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Full Length Research Paper

Nematicidal effects of some plant extracts on egg hatchability and control of *Meloidogyne* spp. in cowpea (*Vigna unguiculata* (L.) Walp)

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The effects of cold and hot aqueous extracts of 5 test plants (Luffa cylindrica, Momordica charantia, Euphorbia hirta, Desmodium scorpiurus and Stachytarpheta cayennensis), wood ash of Gmelina aborea, a synthetic insecticide (Karate –Lambda cyhalothrin) and untreated tap water (control) were evaluated against the egg hatchability and control of *Meloidogyne* spp. in cowpea (Vigna unguiculata (L.) Walp) in the laboratory and greenhouse. The experiments were laid out in a Completely Randomized Design (CRD) with 13 treatments (Hot water extracts (HWE) and cold water extracts (CWE) of *L. cyclindrica*; *E. hirta*, *D. scorpiurus*, S. cayennesis, *M. charantia*, wood ash, *L. cyhalothrin* and control) replicated 3 times. The result of the laboratory experiment showed that the hot and cold water extracts of *L. cylindrica* and HWE *of M. charantia* significantly ($p < 0.05$) inhibited the hatching of nematode eggs. Results obtained from the Greenhouse indicated that HWE of E. hirta, CWE of *S. cayennesis*, CWE and HWE *of L. cylindrica* significantly improved cowpea yield, ($p< 0.05$). The aqueous extracts reduced the number of galls on the roots, nematode populations in the root and soil. The results obtained *G. aborea* extracts did not differ significantly with the synthetic nematicide – *L. cyhalothrin*. The leaf extracts of the test plants have potential as sources of botanical nematicides to reduce the devastating effects of plant-parasitic nematodes in cowpea fields.

Key words: Plant extracts, cowpea, lambda-cyhalothtrin, inoculum, nematomde.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is a dicotyledonous plant belonging to the family Fabaceae (Cronquist, 1988). It is of major importance to the livelihood of millions of people in the tropics (Quin, 1997). In Nigeria and other West African countries, the most grown and eaten legume is cowpea and it is mainly cultivated in the northern states of Nigeria. It is an essential component of sustainable agriculture in marginal lands and drier regions of the tropics, where rainfall is scanty and soils are sandy with little organic matter (Watt et al., 1985). Emechebe (1985) reported that the major problem of cowpea is pests and diseases which do not only cause low yield but also discourage most farmers from cultivating the crop. One of the major limiting factors to the profitable cowpea production is the damage caused by plant-parasitic nematodes especially *Meloidogyne* spp. (Rachie and Lawal, 1975). The extent of damage is influenced by the cultivar, nematode species, level of soil infestation and environment (Ononuju, 1999).

Root-knot nematodes infect roots of cowpea plants resulting in considerable losses. The yield loss is associated with conspicuous galls that disrupt water and nutrient uptake (Ogbuji, 1981). Rose et al. (1989) reported yield losses of more than 90% in high population. Current efforts and campaign being made by

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governments of various countries especially developing countries to improve the standard of living through the production and utilization of cowpea could only be realized if solutions to the problems of pests including plant-parasitic nematodes are found (Ononuju, 1999). The use of synthetic nematicides is considered the most effective practical means of combating the menace of plant-parasitic nematodes in cowpea (Adesiyan, 1992). However, chemical control of root-knot nematodes leads to environmental hazards because of the high toxicity and persistence of the nematicides (Adesiyan et al., 1990). This had led to an increase in the use of naturally occurring pesticides in nematode control (Gulter, 1988).

Plant extracts have been found effective for the control of plant-parasitic nematodes (Hussain et al., 1984; Siddiqui and Allam, 1989). They are easily degraded, pollution-free; leave no harmful residues, are cheaper and not toxic to host plants and humans (Amadioha, 2003). The Current investigation was undertaken to evaluate the nematicidal activity of five plant species: *Luffa cylindrica* (Linn) MJ Roem, *Momordica charantia* Linn, *Euphorbia hirta* Linn, *D. scorpiurus* (Sw.) Desv. and *Starchytarpheta cayennensis* L.C. Rich (Schau), wood ash and a synthetic insecticide (*L. cyhalothrin*) on egg hatachability and control of *Meloidogyne* spp. in cowpea.

MATERIALS AND METHODS

Soil preparation and sterilization

Top moist soil was collected from the farm nursery site of Michael Okpara University of Agriculture. The soil mixture was moistened and then put into a cut drum, covered and heated until it reached a temperature of 80°C and maintained at this temperature for 20 min. After cooling down, 4 kg of soil were separately put in 39 plastic pots of 20 cm diameter.

Extraction of nematode eggs

Eggs were obtained from a culture of nematode infected roots of okra, *Abelmoschus esculentus* (L.) Moench. Galled root pieces of A. esculentus containing egg masses were cut into small pieces and placed in a container of 500 ml capacity with 200 ml of 0.5% chlorox (sodium hypochloride, NaOCl) solution shaken vigorously by hand for 4 min (Hussey and Baker, 1973). This was done in order to digest the gelatinous matrix encasing the eggs. The solution was then poured through two nested sieves, 200 mesh (75 µm) and 500 mesh (25 µm). Eggs in the 500 mesh sieve were washed free of NaOCl solution with slow stream of cold tap water into a container previously marked to contain 1 L. The cut roots in the original container were washed twice with water to obtain additional eggs.

The number of eggs per 1 ml of water was estimated by counting 4 samples of 1 ml each using Domncasters counting dish under a stereomicroscope and a working mean of eggs/ml was estimated for example 99.8 eggs/ml.

Extraction of plant leaf extracts

Leaf extracts were obtained using the method of Amadioha (2001). Collected samples of fresh leaves of each of the plant species were each washed thoroughly in tap water, air dried at 27°C for 1 h and then ground separately with a sterile mortar and pestle to obtain 1 kg paste. Cold water extracts wee prepared by adding 500 ml of water to the pastes and allowing standing for 4 h. This was then filtered through sterile cotton wool to obtain aqueous extracts. The hot water infusion of the test plants were obtained by adding 500 g of the pastes separately in each of 250 ml conical flask containing 100 ml of sterile distilled water. The flasks with their contents were then placed in a water bath set at 80°C for 1 h. Each suspension was then filtered as described earlier. The filtrates obtained were then used as the hot and cold water extracts of the test plants.

Effects of extracts on egg hatchability

The effect of the hot and cold water extracts of the five test plants on the hatchability of eggs of *Meloidogyne* spp. was determined in 39 plastic dishes. Nematode eggs suspension at a concentration of 100 eggs/ml was introduced into each of the 36 plastic Petri dishes followed by the introduction of 5 ml of each of the plant extracts. The same nematode egg suspension was pippeted into 5 ml of tap water contained in 3 separate plastic Petri dishes which served as control. 5 ml of *L. cyhalothrin* 5% a.i and 5 g of wood ash were each added to egg suspension containing 1000 eggs/10 ml in 6 separate Petri dishes.

There were thirteen treatments and each treatment was replicated three times and arranged randomly on incubator shelves at a temperature of 28°C. The set up was left for fourteen days after which the number of hatched eggs was counted.

Greenhouse experiment

Cowpea seeds (IAR-48) developed by the Institute of Agricultural Research, Zaria, Nigeria, were surface sterilized in 0.01% $HgCl_2$ for 1min and rinsed in sterile distilled water. After this 4 seeds per pot were planted and later thinned down to one healthy plant per pot. The pots were arranged in completely randomized design (CRD) on green house benches at a mean temperature of 27°C. After two weeks of planting, each of the cowpea plants was inoculated with 1000 eggs/10 ml egg suspension of *Meloidogyne* spp. Treatments applied using a 10 ml syringe after one week of inoculation were hot and cold water aqueous extracts of the 5 test plants at the rate of 5 ml, 5 g of wood ash of *G. aborea* and 5 ml of *L. cyhalothrin* at 5% (a.i.). There were 13 treatments replicated 3 times. The control plants were treated with 5 ml of tap water. The plants were watered with tap water daily for a period of 3 months.

Estimation of nematode population from the soil samples

Soil (200 ml) samples were taken from each plant at harvest (three months after planting). Nematodes were extracted from soil samples using the modified Baermann technique (Hooper, 1969). Nematode counts were made after 24 h using a stereoscopic microscope.

Estimation of nematode population in root samples

Nematode eggs in the roots were estimated by measuring out 2.0 g each of root samples of the 39 cowpea plants infested with nematode eggs. Galled root samples were cut into small pieces and placed in a beaker of 500 ml, and thereafter processed as previously described in extraction of nematode eggs from soil.

Data collection and statistical analysis

Data collected include plant height, root fresh weight, fresh and dry weights of shoot, pod weight, number of pods and the number of galls. Nematode populations in the soil and root samples were also estimated at the end of the evaluation. All data generated from the trial were subjected to one way analysis of variance (ANOVA) and where significant differences were observed, treatment means were compared

Table 1. Effect of hot and cold water extracts of test plants, wood ash and *Lambda cyhalothrin* on egg hatchability of *Meloidogyne* spp.

Treatment	% Nematode egg hatch
L. cylindrica CWE	1.03^{ef}
HWE	1.50^{def}
E. hirta CWE	3.80^{cde}
HWE	2.53 ^{cde}
D. scorpiurus CWE	2.45 ^{cde}
HWE	3.63^{cde}
M. charantia CWE	5.43^{bc}
HWE	1.95^{cdef}
S. cayennensis CWE	4.43^{cd}
HWE	7.76^{b}
Wood ash	2.60^{cde}
Lambda cyhalothrin	0.00^{f}
Control (Water)	12.30^{a}

*Column means with same letters are not significantly different ($P \leq 0.05$). CWE = Cold water extract; HWE = Hot water extract.

using Duncan's New Multiple Range Test and considered significance at P≤0.05 (Obi, 1990).

RESULTS

Effects of extracts on hatching of Meloidogyne eggs

The effect of the treatments on the hatchability of Meloidogyne eggs is presented on Table 1. The synthetic insecticide, *L. cyhalothrin* completely inhibited egg hatch. However, this was not significantly different from the results obtained from hot water extracts of *M. charantia*, *L. cylindrica* and cold water extract of *L. cylindrica*. These extracts were the most effective in inhibiting egg hatch (Table 1). The least effective were the hot water extract of *S. cayennensis* and cold water extract of *M. charantia*. The water treatments (control) had significantly higher number of eggs that hatched.

Effect of extracts on gall formation and nematode population

The effects of the treatments on number of galls, nematode population in soil and root are shown on Table 2. All the treatments except hot water extracts of *S. cayennensis*, *M. charantia* and cold water extracts of *M. charantia*, and *E. hirta* significantly reduced number of galls compared to control. Similarly, the number of nematodes recovered from the treated plants in soil and root were less when compared with the untreated plants and this was significant.

Effect of treatments on growth of cowpea

The extracts from fresh and dry weights of cowpea shoot (Table 3), did not significantly increase the dry weight of cowpea shoot over the control where as the fresh weight of shoots of treated plants decreased significantly when compared with the control. Extracts of *L. cylindrica* increased the fresh and dry weights of the shoot and this was significantly higher than the other extracts (Table 3). Table 4 shows the effect of treatments on plant height and fresh root weight. Apart from *L. cylindrica* which did not differ significantly from the insecticide and cold water extract of *M. charantia* in plant height, other treatments did not differ from each other but differed significantly from the synthetic nematicide. There was no significant difference in fresh root weight of cowpea treated with cold water extract of *L. cylindrica*, *E. hirta*, *M. charantia*, *S. cayennensis* and *L. cyhalothrin*. These treatments recorded the highest fresh root weight than other treatments.

The lowest fresh root weight was obtained from cold water extract of *D. scorpiurus* which was not significantly different from other remaining treatments.

Table 2. Effect of treatments on number of root galls, nematode population in soil water and root samples.

Treatment	No of galls	Nematode population in 200 ml soil water	Nematode population/2g root sample
L. cylindrica CWE	17.67^{bc}	1200.00^{de}	200.00^{c}
HWE	6.67^{cd}	1133.33^{de}	400.00^{b}
E. hirta CWE	26.00^{abc}	1466.66^{cd}	733.33^{b}
HWE	12.67^{bcd}	2733.33^{b}	533.33^{b}
D. scorpiurus CWE	17.50^{bcd}	2500.00^{bc}	
HWE	12.67^{bcd}	1733.33^{bcd}	266.66^{c}
M. charantia CWE	26.00^{abc}	2200.00^{bcd}	466.00^{b}
HWE	30.00^{ab}	2000.00^{bcd}	800.00^{b}
S. cayennensis CWE	15.00^{bcd}	2733.33^{b}	533.33^{b}
HWE	9.00^{bc}	2200.00^{bcd}	600.00^{b}
Wood ash	9.67^{cd}	2400.00^{bc}	733.33^{b}
L. cyhalothrin	1.66^{d}	200.00^{e}	200.00^{c}
Control (Water)	38.00^{a}	066.67^{a}	1800.00^{a}

*Column means with same letters are not significantly different (P≤ 0.05).

Table 3. Effect of treatments on fresh and dry weights of cowpea shoot.

Treatment	Fresh Weight (g)	Dry Weight (g)
L. cylindrica CWE	30.53^{b}	12.00^{a}
HWE	24.93^{bc}	10.00^{ab}
E. hirta CWE	4.73^{e}	2.20^{c}
HWE	9.90^{cde}	3.50^{bc}
D. scorpiurus CWE	13.05^{cde}	4.50^{bc}
HWE	11.66^{cde}	2.83^{bc}
M. charantia CWE	22.9^{bcd}	3.16^{bc}
HWE	9.90^{cde}	4.40^{bc}
S. cayennensis CWE	7.10^{de}	3.50^{bc}
HWE	6.70^{de}	3.50^{bc}
Wood ash	6.70^{de}	4.50 ^{bc}
L. cyhalothrin	22.06^{bcd}	3.30^{abc}
Control (Water)	57.13^{a}	7.06^{abc}

*Column means with same letters are not significantly different (P ≤ 0.05).

Effect of extracts on yield of cowpea

The number and weights of pods from plants treated with extracts of L. cylindrica, hot water extracts of *E. hirta*, *D. scorpiurus* and cold water extract of *S. cayennensis* significantly increased when compared to other treatments. Only cold water extracts of *L. cylindrica*, *S. cayennensis* and hot water extract of *E. hirta* increased the pod weight significantly. However, most of the treatments did not differ significantly from the control plants in both the number of pods and pod weight (Table 5).

Table 4. Effect of treatments on plant height and fresh root weight.

Treatment	Plant height (cm)	Fresh root weight (g)
L. cylindrica CWE	59.33[a]	3.10[ab]
HWE	53.00[ab]	2.16[b]
E. hirta CWE	37.33[c]	2.93[ab]
HWE	37.33[c]	1.60[b]
D. scorpiurus CWE	41.50[c]	0.93[b]
HWE	40.33[c]	1.86[b]
M. charantia CWE	43.66[bc]	3.06[ab]
HWE	36.00[c]	1.23[b]
S. cayennensis CWE	38.66[c]	2.93[ab]
HWE	36.50[c]	2.40[b]
Wood ash	38.33[c]	1.60[b]
Lambda-cyhalothrin	54.00[ab]	4.73[a]
Control (Water)	35.33[c]	1.50[b]

*Column means with same letters are not significantly different (P ≤ 0.05).

Table 5. Effect of treatment on number of pods and pod weight.

Treatment	Number of pods	Pod weight (g)
L. cylindrica CWE	1.66[abc]	3.06[abc]
HWE	2.29[a]	1.80[bc]
E. hirta CWE	0.47[cd]	0.90 [c]
HWE	2.00[a]	5.33[a]
D. scorpiurus CWE		
HWE	1.43[abc]	1.80[bcd]
M. charantia CWE	0.47[cd]	1.03 [c]
HWE	0.00[d]	0.00 [c]
S. cayennensis CWE	2.96[a]	5.63[a]
HWE	0.00[d]	0.00 [c]
Wood ash	0.47[cd]	0.90[c]
L. cyhalothrin	0.87[bcd]	0.77[c]
Control (Water)	0.47[cd]	0.60[c]

*Column means with same letters are not significantly different (P ≤ 0.05).

DISCUSSION

Various workers have emphasized the advantages and potential use of ingredients of higher plants in controlling plant diseases (Beye, 1978; Renu et al., 1980; Ononuju and Okoye, 2003) but very little work has been done to investigate natural plant products as nematicides, particularly against nematode disease of cowpea. Results of this study revealed the presence of nematicidal substances in extracts of *L. cylindrica*, *M. charantia*, *E. hirta, D. scorpiorus*, *S. cayennensis* and wood ash, which agree with the results reported by other workers on

different pathogens and plants (Al-Abed et al., 1993; Sikora, 1992; Tewari and Nayak, 1991; Onifade and Fawole, 1996; Ononuju and Anyalewechi, 2009). The inhibition of nematode growth and development in vitro and in vivo by cold and hot leaf extracts of the test plants observed in this study strongly suggest the presence of nematicidal substances in the plant leaves and the possibility of using the plant species for control of plant-parasitic nematodes. The use of plant extracts in controlling diseases have several advantages including being pathogen specific.

The differences in the toxicity of different extracts could be attributed to the presence of the active principle(s) or compound(s) in the plant material or even different active principle(s) that may be influenced by several factors such as age of plant, method of extraction and type of extracting solvent (Qasem and Abu-Blan, 1996; Nicolls, 1969). The greater effectiveness of hot water extracts as compared with the cold water extracts may be due to differences in constituent extraction or in heat sensitivity of the bioactive constituent(s). The poor performance recorded by the control experiment (distilled water) in the yield and growth parameters when compared with the treated experiment (plant extract and synthetic nematicide) could be attributed to heavy nematode infestation. Although chemical nematicides may be effective in nematode control (Ononuju and Fawole, 2000), their high costs, non availability at the time of need and the hazards they pose as environmental pollutants discourage most farmers. However, biopesticides of plant origin are preferable because of their non-toxic activities and their environmental friendliness (Reddy et al., 2009). Although the active principles in the plant extracts were not investigated, most of the plants used are known to contain toxic polyphenolic compounds (Brain and Margaret, 1979).

Poor farmers of cowpea need to depend on plant extracts to control nematode diseases for sustainable yield and profitable cropping. The extracts of test plants used in this study which are commonly found locally could be possible replacement for synthetic nematicides for the control of plant-parasitic nematodes especially Meloidogyne spp. The findings of this present investigation are not conclusive. There is need for further study especially in the area of nematode population density that could cause significant damage. Also, the effectiveness of plant extracts in a field environment should also be assessed.

REFERENCES

Adesiyan SO (1992). Chemical Control of nematode pests of some economic crops. In proceedings of the first regional symposium of the Biology and control of nematode pests of food crops in Africa. University of Ibadan, Nigeria, pp. 283-294.

Adesiyan SO, Caveness FE, Adeniji MO, Fawole B (1990). Nematode Pests of tropical crops. Heinemann Educational Books (Nigeria) Ltd., p. 114.

Al-Abed AS,Qasem JR,Abu-Blan HH(1993). Anti-fungal effects of some common wild plant species on certain plant pathogenic fungi. Dirasat. Pure Appl.Sci., 20: 149-159.

Amadioha AC (2001). Fungitoxic Effects of Extracts of Azadirachta indica against *Cochliobolus miyabeanus* causing brown spot disease of Rice. Arch. Phytopathol., 35: 37-42.

Amadioha AC (2003). Evaluation of some plant leaf extracts against Colletotrichum lindemuthianum in cowpea. Actaphyto pathologica et Entomologica Hungarica, 38 (3-4): 259-265.

Beye F (1978). Insecticides for the vegetable kingdom. Plant Res. Dev., 7:13-31.

Brain V, Margaret LV (1979). Plant Products of Tropical Africa. Macmillian Trop. Agric. Hort. Appl. Ecol. Series, pp. 21-28.

Cronquist A (1988). The Evolution and Classification of Flowering plants.2nd Edition. The New York Botanical Garden, New York, ISBN 0-89327-332-5, p. 555.

Emechebe AU (1985). Cowpea pathology. In: Grain Legume improvement programme. Annual Report 1981. IITA, Ibadan, Nigeria, pp. 69-10.

Gulter HG (1988). Natural products and their potential in agriculture. A personal overview In: Gulter HG (Ed.), Biologically Active Natural Products: Potential use in Agriculture, American Chemical Society; Washington, pp. 7-2.

Hooper DJ (1969). Extraction and handling of plant and soil nematode. In: Nematodes of tropical crops. Peachey JE (Ed.). Common Bur. Acliminth. Tech. Common., 4: 20-30.

Hussain SI, Kumar R, Kahn IA, Titov A (1984). Effect of root-dip treatments of eggplant seedlings with plant extracts, nematicides, oil cake extracts and antihelmintic drugs on plant growth and root knot development. Pak. J. Nem., 2(2): 79-83.

Hussey RS, Baker RR (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp. Including a new Technique. Plant Dis. Rep., 57: 1025-1028.

Nicolls JR (1969). Antifungal activity of Passiflora species. Ann. Bot., 34: 229-237.

Obi IU (1990). Statistical methods for detecting differences between treatment means. 2nd ed. Snaap Press, Enugu, Nigeria, pp. 40-42.

Ogbuji RO (1981). The status of root-knot nematodes in the Eastern States of Nigeria. In: Proceedings of the Research Planning Conference on Root-knot nematodes, *Meloidogyne* spp. IITA,Ibadan,June 7-11,1976, pp. 44-47.

Onifade AK, Fawole B (1996). Effect of some plant extracts on the pathogenicity of Meloidogyne incognita on cowpea. Glob. J. Pure Appl. Sci., 2: 9-15.

Ononuju CC (1999). The Use of Natural Nematicides: The best sustainable plant protection option for plant-parasitic nematodes among small scale farmers in Nigeria. Paper presented at the 21st Annual Conf. of Nigerian Society for plant protection. Nigeria institute for oil palm Research (NIFOR), Benin City, Edo State, Nigeria. September, 26th - 30th, 1999.

Ononuju CC, Anyalewechi JA (2009). Nematicidal influence of Pleurotus tuber regiums and Neem leaf extract on the incidence of root-knot Nematodes on Solenostemon rotundisoliua Poir (Hausa potato). Agric. Soc. Nig. J., 40(1): 248-254.

Ononuju CC, Fawole B (2000). Evaluation of Ethoprophos Isazophos, Carbofuran for the control of plant-parasitic nematodes on their effects on plantain growth and yield. Nig. J. Sc., 34: 81-89.

Ononuju CC, Okoye CD (2003). Nematotoxic activity of some common weed extracts against root –knot nematode in soyabean. Afr. J. App. Zoo. Env. Bio., 5: 64-66.

Qasem JR, Abu-Blan HA (1996). Fungicidal activity of some common weed extracts against different plant pathogenic fungi. J. Phytopathol. 144: 157-161.

Quin FM (1997). Importance of Cowpea. In: Advances in Cowpea Research. In Singh BB et al. (eds) pp X-Xii. Printed by Colcocraft, Hong Kong, p. 375.

Rachie KO, Lawal RD (1975). Grain legumes of lowland tropics. In: "Advances in Agronomy". John Wiley and Sons, Chichester, U.K, pp. 47-48.

Reddy KRN, Reddy CS, Muralidharan K (2009). Potential of botanicals and biocontrol agents on growth and aflatoxin production by Aspergillus flavus infecting rice grains. Food control, 20: 173-178.

Renu RO, Tripathi RD, Dexit SN (1980). Fungitoxic properties of Cestrum diurnum. Naturiissenshaften, 67: 150-152.
Rose JP, Nusbaum C, Hircumann H (1989). Soybean reduction by lesion, stunt and spiral nematodes. Phytopathol., 3: 189-207.
Siddiqui MA, Alam MM (1989). Control of stunt nematode by bare root dip in leaf extracts of Margosa (neem) and Persian lilac (barkain) Pak. J. Nematol., 7: 33-38.
Sikora RA (1992). Management of antagonistic potential in agricultural ecosystems for the biological control of pant parasitic nematodes. Ann. Rev. Phytopathol., 30: 245-247.
Tewari SN, Nayak M (1991). Activity of four plant leaf extracts against three fungal pathogens of rice. Trop. Agric. (Trinidad), 68: 378-375.