Nematicidal potential of aqueous extract of *Hyptis suaveolens* in the management of root-knot nematode, *Meloidogyne incognita* of some cowpea cultivars

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Summary

Studies were conducted under field and screenhouse conditions to investigate the potentials of crude aqueous leaf extract of *Hyptis suaveolens* in the management of the root-knot nematode *Meloidogyne incognita* of three cowpea varieties (Sampea 9, 10 and 11). A Randomized Complete Block Design was used in the field while a completely randomized design was used for the screenhouse trials. Results showed that the treatment significantly ($p < 0.05$) improved the growth and yield of the three varieties and also reduced soil nematode population and root galls. It was also observed that all the three varieties were susceptible to the root-knot nematode infestation but Sampea 10 recorded higher yield that were significant in the pot trials. Phytochemical screening revealed the presence of saponins, alkaloids, flavonoids and steroids in the leaves of *H. suaveolens*. For higher yield of the evaluated cowpea varieties in a nematode endemic zone, aqueous leaf extract of *H. suaveolens* is being recommended for infested soil treatment.

Keywords: Meloidogyne incongnita, Hyptis suaveolens, Cowpea cultivars, bio-active ingredients, aqueous extract

Introduction

Globally, annual losses resulting from infestations caused by plant parasitic nematodes are enormous. The root-knot nematodes are the most notorious phytophagous nematodes. Trudgill and Block (2001) reported that the most important plant parasitic nematodes are the root-knot nematodes, *Meloidogyne* spp. since they infest majority of the economically important plant species in the world. This genus contains at least 80 species (Karssen, 2002) and has been reported to cause an estimated US$ 100 billion loss per year worldwide (Oka et al., 2000).
Cowpea (*Vigna unguiculata*) is a very versatile pulse because of its drought tolerant characters, soil resorting properties and multipurpose uses. It is a good source of plant proteins and plays a substantial role as grain and vegetable crop mainly for the rural people of Nigeria and Africa at large. The production of cowpea is hampered by legions of biotic and abiotic factors in developing countries. Among the biotic factors are nematodes, viruses, fungi, bacteria and insects. *Meloidogyne* species are known to be serious pathogens of cowpea in many cowpea growing areas (Umar and Simon, 2008). Successful management of plant parasitic nematodes with synthetic nematicides have been reported by several researchers, but the resultant consequences of their cumulative effect on plants, animals, human and the environment are becoming intolerable. Hence the search for other acceptable alternative control measures with nematicidal potential is essential. Nematicidal activities of some indigenous plants and their products have been reported (Adekunle and Akinlua, 2007; Oyedunmade and Izuogu, 2011; Izuoguet al., 2012). There are many more plants in our tropical forests with anti-microbial activities yet to be exploited. Because there is a dearth of information from literature on the nematicidal potential of *Hyptis suaveolens*, this study was therefore designed to investigate the efficacy of aqueous leaf extracts of *H. suaveolens* against root-knot nematodes infesting some cowpea varieties, as well as to evaluate their response to the nematode infestation.

Materials and methods

Screenhouse trials

Using the method described by Guatam and Goswami (2002), sandy-loam topsoil was sterilized in a drum at 60 ºC for 24 hours. The soil was allowed to sufficiently cool for 72 hours before filling up thirty perforated 8 kg sized buckets and placed on an elevated platform to avoid reinfestation of soil by microorganisms. The experimental design was a completely randomized experiment of six treatments where each treatment was replicated five times. Three cowpea varieties which received treatment and

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their control counterparts that did not receive any treatment served as objects in the study. The arrangement was as follows:

Variety 1 inoculated with nematode; Variety 1 control
Variety 2 inoculated with nematode; Variety 2 control
Variety 3 inoculated with nematode; Variety 3 control

For each variety, ten pots were used and two seeds were planted in each pot; five served as the experimental and the other five as the control. Crude aqueous leaf extract of *H. suaveolens* was prepared by air-drying and grinding the leaves. One kilogram was weighed out and soaked in three liters of boiled water for 24 hours before sieving. The sieved extract served as the crude treatment. One week after planting, seedlings were thinned down to one plant per pot. Then 1000 freshly extracted root-knot nematode (*Meloidogyne incognita*) juveniles were inoculated into the soil at the base of each seedling (Southey, 1986). Immediately after, 100ml of the crude extract were applied to each of the 15 experimental pots. The other 15 pots served as the control. The application of the crude extract was repeated one month after planting.

Field trial

A piece of land measuring 20 m by 30 m on the Teaching and Research Farm of the University of Ilorin was ploughed and harrowed into ridges. Initial soil nematode population was extracted using modified Baerman's tray method as described by Whitehead and Hemming (1965). The nematodes were counted using a stereo-microscope. Thereafter, soil population of root-knot nematode, *Meloidogyne incognita* was increased by incorporating 50 kg of chopped heavily galled roots of susceptible *Celosia argentea* TLV-13 into the soil.
The land was divided into two blocks of 48 plots with 2 m alley separating the blocks and 0.5 m between plots. Each treatment had two ridges replicated four times. Soil samples were randomly collected for initial soil nematode population. The design of the experiment was a randomized complete block design (RCBD). Two seeds were planted per hole and 100 ml of the prepared crude plant extract were applied around the base of each seedling in one of the blocks. The seedlings in the second block which did not receive any treatment served as control. Treatment application was repeated one month after planting.
Phytochemical analyses of the used test plant were carried out in the chemistry department, University of Ilorin, Nigeria using the methods described by Trease and Evans (1989), Harborne (1986) and Sofowora (1982) to test the presence of bio-active ingredients in the leaf of *H. suaveolens*.

Data collection

Data on growth parameters; plant height and number of leaves were collected bi-weekly. The yield data were obtained at harvest. Additional data collected include initial soil nematode population for field trials only and soil nematode population at one month after planting and at harvest for both screenhouse and field experiments. Root gall rating was also carried out at harvest using the method described by Makete (2000).

Data analysis

All numerical data except the root gall rating and population densities in the plots were subjected to ANOVA and where appropriate, the means were separated using Duncan's Multiple Range post-hoc test.

Results and discussion

Generally, all treated plants had significantly higher growth parameters than their control counterparts: plant height, number of leaves and pods (Tables 1-3). Soil nematode population was also reduced in the treated plots (Table 4). Consequently, galling of roots was significantly reduced in all the treated plants compared to the control. The implication of this is that *H. suaveolens* aqueous leaf extract improved the vegetative growth of the cowpea varieties (Tables 1 and 2) and possibly improved their photosynthetic ability with respect to the significantly higher number of leaves recorded. The obtained results corroborate the findings of Adekunle and Fawole (2003) who observed the improved growth of *Meloidogyne* infested cowpea treated with leaf and root extract of siam weed (*Chromolaena odorata*) and neem (*Azadirachta indica*). Less nematode stress on treated plants as a result of the reduced nematode populations and galling of the roots equally contributed to their significantly better performance in terms of growth and yield of the treated cowpea plants compared to the control. The varietal differences played significant roles in plant response to nematode infestation and their environments. Except for Sampea 11, root galling was higher in the control plants grown under pot conditions (Table 5); nematode population was also higher in pot trials (Table 4). This could be a result of a higher reproductive ability which may be traced to a more conducive environment created in the pots by elimination of other soil microorganisms. Some species of soil microorganisms could be nematode competitors in terms of their interaction and thereby limiting nematodes ability to reproduce. Conversely, different forms of nematode interactions with other arrays of soil microorganisms under field conditions may have contributed to the reduced infectivity of nematodes on the plants.

However, all three cowpea varieties were susceptible to root-knot nematode. Though there were significant differences in the measured vegetative parameters, yield was not totally dependent on the vegetative growth, but was determined more by varietal potentials of the test crop.

The bioactive ingredients (saponins, alkaloids, flavonoids and steroids) present in *H. suaveolens* were responsible for the suppression and reduction of population density of the root-knot nematodes infesting the cowpea varieties. In the *in vitro* studies, Izuogu and Oyedunmade (2008 and 2009) implicated the presence of saponins and flavonoids in *Phyllanthus amarus, Morinda lucida, Cymbopogon citratus* as being responsible for juvenile mortality, inhibition of egg hatch and development of infective second stage juveniles (J2) of *Meloidogyne incognita*.

The present study has therefore identified the crude leaf extract of *H. suaveolens* as a potential source of organic nematicide that could be employed in the management of root-knot nematode species infesting cowpea varieties especially Sampeas 9, 10 and 11.

Table 1. Effect of *Hyptis suaveolens* on mean plant height of selected cowpea varieties infected with *Meloidogyne incognita* (cm)

	Field Trial					**Pot Trial**				
Treatments	2WAP	4WAP	6WAP	8WAP	10WAP	2WAP	4WAP	6WAP	8WAP	10WAP
Hyptis	18.00	26.80a	29.20a	31.60a	35.33a	29.00	52.60a	70.20a	73.07	75.80a
Control	19.64	22.60b	24.13b	25.47b	27.00b	27.60	31.20b	47.33b	49.20	52.46b
S.E.D	0.97	0.94	0.92	0.86	0.97	0.92	1.08	1.62	2.93	1.56
L.S.D	1.95	1.93	1.89	1.78	1.97	1.90	1.53	1.86	4.14	2.19
	N.S					N.S				
Sampea 9	19.40b	22.50b	24.60a	26.10b	26.00b	25.00	44.60a	60.20a	64.30a	65.30a
Sampea 10	19.90b	24.20b	25.80b	27.80b	29.70ab	27.00	40.30b	53.10b	53.50ab	58.90b
Sampea 11	22.70a	26.40a	25.80a	31.70a	33.70a	26.00	40.80ab	55.50ab	60.20ab	61.60ab
S.E.D	1.16	2.37	1.12	1.05	1.17	1.55	1.32	1.99	1.89	3.59
L.S.D	2.38	1.16	2.31	2.18	2.41	2.99	1.87	2.81	2.68	5.08
						N.S				

Means in the same column followed by different letter(s) are significantly different at $p<0.05$ according to Duncan's Multiple range tests.
N.S = Not Significant
WAP – Weeks After Planting

Table 2. Effect of *H. suaveolens* on mean number of leaves

	Field Trial Experiment					**Pot Experiment**				
Treatments	2WAP	4WAP	6WAP	8WAP	10WAP	2WAP	4WAP	6WAP	8WAP	10WAP
Hyptis	32.20a	50.87a	67.27a	72.00a	70.53a	12.80	20.04	24.40a	27.60a	30.67a
Control	8.27b	18.73b	29.53b	34.13b	35.40b	13.60	18.07	20.27b	20.00b	24.70b
S.E.D	0.98	0.63	1.39	1.92	2.93	0.93	0.94	0.92	1.06	1.06
L.S.D	1.39	0.89	1.97	2.71	1.15	1.91	1.93	1.90	2.18	2.18
Sampea 9	18.90b	35.30	49.30	52.60	52.00	11.50b	16.10b	17.00b	21.40b	24.10b
Sampea 10	18.90b	34.90	46.00	51.00	51.30	12.00b	18.00b	20.90b	23.10b	26.20b
Sampea 11	22.90a	34.20	49.90	55.60	55.60	17.10b	23.60a	27.10a	29.90a	32.70a
S.E.D	1.20	0.78	1.70	2.35	1.96	1.14	1.15	1.13	1.3	1.29
L.S.D	1.70	1.10	2.41	3.32	2.78	2.34	2.37	2.33	2.68	2.67
		N.S	N.S	N.S	N.S					

Means in the same column followed by different letter(s) are significantly different at $p<0.05$ according to Duncan's Multiple range tests.
N.S= Not Significant
LSD = Least Significant Difference

Table 3. Effect of *M. incognita* on mean number of pods/plant and gall rating

Tresatment	**number of Pods Pots**	**Field**
Hyptis	23.23a	26.20a
Control	13.23b	13.47b
S.E.D	1.01	1.12
L.S.D	2.07	1.59
Sampea 9	17.60b	19.90
Sampea 10	24.20a	20.60
Sampea 11	16.70b	19.00
S.E.D	1.25	1.37
L.S.D	2.57	1.94

Means in the same column followed by different letter(s) are significantly different at P<0.05 according to Duncan's Multiple range tests.
N.S = Not significant

Table 4. Mean soil nematode population

Before planting (Initial)			**One month after**		**Harvest**	
Pot	**Field**		**Pot**	**Field**	**Pot**	**Field**
100 juveniles per pot	365	Treated Sampea 9	96	45	100	53
		Sampea 10	73	61	138	68
		Sampea 11	66	52	102	50
		Control Sampea 9	142	72	270	109
		Sampea 10	175	90	309	135
		Sampea 11	206	65	398	130

Table 5. Effect of *M. incognita* on gall rating

Tresatment	**Field**	**Pots**
Hyptis	1	1
Control	2	3
Sampea 9	1	2.5
Sampea 10	1	2
Sampea 11	2	2

Conclusions

From this study, the efficacy of crude leaf extract of *H. suaveolens* in the management of root- knot nematode (*M. incognita*) infesting three cowpea varieties (Sampea 9, 10 and 11) have been determined. The bioactivity of the crude aqueous extract was as a result of the presence of some phytochemicals such as saponins, alkaloids, flavonoids and steroids in the leaves.

For higher yield of the evaluated cowpea varieties, aqueous leaf extract of *H. suaveolens* is being recommended for infested soil treatment.

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