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Chemical Composition and Bio-Nematicidal Potential of Some Weed Extracts on *Meloidogyne incognita* under Laboratory Conditions

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Abstract: Laboratory experiments were conducted on the effect of leaf extract of some weeds on root knot nematode, *Meloidogyne incognita* and also on the chemical compounds in the leaf extract of some weeds. Leaf extracts were obtained from *Sida acuta* Burm F., *Euphorbia hirta* Linn *Andropogon gayanus* Kunth, *Phyllanthus amarus* Schum and Thomm and *Cassia obtusifolia* L. weeds. Distilled water and ethanol (95%) were used as the extraction media for nematode bio-assay and phyto-chemical analysis experiments, respectively. Concentrations of each leaf extract of the weed were 5, 10, 15 and 20% (w/v). Approximately, 100 *M. incognita* juveniles were dispensed into each Petri-dish containing the graded weed extracts, while *M. incognita* juveniles that were dispensed into distilled water only served as control. Each treatment, including the control, was replicated 10 times. *M. incognita* juvenile mortality rate increased with an increase in test plant extract concentrations and exposure time. In 15 and 20% (w/v) concentrations of *Euphorbia hirta, Phyllanthus amarus* and *Cassia obtusifolia* and 20% (w/v) concentration of *Sida acuta* and *Andropogon gayanus*, there was 100% *M. incognita* juvenile mortality by the 7th day. The result of phyto-chemical analysis revealed that *Euphorbia hirta* contained tannins, saponins, flavonoids and alkaloids; *Andropogon gayanus* contained saponins, flavonoids and alkaloids; *Cassia obtusifolia* contained tannins, flavonoids and alkaloids; *Phyllanthus amarus* contained tannins, saponins, flavonoids and alkaloids while *Sida acuta* contained tannins, saponins, flavonoids and sterols chemical compounds.

Key words: Weed, botanicals, bio-nematicide, root knot nematode, *Melodiogyne incognita*, phyto-chemical analysis

INTRODUCTION

The root knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood belongs to the family Meloidogynidae in the super family Tylenchoidea of the Tylenchida and genus *Meloidogyne*. They possess specialised adaptation (stylet) for parasitism and therefore constitute a major group of plant parasitic nematode of outstanding economic importance. Their distribution is worldwide and *Meloidogyne incognita*, is capable of attacking virtually every type of field crops, therefore causing considerable losses in the yield and quality of the produce (Adesiyan *et al*., 1990; Maniya, 1970). The root knot nematodes, *Meloidogyne* species, are probably the major obstacle to crop production in the tropics (Caveness, 1967; Sasser, 1989; Olabiyi, 2004).

In the past, root knot nematode diseases had been effectively and mostly controlled by synthetic nematicide. But synthetic nematicide had been confirmed to be a source of water, food and environmental pollution. Agricultural crop consumers are poisoned and in most cases price of synthetic nematicides are very high. All these problems have greatly limited the use of nematicides (Thomas, 1996; Anonymous, 2000; Olabiyi, 2005).

In the recent years, some natural plants (botanicals) have been effectively used as soil amendments, plant extracts and compost in the control of root knot nematode

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disease in the laboratory, greenhouse and screen house and on the field (Abid and Maqbool, 1991; Akhtar and Mahmood, 1994; Abadir *et al*., 1996; Olabiyi and Oyedumade, 2005; Olabiyi *et al*., 2007).

With current worldwide drive towards organic agriculture which will subsequently, leads to the production of organic foods, it is envisaged that search should continue on natural plants (botanicals) which could be used instead of synthetic nematicides in crop protection. Weeds are considered as plants growing out of place. Majority of the common weeds are harmful to crop, soil nutrient competitors, some are even host to pathogenic organisms, while some are quite beneficiary and found useful in both human and herbal medicine (Akubundu and Agyakwa, 1998; Olabiyi and Adesina, 2006). Therefore, search for weed that could be used in the control of nematode disease either in the laboratory or field should be a welcome idea.

In this study, attempts were made to carry out phyto-chemical analysis on *Sida acuta*, *Euphorbia hirta andropogon gayanus*, *Phyllanthus amarus* and *Cassia obtusifolia* weeds and also to assessed the aqueous leaf extract of each weed on the second stage juveniles (J2) of *Meloidogyne incognita* under the laboratory conditions.

MATERIALS AND METHODS

Second stage juveniles (J2) of the root knot nematode, *M. incognita* (Kofoid and White) Chitwood was the test pathogen. Five common weeds used as botanical against *M. incognita* were *Sida acuta*, *Euphorbia hirta andropogon gayanus*, *Phyllanthus amarus* and *Cassia obtusifolia*. The leaves extract of the selected weeds were used for both nematode bio-assay and phyto-chemical analysis laboratory experiments.

Collection and preparation of the test plant: Before the blooming stage, the fresh green leaves were separately harvested with sickle from *Sida acuta*, *Euphorbia hirta andropogon gayanus*, *Phyllanthus amarus* and *Cassia obtusifolia* weeds at different location within the premises of University of Ilorin, Ilorin, Nigeria. The leaves were air-dried on the laboratory benches in the Department of Crop Protection, University of Ilorin, Ilorin, Nigeria for 6 weeks at a temperature ranging between 26-30°C. Each separately packed weed was ground into fine powder (1.0 mm) using attrition mill.

Ethanol extraction of the test plant (weed) and phyto-chemical analysis: The phyto-chemical analysis of the test plants (weeds) was carried at the Chemistry Department of Ladoke Akintola, University of Technology, Ogbomoso, Nigeria.

Two-hundred and fifty gram of each test plant (weed) in powder form was weighed with sensitive electric weighing balance (4 digital) in the laboratory. Each quantity of the test plant was separately soaked into 500 mL ethanol (95%) for 3 days in a closely air-tight 1 L sized measuring cylinder. The content was filtered using No. 1 Whiteman filter paper. The filtrate (ethanol extract) was used for the phyto-chemical analysis.

Test for flavonoids:

- Aliquot of 4 mL of aqueous NaOH was added to 2 mL of each of ethanol extract. If a yellow precipitate was observed, it indicates the presence of flavonoids in the extracts. But if otherwise, it indicates the absence of flavonoids.
- Shinoda test: A little amount of magnesium powder and 3 drops of concentrated HCl were added to 4 mL of each of the ethanol extract. If a red colour was observed, it indicates the presence of flavonoids. If otherwise, it shows the absence of flavonoids.

Test for sterols: Aliquot of 2 mL concentrated H_2SO_4 was added to 1 mL of each ethanol extract. If a brownish red colour was observed, it shows the presence of sterols. But, if otherwise, it shows the absence of sterols.

Test for glycosides: Aliquot of 10 mL of 50% H_2SO_4 was added to 1 mL of each ethanol extract. The mixture was heated on water bath for 15 min. Then, 5cm^3 each of fehling solutions A and B was added and boiled. If a red precipitate was observed it shows the presence of glycoside. But if otherwise, it shows the absence of glycosides.

Test for alkaloids: Aliquot of 2 mL of each of the ethanol extract was stirred with 5 mL of 1% aqueous HCl acid on a steam bath. One mililiter of the filtrate was treated with 2 drops of Mayer's reagent. The second 1 mL portion was treated with Wagner's reagent. If a creamy white (Mayer) and reddish brown (Wagner) precipitates were observed. It indicates the presence of alkaloids. But if otherwise, it indicates the absence of alkaloids.

Test for tannins: To 2 mL of each of the ethanol extract was added 5 drops of ferrous chloride solution. If a dirty green precipitate was observed, it indicates the presence of tannins. But if otherwise, it indicates the absence of tannins.

Test for saponins

Frothing test: Aliquot of 2 mL of each ethanol extract was shaken vigorously in test tube for 2 min, frothing shows the presence of saponins. But if otherwise, it indicates absence of saponins.

Emulsion test: Aliquot of 5 drops of olive oil was added to 3 mL of the weed extract in test tube and mixture was vigorously shaken. If a stable emulsion is formed, it indicates the absence of saponins. But if froth is formed, it indicates presence of saponins.

Root knot nematode bio-assay: Root knot nematode bio-assay and preparation of the botanical was carried at the Department of Agronomy Research laboratory, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

Preparation of the test organism (root knot nematode): Cowpea, *Vigna ungiculata* cv 3236, was grown in a steam sterilised soil and inoculated with pure stock culture of *Meloidogyne incognita* obtained from previous experiment (Olabiyi *et al*., 2006). Fifteen weeks after planting (15 WAP), eggs of *Meloidogyne incognita* were extracted, using sodium hypochlorite (Hussey and Barker, 1973) from cowpea galled roots. The eggs were incubated at temperature of 25°C for 24 h. The freshly hatched second stage nematode juveniles were collected. Juveniles of *M. incognita* in the distil water suspension was concentrated and standardized so that each 1 mL suspension contained approximately 100 juveniles.

Effect of the leaf extract of weed on *m. Incognita* juveniles (nematode bio-assay): Leaf extract of each weed was prepared into 5, 10, 15 and 20% (w/v) concentrations in the Agronomy Research Laboratory, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. Aliquot of 50 mL of each aqueous leaf extract was dispensed into Petri-dish and thereafter, 1 mL each of nematode suspension containing approximately 100 *M. incognita* population was added. The Petri dish containing only 1 mL juvenile suspension containing approximately 100 *M. incognita* juveniles in 50 mL distilled water only served as the control. Each treatment, including the control, was replicated ten times. The experimental design was complete randomised design. A count of live nematodes was made and cumulative numbers of dead nematodes were put on record on daily basis for a period of 7 consecutive days.

Statistical analysis: Data obtained on the effect of leaf extract of weed on *M. incognita* juveniles was subjected to statistical analysis (analysis of variance) and differences between the treatments were partitioned using Duncan's multiple range tests at 5% probability level.

RESULTS

Effect of leaf extract of weed on the mortality rate of *M. incognita* juveniles: Leaf extract of weed at 5, 10, 15 and 20% (w/v) concentrations resulted into mortality of *M. incognita* juveniles (Table 1). As leaf extract concentrations and numbers of day increase, mortality rate of *M. incognita* increases. At 15 and 20% (w/v) concentrations of *Euphorbia hirta, Phyllanthus amarus* and *Cassia obtusifolia* and 20% (w/v) concentration of *Sida acuta* and *Andropogon gayanus,* there was 100% *M. incognita* juvenile mortality by the 7th day. Higher concentrations of each leaf extract resulted into significantly higher mortality rate of nematode juveniles (Table 1). Significantly, reduced mortality rate was observed in the control experiment where no leaf extract of weed was applied. Significantly high mortality rate of root knot nematode juvenile in all the leaf extract of weed shows that the extracts were toxic to *M. incognita* juveniles under the laboratory condition.

Result of phyto-chemical screening of the leaf extract: The result of the phyto-chemical analysis revealed that different weeds contained different chemical compounds (Table 2). Presence of tannins, saponins, flavonoids, alkaloids, sterols and glycosides were assessed in *Sida acuta*, *Euphorbia hirta andropogon gayanus*, *Phyllanthus amarus* and *Cassia obtusifolia.* The result of phyto-chemical analysis revealed that *Euphorbia hirta* contained tannins, saponins, flavonoids and alkaloids;

Table 1: Result of phyto-chemical analysis of *Sida acuta, Euphorbia hirta, Andropogon gayanus, Phyllanthus amarus* and *Cassia obtusifolia*

Chemical constituents	*S. acuta*	*E. hirta*	*A. gayanus*	*P. amarus*	*C. obtusifolia*
Tannins	+	+	-	+	+
Saponins	+	+	+	+	-
Flavonoids	+	+	+	+	+
Alkaloids	-	+	+	+	+
Sterols	+	-	-	-	-
Glycosides	-	-	-	-	-

Keys: - indicates absence, + indicates presence

Table 2: Effect of leaf extract of different weed species on the cumulative mortality of the second stage juveniles of *Meloidogyne incognita*

Weed species	Leaf extract concentrations in % (w v^{-1})	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Sida acuta	5	9_{g}	12_{g}	20_{g}	22_{g}	29_{f}	40_{f}	55_{e}
	10	13_{f}	29_{f}	36_{f}	45_{e}	53_{e}	64_{d}	78_{c}
	15	32_{d}	42_{d}	50_{d}	65_{c}	70_{d}	81_{b}	90_{b}
	20	49_{bc}	62_{b}	67_{c}	73_{b}	79_{c}	88_{b}	100_{a}
E. hirta	5	7_{g}	14_{g}	20_{g}	25_{g}	34_{f}	50_{e}	59_{de}
	10	24_{e}	33_{e}	48_{e}	56_{d}	69_{d}	82_{b}	96_{a}
	15	36_{d}	68_{b}	77_{b}	85_{a}	90_{b}	94_{a}	100_{a}
	20	52_{b}	81_{a}	89_{a}	94_{a}	98_{a}	100_{a}	100_{a}
A. gayanus	5	5_{g}	10_{g}	16_{g}	20_{g}	30_{f}	37_{f}	50e
	10	16_{f}	26_{f}	38_{f}	53_{d}	59_{e}	68_{d}	78_{c}
	15	40_{d}	47_{d}	56_{d}	64_{c}	72_{c}	86_{b}	92_{a}
	20	51_{b}	56_{c}	62_{c}	66c	70_{cd}	90_{b}	100_{a}
P. amarus	5	9_{g}	15_{g}	22_{g}	28_{g}	39_{f}	52_{e}	60_{de}
	10	45_{c}	46_{d}	48_{e}	52_{d}	60_{e}	70_{c}	86_{b}
	15	56_{b}	62_{b}	73_{b}	81_{a}	94_{a}	97_{a}	100_{a}
	20	62_{a}	76_{a}	80_{b}	89_{a}	93_{ab}	99_{a}	100_{a}
C. obtusifolia	5	6_{g}	10_{g}	25_{g}	32_{f}	37_{f}	51_{e}	58_{de}
	10	42_{d}	44_{d}	56_{d}	69_{bc}	72_{c}	82_{b}	88_{b}
	15	53_{b}	68_{ab}	69_{c}	70_{bc}	73_{c}	94_{a}	100_{a}
	20	65_{a}	70_{ab}	72_{b}	73_{b}	82_{b}	96_{a}	100_{a}
Control	0	0_{h}	0_{h}	0_{h}	0_{h}	1_{g}	4_{g}	9_{f}

Figure(s) followed by the same alphabet(s) along the same column is not statistically different at 5% probability level

Andropogon gayanus contained saponins, flavonoids and alkaloids; *Cassia obtusifolia* contained tannins, flavonoids and alkaloids; *Phyllanthus amarus* tannins, saponins, flavonoids and alkaloids while *Sida acuta* contained tannins, saponins, flavonoids and sterols chemical compounds.

DISCUSSION

Application of different leaf extract of weed on root knot nematode, *M. incognita* juveniles in the laboratory resulted into significantly high mortality rate. *M. incognita* juvenile mortality rate increased with an increase in test plant extract concentrations and exposure time. In 15 and 20% (w/v) concentrations of *Euphorbia hirta*, *Phyllanthus amarus* and *Cassia obtusifolia* and 20% (w/v) concentration of *Sida acuta* and *Andropogon gayanus*, there was 100% *M. incognita* juvenile mortality by the 7th day. Olabiyi *et al.* (2006) reported that flavonoid concentrations between 30 and 70% resulted into 100% root knot nematode, *M. incognita*, juvenile mortality within 24 h *in vitro*. Moreover, Oyedumade and Olabiyi (2004) opined that bitter leaf, *Vernonia amygdalina* was toxic to root knot nematode, *M. incognita* under both laboratory and field conditions. Onifade and Fawole (1996) also reported that extracts of *Anarcardium occidentale* and *Gmelina arborea* effectively controlled *M. incognita* in the laboratory with percentage variations on egg inhibition and juvenile survival in different plant extracts. The results also corroborate the earlier findings of Pandey (1990) that reported the phytonematoxic properties in the extract of some aromatic and medicinal plants. It was reported that shoot and root aqueous extracts at 100% concentration of *Lactua sativa*, *Ammi majus*, *Artemisia pallens* and *Artemisia annua* resulted in 100% *M. incognita* juvenile mortality within 24 h of exposure *in vitro*. Adegbite and Adesiyan (2005) demonstrated that 100% concentration of root extracts of siam weed and neem exhibited 100% inhibition of *M. incognita* juvenile mortality under laboratory condition.

The present findings also indicated that different leaf extract of weeds contained different chemical compounds. The result of phyto-chemical analysis revealed that *Euphorbia hirta* contained tannins, saponins, flavonoids and alkaloids; *Andropogon gayanus* contained saponins, flavonoids and alkaloids; *Cassia obtusifolia* contained tannins, flavonoids and alkaloids; *Phyllanthus amarus* tannins, saponins, flavonoids and alkaloids while *Sida acuta* contained tannins, saponins, flavonoids and sterols chemical compounds. Olabiyi (2004) reported that leaves of African marigold, nitta and basil plants contained saponins and flavonoids; nitta root, rattle weed (leaf and root) contained saponins; roots of African marigold and basil plants contained flavonoids. Schmutterer (1990) opined that natural plant products have the ability to produce environmentally less harmful but efficacious chemical substances. Jackai *et al.* (1992) and Olabiyi (2004) reported their hope and look forward for natural plants (botanicals) that would replace the prohibitive synthetic nematicides in the plant parasitic nematode control.

CONCLUSION

Weeds are readily available, covering virtually every terrestrial piece of land in Nigeria. Since, the present research proved that some weeds species could be used as botanical in the control of *M. incognita* under laboratory condition, we propose to carry out similar research on the field and also on the use of weed species as composted material for the control of nematode pest.

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