CHEMICAL COMPOSITION AND BIO-NEMATICIDAL POTENTIAL OF SOME PLANT EXTRACTS ON *Meloidogyne incognita* (Kofoid and White)

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Article received: 22nd April, 2015; Article accepted: 5th July, 2015.

ABSTRACT
Phytochemical screening of ten selected plants (Moringa oleifera, Hyptis suaveolens, Phyllanthus muellerianus, Chromolaena odorata, Peperomia pellucida, Jatropha curcas, Euphorbia heterophylla, Tithonia diversifolia, Cochlospermum planchonii and Solanum torvum) was carried out twice to determine the presence of secondary metabolites in their leaves and also their nematicidal activity on root-knot nematode Meloidogyne incognita eggs and juveniles. Different levels of six out of the ten test plants were applied on the eggs and juveniles at the rate of 5%, 10%, 15% and 20% concentrations and replicated three times for each plant for aqueous and methanol extracts of the plants. Result of the phytochemical screening showed the presence of tannins, saponins, alkaloids, flavonoids, steroids,and reducing sugar. For egg hatchability, laboratory investigations revealed that all the tested plant extracts of either aqueous or methanol solvent at different concentrations used were capable of causing 100% egg hatch inhibition. For Juvenile mortality, the result showed that four of the plant extracts (Moringa oleifera, Jatropha curcas, Tithonia diversifolia and Chromolaena odorata) were more effective against root-knot nematode juveniles mortality than (Euphorbia heterophylla and Peperomia pellucida) at the same levels. Since the plant species screened against M. incognita proved quite effective, the use of these plant extracts instead of the harmful synthetic chemicals to control root-knot nematode holds a good promise for the future of crop protection.
***KEYWORDS**:* Meloidogyne incognita, Phytochemical screening, Test plants, Nematicidal activity, Egg hatchability, Juvenile mortality, Synthetic chemicals and Crop protection.

INTRODUCTION

Plant disease has been defined as the series of invisible and visible responses of plant cells and tissue through the pathogenic organisms or environmental conditions that results in adverse changes in form, function or integrity of plants (Agrios, 2005). Plants can be affected by diseases through pathogenic microbes which disturb the metabolism of plant cells through enzymes, toxins, growth regulators and other substances. They secrete toxic substances and also by absorbing food stuff from the host cells for their own use cause a great loss to agricultural products. The type of plant cells and tissues that become affected by these pathogenic microbes determine the type of physiological functions that will be disrupted. Examples of pathogens that cause infectious disease on plants include fungi, bacteria, viruses, viroids (virus-like organism), protozoa, nematodes, parasitic plants e.t.c. They are found in every part of the earth's lithosphere (Borgonie *et al.*, 2011). Of the nematodes, the most common are the root-knot nematodes, *Meloidogyne* spp. *Meloidogyne* spp are obligate parasites which are a problem in tropical and subtropical regions of the world where they affect major food crops, fruits, ornamental plants, vegetables e.tc (Jonathan and Hedwig, 1991). Root knot nematodes are able to cause radical changes in root cells in order to facilitate their lifestyle. They possess specialized adaptations for parasitism and therefore constitute a major group of plant parasitic nematodes of outstanding economic importance. In the history of the management of plant parasitic nematodes, the reduction in crop damage caused, has been achieved through the adoption of many control measures e.g. cultural, biological, quarantine, preventive, chemical control etc. Of all the tested approaches, chemical control has proved to be the most common and effective means of reducing or suppressing the effects of plant diseases but not without its attendant problems when not properly administered such as its toxicity to plants and soil, as well as being hazardous to the environment and its users (Egunjobi and Onayemi, 1981). Also, high cost of purchase, limited availability since most of them are imported, the residual effects to the environment causing pollution both to the atmosphere and underground waters with its continual effect of rendering the earth unsafe for mankind (Rotimi and Moens, 2002) constitute additional challenges of synthetic chemicals. Natural plant products are at present in the focus of research efforts because of their ability to produce environmentally safe and efficacious chemical substances. In this study, attempts were made to carry out phytochemical analysis on *Jatropha curcas, Moringa oleifera, Chromolaena odorata, Euphorbia heterophylla, Hyptis suaveolens, Peperomia pellucida, Phyllanthus muellerianus, Tithonia diversifolia, Solanum torvum* and *Cochlospermum planchonii.* Also tests were performed to assess the aqueous and methanol leaf extracts of six of the ten plants on egg hatchability and juvenile mortality of *Meloidogyne incognita* under laboratory conditions.

It is hoped that this study will stimulate interest for the search of more botanicals that would minimize the use of synthetic nematicide.

MATERIALS AND METHODS

These investigations were conducted in February and repeated in August 2012.

Ten Selected plants used include: *Peperomia pellucida, Cochlospermum planchomi, Hyptis suaveolens, Chromolaena odorata*, *Moringa oleifera*, *Jatropha curcas*, *Euphorbia heterophylla*, *Phyllanthus muellerianus*, *Solanum torvum*, and *Tithonia diversifolia.*

The phytochemical screening of the selected plants were carried out at the chemistry department, faculty of science, University of Ilorin, Kwara State while the *in-vitro* tests were carried out at Ladoke Akintola University of Technology, Crop and Environmental Protection Department, Faculty of Agriculture, Ogbomoso, Oyo State.

Collection and Preparation of Test Plants

Some of the plants were collected at different locations within University of Ilorin, Kwara State, Nigeria and the others were collected from Nigeria Institute of Horticulture (NIHORT) Ibadan, Oyo State, Nigeria.

The plants were identified in the department of Plant Biology, Faculty of Science, University of Ilorin. The leaves of each plant were sorted, washed and air dried for two weeks at temperatures ranging between 26^0c and 30^0c. Each was separately packed and ground into coarse powder using attrition mill (blender).

Two hundred and fifty grams (250gm) of each tested plants powder was extracted with methanol by soaking in the solvent for 48hours. The content was filtered using No 1 Whatman filter paper, each extract was concentrated using rotary evaporator. After concentration, the extract was used for phytochemical analysis.

Phytochemical Analysis

Using standard methods according to Debella, (2002), each of the 10 plant extracts was assessed for the presence of saponins, tannins, alkaloids, flavonoids, glycosides, steroid, reducing sugars and anthraquinones.

Root Extraction (Bearman method, 1965)

Extraction of *Meloidogyne incognita* eggs *M. incognita* was obtained from pure culture maintained on *Celosia argentea*. The entire root gall with soil was immersed into water and the soil was removed gently, without dislodging egg masses. After washing, the roots were chopped into pieces. Eggs were extracted by shaking the chopped gall roots in a 0.5%NaOCl solution for 3-5minutes.

Plant debris and tiny or small stones were gotten rid off with mesh sieves (Hussey and Baker, 1973). The mesh sieves of 120mm nested over 90µm, 60µm, 38µm and finally on 28µm were used to harvest the eggs. *Meloidogyne incognita* eggs that were finally collected were washed with several changes of the distilled water in order to remove all traces of NaOCl.

The number of eggs in the extraction was viewed under stereomicroscope. Approximately 100eggs per 1ml of solution was used in all the treatments and their replicates. The experimental trials were set up in Petri-dishes and the plant extracts were serially diluted and added in various concentrations of 5%, 10%, 15%, 20% and replicated three times for each treatment.

Serial Dilutions;

5% = 1ml of concentrated plant extract + 19ml of water

10% = 1ml of concentrated plant extract + 9ml of water

15% = 1ml of concentrated plant extract + 5.7ml of water

20% = 1ml of concentrated plant extract + 4ml of water

The experimental set-up was observed for 10days and counts of hatched juveniles were made every 24hours.

Extraction of *Meloidogyne incognita* juveniles

Freshly extracted eggs were incubated at 27± 2^oc for 72 hours in the incubator to hatch out the second stage juveniles. Standardization of the number of the juveniles per unit volume was done so that 1ml juvenile suspension contained approximately 100ml hatched juveniles.

RESULTS

The results of the phytochemical screening and bio-nematicidal potential of some plant extracts on root-knot nematode, *Meloidogyne incognita* eggs and juveniles *in-vitro*, were generally similar, and followed the same trend in the two trials. Results were pooled together as shown in Tables 1-5.

Phytochemical Analysis of Some Plants Extracts

Chemical composition of the leaves of *Moringa oleifera, Hyptis suaveolens, Phyllanthus muellerianus, Chromolena odorata, Peperomia pellucida, Jatropha curcas, Euphorbia heterophylla, Tithonia diversifolia, Cochlospermum planchonii* and *Solanum torvum* are shown in Table 1

Egg-hatch Experiments

Effects of aqueous plant extracts on root-knot nematode eggs

The result of the effect of aqueous extracts of *Moringa oleifera*, *Jatropha curcas, Peperomia pellucid, Euphorbia heterophylla, Tithonia diversifolia,* and *Chromolaena odorata* on egg hatch assessed for ten (10) consecutive days in the laboratory. Table 2 shows that all the plants extracts inhibited egg hatch within 10 days, whereas there were progressive hatching of root-knot nematode eggs in the control experiment (Distilled water)

There was 72.67% hatch in the control experiment while there was 0% egg hatch in the treatments where plant leaf extracts were applied.

Effects of Methanol Plant Extracts on Root-Knot Nematode Eggs

There was also 100% egg-hatch inhibition with respect to methanol plant extracts as shown in Table 3

Juvenile Mortality Experiments

Effects of Aqueous Plant Extracts on Root-Knot Nematode Juveniles

The result of the effect of aqueous plant extracts of *Moringa oleifera*, *Jatropha curcas, Peperomia pellucid, Euphorbia heterophylla, Tithonia diversifolia* and *Chromolaena odorata* on Juvenile mortality rate (Table 4). After 24 hours *Euphorbia heterophylla*, had 95% mortality, *Pepermoia pellucida* had 85% mortality while all other plant extracts recorded 100% mortality. On day 2, *Euphorbia heterophylla* had 98% mortality; *Peperomia pellucida* had 95% while others still maintained 100% mortality. On day 3 all plant extracts recorded 100% juvenile mortality.

In the control (Distilled water), all the juveniles were still alive and active, on the 2nd day, there was 5% mortality and on the 3rd day, there was 7% mortality

Effects of Methanol Plant Extracts on Root-Knot Nematode Juveniles

It was observed that all the tested plant extracts and the control (methanol) Table 5 had 100% mortality rate on the juveniles of root-knot nematodes within 24 hours.

DISCUSSION AND CONCLUSION

In recent years, some natural plants have been discovered to have nematicidal properties which their extracts could be effectively used in the control of root-knot nematode disease both on the field, green/screen house and in the laboratory (Oyedunmade and Olabiyi, 2005; Olabiyi *et al.*, 2007). Example of one of such botanicals that has been worked on is *Phyllanthus amarus* which is found to contain certain phytochemicals like tannin, saponin, flavonoid and alkaloid (Izuogu, 2009), this justifies their ability of being able to act as an anti-nematicidal plant. Others include *Moringa oleifera*, *Cassia spp* lemon grass (Izuogu and Oyedunmade, 2009).

The result of the phytochemical analysis from this study showed that the plant extracts screened contain basic secondary metabolites such as tannins, saponins, alkaloids, flavonoids, deoxysugar cardenolides (steroids) and reducing sugars which could be bio-nematicidal.

The results of the *in-vitro* studies on nematicidal potentials of *Moringa oleifera*, *Jatropha curcas*, *Peperomia pellucida*, *Euphorbia heterophylla*, *Tithonia diversifolia*, and *Chromolaena odorata* revealed that aqueous and methanol extracts were effective in inhibiting egg hatch and causing mortality of the second stage juveniles of *Meloidogyne incognita* at the four concentrations of 5%, 10%, 15% and 20%. For egg hatchability, laboratory investigations showed that all the test plant extracts (aqueous and methanol) concentrations (5%, 10%, 15% and 20%) were capable of causing 100% egg hatch inhibition.

The result further revealed that *Meloidogyne incognita* juveniles had 100% mortality when exposed to *Jatropha curcas*, *Moringa oleifera*, *Tithonia diversifolia*, and *Chromolaena odorata* aqueous leaf extracts at concentrations between 5-20% within 72hours. However, there were variations in the nematicidal activity exhibited in terms of juvenile mortality of *M. incognita* with the aqueous leaf extracts of *Peperomia pellucida* and *Euphorbia heterophylla* at concentrations between 5-20% within 48hours but at 72hours, there was 100% mortality of the juveniles of *M.incognita* in all the tested leaf extracts. For methanol extracts of the six plants, they recorded faster mortality of juveniles of *M.incognita* from the 1st day to the 3rd day. The nematicidal activity observed in these leaf extracts of *Moringa oleifera*, *Jatropha curcas*, *Peperomia pellucida*, *Euphorbia heterophylla*, *Tithonia diversifolia*, and *Chromolaena odorata* might be due to the presence of active ingredients; Phytochemicals.

Table 1: Phytochemical Analysis of Plant Extracts

Plants	Saponins	Tanins	Alkaloids	Glycosides	Flavonoids	Steroids	Reducing sugar	Anthraquinones
Solanum torvum (Prickly Solanum) or turkey berry	+	-	+	-	-	+	-	-
Chromolaena odorata (Siam weed)	+	+	+	-	-	-	-	-
Cochlospermum planchonii (cotton plant)	+	-	+	-	-	-	-	-
Tithonia diversifolia (tree marigold)	+	+	-	-	+	-	-	-
Moringa oleifera (moringa or Benzolive tree)	+	+	+	-	-	+	+	+
Jatropha curcas (Physic nut)	+	-	+	-	+	+	+	-
Phyllanthus muellerianus (Myrobalan)	+	+	-	-	+	+	-	-
Hyptis suaveolens (Wild spikenard)	+	-	+	-	+	+	+	-
Euphorbia heterophylla (Milk weed)	+	+	+	-	+	-	+	-
Peperomia pellucida (Shiny or silver bush)	+	+	+	-	+	-	-	-

Key: + indicates present - indicates absent

The present study corroborates the earlier findings of some investigators. Izuogu and Oyedunmade, (2009) reported that the methanolic extracts from the leaves of Brimstone, Cassia, Lemon grass and Chanca prevented *Meloidogyne incognita* egg-hatch and juvenile survival *in-vitro*. Oyedunmade (2000) reported that African marigold (Tagetes erecta) compared favorably with Carbofuran (Furadan) and improved the vegetative growth (number of leaves/plant and plant height) and yield (number of fruits and weight of fruits/plant) of Okra. . Kimpinski *et al.,* (2000) reported that *Tagetes tenuifolia*, *T.patula* and *T.erecta* were found to significantly reduce the population of *Pratylenchus penetrans* on potato field.

These phytochemicals had been reported to confer pesticidal abilities in plants (Fatoki and Fawole, 2000; Chitwood, 2002; Adekunle and Okoli, 2002; Adeniyi *et al.,* 2010). Some of these phytochemicals such as tannins, saponins, amongst others are reported to have nematicidal properties and had led to disruption of membranes in organisms thereby facilitating penetration of toxic principles to the detriment of such organisms (Agrios, 2005; D'Addabbo *et al.,* 2010). Saponins, also called saponocydes, have been reported to possess cell membrane breaking property. In particular, saponins bind with the lipid membrane of cells, making the cells more permeable and at the same time more fragile, enabling a loss of cell contents through leakages (Bassetti and Sala, 2005). Bassetti and Sala (2005) posited that inhibition of egg-hatch inhibition and mortality of 2nd-stage juveniles of *M. incognita* due to application of plant extracts might be due to disruption of membranes that facilitated penetration of lethal ingredients i.e phytochemical.

Table 2: Effects of Aqueous Plant Extracts on Root-Knot Nematode Eggs

Plant extract	Application rate (%)	Number of eggs hatched on day:									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Jatropha curcas (physic nut)	5	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0
Euphorbia heterophylla (Milk weed)	5	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0
Tithonia diversifolia (Tree marigold or Mexican sunflower)	5	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0
Moriga oleifera (moringa or Benzolive tree)	5	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0
Chromoleana odorata (Siam weed)	5	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0
Peperomia pellucida (shiny bush or silver bush)	5	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0
Control (Water)		0	1	9	11.33	24	33.67	42.67	55.67	64.33	72.67

Table 3: Effects of Methanol Plant Extracts on Root-Knot Nematode Eggs

Plant extract	**Application rate (%)**	**Number of eggs hatched on day:**									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Jatropha curcas (physic nut)	5	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0
Euphorbia heterophylla (Milk weed)	5	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0
Tithonia diversifolia (Tree marigold or Mexican sunflower)	5	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0
Moriga oleifera (moringa or Benzolive tree)	5	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0
Chromoleana odorata (Siam weed)	5	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0
Peperomia pellucida (shiny bush or silver bush)	5	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0
Contro(Methanol)		0	0	0	0	0	0	0	0	0	0

Table 4: Effects of Aqueous Plant Extracts on Root-Knot Nematode Juveniles

Plant extract	Application rate (%)	Percentage cumulative juvenile mortality at day:		
		1	**2**	**3**
Jatropha curcas (physic nut)	5	100	100	100
	10	100	100	100
	15	100	100	100
	20	100	100	100
Euphorbia heterophylla (Milk weed)	5	90	95	100
	10	92	96	100
	15	93	97	100
	20	95	98	100
Tithonia diversifolia (Tree marigold or Mexican sunflower)	5	100	100	100
	10	100	100	100
	15	100	100	100
	20	100	100	100
Moriga oleifera (moringa or Benzolive tree)	5	100	100	100
	10	100	100	100
	15	100	100	100
	20	100	100	100
Chromoleana odorata (Siam weed)	5	100	100	100
	10	100	100	100
	15	100	100	100
	20	100	100	100
Peperomia pellucida (shiny bush or silver bush)	5	75	85	100
	10	80	85	100
	15	80	90	100
	20	85	95	100
Control		0	5	7

Table 5: Effects of Methanol Plant Extracts on Root-Knot Nematode Juveniles

Plant extract	**Application rate (%)**	**Percentage cumulative juvenile mortality at day:**		
		1	**2**	**3**
Jatropha curcas (physic nut)	5	100	100	100
	10	100	100	100
	15	100	100	100
	20	100	100	100
Euphorbia heterophylla (Milk weed)	5	100	100	100
	10	100	100	100
	15	100	100	100
	20	100	100	100
Tithonia diversifolia (Tree marigold or Mexican sunflower)	5	100	100	100
	10	100	100	100
	15	100	100	100
	20	100	100	100
Moriga oleifera (moringa or Benzolive tree)	5	100	100	100
	10	100	100	100
	15	100	100	100
	20	100	100	100
Chromoleana odorata (Siam weed)	5	100	100	100
	10	100	100	100
	15	100	100	100
	20	100	100	100
Peperomia pellucida (shiny bush or silver bush)	5	100	100	100
	10	100	100	100
	15	100	100	100
	20	100	100	100
Control (Methanol)		100	100	100

Alkaloids in plants have been reported to exhibit nematicidal activity on root-knot nematodes decreasing galling in roots, but its mode of action is under investigation. The mechanisms of many plants extracts may include denaturing and degrading proteins, inhibition of enzymes and interfering with the electron flowing respiratory chain with ADP phosphorylation (Claudius-cole *et al.,* 2010).
Alkaloids are also known to play some metabolic roles and control development in living system (Edeoga *et al.,* 2006). The compound have protective role in animal and it is used in medicine especially the steroidal alkaloids which constitutes most of the valuable drugs. Additionally, plant extracts showing active trypanocidal activity was found to contain alkaloids, flavonoids, phenolic and/or terpenes (LeGrand, 1989).
Edeoga *et al.,* (2006) also reported that saponins are glycocide of both triterpenes and sterols and are used as expectorant and emulsifying agent. Additionally, saponin is equally used in medicine and pharmaceutical industries because of its foaming ability with the production of frothy effect. Saponin is used in the preparation of insecticides, various drugs and synthesis of steroid hormones (Okwu, 2003).
It has been reported that the chloroform extracts from dried leaves of *P. Pellucida*, particularly apiol and pachypophyllin have antifungal activity against *Trichophyton mentagrophytes*. Antibacterial *in-vitro* data of *P. pellucida* against numerous species, including *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

RECOMMENDATION

Since the plant species screened against *M. incognita* were effective against juvenile mortality and egg hatch, the use of plant extracts instead of the harmful/prohibitive synthetic chemicals to control root-knot nematode holds a good promise for the future of crop protection and should be encouraged by the government at all levels.
It is a safe control measure that should be adopted in accordance with the concept of organic farming since it poses no harmful effect on plants and environment, it is equally cheaper compared than synthetic nematicides. However further field triads are recommended to buttress our findings.

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