Research Article

Copy Right ©KY Publications

Journal of Advanced Studies in Agricultural, Biological and Environmental Sciences (JABE)

A Peer Reviewed & Refereed, International Open Access Journal

www.jabe.in **Vol.2.Issue.2.2015, ISSN:2394-2606**

NEMATICIDAL PROPERTIES OF AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF *EUPHORBIA HETEROPHYLLA Linn*. AGAINST THE NEMATODE ON *CERATOTHECA SESAMOIDES Endl*.

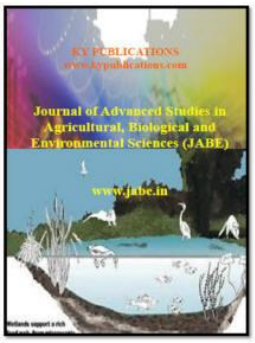
ANIH, A.C*[1]., NDANA, R.W[2]., Kela, S.L[3]., Anjorin S.T.[4]

[1]Department of Biological Sciences, Nigeria Police Academy Wudil, Kano, Kano State. [2]Department of Biological Sciences, University of Abuja,

[3]Biological Science Dept.,Federal University Kashere, Gombe state.,

[4]Department of Agriculture, University of Abuja.

*E-mail: esath_agatha@yahoo.com



ABSTRACT

Aqueous and ethanolic crude extracts of *Euphorbia heterophylla* (L.) were used in control of root-knot nematode on "Vegetables '*Ceratotheca sesamoides Endl*." The extract contains phytochemicals which are toxic to nematode and serve as nutrient in the soil making it suitable for both human consumption and commercial purposes. 2kg of powered air dried leaves were soaked in 10 litres of distilled water or 80% ethanol for 12 hours respectively, then it was strained, concentrated and frozen dried to obtain a powdered stock. Serial dilution with distilled water was made from stock solution (100%) to obtain, 75%, 50%, 25% solutions made by w/v of 100ml of both the aqueous and ethanolicextracts. These extracts were applied in a ring–round of each of the plants in the pots. The obtained result showed that all the phytochemicals present in ethanolic extract are also present in aqueous extract, but not all of them present in aqueous are met in ethanolic extract showing that water is capable of extracting all the components contained in the leaves. The aqueous extracts showed more activity in the antinematodal effect on the nematode on *Ceratotheca sesamoides* as this can serve as high nutritional food and cash crop. At p=0.05 level of significance, the treatment and concentration is significantly different.

Keywords: *Ceratotheca sesamoides Endl*., *Euphorbia heterophylla Linn*., Crude extracts, Ethanolic and Aqueous, photochemical

Cite this article: **ANIH AGATHA C et al.,** *NEMATICIDAL PROPERTIES OF AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF EUPHORBIA HETEROPHYLLA Linn. AGAINST THE NEMATODE ON CERATOTHECA SESAMOIDES Endl. Journal of Advanced Studies in Agricultural, Biological and Environmental Sciences, 2(2): 2015, 38-45*

INTRODUCTION

The most limiting factor to profitable production of *Ceratotheca sesamoides* is the damage causing by root knot nematodes, *Meloidogyne* species. They cause conspicuous root galls and considerable reduction in the yield of several host plants. The symptoms of these diseases are characterized by numerous swellings or galls on roots of the host plants, leading to disruption of water and nutrients uptake, stuntedness, patchiness and chlorosis. Adesiyan (1992) recognized commercial nematicides as the most efficient method in reducing nematode-induced losses on economically important crops. The increased awareness of the prohibitive costs and hazards associated with some of these available compounds according to Jordana *et al.,* (1994), however

Copy Right ©KY Publications
Journal of Advanced Studies in Agricultural, Biological and Environmental Sciences (JABE)
A Peer Reviewed & Refereed, International Open Access Journal
www.jabe.in **Vol.2.Issue.2.2015, ISSN:2394-2606**

has introduced a sense of urgency in the searching for alternative nematode management strategies. Research on natural plant products capable of controlling the development and biology of certain nematodes either their direct action on the life cycle, or an improvement of species diversity, has always been considered a good approach to solving the problem. Plants extract have been found to be effective in the control of plant parasitic nematodes (Chatterjee and Sukui, 1980). Extracts from neem*(Azadiractaindica)* and bakain leaves mustard seeds(Siddiqul and Alam, 1989) were found to be highly toxic to juvenile nematodes such extracts also inhibited egg-hatching of root-knot nematode (Hussain *et al*., 1984).Plant extracts have the advantages of being cheap and readily available in the relation to the conventional nematicides. Their environmental safety (Egunjobi, 1974., Egunjobi and Onayemi, 1981., Zureen and Khan, 1984) in an environmentally conscious world also holds promise for their acceptability and use by resource–poor farmers, plant extracts have been found effective for the control of plant parasitic nematode (Chatterjee and Sukui, 1980). The plant extracts are also easily degraded, pollution free, leave no residue, they are cheaper and less toxic. Because *Cerathotecasesamoides* is the important indigenous crop, commercially, medicinally and nutritionally use, there is therefore a need to save this important crop from nematode infection.

There is also the need to search for suitable extract which at certain concentration would be high yielding and even lethal to plant parasitic nematodes, particularly the root-knot nematode which is real nuisance in vegetable production aiming to obtain the maximum nutritional value of the vegetables

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Euphorbia heterophylla* were freshly collected from a matured open field at Garki Abuja. They were brought to the laboratory and were identified using a standard key of Daziel, (1937). Samples of the plant were dried for 14 days under shade at ambient room temperature that oscillated from 25̊C to 30̊C.

Preparation of Plant Extracts

The air dried leaves were pulverized into powder by the use of wooden mortar and pestle according to the method of Kela*et al;* (1985, 1989).2kg of the processed plant leaves was weighed and soaked in 10 litres of distilled water or 80% ethanol in the ratio of 1:5w/v , this was stirred was kept for twelve hours. Filtration was done using first a muslin cloth and later the filter paper. The filtrate was concentrated by heating at 40̊c it was labeled and stored at 4̊c until needed.

Pre-Planting Operation

Sandy-loam soil used for planting; was sterilized by heating twice at an interval of 12 hours at 100̊C for 4 hours according to method of Gautam and Goswami (2002). The soil was left to cool for twenty four hours and stirred for aeration before putting it into perforated experimental pots in the quantity of ten kilogram per pot. The pots were placed on a metal stand to avoid nematodes or microbial reinfestationand were kept in a control room for three days to before planting operation.

Planting Operation

The seed of *Cerathotecasesamoides* was obtained from agro-chemical store was taken to the laboratory for test for viability, sowing of the seeds was carried out in July and October 2008.The sand-filled pots were arranged in four replications by five columns and two groups of the ethanolic extracts and aqueous extracts. This was labeled accordingly as L_1 and L_2 respectively for aqueous and ethanolic extracts while T_1-T_5 for different concentrations of the extracts application

Nematode Extraction, identification and Inoculation

Galled root of 'Ewedu' plant as mentioned by Olorode (1991) (*Chochorusolitorus*) was also collected from a local farm of University of Abuja Garden. They were washed gently in pond water with a hair brush to remove the sand and plant debris. And the root was brought to the laboratory of biological science for extraction of the nematode and identification.

Research Article

Copy Right ©KY Publications

Journal of Advanced Studies in Agricultural, Biological and Environmental Sciences (JABE)

A Peer Reviewed & Refereed, International Open Access Journal

www.jabe.in **Vol.2.Issue.2.2015, ISSN:2394-2606**

The extraction of the nematode was done using the method of White-head and Hemming (1965). In the method, two thin layer tissues were placed in a wire netted plastic plate. The root samples was thinly spread on the tissue paper and water poured gently to saturate it. Samples were kept for about 24 hours to allow the active nematode from to move the moist into collecting plate containing water. The suspension was collected in a beaker to allow for the nematodes to settle for few hours. The collected juveniles were viewed under a binocular microscope.

The root was chopped into bits and five grams of infected roots galls was applied in to the soil round the crop four weeks after planting (4WAP).

Application of the Crude Extracts

Ten days after nematode inoculation, the plants were subsequently treated with the crude leaf extracts of *E. heterophylla*rangingat various doses levels including that of 0% concentration (distilled water only). The different concentrations of extract were the stock diluted serially with distilled water from the 100% for the stock solutions, 75% for 75mg/25ml, 50% for 50mg/50ml and 25% for 25mg/75ml were made by serial dilution with distilled water according to the method of Oyedumade (1998). 100ml of both the aqueous and ethanolic crude extracts concentration was applied ring–round of each pot with the plant.

***In-vitro* nematicidal Screening of*E.heterophylla*linn.**

This was carried out in the Laboratory of University of Abuja. The extracts were obtained from the stored aqueous and ethanolic crude extracts of *E. heterophylla*. Nematode eggs were obtained from a culture of *Meloidogyne incognita* on Ewedu (*Chochorusolitorus* Linn.) Water and ethanol was used to extract toxic principles from the air dried leaves according to (Olabiyi*et al.,* 1998)

Extraction Techniques

One hundred grams of leaf of the *E. heterophylla* was boiled in 100 ml of distilled water for about one hour, allowed to cool and filtered. The filtrate obtained was designed as 100,000 ppm (part per million). Serial dilution was carried out by mixing 10 mls of the stock solution in 90 mls ofdistlled water to produce 100, 000, 1, 000, and 100 ppm solutions. This extraction procedure was repeated using ethanol. Distilled water served as the control.

Extraction of Nematode Eggs

Ewedu *(Chocorusolitorus Linn.*) root pieces containing egg masses were shaken with 0.5% sodium hypochlorite for 3 minutes in a 1 litrekilner jar according to the methods of (Barker, 1978) in order to digest the gelatinous matrix encasing the eggs.

Application of Plant Extract on Egg Hatch

Aliquots of 3ml,of both aqueous and ethanol plant extracts (100,000, 10,000, 1,000, 100 ppm) and distilled water (control), were dispensed into transparent glass blocks containing 50 fresh root-knot nematode eggs. The eggs treatments, including control were replicated three times. The eggs were incubated at 28±2°C.Counting of hatched juveniles were made every 24 hours for 10 days. This counting was done with use of binocular microscope at x40 magnification.

Data Collection

Data on the following parameters were obtained from the planted false sesame (*Ceratotheca sesamoides Endl.*) plants: Plant height was measured weekly starting from third (WAP), number of leaves at flowering, number of days to flowering, stem weight, root gall indices, number of fruits, weight of fruits, root weight, number of nematodes in 100ml of soil, number of nematode in 5gram of root using the method of White-head and Hemming (1965). Number of eggs incubated and number of eggs hatched after incubation, fresh weight of plant parts at harvest and dry weight of plant parts at harvest (plant parts include the leaf, stem, root and fruit)

Research Article

Copy Right ©KY Publications

Journal of Advanced Studies in Agricultural, Biological and Environmental Sciences (JABE)

A Peer Reviewed & Refereed, International Open Access Journal

www.jabe.in **Vol.2.Issue.2.2015, ISSN:2394-2606**

Statistical Analysis

The experimental data was subjected to Statistical analysis and differences were defined using Least Significance Differences with the appropriate to difference between treatments and means were compared using Duncans New Multiple Range Test (DNMRT) at P<0.05 level of significance.

RESULTS

Effects of Crude Extracts of *E. heterophylla* Linn. on the Population of nematode on *Ceratotheca sesamoides Endls.*

The result showed that control has the highest number of nematodes surviving in the root and in the soil this decreases as the concentration of the extract increases and the number of nematodes in root of the crop treated with the ethanolic extract decreased more than the crop treated with aqueous extract. There is significant difference in both the application and concentration of the extracts in the population of nematodes both in the root of the crop and in the soil.

Table 1 Mean number of nematode and nodes in the soil and root of *Ceratotheca sesamoides Endls.*

Treatment	Concentration of the extracts	No of juveniles nematodes in 5gram of root	No of nematode in 100 ml of soil	Root gall indices
Control	0%	25^{c}	1011^{c}	20^{d}
Aq. Ext.	25%	18^{b}	500^{b}	16^{d}
	50%	13^{a}	507^{b}	14^{cd}
	75%	19^{ab}	300^{ab}	14^{cd}
	100%	23^{b}	185^{a}	13^{c}
Control	0%	25^{c}	1011^{c}	20^{d}
Eth. Ext.	25%	12^{a}	200^{ab}	13^{c}
	50%	17^{ab}	170^{a}	12^{c}
	75%	4^{a}	35^{a}	10^{c}
	100%	7^{a}	18^{a}	10^{c}

Means in the same column followed by the same letter do not differ significantly at p=0.05 (DMRT)

Mean value of four replicates

The Effect of *Euphorbia heterophylla Linn*. Leaf Extract on the Hatchability of Meloidogyne incognita Eggs at 28±2°C

The egg-hatching on concentration with the application of the extracts,in relation to the control which was distilled water only, the egg-hatching increases as day goes by. But on serial dilution of the extract applied, the egg-hatching suppressed at the highest concentration of 100,000ppm, and on 100ppm it decreases as the extract is being introduced on the application of the extracts, ethanolic extract showed more activity on suppressing the hatchability of the egg of nematode than the aqueous extract. This shows that the activity of the ethanolic extract carried out in-vitro is effective on the nematode than in the field. The reason could be that nematodes strive to survive in an environment where all the factors are adequately supplied.

Research Article

Copy Right ©KY Publications

Journal of Advanced Studies in Agricultural, Biological and Environmental Sciences (JABE)

A Peer Reviewed & Refereed, International Open Access Journal

www.jabe.in **Vol.2.Issue.2.2015, ISSN:2394-2606**

DISCUSSION

The increased growth of false sesame grown with treatment of both aqueous extract and compared to ethanolic extract may be attributed to, among others, the decaying potential of the aqueous extract and the increase in nutrient supply to the soil resulting from addition of the extracts which still served as organic manure. The addition of manure to soils leads to a better environment for the growth of roots and canopy. This enhances the utilization of soil nutrient on a consequence of which the nematode damage might have been markedly reduced as observed by Vander Borgett*et al;* (1994). The increased growth may also be attributed to the decrease in nematode number in the soil. The reduction in nematode number may also be responsible for the observed decrease in root–knot indices and increase in yield of false sesame. The decrease in number nematode of high concentration of ethanolic and aqueous extract which led to increased growth and yield as shown in Tables 1 and 2 are suggestive of the nematicidal potentials of these crude extract (Alam et al.,1994).Similar observations on the increase in growth of crops and decrease in number of nematodes as a result of application of the extracts was reported by Babatola (1990) and Akhtar and Alam (1990,1992).This study further establishes the effectiveness of ethanolic and aqueous extracts of *E. heterophylla*Linn. on False sesame which has not been earlier investigated. From the result, it showed that:

- Aqueous and ethanolic extracts has nematicidal potential but aqueous crude extract is more effective in controlling nematode than that of ethanolic
- Aqueous crude extracts contain more phytochemicals than ethanolic crude extracts which might be responsible for the nematicidal potential
- Aqueous extract can be used at any concentration which has the ability of reducing the nematode population by inhibiting their reproduction or/and killing them totally, serving as an organic nutrient to the soil which helps the production of the crop and inducing nutrient in the crop to compare that of ethanolic extracts which does not decay as fast as aqueous extract and also does not contain some phytochemicals that may be responsible for the up growth of this important crop.

Table 2: The Effect of *Euphorbia heterophylla Linn*. Leaf Extract on the Hatchability of Meloidogyne incognita Eggs at 28±2°C Juvenile count after:

Extraction medium	Concentration (ppm)	1 day	5 days	10 days	15days
Aqueous	0	16^{b}	24^{b}	30^{b}	48^{b}
	100	0^{a}	12^{b}	15^{a}	30^{b}
	1,000	0^{a}	7^{a}	9^{a}	12^{b}
	10,000	$0^{a}0^{a}$	5^{a}	6^{a}	
	100,000	$0^{a}0^{a}0^{a}$	2^{a}		
Ethanol	0	16^{b}	24^{b}	30^{b}	48^{b}
	100	1^{a}	5^{a}	7^{a}	15^{a}
	1,000	0^{a}	$5^{a}5^{a}$	9^{a}	
	10,000	0^{a}	2^{a}	$4^{a}4^{a}$	
	100,000	$0^{a}0^{a}0^{a}0^{a}$			

*Means in the same column followed by the same letter do not differ significantly at p=0.05 (DMRT)

*Mean value of three replicates

REFERENCES

Adesiyan, S.O. (1992). Chemical control of nematode pests of some economic crops. In: The Biology and Control of nematode pests of food crops in Africa.

Barker, K.R. (1978). Determining nematode population responses to control agents. **In**: Methods for Evaluating Plant fungicides, Nematicides and Bacteriacides. *Amer. PhytoPathol. Soc;* St. Paul Mimesota

Chatterjee, A. and Sukul, N.C. (1980). Nematicidal action of three wild plants. *Nematologica*, **26**: 500-501.

Daziel, J.M. (1937). The useful plants of West Africa. Crown agents for overseas Government Londons Pp. 24-27.

Egunjobi, O. A. (1974). Nematodes and Maize growth in Nigeria. 1. Population dynamics of *Pratylencusbrachyurus* in and about the root of maize and its effects on maize production at Ibadan. *Nematologica*, **20**:181-186.

Egunjobi, O. A. andOnayemi, S.O. (1981). The efficacy of water extract of neem *(Azadirachtaindica* L.) Leaves as a systemic nematode. *Nigerian Journal of Plant Protection*, **5:** 70-74.

Guatam, C. and Goswami, B. K. (2002). Different combinations of neem cake and carbofuran against*Meloidogyne incognita* on *Vigna radiate. International journal of nematology.* Vol. **12,** No 1, Pp 106-110.

Hussain, S.I., Kumar, R. Khan, T.A. and Titov, A. (1984). Effect root dip treatment of egg plant seedlings with plant extracts, nematicides oil-cake extracts and anthelminthic drugs on plant growth and root-knot development *Pakistan Journal of Nematology.*

Jordana, R.J., Garcia-Mina, M., Hernandez, M.A., De Portugal, D.C. and Arino, A.H. (1994). Effects of an organic soil amendment on the parasitism of *Meloidogyne sp*. On tobacco in Spain. *Afro-Asian J. Nematology.* 4 **(1)**:88-95

Kela, S.L. and Bowen, I.D. (19985). "Histopathological effects of Deteriummicrocarpum, a naturally occurring plant molluscide on the mid gut and digestive gland of*LymneaStagnalis"*. *Cell Biol. Inter*, **19 (3):** 175-181

Kela, S.L., Ogunsusi, R.A., Ogbogu,V.C. and Nwude, N. (1989a). Susceptibility of 2-week old *Lymneanatalensis* to some plant extracts.*Rev. Elev.Med. pays Trop.***42(2):**189-195

Olabiyi, T.I., Babatola, J.O. and Oyedunmade, E.E.A. (1998). In-vitro assessment of some plant extracts for their nematicidal properties. *Nigerian J. of Plant Protection* 28. Pp. 311.

Olorode, O. (1991). The Taxonomy of West African flowering plants. Page 130

Oyedumade, E. E. A. (1998). Control of nematode pests of cowpea with mecarp (Ethoprop), leaf residues of neem*(Azardirachtaindica)*. Rattle weed (*Crotalaria retusa)* and *Hyptissuaveoleus),*Cenbtre point Vol. **8** No 1 Pp 57-63.

Siddiqui, M. A. and Alam, M. M. (1989). Control of stunt nematode by bare-root dip in leaf extract of Margosa (neem) and persionlilac (bakain) *Pakistan Journal Nematology***7**:33-38.

Whitehead, A.G. and Hemming, J.R. (1965). A comparison of methods of extracting small vermiform nematode soil. *Annals Applied Biology*. **55**:25-28.

Wiesmann and Chapagain, B.P. (2006). Larvacidal activity of Saponin containing extracts and fractions of fruit mesocarp of *Balanitesaegyptica. Fitoterpia***77:** 420-424

Zurren, S. and Khan, M. I. (1984). Nematicidal activity in some plant lactces. *Pakistan Journal Nematology*. **2(2)**:69-75