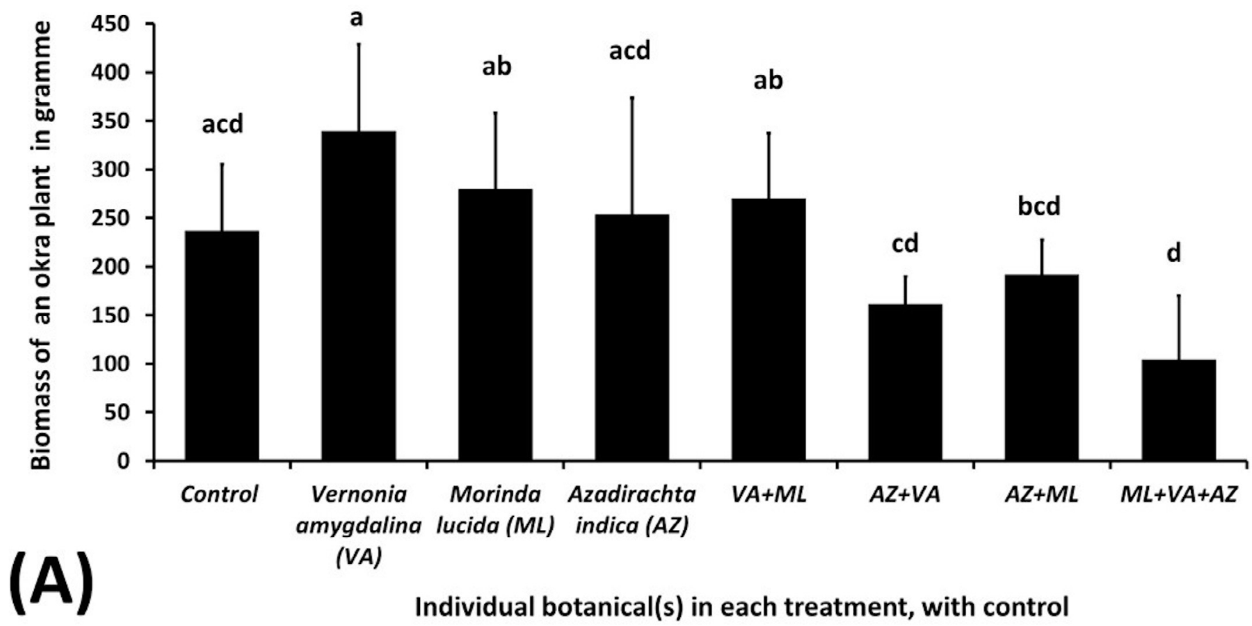


Figure 2: *Observations indicating: (A) numbers of overall PPNs (okra pant)$^{-1}$ for each of the individual treatments, with control; (B) percentage of overall PPNs suppressed by each number of medicinal botanicals combined in each treatment. According to Dunn's test at P ≤ 0.05, different lowercase letters on top of bars denote significant differences in observations between treatments.*

Although numbers of OPPNs were significantly ($P < 0.05$) reduced across all treatments that had botanicals (Figure 2A), none of the categorical factors caused variation in percentage of OPPNs suppressed (P_t) (Table 1). In effect, values of P_t across the individual treatments were similar (Figure 2B) in such a way that single, two-combined and three-combined botanicals in treatments had P_t values to be 84±6, 85±7 and 91±5%, respectively (Figure 2B). Overall, increasing P_t had an extremely weak positive correlation, ($r = 0.063$; P-value = 0.737), with increasing number of medicinal botanicals combined in treatments.

Effects of multiple combinations of fresh green leaves of the botanicals in treatments on biomass of an okra plant

None of the categorical factors was a source of variation in an okra plant biomass (OPB) (Table 1). The mean±SE value of OPB in the treatment of *V. amygdalina* was not significantly ($P > 0.05$) higher than that of control (Figure 3A). Also, the mean±SE-values of OPB in treatments of *M. lucida*, *A. indica* and '*V. amygdalina* + *M. lucida*' were similar to that of control (Figure 3A). Nevertheless, treatments of *V. amygdalina*, *M. lucida* and '*V. amygdalina* + *M. lucida*' had significantly ($P < 0.05$) higher OPB than that



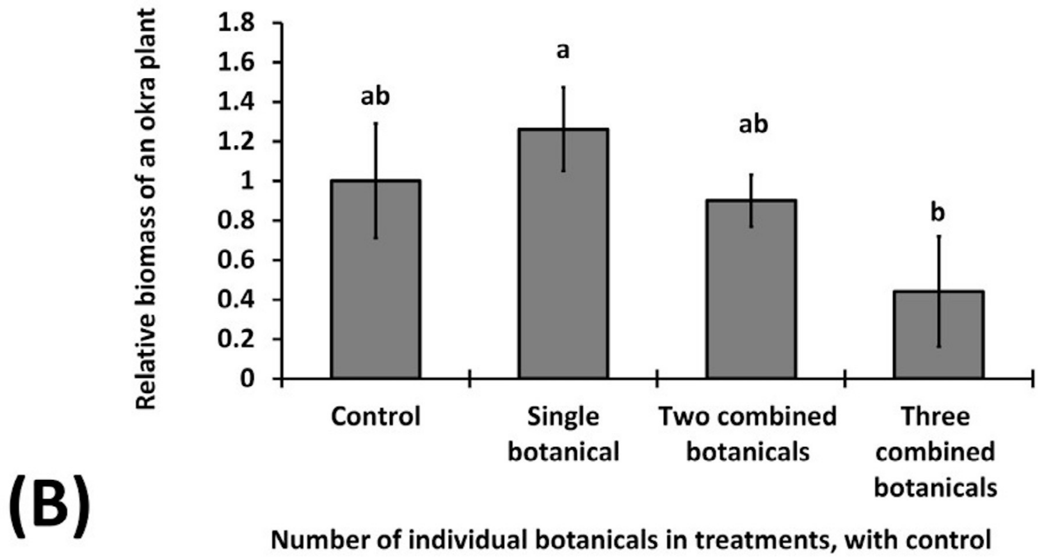


Figure 3: *Observations showing: (A) biomass of an okra plant for the individual medicinal botanical(s) in each treatment, with control; (B) relative biomass of an okra plant for each number of medicinal botanicals combined in each treatment, with control. According to Dunn's test at P ≤ 0.05, different lowercase letters on top of bars represent significant differences in observations between treatments.*

The mean±SE value of OPB in treatment of *A. indica* in the treatment of '*A. indica* + *V. amygdalina*' or '*M. lucida* + *V. amygdalina* + *A. indica*' (Figure 3A). alone, '*A. indica* + *V. amygdalina*', '*A. indica* + *M. lucida*' or '*M. lucida* + *V. amygdalina* + *A. indica*' was similar to that of control (Figure 3A). Also, the mean±SE value of OPB in treatment of '*A. indica* + *M. lucida*' was not significantly ($P > 0.05$) higher than that in the treatment of '*M. lucida* + *V. amygdalina* + *A. indica*' (Figure 3A). Relative to control, *V. amygdalina* alone, *M. lucida* alone and *A. indica* alone in treatments achieved 43.4, 18.22 and 7.32% increase in OPB, respectively (Figure 3A).

In Figure 3B, the values of relative biomass of an okra plant (RBAOP) in all treatments of single botanicals were not significantly ($P > 0.05$) higher than those in either control or 'two botanicals combined in treatments. Also, the mean±SE value of RBAOP in 'two botanicals combined in treatments' was not significantly ($P > 0.05$) higher than values of RBAOP in 'three botanicals combined in treatments' (Figure 3B). Using the control as a reference for RBAOP, the single, two-combined and three-combined botanicals in treatments had mean±SE values of RBAOP to be 1.261± 0.212, 0.9± 0.131 and 0.44± 0.209, respectively (Figure 3B). Therefore, excluding control, the RBAOP was negatively correlated (r =-0.34; P-value = 0.05615) with number of medicinal botanicals combined in treatments.

Discussion

Nematicidal properties of phytochemicals in Azadirachta indica, Morinda lucida, Vernonia amygdalina and other botanicals

Carbohydrates, saponins, terpenes, terpenoids, sterols, steroids and flavonoids are common in leafy parts of *M. lucida*, *A. indica* and *V. amygdalina* (Ujah *et al.*, 2021; Okechalu *et al.*, 2021; Okhale *et al.*, 2024). However, alkaloids, glycosides and polyphenols are additional phytochemical constituents in green leaves of *A. indica* and *V. amygdalina* only (Okechalu *et al.*, 2021; Ujah *et al.*, 2021). Apart from all the aforementioned phytochemicals, tannins and oxalic acid are other unique ones in the leaves of *A. indica* (Ujah *et al.*, 2021), whereas resins are exceptional extra constituents in the leaves of *V. amygdalina* (Okechalu *et al.*, 2021). Thus, phytochemical contents in green leaves of the three medicinal botanicals used for the study vary.

Several phytochemicals from *Artemisia* species have been effective in causing death of crop plant-infecting second-stage juveniles (J2) and/or inhibiting egg-hatching of root-knot nematodes (RKN; *Meloidogyne incognita*) (Khan *et al.*, 2020). Consequently, a significant decline in severity of *M. incognita*-induced galls on tomato (*Lycopersicon esculentum* L.) plant roots was achieved (Khan *et al.*, 2020). In recent report of Mezerket *et al.* (2025), the use of essential oil from Algerian wild (*Origanum vulgare* subspecies *glandulosum* Defs) for both preventive and curative treatment techniques significantly reduced both *M. incognita* J2 and tomato plant root-galling severity. Consequently, a significant increase in tomato plant biomass was observed across treatments (Mezerket *et al.*, 2025). A phytochemical profile study on Algerian wild revealed that its essential oil contains a mixture of biologically-active compounds, which include glycosides, flavonoids, saponins, gallic tannins and tannins (Mezerket *et al.*, 2025). Some of the bioactive chemical compounds in green leaves of *M. lucida*, *A. indica* and *V. amygdalina* are similar to those in the Algerian wild, *Artemisia* spp., *Arisaema erubescens* (Wallich), *Medicago sativa* (L.) and other numerous medicinal botanicals, which have been suppressive to PPNs (D'Addabbo *et al.*, 2011; Du *et al.*, 2011; Chin *et al.*, 2018; Mezerket *et al.*, 2025). This is because reports have shown that flavone-*C*-glycosides, flavonoids, saponins, phytoterpenoids and tannins from diverse medicinal botanicals suppress PPNs (D'Addabbo *et al.*, 2011; Du *et al.*, 2011; Abdel-Rahman *et al.*, 2013; Chin *et al.*, 2018; Greiffer *et al.*, 2022; Mezerket *et al.*, 2025).

Extracted forms of phytochemicals showed dissimilar modes of actions to suppress PPNs (Chin *et al.*, 2018; Bano *et al.*, 2020). In a recent study, about 39 plant-based different chemicals damaged all the internal organs of a root-lesion nematode (*Pratylenchus penetrans* Cobb) (Barbosa *et al.*, 2024). According to Greiffer *et al.* (2022), tannins suppress PPNs by making their cuticle to become rigid and thickened, thereby impairing nematode's mobility towards hosts. Plants naturally use flavonoids to defend themselves against harmful microorganisms that cause parasitic and pathogenic disease to them (Chin *et al.*, 2018). According to reports (Chin *et al.*, 2018; Bano *et al.*, 2020), the major suppressive functions of flavonoids against PPNs include (i) being effective in repelling phytonematodes, (ii) reducing hatchability of nematode eggs, (iii) causing improper coordination in movement of nematodes and (iv) being toxic to directly kill crop-damaging phytoparasitic ones. Several other bioactive compounds in botanicals are also toxic to cause death of PPNs or suppress their egg-hatching potential in the field (Khan *et al*., 2020).

Combining green leaves of Azadirachta indica, Morinda lucida and Vernonia amygdalina in treatments was antagonistic to visual root-galling severity (VR-GS)

We observed that visual root-galling severity (VR-GS) increased in treatments where two or three botanicals were combined better than treatments that had single botanicals. Plant-feeding mites, phytopathogens and other diverse microbes in rhizosphere use various mechanisms to suppress plant defense or root immune system (PDRIS) (Lehr *et al*., 2007; Chung *et al*., 2013; Blaazer *et al*., 2018; Ordon *et al*., 2025). Moreover, PPNs, including the topmost-ranked world-wide crop-damaging RKN, often secrete numerous effectors to weaken PDRIS (Sharma and Chaubey, 2023). Possibly, phytotoxins in green leaves of combined botanicals in treatments could weaken the okra PDRIS. In this case, any releases of effectors by RKN will further weaken the already weakened okra PDRIS, thereby allowing development and formation of root galls by *Meloidogyne* spp. in our field trial. In effect, all the pre-treatments that had *M. lucida* only, *A. indica* alone or *V. amygdalina* alone suppressed VR-GS better than pre-treatments that had two or three combined botanicals, except field trial that involved combination of "*A. indica* + *V. amygdalina*". Thus, studies are needed to understand the correct interactive mechanisms that might have facilitated an increase in VR-GS in treatments that had combined botanicals. Although the treatments for two or three combined botanicals had an increase in VR-GS, the observed numbers of J2 of RKN were generally low in this study. Perhaps, phytochemicals in these combined botanicals could synergistically inhibit egg-hatching of RKN in MBFM, similar to the way those of *M. incognita* were significantly suppressed by phytochemicals from *Artemisia* spp. (Khan *et al*., 2020). Again, studies are needed to understand how pre-treatments with combinations of green leaves of *M. lucida*, *A. indica* and *V. amygdalina* suppress hatching J2 from RKN eggs in galled plant roots.

In terms of suppressing root galls, Heve *et al*. (2025) observed that *V. amygdalina* alone, *M. lucida* alone and *A. indica* alone in treatment caused similar decrease in visual root-galling severity (VR-GS) of tomato plants in a field trial. Hence, each of the three botanicals was more promising. In the current study, *V. amygdalina* alone, *M. lucida* alone and *A. indica* alone in treatments achieved similar low VR-GS, thereby suggesting that any of the three botanicals can be used to effectively reduce plant root-galling severity. Our observations in the current study are in agreement with recent report of Heve *et al*. (2025).

Combining green leaves of Azadirachta indica, Morinda lucida and Vernonia amygdalina in treatments suppressed overall phytoparasitic nematodes (PPNs) better

In the current study, individual combinations of green leaves of *M. lucida*, *A. indica*, *V. amygdalina* across treatments significantly suppressed the overall PPNs in field trial. However, no significant differences in efficacies were observed between treatments that had single, two and three combined botanicals. Of course, combinations of numerous bioactive phytochemical compounds in the combined green leaves should be expected to exhibit individual different modes of actions to synergistically suppress PPNs better. This is because several factors can be considered for this phenomenon to occur. Firstly, all bioactive compounds reported in *M. lucida*, *A. indica*, *V. amygdalina* or any other medicinal botanicals naturally exist in combinational form (Ujah *et al*., 2021; Okechalu *et al*., 2021; Okhale *et al*., 2024; Mezerket *et al*., 2025; Ogwudire, 2025). Secondly, the bioactive compounds in combined green leaves of different botanicals often manifest differential modes of actions (D'Addabbo *et al*., 2011; Du *et al*., 2011; Abdel-Rahman *et al*., 2013; Chin *et al*., 2018; Khan *et al*., 2020; Greiffer *et al*., 2022). Thirdly, some phytochemicals inhibit nematode egg-hatching (Chin *et al*., 2018; Khan *et al*., 2020), whereas others cause destruction of vital internal tissues of infectious PPNs (Barbosa *et al*., 2024). Fourthly, tannins cause disruption of nematode cuticle to impair mobility of PPNs (Greiffer *et al*., 2022). Fifthly, some bioactive phytochemicals have been highly nematotoxic to cause death of infective nematodes (D'Addabbo *et al*., 2011; Du *et al*., 2011; Abdel-Rahman *et al*., 2013; Chin *et al*., 2018). Sixthly, resins and flavonoids potentially protect plants against numerous harmful biological entities in either aquatic or terrestrial ecosystems on Earth (Söderberg *et al*., 1996; Chin *et al*., 2018; Bhattacharya and Chakraborty, 2024). Probably, the varying modes of nematicidal actions of different phytochemicals in green leaves of *M. lucida*, *A. indica* and *V. amygdalina* might have contributed to significantly suppress soil-borne PPNs across all treatments in similar manner. Hence, the percentages for the suppression of overall PPNs were similar across all the individual treatments in this study.

In terms of suppressing overall PPNs on tomato plants in previous field trial by Heve *et al.* (2025), *V. amygdalina* was more promising. In the current study, *V. amygdalina* alone, *M. lucida* alone and *A. indica* alone in treatments significantly ($P < 0.05$) suppressed PPNs in similar manner, thereby suggesting that any of the three botanicals can be used to effectively manage PPNs on okra plants. However, field trials will be required to measure optimum efficacy of *M. lucida*, *A. indica* and/or *V. amygdalina* so that suggestions for the use of any of these medicinal botanicals will sustain maximum suppression of PPNs.

Combining green leaves of Azadirachta indica, Morinda lucida and Vernonia amygdalina in treatments suppressed okra plant biomass

According to reports (Adibah and Azzreena, 2019; Griffiths *et al*., 2021; Vezikov and Simpson, 2023), high doses of naturally-occurring alkaloids in botanicals can be toxic to both animals and plants. Green leaves of botanicals that have higher levels of heavy metals can be phytotoxic to okra plant roots (OPRs) (Ali and Gill, 2022). According to Choudhary *et al.* (2023), knowledge in allelopathic interactions between plants in the field can be explored for management of noxious weeds across cultivated fields. This is largely because environmental factors cause plants to release several powerful allelopathic phytotoxic secondary volatiles (APSV) into soil environments. The APSV often modify soil ecosystem to become phytotoxic to neighbouring plants in the same vicinity (Choudhary *et al*., 2023). Any toxified soil environments generally inhibit seed germination, retard plant growth and suppress physiological and biochemical processes in plants (Choudhary *et al*., 2023; Angon *et al*., 2024; Baah *et al*., 2024). Perhaps, incorporating green leaves of two or three botanicals into soil might become phytotoxic to OPRs in the field. In effect, toxified soil environment for OPRs in "two or three combined botanicals" in treatments could reduce okra plant growth and biomass. Thus, relative to control, all the individual single botanicals in treatments achieved an average of 26.13% increase in okra plant biomass (OPB), whereas two and three botanicals combined in treatments caused 10 and 56% decrease in OPB, respectively. Therefore, we concluded that single botanicals in treatments will improve yield of okra plants, whereas the combined botanicals will cause significant economic losses at a larger scale.

Among the single botanical treatment, we observed that *V. amygdalina* alone in treatments will most likely increase okra yield by 43.4% better than *M. lucida* alone or *A. indica* alone in treatments will achieve. However, in previous field trial by Heve *et al.* (2025), *V. amygdalina* alone, *M. lucida* alone and *A. indica* alone in treatments caused similar percentage increase in tomato plant root weight, thereby suggesting that any of the three botanicals can be used to improve plant biomass, while suppressing PPNs. Our observations in the current study are partially in agreement with recent report of Heve *et al.* (2025).

Conclusions

An increase in number of botanicals combined in treatments caused an increase in visual root-galling severity (VR-GS). All treatments that had single, two-combined and three-combined botanicals effectively suppressed overall phytoparasitic nematodes (PPNs). However, all single botanicals in treatments triggered an increase in okra plant biomass (OPB), whereas two-combined or three-combined botanicals in treatments caused a decrease in OPB. Among the single botanicals in treatments, green leaves of *V. amygdalina* achieved a 43.4% increase in OPB higher than that of *M. lucida* or *A. indica* achieved. Moreover, *V. amygdalina* effectively suppressed overall PPNs, similar to the way *M. lucida*, *A. indica*, two-combined or three-combined botanicals did. Therefore, field trials for assessment of optimum efficacy of *V. amygdalina* will be useful to maximise suppression of PPNs.

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Novelty Statement

Our field trial demonstrated that multiple combinations of fresh green leaves of medicinal brimstone, neem and bitter leaf trees effectively suppressed diverse invasive soil-borne crop-damaging phytoparasitic nematodes (PPNs) in rhizospheres of okra plants. Nevertheless, the protected growing okra crops lost potential to produce desired bio-mass, thereby suggesting that such a decline in output of okra crops on a larger scale can threaten both "global food security" and "sustainable development".

Author's Contribution

Dr. William K. Heve: Conceived the research idea, expertly developed procedures for field trial, methodically supervised data collection, carefully identified PPNs in nematode suspensions, analysed data using R software, drafted the manuscript and carefully revised it for publication. Nechamah N. O. Aryee and Abigail M. Norvor: Executed the field trial, collected samples from the study site and then extracted diverse PPNs, under the auspices of Dr. William K. Heve.

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Human and animal rights
The authors did not use humans, animals or both as objects or materials for this study.

Generative AI and AI-assisted technology statement
The authors have declared that no AI and no AI-assisted technology were used for this study. Rather, observations in the authors' current study were from a carefully-executed field trial at a place, located at (6° 3'0.66" N, 0° 0'4.64" W).

Conflict of interest
The authors have declared no conflict of interest.

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