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Chemical Compositions and Nematicidal Properties of *Hyptis suaveolens* and *Chromolaena odorata* on Root-Knot Nematode Eggs and Juveniles

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Abstract

Laboratory experiments were conducted to investigate the chemical compositions and nematicidal properties in the leaf of Bush tea, *Hytis suaveolens* and Siam weed, *Chromolaena odorata* and on the root-knot nematode eggs and juveniles at different concentration levels. The treatments as different concentration levels were 25% stock solution of bush tea leaf extract, 50% stock solution of bush tea leaf extract, 75% stock solution bush tea leaf extract, 100% (stock) solution bush tea leaf extract, 25% stock solution siam weed leaf extract, 50% stock solution siam weed leaf extract, 75% stock solution siam weed leaf extract, 100% (stock) solution siam weed leaf extract, distilled water (0% stock) served as the control. The different phyto-chemical concentration levels have varying levels of egg hatch inhibition and juvenile mortality as compared with the control (0% stock). Each treatment was replicated three times and fitted into Complete Randomized Design. The results show that the higher the concentration level of each leaf extracts the more the egg hatch inhibition and juvenile mortality. Low egg hatch inhibition and juvenile mortality were observed in the control (distilled water) and 25% plant extracts. The phyto-chemical results revealed the presence of alkaloids, flavonoids, saponins, polyphenols, steroids, coumarins, terpenoids, glycosides and tannins in the leaves of Bush tea and Siam weed. Anthraquinones, anthocyanins and quinones was absent.

KEY WORDS: Root-knot nematode, egg, juvenile, bush tea, siam weed

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1. Introduction

Plant parasitic nematodes are microscopic worms, living in the soil, which feed on plant roots and cause diseases on crops. Due to their small sizes, it is not possible to see them with the naked eye unless with the aid of microscope. Some nematodes feed from the outside of plants, others enter the plant. Feeding of nematodes on crops (plants) resulted into various damages and diseased conditions. Greater damages can occur if viruses or fungi enter the plant as a result of the injuries caused by the nematodes, and then proceed to cause devastating diseases, and eventually lead to plant death (Umar et al., 2014). Nematodes generally are regarded as hidden enemies, they cause yield losses of about 30% in tomato in the tropics (Olson, 2016). *Meloidogyne* species have more than 2000 hosts among which tomato is one (Amer-Zareen, Zaki et al, 2003; De Lannoy, 2001). The root-knot nematode is estimated to cause losses ranging from 10% to 69% in Nigeria (Olabiyi and Ayeni, 2016).

There is an important need to develop effective and sustainable management strategy for nematodes. Following this, the biological nematicidal potential of some plants have been evaluated and few are found effective in the management of root-knot nematode disease (Adegbite and Adesiyan, 2005); Olabiyi et al., 2007). In addition, biological nematicides have been found to be cheaper, less harmful to man and effective in the management of plant parasitic nematodes (Khan et al., 2005; Oka et al., 2000). It had been reported by Khan et al. (2005) that biological bio-nematicides have consistent beneficial effects on soil biological activity and thereby improving the health of plant and reducing the population of plant parasitic nematode. The beneficial effect of biological nematicides with respect to the management of plant parasitic nematodes has been

recognized in recent years. Today there is an increasing interest in discovering nematostatic compounds from the plant products in nematode management (Chitwood, 2002; Khan et al., 2008).

Nematodes infestation and transmission can occur in many ways: via infected plant material, tools, rainwater and irrigation water, strong winds (which carry infested soil particles), and contaminated soil carried on shoes, or animal feet. Nematodes will survive in soil as long as it stays moist (Umar et al., 2014). The use of plant extracts is one of the methods for nematode control. They are cheap, easy to apply, produce no pollution hazards and have the capacity to improve the soil health (Simone, 2018). Root-knot nematodes infect a wide range of important crop plants and are particularly damaging to vegetable crops in tropical and subtropical countries (Sikora and Fernandez 2005). There are more than 90 described species in the genus Meloidogyne but the four most commonly occurring species are *Meloidogyne incognita*, *M. arenaria*, *M. javanica* and *M. hapla* (Karssen 2000; Hunt et al., 2005). The short life cycle of 6 to 8 weeks enables root knot nematode populations to survive well in the presence of a suitable host and their populations build up to a maximum usually as crops reach maturity (Shurtleff and Averre, 2000).

Application of chemical nematicide is the primary method used to control root-knot nematodes in vegetables in Africa and worldwide. However, apart from its very high cost, there is growing public uncertainty about the routine use of pesticides, including nematicides, in global agriculture. Concern about pesticides use has stimulated interest in the development of alternative pest management strategies (Claudius-Cole et al., 2001). In recent times, researchers have paid more attention to the study of natural pesticides in nematode control (Adegbite and Adesiyan, 2005). Results available from these studies show great promises. Several plants have been identified with nematicidal or nematostatic properties either in their seeds, fruits, roots, leaves, barks or in their root exudates and have been used in rotational practices to control root-knot nematodes (Claudius-Cole et al., 2001). Bush tea (*Hyptis suaveolens*) and Siam weed (*Chromolaena odorata*) leaf powders have shown promise as bio-control agents against nematodes (Omobolanle et al., 2020; Nyong et al., 2023).

Chromolaena odorata is a tropical and subtropical species of flowering shrub in the family Asteraceae. It is native to the Americas, from Florida and Texas in the United States south through Mexico and the Caribbean (Nesom, Guy, 2006) to South America. (King and Rob, 2017) It has been introduced to tropical Asia, West Africa, and parts of Australia (Atlas of Living, 2021). Common names include Siam weed, rouge plant, Christmas bush, jack in the box (Nesom, Guy, 2006) devil weed, common floss flower, hagunoy (Cebuano language), rompesaragüey (Spanish), Abani di egwu or Nsiibilibe (Igbo language**),** ewé Akíntọ́lá (Yorùbá) and triffid (Gunasekera, 2009).Siam weed belongs to kingdom plantae, order *Asterales*, family*Asteraceae*, genus *Chromolaena*, species *C. odorata.*

Hyptis suaveolens, commonly known as nitta, is a plant that has garnered significant attention in various research fields due to its diverse pharmacological properties and potential applications. Studies have highlighted the plant's antimicrobial (Andrade et al.,2017), larvicidal (Aïzoun et al., 2022), insecticidal (Aliyu et al.,2022), and medicinal properties (Mokwenye et al.,2023). The plant is rich in essential oils with antibacterial, antiseptic, and antiulcer properties (Hsu et al., 2023). Additionally, it has been found to possess bioactive chemical constituents such as rosmarinic acid and caffeic acid (Thomford et al.,2016). The plant has been explored for its potential in nanoparticle synthesis (Oumarou et al., 2021), larvicidal activities against malaria vectors (Aïzoun et al., 2022),and as a biopesticide (Ahmed et al., 2022). Studies have also investigated its efficacy in controlling mosquito populations (Abagale et al., 2017), with extracts showing adulticidal activity against *Anopheles gambiae* (Abagale et al., 2017). Additionally, theplant has been evaluated for its repellent properties against Anopheles mosquitoes (Jidere and Oluwatayo, 2018;Cruz-Torres et al.,2020)**.** *Hyptis suaveolens* has been utilized in various applications, ranging from medicinal uses to food science. It has been studied for its potential to control pests in agricultural settings (Sumitha and Thoppil, 2015**),** as well as its thermal and emulsifying properties in food processing. Nitta (*Hypis suaveolens*), a plant species of interest, contains various phytochemicals such as alkaloids, flavonoids, and terpenoids, which have been associated with antimicrobial and nematicidal properties in previous research (Leelarasamee et al., 2018; Soliman et al., 2021). Understanding the specific phyto-constituents of Nitta and their interactions with root-knot nematodes can provide valuable insights into the mechanisms underlying plant-nematode interactions and potential applications in nematode management strategies. Phyto-constituents of bush tea and siam weed play a crucial role in the management of root-knot nematodes, particularly *Meloidogyne* species. The objective of the current research is determination of the chemical composition and assessment of the nematicidal properties of *Hyptis suaveolens* and *Chromolaena odorata* on root-knot nematode eggs and juveniles.

2. Materials and Methods

2.1 Experiment 1: Phytochemical analysis of Bush tea and Siam weed

(a) Qualitative phyto-chemical analysis:

The phytochemical components of *Hyptis suaveolens* and *Chromolaena odorata* leaf extracts were evaluated using a standardized protocol described by Shaikh and Patil, (2020). Saponins, alkaloids, flavonoids, tannins, coumarins, steroids, terpenoids, glycosides, quinones, anthraquinones, anthocyanin and phenol were among the phytochemicals investigated.

Test for Alkaloids: Mayer's and Wagner's reagent test: To 2 ml of plant extract in different tube, 2 ml of 2% hydrochloric acid was added. Then 3 drops of Mayer's and Wagner's reagent were added. Presence of a creamy white/yellow precipitate was observed for Mayer's and a brown/reddish precipitate for Wagner's signifies the presence of alkaloids.
Test for Tannins: Braymer's test: To 2 ml of plant extract, 2 ml of water was added with 3 drops of 10% ferric chloride. The formation of blue-green color precipitate indicates the presence of tannins.
Test for Saponins: Frothing Test: To 2 ml of plant extract, 2 ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1 cm layer of foam is observed which shows the presence of saponin.
Test for flavonoids: Alkaline reagent test: To 2 ml of plant extract, 2 ml of 2% sodium hydroxide was added and few drops of dilute hydrochloric acid. An intense yellow color becomes colorless on addition of diluted acid shows the presence of flavonoids.
Test for Quinones: Sulphuric acid test: To 2 ml of plant extract, 2 ml of isopropyl alcohol was added and 2 ml concentrated sulphuric acid. A red color was formed and this indicates the presence of quinones.
Test for Glycosides: Sulphuric acid test: To 2 ml of plant extract, 2 ml 50% sulfuric acid was added. The mixture was heated in boiling water for in boiling water for 15 minutes. Fehling solution was then added and the resulting mixture was heated to boiling. A brick-red precipitate indicates the presence of glycosides.
Test for Terpenoids: To 0.5 ml of plant extract, 2 ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicates the presence of terpenoids.
Test for Phenols: Ferric chloride test: To 2 ml of plant extract, 2 ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of dark green/bluish black color indicates the presence of phenols.
Test for Coumarin: Sodium hydroxide test: To 2 ml of plant extract, 2 ml of 10% NaOH was added. Formation of yellow color indicates the presence of coumarin.
Test for Steroids: Salkowski test: 2 ml of plant extract was mixed with 2 ml of chloroform, followed by the addition of 10 drops of acetic anhydride and 3 drops of concentrated sulfuric acid. The emergence of a red-rose color indicates the presence of steroids.
Test for Anthocyanins: HCl test: To 2 ml of plant extract, 2 ml of 2N HCl and 3 drops of ammonia solution was added. Pink-red sol. Which turns blue-violet after addition of ammonia indicates the presence of anthocyanin.
Test for Anthraquinones: Borntrager's test: To 2 ml of plant extract, 2 ml drops of 10% ammonia solution was added then shake vigorously for 30 seconds. A pink, violet or red colored solution indicates the absence of anthraquinone.

(b) Quantitative phytochemical analysis: The quantity of saponin, alkaloid and phenol in Bush tea and Siam weed were measured. The Saponins and Alkaloids in Bush tea and Siam weed were measured in percentages, while the Phenol was quantified using Garlic Acid Equivalent (GAE) in Milligram (mg) per gramme (g).

Determination of total alkaloid contents: The alkaloid content was determined using the method described by Van Tans (2018). A 5g sample was mixed with 200 ml of 10% acetic acid in ethanol in a 250 ml beaker. The mixture was filtered for 4 hours, and the resulting extract was then concentrated in a water bath to approximately 25% of its initial volume. Concentrated ammonium hydroxide was gradually added until precipitation occurred. The precipitate was then treated with a diluted ammonium hydroxide solution and filtered. The final product was obtained as a weighed and dried alkaloid.
Determination of total saponin contents: The methodology described by Adepoju *et al.,* (2024) was followed precisely. A 20 g sample of each was macerated with 100cm^3 of 20% aqueous ethanol in a conical flask and heated at 55^{0}C for 4 hours using a hot water bath. The mixture was then filtered and the residue was further extracted with 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml using a 90^{0}C water bath, followed by the addition of 20 ml diethyl ether. The aqueous layer was separated and washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated to a constant weight, and the saponin content was determined
Determination of total phenolic content: Siddiqui *et al.,* (2017) described a method for determining the total phenolic content in plant extracts using spectro-photometric analysis with the Folin-Ciocalteu test. The procedure involves preparing a reaction mixture in a 25 ml volumetric flask by combining 1 ml of plant extract, 1 ml of Folin-Ciocalteu reagent, and 9 ml of distilled water. After a 5-minute reaction time, 10 ml of a 7% sodium carbonate solution are added. Gallic acid standards (20-100 µg/ml) are prepared using the same

procedure. The absorbance of the test and standard solutions is then measured at 550 nm using a UV/Visible spectrophotometer after a 90-minute incubation period at room temperature. The total phenol content is expressed as milligrams of gallic acid equivalent (GAE) per gram of extract.

2.2 Experiment 2: Nematicidal Properties of Bush Tea and Siam Weed on Root-Knot Nematode Eggs

Extraction of root-knot nematode eggs: Galled roots were collected from *Celosia argentea* on which a pure culture of *Meliodogyne incognita* was raised. The galled roots were washed properly under the running tap in order to get rid of attached soil. The roots were cut into small pieces and shaken with 0.5% sodium hypochlorite solution for four minutes in a Kilner's jar (Hussey and Barker, 1973) in order to digest the gelatinous matrix encasing the eggs. The content was sieved through a 200 mesh sieve nested over a 500 mesh sieve. The 500 mesh sieve containing the eggs was placed under a running water tap in order to remove residual sodium hypochlorite solution. Egg suspension was made with distilled water inside a 500 ml sized beaker. The number of eggs in 1 ml egg suspension was standardized so that 1 ml egg suspension contained approximately 100 fresh *Meloidogyne incognita* eggs.

***Meloidogyne incognita* egg hatch test:** Aliquots of 1 ml of *Meloidogyne incognita* egg suspension containing count of a hundred (100) fresh *Meloidogyne incognita* eggs were dispensed into each of the transparent glass petri-glass containing 10 ml of different extracts (bush tea leaf and siam weed leaf) and control (distilled water) in the laboratory. A hundred (100) fresh *M. incognita* eggs dispensed into distilled water served as the control. The treatments including control were replicated 4 times and incubated at ambient temperature. The glass petri dishes were covered with glass covers to prevent evaporation. The experimental design was a completely randomized one with 35 treatments. Counts of un-hatched eggs were made and recorded at every 24 hours for 7 days under the stereo microscope.

2.3 Experiment 3: Nematicidal Properties of Bush tea and Siam weed on root knot nematode juveniles

Source of root-knot nematode juveniles: Freshly extracted *M. incognita* eggs were incubated at 27 – 30^{0}C for 72 hours. *M. incognita* juveniles that were hatched out from the eggs were poured into 200 ml sized conical flask. The content was allowed to rest and later decanted. Standardization of number of juveniles per unit volume was done, so that 1 ml juvenile suspension contained a count of approximately 100 juveniles.
Effect of Bush tea and Siam weed on the activity and survival of *Meloidogyne incognita* juveniles: Aliquots of 1 ml of *M. incognita* juveniles suspension containing a count of approximately 100 freshly hatched juveniles were pipetted into each of the transparent glass Petri-dishes containing 25% stock solution bush tea leaf extract, 50% stock solution bush tea leaf extract, 75% stock solution bush tea leaf extract, 100% (stock) solution bush tea leaf extract, 25% stock solution siam weed leaf extract, 50% stock solution siam weed leaf extract, 75% stock solution siam weed leaf extract, 100% (stock) solution siam weed leaf extract, distilled water (control). The Petri-dish that contained 100 *M. incognita* juveniles in distilled water only served as the control. The glass petri-dishes were covered to prevent evaporation and other pathogen interference.
All the glass Petri-dishes were labeled accordingly and arranged randomly on the bench at the Crop Protection Laboratory, Bee House, Ladoke Akintola University of Technology, Ogbomosho, Nigeria. The experiment was a completely randomized design with three replicates. Percentages of *M. incognita* that were active (life) and dead were put on record after 24 hours each day for 7 days consecutively.

3. Results

It was evident that alkaloids were highly present in Bush tea while Flavonoids, Saponins, Polyphenols, Sterols, Coumarins, Terpernoids, Glycosides and Tanins are moderately present. Siam weed leaf has Flavonids and Coumarins to be highly present while Alkaloids, Saponins, Polyphenols, Sterols, Terpernoids, Glycosides and Tanins were moderately present. Be as it is, Anthocyanins, Anthraquinones and Quinones were absent (Table 1).

Table 1: Qualitative phytochemical screening of Bush tea and Siam weed leaves

Phytochemicals	Bush tea leaf	Siam weed leaf
Alkaloids	+++	++
Flavonoids	++	+++
Saponins	++	++
Polyphenols	++	++
Steriods	++	++
Coumarins	++	+++
Terpenoids	++	++
Glycosides	++	++
Anthraquinones	-	-
Anthocyanins	-	-
Tannins	++	++
Quinones	-	-

Foot note: Slightly present (+), Moderately present(++), Highly present(+++) and Absent (-)

Table 2 shows the result of the quantitative phytochemical screening of Bush tea and Siam weed leaves. It shows that, Saponin in the leaf of bush tea was 2.21±0.02%, alkaloid was 4.19±0.01%. and Phenol was 29.54±0.00 GAE/mg/g.

The Siam weed leaf sample tested showed that it contained 1.83±0.17% Saponin, 13.02±0.17% Alkaloid. 25.52±0.07 GAE/mg/g Phenol.

Table 2: Quantitative phytochemical screening of Bush tea and Siam weed leaves

Leaf sample	% Saponin	% Alkaloid	Phenol (GAE/mg/g)
Bush tea leaf	2.21±0.02	4.19±0.01	29.54±0.00
Siam weed leaf	1.83±0.17	13.02±0.17	25.52±0.07

Foot note: Mean ± SD (n=3); GAE = Garlic Acid Equivalent; QE = Quercetin Equivalent

Table 3 elicits the inhibitory effect of aqueous extract of Siam weed and Bush tea leaves on the hatchability of root-knot nematode eggs. It was evident that the percentage egg hatch was 66% in the control (0%) at day 1, 93% at day 2, while it was 100% at day 3 till day 7. In 25% aqueous extract of Siam weed, there was 10% egg hatch. Egg hatchability was totally inhibited from day 2 to day 7. At 50%, 75%, 100% there was no egg hatch from day 1 to day 7. This shows that 50%, 75%, and 100% concentrations of the aqueous extract of Siam weed, resulted to 100% egg hatch inhibition.

Table 3 also shows that in the aqueous extract of bush tea, egg hatch were 68% (Day 1), 97% (Day 2), 100% (Day 3 - 7). Only 8 eggs were hatched at day 1 which was treated with 25% Bush tea, there was total egg hatch inhibition between day 3 and 7. There were no egg hatch in higher concentration for day 7.
The lowest egg hatchability was observed in 0%, while the highest was recorded at 100% in both samples.

Table 3: Aqueous extract of Siam weed and Bush tea leaves on the hatchability of root-knot nematode eggs

Plant part	Concentration	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Siam weed	0%	66^{c}	93^{b}	100^{b}	100^{b}	100^{b}	100^{b}	100^{b}
leaf	25%	10^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	50%	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	75%	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	100%	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Bush tea	25%	8^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
leaf	50%	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	75%	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	100%	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}

Same superscript (alphabet) along same column indicates no significant difference

Table 4 shows the effect of aqueous extract of Siam weed and bush tea leaves on the root-knot nematode juveniles' mortality. The results show that Siam weed leaf at 0% (control) there were 100 live nematodes on day 1 and 2. On day 3, 4, 5, 6, 7 the number reduced to 95, 80, 59, 57 and 44 respectively.

It was observed that there was low mortalities at no and low aqueous extract of Siam weed leaf concentration. At 25% concentration of Siam weed extract, there were 36 live nematode juveniles on day 1, by day 2, 11 live nematodes were left. From day 3 – 7, 100% mortalities were observed.

The higher the concentration of the aqueous extract of Siam weed leaf, the higher the mortality rate. At 50%, 75%, 100% concentrations, it was observed that there were total juvenile mortality throughout the exposure time (1 -7 days).

Table 4 revealed that Bush tea at 25% concentration has 41 live nematodes on day 1 which significantly reduced to 15 on day 2. From day 3 to day 7, mortality rates were significantly increased causing total nematode mortality. At the higher concentration of 50%, 75%, 100%, the total nematode mortality were recorded during the exposure (1 – 7 days).

Table 4: Aqueous extract of Siam weed and bush tea leaves on the root-knot nematode juveniles' mortality

Plant part	Concentration	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Siam weed	0%	100^{d}	100^{c}	95^{b}	80^{b}	59^{b}	57^{b}	44^{b}
leaf	25%	36^{b}	11_{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	50%	9^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	75%	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	100%	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Bush tea	25%	41^{b}	15^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
leaf	50%	11^{a}	2^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	75%	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	100%	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}

Same superscript (alphabet) along same column indicates no significant difference

4. Discussion

The active ingredients in Siam weed and Bush tea leaves were Alkaloids, Flavonoids, Saponins, Polyphenols, Steroids, Terpenoids, Glycosides, Coumarins and Tannins have been reported to have implications for nematode control (Omobolanle et al., 2020; Nyong et al., 2023). The study on the phyto-constituents of bush tea (Nitta) and siam weed leaf and their in vitro assessment on root-knot nematodes holds significant promise for advancing knowledge in plant-nematode interactions and could potentially lead to the development of effective and sustainable solutions for nematode control.

The various levels of bush tea leaf and siam weed leaf extract inhibited the egg hatch and juvenile survival of *M. incognita* in vitro. This inhibitory level increased with the concentration of bush tea leaf and siam weed leaf extract and exposure time. The results of this research corroborate the findings of Oyedunmade and Olabiyi (2004) who reported that extracts from *Vernonia amygdalina* inhibited root-knot nematode juveniles and egg hatch with percentage inhibition varying at different levels of concentration. Egg hatching and juvenile survival was significantly influenced by the concentrations of the extract and duration of exposure.

5. Conclusion

The study was conducted to evaluate the effects of plant-derived biological nematicides on root-knot nematode. The study has demonstrated that the use of plant-derived biological nematicides can be effectively explored as alternative management strategy for root-knot nematode. The application of plant-derived biological nematicides is therefore recommended for sustainable management of root-knot nematode.

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