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Effect of botanical extracts on root-knot nematode (*Meloidogyne incognita)* infection and growth of cacao seedlings

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ABSTRACT

Objectives: To determine the effects of *Bridelia micrantha, Mallotus oppositifolius, Hunteria umbellata* and *Citrus medica* leaf extracts on growth and root-knot infection caused by *M. incognita* on cacao.

Methodology and results: The effect of four rates (0, 10, 25, 50 and 100%) of leaf extracts was tested. Water extract of all the test plants significantly inhibited egg hatching of nematode and caused 100% mortality of the second juveniles of *M.* incognita *in vitro* after 12h of exposure. Undiluted crude leaf extracts of *H. umbellata* and *M. oppositifolius* exhibited 100% inhibition of egg hatch and larva mortality, while undiluted leaf extracts of *B. micrantha* and *C. medica* exhibited 92 and 93.2% inhibition of egg hatch and 62.1 and 73% larval mortality respectively. Egg inhibition and larval mortality decreased with increase in dilution of all the extracts. Juvenile mortality increased corresponding to an increased time of exposure. The leaf extracts of individual plant significantly enhanced the growth of cacao seedlings in the presence of the nematode in the nursery when compared to the control ($p<0.05$). There was a significant increase in plant height (68.2, 70.2, 65.9 and 65.7 for *Bridelia micrantha, Mallotus oppositifolius, Hunteria umbellata* and *Citrus medica* respectively), shoot weight and root weight of the seedlings treated with all the leaf extracts even at the lowest concentration of 10% compared to the rest.

Conclusion and application of results: This study showed that the test plants which are readily available to farmers at no cost have the ability to reduce nematode below economic threshold, thus this finding is important from the point of view of controlling root-knot nematodes affecting cacao seedling without the use of nematicides in view of the environmental pollution likely to cause. Farmers are therefore advised to apply the undiluted crude extract of the plants to cacao plants after transplanting to field as plant-parasitic nematodes have been implicated for poor seedling establishment. There is need for further studies in identifying new classes of pesticides from natural plants to replace the synthetic dangerous and expensive chemicals used at present.

Key words: Cacao seedlings, leaf extracts, *Meloidogyne incognita,* egg hatching, larval mortality

INTRODUCTION

Plant parasitic nematodes, are capable of reproducing on over 2,000 species of plants (Sasser and Freckman, 1987) and are responsible for approximately 50% of overall damage (Abbasi *et al.,* 2008). Root-knot disease caused by *Meloidogyne* spp. is a well-known disease of many tropical and sub-tropical crops. Earlier studies in Brazil, the Congo, Ghana and Nigeria have shown that they are the most important nematodes of cacao due to their pathogenecity and wide

distribution in cocoa producing regions. It is a common pest of cacao in West Africa (). (Whitehead, 1969; Asare-Nyako & Owusu, 1979; Afolami and Caveness, 1983; Campos & Villain, 2005 and Fademi et al., 2006).

The symptoms of nematode infection include formation of root galls which results in growth reduction, nutrient and water uptake reduction, increased wilting, mineral deficiency, weak and poor yielding plants (Abad *et al.,* 2003). Although the application of chemical nematicides has been found to be an effective measure for the control of nematodes, the highly toxic residual effect of chemical on the environment and particularly on non-target organisms (Anastasiadis *et al.,* 2008), require an urgent need to develop alternative strategies for the control of nematodes.

Crops and weeds may exhibit biochemical mechanisms to counteract the activity of nematodes. Numerous plant species, representing 57 families, have been shown to contain nematicidal compounds (Sukul, 1992).

There is a need to develop naturally occurring nematicides, which may be less toxic to man and animals but as effective against nematodes of various crops as synthetic ones. The toxicity of root extracts of different plants against nematodes has been reported previously (Onifade and Egunjobi, 1994; Adegbite and Adesiyan, 2005). This research was undertaken to evaluate the effectiveness of leaf extracts of *Bridelia micrantha, Mallotus oppositifolius, Hunteria umbellata* and *Citrus medica* to control nematodes on cacao.

MATERIALS AND METHODS

Extract preparation: Fresh leaves of *Bridelia micrantha, Mallotus oppositifolius, Hunteria umbellata* and *Citrus medica* were obtained from Idi-Ayunre on a farmer's plantation. They were washed under running tap water and sterile distilled water. Leaf extracts of each plant were prepared by blending 25g of chopped roots in 100ml-distilled water with a warring blender. Thereafter, the suspension was filtered through sterile muslin cloth. Suspensions of concentrations of 10, 25, 50 and 100% were prepared with distilled water (Orisajo *et al.,* 2007).

Preparation of root-knot nematodes cultures: Roots of cacao plants naturally infested with root-knot nematodes were collected from CRIN farm. The root-knot nematodes species were identified with the help of perennial pattern as described by Taylor and Netscher (1974). The root-knot nematode *Meloidogyne incognita* was cultured on *Celosia argentea* seedlings in the screen house from a single egg mass. Nematode (*M. incognita)* eggs were extracted from infected roots using a 2% NaOCl solution and the eggs released from the roots were collected using the modified technique described by McClure *et al.,* (1973). The egg suspension was poured on a cotton-wool filter paper and incubated at 28±2^{0}C to obtain freshly hatched juveniles (J2). Juveniles were collected and used within 48hrs.

Hatchability test: Eggs of *M. incognita* were collected by the method of Hussey and Barker (1973). A suspension of eggs in distilled water was prepared. 1 ml of egg suspension (40-55 eggs/ml) and 1 ml of leaf extract was transferred in glass cavity blocks and kept at 27^{0}C. Each treatment was replicated thrice. The glass cavity containing 1 ml egg suspension and one ml distilled water served as control. After four days of exposure, the number of hatched eggs was counted under a low power (6X) stereomicroscope. The toxicity of plant extract was assessed as the mean percentage of the dead nematodes. Nematodes were considered dead if they did not move when probed with a fine needle (Waseem Abbasi *et al.,* 2008).

Mortality test of nematode larvae: Eggs and eggs masses of *M. incognita* were placed in distilled water and incubated at 28±2^{0}C. After hatching, the juveniles were collected and a suspension of juveniles in distilled water was prepared. 1 ml of egg suspension (50-56 juveniles/ml) and one ml of root extract for each plant were transferred into different glass cavity blocks and kept at 27^{0}C. Each treatment was replicated thrice. The glass block containing 1 ml distilled water served as control. Percentage mortality was calculated after 12, 24 and 48 hours of exposure, the number of killed juveniles was counted under a low power (6X) stereomicroscope. The toxicity of root extract was assessed based on the mean percentage of the dead nematodes. Nematodes were considered dead if they did not move when probed with a fine needle. (Waseem Abbasi *et al.,* 2008).

Pathogenecity test: Sandy-loam top soil (sand 60.8%, silt 34%, clay 5.2% and organic matter 6.8%) normally

used for raising cacao seedlings was obtained from the field of Cocoa Research Institute of Nigeria. Soil was dispensed into 185mm diameter pots,–the usual one for raising cacao seedlings commercially. All pots were planted each with two seeds of *Theobroma cacao* cv. F_3 Amazon, which was later thinned down to one per pot a week after emergence. Each pot received 5000 freshly hatched second stage juvenile introduced as drench in holes made around the roots of each plant; and was amended with 40ml of the root extracts by pouring the aqueous solution of the extracts into holes made around the seedlings in each pot. Unamended and uninoculated pots served as positive control, while unamended and nematode inoculated pots served as negative controls. Three replicate pots per treatment and controls were arranged in a completely randomized design in a screen house and watered daily.

Effects of leaf extract: Regular observations were made to record data on phytotoxic effects of treatment on plants and disease symptoms. The experiment was terminated 26 weeks after planting. The growth parameters, i.e. plant height, stem girth and, numbers of leaves were taken. Leaf areas were recorded with the aid of electronic leaf meter. The roots were carefully freed of soil, washed under a gentle stream of tap water, mopped and galls counted using a hand lens at 3-5 times magnification. Root galling was assessed by using the 0-5 gall index (Sasser *et al.,* 1984). Nematodes were extracted from 1g of roots using sodium hypochlorite method (NaOCl) of McClure *et al.,* (1973) and all the developmental stages of the nematode were counted. An aliquot of 175cm^3 soil from each pot was assayed for juveniles of *M. incognita* using Whitehead and Hemming (1965) tray modification of the Baermann technique. Nematode suspensions were concentrated to a 20ml of water and a 2-ml aliquot in a nematode counting dish with the aid of a stereomicroscope.

Statistical analysis: Data sets were subjected to analysis of variance (ANOVA) and means separated with SAS-SNK test ($p<0.05$) (SAS Institute, 1990).

RESULTS

Hatchability: The results indicated that undiluted leaf extracts of *H. umbellata* and *M. oppositifolius* gave the highest inhibition of egg hatching (100%), followed by *B. micrantha* and *C. medica* with 92 and 93.2% inhibition respectively (table 1). Other dilutions i.e. 50, 25 and 10% though significant, were less effective as compared to the undiluted extracts.

This inhibitory effect of the extract may be due to the chemicals present in the extracts that possesses ovicidal and larvicidal properties.

Several authors have reported that extracts of *Azadirachta indica* and *Ricinus communis* contained alkanoids, flavonoids, saponins, amides, including benzamide and ketones that singly and in combination inhibited hatching (Adegbite and Adesiyan, 2005).

Mortality: Results in Table 1 show the effect of the concentration of extracts of leaves of the test plants on larval mortality over time. The leaves extracts were effective in causing larva mortality with 100% concentration being more efficacious. It showed a highly significant difference than the other concentrations. Undiluted extracts of *H. umbellata* and *M. oppositifolius* showed 100% mortality even after 12h of exposure time, while 100% of *B. micrantha* and *C. medica* showed 73 and 62.1% mortality, respectively, after 12h of exposure time. The juvenile mortality increased with increase in exposure time.

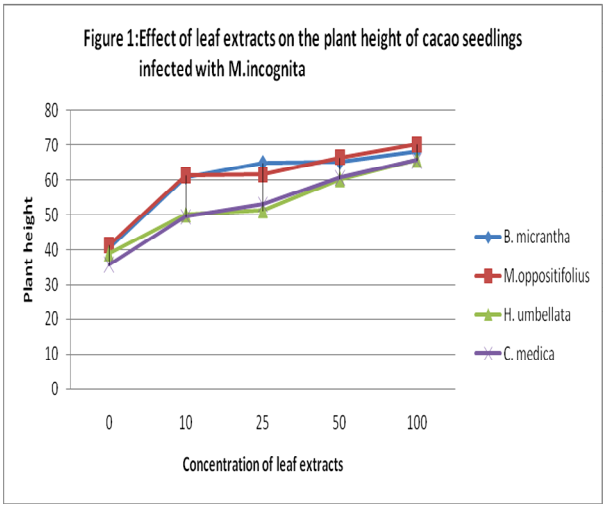
Table 1: Effects of different concentrations of plant leaf extracts on *Meloidogyne incognita* egg hatch, and exposure time on larval mortality.

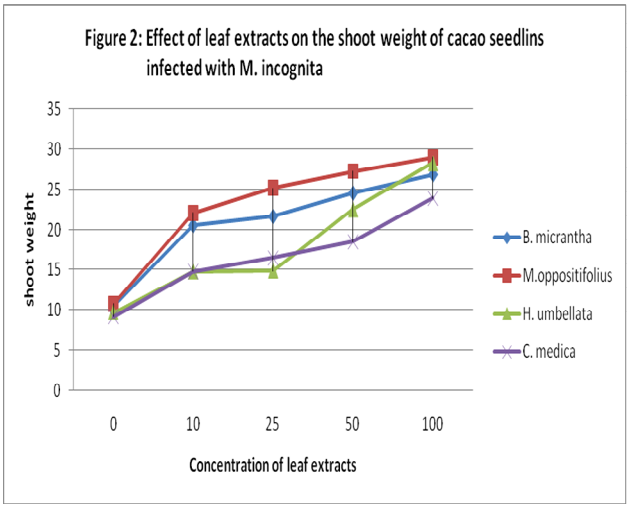
		Egg Hatch		Larval Mortality (%) after		
Plant	**Concentration**	**No of eggs**	**Hatch inhibition After 7 days (%)**	**12h**	**24h**	**48h**
B. micrantha	100	21.5a	92.0a	73.0a	76.6a	87.5a
	50	16.0b	71.5b	15.1b	25.8b	49.0b
	25	10.5c	61.6c	12.1c	12.5c	23.7c
	10	10.5c	50.9d	3.3d	5.7d	12.5d
	0	19.0a	11.0e	0.0d	0.0e	0.0e
M. oppositifolia	100	20.1a	100a	100.0a	100.0a	100.0a
	50	17.4a	87.6b	80.0b	88.0b	90.0b
	25	12.7b	66.3c	11.3c	26.9c	44.9c
	10	11.7b	65.9c	1.7d	5.8d	13.6d
	0	12.0b	13.1d	0.0e	0.0e	0.0e
H. umbellata	100	26.9a	100.0a	100.0a	100.0a	100.0a
	50	19.9b	89.2b	90.0b	97.8a	96.0a
	25	18.0b	69.9c	13.6c	35.9b	55.7b
	10	15.4c	69.8c	4.0d	6.8c	14.9c
	0	21.3b	16.3d	0.0e	2.5d	2.5d
C. medica	100	16.3a	93.2a	62.1a	68.3a	75.6a
	50	19.5a	73.4b	23.4b	26.9b	38.9b
	25	19.2a	54.7c	13.2c	22.6c	25.8c
	10	18.3a	41.5d	5.7d	8.0d	25.8c
	0	10.3b	11.9c	0.0e	0.7e	1.2e

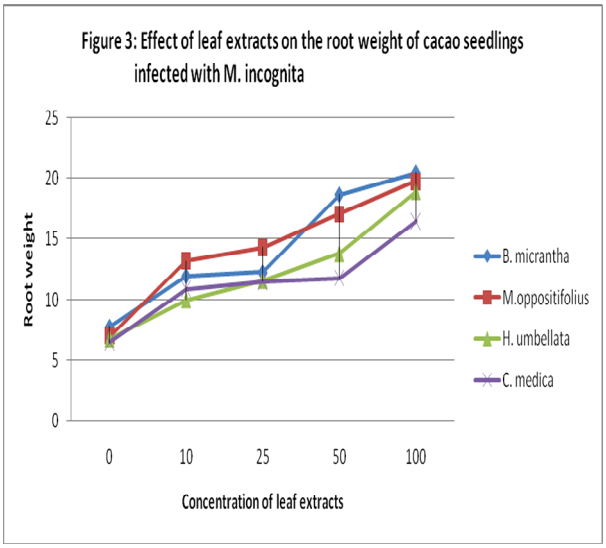
100 = undiluted extract, 50, 25 and 10 represent extract that was diluted 10, 25 and 50 times, respectively; 0 = distilled water

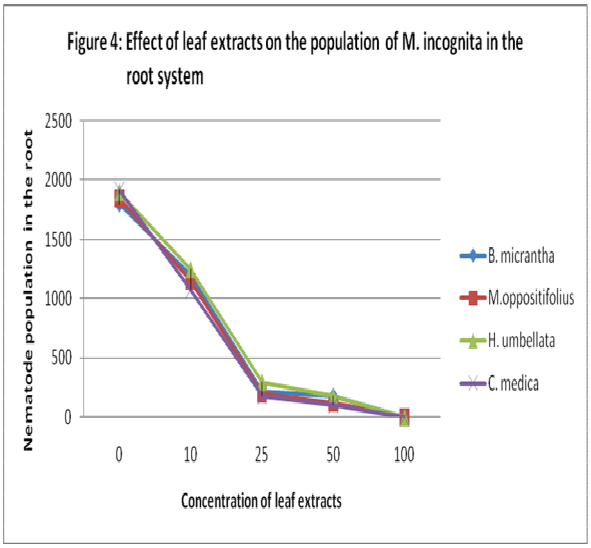
Means for each plant within a column followed by the same superscript(s) are not significantly different by SAS-SNK test ($P<0.05$).

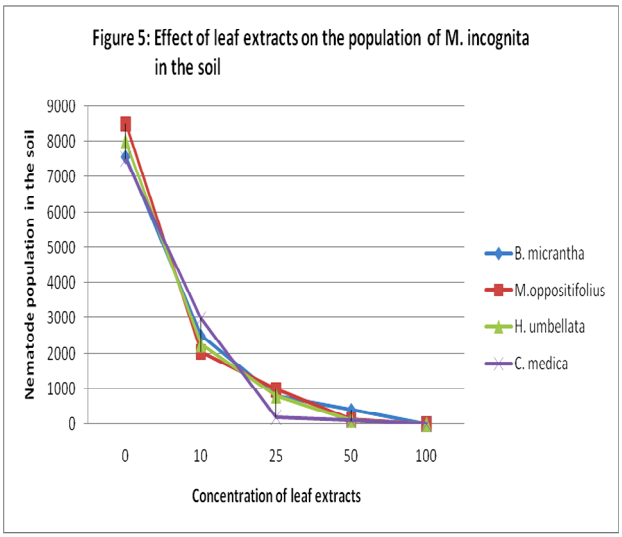
Effects of leaf extracts on cacao seedlings: The growth of cacao seedlings was significantly enhanced by all the extracts used in the presence of nematode compared to the control (P<0.05). However, there was a significant increase in the plant height of the seedlings treated with the extracts of *M. oppositifolius* and *B. micrantha* at 10% when compared to the rest (Figure 1). No phytoxic effects were observed on the cacao seedlings, stunted growth characterized by chlorotic symptoms were observed in the negative controlled experiment The leaf extracts also resulted in an increase in the shoot weight of cacao seedlings with weight of the seedlings treated with *M. oppositifolia* at 25% having a significantly higher weight when compared to the other treatment at the same levels (Figure 2), but at 100% there was no significant difference in their weights.The leaf extracts of all the plants enhanced the root weight of cacao seedlings significantly in the presence of the nematode when compared to the control (P<0.05) (Figure 3).A general trend of increased nematicidal activity with a corresponding increase in the concentration of the plant extracts was observed. The densities of *M. incognita* in both soil and root were significantly reduced at 100% concentration compared to the untreated (Figure 4 and 5). At this level of application gall formation was inhibited (Figure 6).











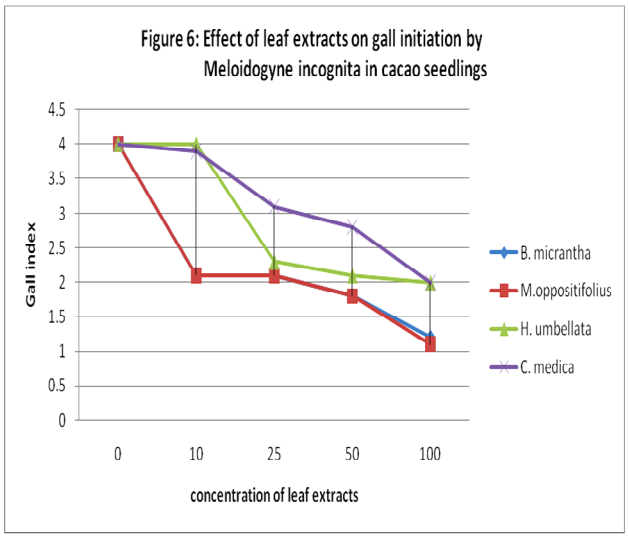


Figure 1-6: Effect of plant leaf extracts on growth of cacao plants and infection by *Meloidogyne incognita.*

DISCUSSION

The potential of using plant extracts in controlling plant parasitic nematodes has been shown by several authors (Adegbite, 2005; Opareke *et al.*, 2005; Orisajo *et al.,* 2007 and Abbasi *et al.,* 2008;). This study has shown that the leaf extracts of *Bridelia micrantha, Mallotus oppositifolius, Hunteria umbellata* and *Citrus medica* has some nematicidal effects on *M. incognita* in cacao seedlings. The inhibitory effect observed in egg hatching according to Adegbite and Adesiyan (2005), might be due to the chemicals present in the extracts that possesses ovicidal and larvicidal properties. Opabode and Adeboye (2005), showed that Nigeria is endowed with many indigenous plant species, some of which are used for herbal medicine. The use of leaf extract is suggested as a potential substitute for synthetic nematicides used in the management of root-knot disease of cacao seedlings (Orisajo *et al.,* 2007). Further studies will be conducted in the field to ascertain the nematicidal ability of the extracts in the soil.

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