Journal of Agriculture and Veterinary Sciences
Volume 5, Number 2, 2013
ISSN: 2277-0062

Comparative Evaluation of Some Botanicals and Carbofuran in the Control of Root-Knot Nematode, *Meloidogyne spp*. on Cowpea (*Vigna unguiculata*)

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Abstract: The nematicidal effect of leaves of three selected plants, *Calotropis procera*, *Crotalaria retusa*, and *Hyptis suaveolens* were compared with a synthetic nematicide, Carbofuran 3G applied singly to control root knot nematode infecting cowpea. A ploughed, harrowed and ridged plot of land was divided into 3 blocks, each having 8 treatments but replicated 3 times in a randomized complete block design form to give a total of 24 subplots. All subplots received the same level of root knot nematode inoculation. The leaves of each test plant were applied at two rates (3.0 tonnes/ha and 1.5tonnes/ha) while the Carbofuran was applied at 3kgai/ha. All the test plant leaves applied at higher rates performed significantly higher (P=0.05) and were almost as effective as Carbofuran at 3kgai/ha in terms of plant height, leaf number, seed weight, and days to 50% flowering. At harvest, galling was significantly lower in Carbofuran treated plots. The control plants were most galled with poor yield. The lower rate of botanicals (1.5tonnes/ha) performed significantly better than control plants in suppressing the effects of root-knot nematode infecting cowpea. These suggest that the tested botanicals are effective in the management of root-knot nematode population. The application of the tested botanicals at 3tonnes/ha can be used as substitutes for Carbofuran or other nematicidal synthetic chemicals in controlling root-knot nematode population in farmlands.

Keywords: *Meloidogyne spp*., *Calotropis procera*, *Crotalaria retusa*, *Hyptis suaveolens*, Carbofuran, Botanicals.

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is an important legume grown by both small and commercial farmers in Nigeria. The importance of cowpea lies in its high protein content and the fact that it fixes atmospheric nitrogen into the soil. It has been estimated that 3.3 million tonnes of dry grains of cowpea are produced worldwide with Nigeria producing 2.1 million tonnes making it the world largest producer (IITA, 2002). Plant parasitic nematode cause diseases of plant and are responsible for approximately 50% of overall plant damage (Abbasi *et al*., 2008). Root-knot nematodes (*Meloidogyne spp*.) are found in all temperate and tropical areas, and are among the most damaging plant pathogens worldwide (Trudgil and Blok, 2001). The damage they cause to plants usually increase with the number of nematodes present (Ploeg, 2001). The symptoms of nematode infection include formation of root galls which results in stunting, nutrient and water uptake reduction, increased wilting, mineral deficiency, weak and poor yielding plant (Abad *et al*.,2003). Various control measures have been adopted for controlling root-knot nematodes. However, the application of chemical nematicide has been found to be more effective measure for control of nematodes, but the highly toxic residual effect of chemicals on environment and particularly on non-target organism (Anastasiadis *et al*., 2008), demands an urgent need to develop alternative strategies for the control of nematodes. In search for more benign acceptable alternatives, the possibilities are being investigated of exploiting nematode antagonistic botanicals for the management of plant parasitic nematodes (Chitwood, 2002; Aktar, 2004). Botanicals (plant-based pesticidal chemicals) have found favour as alternatives to pesticides in recent times. Some of these botanicals are already being exploited commercially in pest management. Different plant species are being tested to identify the sources of nematicidal substances and many of them have shown promising results in the control of plant parasitic nematodes within and outside Nigeria. Therefore, this study tends to evaluate and compare the efficacy of some botanicals and a synthetic nematicide, carbofuran in controlling root-knot nematode.

Materials and Methods

Study Area

The experiment was carried out at the Faculty of Agriculture Teaching and Research Farm, University of Ilorin, Kwara state, Nigeria. A piece of land measuring 30m x 24m was ploughed, harrowed and ridged as experimental plot. Experimental layout/ field preparation: The land was divided into 3 blocks. Each block comprises 8 treatments which were replicated three times giving a total of 24 subplots in a randomized complete block design (RCBD) form. Alley ways measuring 1m were in-between the subplot and 1.5m in-between blocks. This was done to serve as a natural drainage and to prevent treatment interactions. Weeding of the plots was done manually with hoe.

Plant Materials

Plant materials used for the experiment as organic soil amendments are the leaf of *Crotalaria retusa*, *Calotropis procera* and *Hyptis suaveolens*. They were obtained from uncultivated land on the University of Ilorin Campus. The synthetic nematicide, Carbofuran 3G was bought from an agro-chemical shop in Ilorin. Soil inoculation with root-knot nematodes: Chopped roots of nematode infested *Celosia agentea* plant were incorporated into the soil to build up the initial root-knot nematode population in the field.

Determination of Root-Knot Nematode Population in Soil

Prior to the incorporation of plant leaves into the soil, the initial population of the root-knot nematode was determined. Ten core soil samples were randomly taken from each of the sub-plots using a soil auger at 10-15cm depth and mixed in a polythene bag to form a composite core sample. A total of 24 bags of composite soil samples were taken to the Laboratory for nematode extraction. Using the modified Baermann's technique by Whitehead and Hemming, (1965), nematodes were extracted from 200ml soil of each composited sample. The population was counted using stereoscopic microscope.

Treatment Application

A week after soil nematode build-up, leaves of *Crotalaria retusa*, *Calotropic procera* and *Hyptis suaveolens* were incorporated into the soil at the rates of 3.0tonnes/ha and 1.5tonnes/ha. The synthetic nematicide Carbofuran was applied at the recommended rate of 3kgai/ha. This was left for another one week to allow decomposition in the soil before planting operation. Treatments used are as shown:

Treatment 1 = *Calotropis procera* at 3.0 tonnes/ha + Inoculum.
Treatment 2 = *Calotropis procera* at 1.5 tonnes/ha + Inoculum.
Treatment 3 = *Crotalaria retusa* at 3.0 tonnes/ha + Inoculum.
Treatment 4 = *Crotalaria retusa* at 1.5 tonnes/ha + Inoculum.
Treatment 5 = *Hyptis suaveolens* at 3.0 tonnes/ha + Inoculum.
Treatment 6 = *Hyptis suaveolens* at 1.5 tonnes/ha + Inoculum.
Treatment 7 = Carbofuran at 3kgai/ha + Inoculum.
Treatment 8 = Root-knot nematode inoculation only (Control)

Planting Operation

Cowpea variety (TT97kd-568-18) was sown in two rows per ridge at spacing of 30cm apart using 3-4 seeds per hole a week after treatment application. Germination took place 4 days after planting. Supplying of ungerminated seeds was done immediately. The seedlings were later thinned down to 2 plants per stand.

Data Collection

Data on the Initial and post harvest nematode population density were collected.

Growth/Yield Parameters

Visual observation and measurement were taken from plant in each plot and data recorded. The parameters studied include plant height, number of leaves, and time to 50% flowering, days to appearance of 1st pod, pod length, pod weight and weight of seeds per pod.

Root Knot Index

Rating of galls was obtained by observing the number of galls on the roots of cowpea after harvest. The assessment was done on 1-5 scale (Taylor and Sasser, 1978).

Score	Rating	Reaction
0	0% of root galled	Immune
1	1-5% of root galled	Resistant
2	6-25% of root galled	Fairly resistant
3	26-50% of root galled	Fairly susceptible
4	51-75% of root galled	Susceptible
5	76-100% 0f root galled	Very susceptible

Rating of Root Galls by Taylor and Sasser (1978)

Data analysis**:** All numerical data collected were subjected to analysis of variance and significantly different means were further separated using the Duncan's Multiple Range Test (DMRT) at P=0.05.

Results

Table 1**:** Shows the effect of different treatments on mean plant height of cowpea plant infested with root-knot nematode for 10weeks. There were no significant differences at the 1st and 2nd week while there were significant differences in plant height among the treatments used from 3rd – 10th week. Plant heights of treatment with Carbofuran 3kgai/ha, *Calotropis* and *Hyptis* at 3.0tonnes/ha was significantly higher than other treatments of lower rates (1.5t/ha). However the lower rates (1.5t/ha) of leaves treatment gave significantly higher height than the control plants.

Table 1: Showing the Effect of Treatments on Mean Height of Plant Per Week (cm)

Treatment	1wk	2wk	3wk	4wk	5wk	6wk	7wk	8wk	9wk	10wk
Calotropis Procera 3t/ha	7.4	11.0	19.1b	28.0b	32.8b	36.1b	43.9b	48.2a	51.2a	54.5a
Calotropis procera 1.5t/ha	7.6	11.1	17.1c	25.1d	28.5c	34.0c	39.0c	43.3c	45.7b	47.4b
Crotalaria retusa 3t/ha	7.4	11.0	19.0b	27.1c	31.0c	35.0bc	43.4b	46.9b	49.0b	53.4a
Crotalaria retusa 1.5t/ha	7.5	11.0	17.4a	25.2d	28.6c	32.9c	38.9c	41.0b	43.1bc	45.7b
Hyptis suaveolens 3t/ha	7.6	11.1	19.7a	28.2b	32.3b	36.9b	45.0a	48.3a	53.7a	54.8a
Hyptis suaveolens 1.5t/ha	7.6	11.0	17.5c	25.3d	28.4c	34.9c	39.2c	44.5c	46.3b	49.4b
Carbofuran	7.4	10.9	19.8a	28.5b	33.5a	39.9a	45.2a	50.0a	53.8a	55.8a
Control	7.6	11.0	15.3d	23.6e	26.d	31.3d	36.7d	38.0d	41.3c	44.7c
S.E	**0.1 N.S**	**0.06 N.S**	**0.5**	**0.8**	**1.2**	**1.9**	**2.1**	**2.3**	**2.6**	**2.2**

Mean followed by different letters are significantly different according to Duncan's Multiple range test at P = 0.05

Table 2: Shows that treated plants with higher rates (3.0t/ha) of botanicals gave significantly higher number of leaves from the 3rd to 10th week. Followed by treatments with lower rates (1.5t/ha) of plant leaves of botanicals. The control plants produced significantly fewer leaves comparatively. However, the mean number of leaves of plants treated with botanicals was significantly lower than the carbofuran treated plants.

Table 2: Effect of Treatments on Mean Number of Leaves Per Week

Treatment	1wk	2wk	3wk	4wk	5wk	6wk	7wk	8wk	9wk	10wk
Calotrpis Procera 3t/ha	4.3	8.3	18.2b	21.7b	25.3a	28.4b	31.4b	33.0b	36.3b	30.3c
Calotropis procera 1.5t/ha	4.3	8.4	14.4cd	17.7c	22.0c	24.7c	24.8d	27.8c	29.0c	27.0d
Crotalaria retusa 3t/ha	4.3	8.3	18.4b	21.8b	24.7b	27.4b	30.8c	32.5b	35.4b	33.2b
Crotalaria retusa 1.5t/ha	4.0	8.1	15.2c	17.3c	21.7c	24.1c	26.1c	29.0c	31.5b	27.5d
Hyptis suaveolens 3t/ha	4.4	8.1	19.0b	21.7b	25.6a	29.0a	33.0b	35.4a	39.0a	34.8b
Hyptis suaveolens 1.5t/ha	4.3	8.3	15.7c	18.2bc	22.6c	25.0c	28.6d	30.0c	32.8b	30.5c
Carbofuran	4.3	8.3	20.0a	23.3a	26.4a	29.8a	35.1a	39.5a	42.1a	38.2a
Control	4.3	8.3	14.0d	16.2d	19.6d	22.4d	24.4f	27.1d	28.3d	25.3e
S.E	**0.1 N.S**	**0.3 N.S**	**0.6**	**0.7**	**1.03**	**1.38**	**1.57**	**1.73**	**1.98**	**2.13**

Mean followed by different letters are significantly different according to Duncan's Multiple range test at P = 0.05

Table 3**:** shows effect of treatment on mean pod length, pod weight and pod number per plant. The table shows that there was no significant difference in the measured parameters per plant treated with carbofuran and plants treated with *Calotropis procera, Hyptis suaveolens* and *Crotalaria retusa* at 3.0t tonnes/ha. They gave significantly higher pod size and number than the cowpea plants treated with lower rates of botanicals at 1.5 tonnes/ha and these in turn performed significantly higher than the control plants. Mean number of pods

treated with *Calotropis* at 1.5 tonnes/ha, gave significantly higher pod number compared to higher rates of botanicals.

Table 4: shows the effect of treatments on mean number of seeds per pod, seed weight per pod and seed weight per plant. Treatment with carbofuran at 3kgai/ha, *Hyptis, Calotropis* and *Crotalaria* at 3 tonnes/ha gave maximum yields. Lower rates of botanicals gave significantly lower yield than the plants treated with higher rate of botanicals and carbofuran. The least yields were obtained from control plants.

Table 3: Shows the Mean Pod Length, Pod Weight and Number of Pods Per Plant

TREATMENT	POD LENGTH (cm)	POD WEIGHT (g)	NUMBER OF POD
Calotrpis Procera 3t/ha	12.53a	1.55a	10.50a
Calotropis procera 1.5t/ha	10.60bc	1.08bc	10.50a
Crotalaria retusa 3t/ha	12.50ab	1.46a	10.33a
Crotalaria retusa 1.5t/ha	10.20c	0.97bc	7.16b
Hyptis suaveolens 3t/ha	13.70a	1.46a	10.67a
Hyptis suaveolens 1.5t/ha	11.20b	1.13b	7.50b
Carbofuran	12.95a	1.54a	10.66a
Control	7.40d	0.63d	4.66c
S.E	**0.78**	**0.6**	**0.4**

Mean followed by different letters are significantly different according to Duncan's Multiple range test at P = 0.05

Table 4: Shows Mean Number of Seeds Per Pod, Seed Weight Per Pod, and Seed Weight Per Plant

Treatment	**Number of Seeds Per Pod**	**Seed weight per pod**	**Seed Weight Per Plant**
Calotrpis Procera 3t/ha	10.62a	1.33a	10.92a
Calotropis procera 1.5t/ha	7.75b	0.93b	8.49b
Crotalaria retusa 3t/ha	9.75a	1.28a	10.79a
Crotalaria retusa *1.*5t/ha	7.62b	0.91b	8.27b
Hyptis suaveolens 3t/ha	11.00a	1.37a	11.18a
Hyptis suaveolens 1.5t/ha	8.62b	0.99b	9.38b
Carbofuran	9.50a	1.41a	11.70a
Control	6.25c	0.72c	7.73c
S.E	**4.56**	**0.04**	**1.71**

Mean followed by different letters are significantly different according to Duncan's Multiple range test at P = 0.05

Table 5: Shows effect of treatment on mean initial and final nematode populations and gall rating. From the table, there was no significant difference among treatments for the initial nematode population. While at harvest, there

was significant reduction in the final nematode population in treated plants. The synthetic nematicide, carbofuran recorded least nematode population and least galling at harvest followed by plants treated with higher levels (3.0 tonnes/ha) of plant leaves of *Hyptis, Calotropis* and *Crotalaria*. Plants treated with 1.5t/ha of *Hyptis*, *Calotropis* and *Crotalaria* gave significantly more reduced nematode population and galling at harvest than the control plants at harvest.

Table 5: Effect of Treatment on Mean Initial Nematode Population and Gall Rating

Treatment	Initial Nematode Population	Final Nematode Population	Gall Rating
Calotrpis Procera 3t/ha	445	336c	1.2b
Calotropis rocera 1.5t/ha	557	392b	2.1c
Crotalaria retusa 3t/ha	560	338c	1,3b
Crotalaria retusa 1.5t/ha	561	381b	2.1c
Hyptis suaveolens 3t/ha	524	330c	1.2b
Hyptis suaveolens 1.5t/ha	528	413b	2.1c
Carbofuran	531	105a	0.0a
Control	533	827d	3.2d
S.E	**0.2 N.S**	**5.2**	**0.8**

Means followed by different letters are significantly different according to Duncan's Multiple range test at P = 0.05

Table 6: Shows the effect of treatments on the number of days to 50% flowering and days to appearance of the first pod. Early flowering was observed for plants treated with *Hyptis* leaves at 3tonnes/ha. This was significantly shorter than those of Carbofuran 3kgai/ha, *Calotropis* and *Crotalaria* at 3 tonnes/ha. This was followed by plants leaf treated with lower rates 1.5 tonnes/ha of botanicals, whereas the control plants recorded significantly higher number of days to 50% flowering.

There was No significant difference in appearance of first pod between the treatment of Carbofuran 3kgai/ha and *Hyptis suaveolens* at 3 tonnes/ha. Also, *Calotropis* and *Crotalaria* treatment level at 3.0t/ha were not significantly differed in days to appearance of 1st pod from leaf treatments of botanicals at lower rate of 1.5 tonnes/ha. The control plants have longest days to appearance of first pods.

Table 6: Effect of Treatment on Mean Number of Days to 50% Flowering and Days to Appearance of 1st Pod

Treatment	Days to 50% Flowering	Days to Appearance of 1st Pod
Calotrpis Procera 3t/ha	50b	58bc
Calotropis procera 1.5t/ha	50b	60b
Crotalaria retusa 3t/ha	52b	59c
Crotalaria retusa 1.5t/ha	52c	59c
Hyptis suaveolens 3t/ha	49a	57a
Hyptis suaveolens 1.5t/ha	52c	59c
Carbofuran	50b	57a
Control	54d	65d
S.E	**0.03**	**10.92**

Mean followed by different letters are significantly different according to Duncan's Multiple range test at P = 0.05

Discussion

The results obtained showed that cowpea plants treated with carbofuran and higher concentration of botanicals reduced nematode population density in the soil, establishing the botanicals as nematicidal. However, it is evident from the results that the cowpea variety (TT97kd-568-18) used was susceptible to root-knot nematode infection.

The general increase in plant height and number of leaves (Tables 1 and 2) may be attributed to the fact that incorporation of soil with botanicals provided organic matter and therefore additional nutrient that improved the growth and yield of the cowpea plant. Also the significantly better results recorded in plants treated with higher rates of botanicals tended to encourage the multiplication of soil inhibiting nematode antagonists which further suppressed the nematode population in the soil.

Variation in the effects of treatments against root-knot nematode infestation, growth and yield of cowpea plant could be due to differences in the chemical compositions of the botanicals. *Hyptis* for example has been found to contain tannin, flavonoid, alkaloid, etc., effect of tannin on nematode at higher rates causes' high toxicity. Such results have also been reported by Oyedunmade *et al.,* (2001) on the use of plant extracts to control plant root-knot nematodes and Shaukat *et al*.,(2004) who demonstrated that extract of certain weeds are able to cause substantial mortality of plant parasitic nematode thereby improving plant growth and yield.

Observed symptoms such as reduction in yield of untreated plants, galled roots and stunted growth in some of the plants could be traced to higher population of nematode on the untreated plants and plants treated with lower rate (1.5t/ha) of botanicals, since no or little nematicide was applied. The population build up may result in increased penetration of juvenile stages into the root system to induce parasitic attack and formation of galls. These findings are in consonance with those of Abad *et al*., 2003.

Galls ranging from slight to severe galling were observed in some plants in the experiment (Table 5) indicating the proliferation of nematodes and their active penetration due to the absence or lower rate of nematicidal materials. Reduction in root-knot index may also be attributed to the toxicity or high concentrations of the plant materials. This results are in conformity with those of Azhar and Seddiqu, (2007) that showed plant extract of basil, marigold, pyrethium, neem, and China berry proved effective in reducing galling of root-knot nematode Meloidogyne Incognita.

However, the population of root-knot nematode(*Meloidogyne* spp.) in the final soil samples were substantialy low (Table 5) confirming the effect of treatments on nematode population. The difference in final nematode population from the initial population might also be due to direct toxicity of the treatments to eggs and /or juveniles and thus reducing the root-knot nematode population density. This was also reported by Azhar *et al*., (2007) on the reduction of nematode population in the soil treated with some botanicals.

Conclusion

The present study revealed that, the leaves of *Hyptis suaveolens*, *Calotropis procera* and *Crotalaria retusa* have nematicidal effect on root-knot nematode *Meloidogyne* species in cowpea plant. The higher levels which were more effective in controlling the nematodes compared favourably to the synthetic nematicide Cabofuran 3G. From the findings it can be concluded that the incorporation of plant leaves into the soil as organic amendment to control root-knot nematode *Meloidogyne* spp. could provide a suitable and cheaper alternative for management of *Meloidogyne* spp. and will reduce much dependence on the use of synthetic nematicide.

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Reference to this paper should be made follows: Yakubu, L.B. and Izuogu, N.B. (2013), Comparative Evaluation of Some Botanicals and Carbofuran in the Control of Root-Knot Nematode, *Meloidogyne spp*. on Cowpea (*Vigna unguiculata*). *J. of Sciences and Multidisciplinary Research,* Vol. 5, No. 2, Pp. 105 – 115.