NEMATICIDAL INFLUENCE OF *P.tuberreguim* AND NEEM EXTRACT ON THE INCIDENCE OF ROOT-KNOT NEMATODE ON HAUSA POTATO (*Solenostemon rotundifolius Poir).*

ANYALEWECHI, J. A[1] ONONUJU, C.C[1] and OKWUJIAKO A.I[2]

[1] Department of Plant Health Management Michael Okpara University of Agriculture, Umudike Umuahia, Abia State Nigeria. E-mail: agu4all2@yahoo.com Tel: +2347034549425
[2] Department of Biological Sciences, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria.

ABSTRACT
Efficacy of Pleurotus tuberregium, Neem-leaf ash, carbofuram and combination of the three above were tested in the control of root-knot nematode (Meloidogyne incognita) on Hausa potato. In the green house, twenty-five polythene bags were filled with 10kg of steam-sterilized sandy loam soil and Hausa potato seedlings were planted in each of them. Four weeks after planting 5000 eggs of (Meloidogyne incognita) were inoculated round the plants in each of the bag. The treatments which included Pleurotus tuberregium, Neem-leaf ash, Carbofuran (a synthetic nematicide), and a combination of the three treatments above as a combined approach treatment were applied into the bags around the plants to ascertain the nematicidal influence of each.
A control plot where nematode eggs were not inoculated and no treatment application was maintained and observed. Influence of these treatment were further observed in the field which is naturally infested with nematode (Meloidogyne incognita.) The field study was laid out in a Randomized Complete Block Design (RCBD) replicated five times. Both the green house study and the field study shows that all the materials used as treatments in this study have nematicidal influence as they significantly (P=0.05) reduced nematode population and their damaging effects. Combined approach treatment (i.e. P. tuberregium, plus Neem-leaf ash, plus carbofuran) gave better result than the single treatments.

KEY WORDS: Root-Knot nematode, control, Hausa potato.

INTRODUCTION

The crop Hausa potato (*Solenostemon rotundifolius)* belongs to the family labiatea (Allemann, 2006) It has shallow, fibrous root system, some of which develops into tubers with diverse shapes, sizes, and mature for harvesting in six months (150-200) days after planting. At this time the plant has flowered and aerial parts have become senescent (Jansen, 1996). The tubers are cooked in combination with other foods such as beans and vegetables. They can also be roasted, baked or fried and served as a delicious dish or snacks (Apobol, 1997). The tubers are also used as row materials for making alcoholic drinks while the leaves are occasionally used as a pot-herb and more often in traditional medicines eg for the treatment of dysentery in Nigeria (Schippers, 2000). The plant is also used to treat blood-in-urine as well as eye disorders. It has various socio-cultural uses (Jansen, 1996). According to Allemann, (2002) a standard serving dish of Hausa potato provides a large percentage of the daily requirements of calcium and vitamin A as well as more than the daily need of iron. The tubers contain 5-13 percent protein (calculated in a dry weight basis) or up to twice the amount found in sweet potato and Irish potato (Allemann, 2002)

Parasitic nematode problems are pronounced and prevalent in tropical environment where higher temperatures, longer growing season, higher annual nematode population tune-over, wider-host-ranges and many disease complexes abound (Mai *et al,* 1996). In the field. root-knot nematodes are said to exist in pockets around roots of susceptible crop plants and at various population densities (Mai *et al* ,1996). Root -Knot nematodes (*Mleliodogyne spp*) are economically important pathogens world wide. They are obligate parasites of thousands of plants including major food crops. Potatoes are very susceptible to root-knot nematodes which

have a broad host range and are the most economically damaging of all the nematode species to agricultural crops (Gordon Berg, 2006). Plant parasitic nematode damage is an important factor in tuber quality reduction and yield loss in the field and in storage (Adegbite *et al* ,2005). According to Tetteh and Guo (1993) nematode Infection may lead to large losses in Hausa potato production in Ghana, a West African Country. Okorocha *et al*, (2006) also reported that at high nematode population density, a significant damage was observerd on Hausa potato in Umudike, Nigeria. It therefore become necessary to find a cheap, reliable and environmentally friendly measures to control root- knot nematodes to enhance the production of this all important food crop.

The control of plant-parasitic nematodes is very necessary to reduce their negative effect on plant food production. Adoption of any management practice depends on urgency of the need, cost effectiveness, reliability of the chosen option, and their environmental implications (Hemong and Garbara, 1992). Of all control options recommended against root-knot nematode disease, chemical control measures are most effective because of its quick action. Chemical control of root-knot nematodes has proved useful over the years but its known phytotoxicity, health hazards and prohibitive cost associated with its usage has prompted current search for easily, available, cheap and environmentally friendly alternatives (Oyedunmade and Fatoki, 1995).

Studies have shown the importance of natural nematicides as possible sources of non-phytotoxic and easily biodegradable alternative pesticides (Amadioha, 2000). Natural plant products and their analogues are important sources of new agricultural nematicides used in the control of pests and plant diseases (Emosairue and Ukeh, 1997).

Hausa potato is nutritionally, medicinally and socio-culturally important crop, unforunatelly root-knot nematodes *(M. incognita)* attack on the crop limits its production. This study therefore is to :

i. determine the nematicidal influence of *Pleurotus tuberregium,* Neem-leaf ash and carbofuran (synthetic nematicide) on the control of root-knot nematode *(M. incognita)* and to compare the effect of *P. tuberreguim*, Neem leaf ash and Carbofuran (synthetic nematicide) on the control of root-knot nematode.

MATERIALS AND METHODS

RAISING OF *Plenrotus tuberregium* (the bioagent): *Pleurotus tuber-regium* was raised in the mushroom house of Department of Biological Sciences of Micheal Okpara University of Agriculture Umudike using rice straw collected from rice- farm site. The rice straw was cut into pieces of 3-4cm and soaked in water overnight. The straw was removed from water, pressed and packed into plastic containers with perforated sides and bottom. This was kept for water to drip off. The container and their straw contents were steam sterilized by heating at 80^{oc} for one hour using a gas burner device in the mushroom house After cooling, the rice straw was inoculated with surface sterilized *sclerotium* of the fungus *(Pleurotus)* already cut into pieces weighing 25g into each container. This was allowed to remain for four weeks when *P. tuber-regium* has reasonably grown in such environment devoid of contaminants.

In the green house, twenty-five polythene bags were surface sterilized using laboratory alcohol. The bags were then filled with 10kg of steam sterilized sandy loam soil. Two seedlings of Hausa potato were planted into each of the bags. At two weeks after planting (2WAP), the seedlings were thinned down to one seedling per bag. Twenty grammes of NPK 15:15:15 fertilizer was applied per bag. The experimental design is Completely Ramdomized Design (Crd) with five treatments replicated five times. At four weeks after planting (4WAD),5000 eggs of root-knot nematode (*Meloidogyne incognita)* extracted from Indian Spinach (Basela rubra) roots using Sodium Hypochlorite (NaOCl) method (Hussey and Barker, 1973) were inoculated into a depression made around each seedling with the aid of a syringe. One milliliter of the inoculum suspension contained 200eggs approximately so, 25milliliter of the inoculum suspension was introduced into each bag to give the 5000 eggs per bag. Six hours after inoculation the treatments were weighed out in the laboratory using electronic weighing device and applied into a groove or depression made around the seedlings and subsequently covered with soil. The applied treatments included:

50gms *Pleurotus tuberregium* (pt)

50gms Neem-leaf ash (Nla)

0.16gms carbofuran ©

25gms pt + 25 gms nla + 0.08gm c
Control plot (no treatment applied

Six months after planting (6WAP), the experiment was terminated. Data collected included number of tuber per plant, fresh tuber weight per plant, fresh shoot weight per plant, dry shoot weight per plant, number of nematodes in tubers, no. of nematodes in soil, number of galls on roots. These data were subjected to analysis of variance and the means separated by LSD.

In the field- a naturally infested soil with nematode *(M. incognita) was used.* The secondary vegetation was manually cleared and tilled. The experiment was laid out in a Randomised Complete Block Design (RCBD) with five treatments replicated five times. Each of the blocks contained five mounds at 1m apart. Five soil samples were randomly collected from each of the mounds, to estimate the initial root-knot nematode population in the field before planting. Nematodes were extracted from 200millilitre (200ml) of each of the twenty-five soil samples using the piepan modification of the Bearman Funnel method (Hopper,1969). Two milliliter (2ml) from each sample suspension was viewed under light microscope for four times to identify the nematode and calculated the total nematode population in the soil samples. After the soil sampling as stated above, two seedlings of Hausa potato were planted on each mound and thinned down to one seedling per mound two weeks after planting (2WAP) followed by twenty grammes of N P K 15:15:15 fertilizer application to each of the mounds. Four weeks after planting (4WAP), the five treatment as in the green house were applied. Six months after planting (6WAP), the experiment was terminated and data collected included: number of tubers per plant, fresh-tuber weight per plant, fresh shoot weight per plant, Dry shoot weight per plant, Number of galls on roots, number of root-knot nematode in the soil (at planting and at harvest) as second soil sampling was done immediately after harvest and nematode extraction done as above to estimate nematode population in the soil after harvesting. All the collected data were analyzed by analysis of variance (ANOVA) and means separated by LSD.

RESULTS

The result of treatment on the incidence of root-knot nematode (*Mebidogyne incognita*) on Hausa potato in green house is shown in table I. All the treatment applied significantly (P=0.05) reduced the number of nematodes in tubers, roots and soil. Similarly number of galls on root was significantly reduced by all the treatment. Nematode-alone treatment had the highest number of galls on the roots. Nematode population in the field at harvest as influenced by the treatments as well as number of nematodes in tubers, roots and gall incidence are presented in tables 2 and 3. All the treatments in the field significantly reduced root-knot nematode population as compared with plants inoculated with nematode alone. The combined treatment, carbofuran, *P. tuberregium* and neem-leaf ash treatment gave 53%, 50%, 45% and 44% reduction respectively of root-knot nematode population in the soil. Nematode population increased in plots without the control treatments. The least number of rootknot nematodes in tuber, roots, and number of galls were found from the combined treatment which differed significantly (P = 0.05) from other treatments except carbofuran treated plants (Table 3). Effects of treatments on the number of tubers, fresh tubers weight, fresh and dry shoot weights in the field is shown in table 4. Combined treatment, Neem-leaf ash, carbofuran or *P. tuberregium* improved number of tuber per plant although the effect was not significantly (p = 0.05) different from each other. Combined approach and Neem-leaf ash gave better fresh tuber weights than carbofuran and P*lenrotus tuberregium* but they did not significantly differ. Combined approach and *p. tuberregium* treatments effected better fresh and dry shoot weights

Table 1: Effects of *P.tuberegium and Neem extract* on the incidence of root-knot nematode *(M. incognita*) on Hausa potato in green house.

Treatments	Mean No. of nematodes in tubers.	Mean No. of nematodes in roots	Mean No. of Nematodes in soil	Mean No. of galls on roots	Mean wt. of galled roots
P. tub erregium	33.80	31.80	32.00	10.20	0.008
Neem-leaf ash	31.80	187.00	236.60	5.40	0.022
Carbofuran	32.00	180.00	214.20	5.00	0.006
Combined approach				4.40	0.040
Nematode alone	32.40	187.00	211.00		
Control (no nematode)				164.40	0.182
	164.00	1301.32	575.60	0.00	0.00
	0.00	0.00	0.00		
LSD (0.05)	9.92	247.2	121.80	9.66	0.035

Table 2: Nematode population at harvest as influenced by the application of treatments in the field.

Treatments	No. of nematodes in soil at planting	no. of nematodes in soil at harvest	Percentage reduction (%)
P. tuberregium	762.60	421.00	44.80
Neem-leaf ash	761.60	425.00	44.20
Carbofuran	804.60	402.00	50.00
Combined approach	821.20	384.00	53.20
Control (nematode alone)	826.00	1078.00	-131.40
LSD $_{(0.05)}$	162.8	276.20	

Table 3: Effects of *P.tuberegium and Neem extract* on number of root-knot nematodes and galls in Hausa potato tubers and roots in the field.

Treatments	Mean no. of nematodes in tubers	Mean no. of nematodes in roots	Mean no. of galls	Mean wt. of galled roots (g/pit)
p. tuberregium	255	44.60	15.80	27.20
Neem-leaf ash	285	49.20	15.60	27.00
Carbofuran	194	30.60	13.00	18.40
Combined approach	180	17.20	11.80	12.00
Control (nematode alone)	362	149.60	123.80	59.40
LSD$_{(0.05)}$	89.4	33.28	12.37	7.70

Nematicidal ability of P. tuberregium, Neem-Leaf Ash, on Hausa potato.

Table 4: Effects of *P.tuberegium and Neem extract* on yield and yield components of hausa potato in the field.

Treatments	Mean no. of tubers per plant	Mean fresh tuber wt. (kg/plt)	Mean fresh shoot wt. (kg/plt)	Mean dry shoot wt. (kg/plt)
P. tuberregium	30.40	0.284	5.934	1.124
	37.60	0.388	4.316	0.690
Neem-leaf ash	35.00	0.234	4.518	0.690
Carbofuram	45.40	0.430	6.414	10146
Combined approach		0.114	5.522	1.044
Control (nematode alone)	19.20			
$LSD_{(0.05)}$	NS	NS	NS	NS

DISCUSSIONS

All the plant materials applied in this study significantly reduced the incidence of root-knot nematodes. In view of the ability of some of the treatments applied to have reduced the number of nematodes in tubers, roots and soil and also reduced the galling incidence on roots indicates that *P. tuberregium* and Neem-leaf ash have nematicidal potentials. The observation with *Pleurotus tuberregium* as being nematicidal confirms the report by Tzean and Liou, (1993). *P. tuberregium,* is a nematophagous fungus which uses adhesive knobs, nets, and paralyzing toxin droplets etc. to trap and consume nematodes.

Hutchison *et al.,* (1995) also reported that toxic droplets flow from secretary cell of *Pleurotus spp.* Kwok *et al,* (!992) observed an interaction between nematodes and toxin producing nematophagous fungus *Pleurotus osereatus* which produced a toxin-decentiotic identified as trans-2-acid which immobilized the nematode Panagrellus redivivus. The ability of Neem-leaf ash to control nematodes is confirmed by Nwauzor *et al* (2005) who reported that if Neem-leaf ash or powder is applied at the base of sweet potato plants, at the rate of 10-30g/plant will reduced pest and disease damages on potato tuber-rot by up to 27%. Anand *et al* (1997) further stated that Neem has been proven to have nematicidal properties and that various parts of Neem plant have been applied in various forms to control pests and disease problems in agriculture. Combined approach treatment, Neem-leaf ash, carbofuran and *P. tuberregium* treatments improved tuber yield. This implies that they reduced nematode populations beyond the level at which they could have caused economic damages. This fact is confired in the result from the plots with nematode alone which gave the least yield. This is a proof that the materials used as treatment have the potential and can be used to control root-knot nematodes. Combined approach competed favourably with carbonfuran (the synthetic nematicides) not only on tuber yield but also in all other parameters under survey meanng that the combined approach treatment which is cheaper and more envinmentally friendly is an effective substitute for carbofuran which is costly and environmentally unfriendly. Neem-leaf ash also competed favourably with carbofuran both in the green house and in the field. *Pleurotus tuberregium* in the same trend improved number of tuber yield, fresh tuber weight, fresh and dry shoot weights in the field.

CONCLUSION AND RECOMMENDATION

Comparing the nematicidal ability of *P. tuberrgium,* Neem-leaf ash, carbofuran and a combined approach treatment in controlling nematodes (*M. incognita*) in this study, it appears that all these treatments could be used effectively in controlling nematode as substitutes for Carbofuran (a Synthetic nematicide). Combined approach treatment which performed better than others is a sure way of reducing the quantity of synthetic

nematicides applied to our agricultural soil in a bid to reduce its adverse effect on the crops, soil and man. However, further studies are needed to determine the best method and time of application for more efficient result.

REFERENCES:

Adegbite, A.A Adesiyan, S.O. Agbaje, G.O.Omoloye, A.A. (2005). Host suitability of crops under yam intercrop to root-knot nematode (*Meloidogyne incognita* race 2) in South Western Nigeria. J. Agric. Rural Develop Trop. Subtrop 106(2)-113-118.

Allemann, J.(2002) Evaluation of *Plectranthus esculentus* N.E.Br. As a potential vegetable crop. PhD Thesis, University of Pretoria, South Africa. Pp40

Allmanm J.(2006) Lost Crop of Africa : volume II vegetables. Pp268. The National Academics Press

Amadioha, A.C and V.I Obi (1998). Fungi toxic activity of extracts from *Azadirachta indica* and *Xyllopia* on *Collectotrichum Lindenuthianum* in cowpea. Jornal of herbs, spices and medicinal plants 6(2). Harworth press. Pp.33-40.

Anand Prakash and Jafadiswari Ruo, (1997). Botanical Pesticides in Agriculture, Lewis Publications 40pp.

Apobol, R.R (1997) Assessment of the performance of some Frafra potato (*Coleus Dysentericus Baker*) accessions in Nyankpala area of Ghana. Dissertation. University of Development studies Nyankpala, Tamale, Ghana.Pp57

Emosairue S.O.and D.A Ukeh, (1997). Field trial of Neem for the control of Okra Flea beetle in South Eastern Nig. Global Journal of pure and Applied Sci. 3(1).

Gordon Berg, Knoxfield (2006). Root-Knot nematodes of potato: In Jillian Hinch, La Trobe University Bundoora. Pp.1-3.

Hemeng, O.B. and Barbara, M.S.(1992). "Control strategies appropriate for nematode of food crops in Africa" Pp. 137-142. In: Procs of the first Regional Symposium on the Biology and Control of nematode pests of food crops in Africa. University of Ibadan, Ibadan Nig.July, 26-29, 1992

Hopper, d. j. (1969). Extraction and handling of plant and soil nematodes. pp. 20-30 in nematodes of tropical crops. Peachey J. e. (Ed). Nematodes of tropical crops. Common w. bar. Helminthel tech. common no.4 pp 20-36

Hussey, r. s. and baker, K. R. (1993). A comparison of method of collecting eggs of *meloidogyne spp* including a new technique. Plant disease reporter, 57: 1025-1028.

Hutchinson, l. j; madzia, s. e; barron, g. l. (1995). The presence and antifeedant function of toxin producing secretary cells on hyphae of the lawn-inhabiting. Agric conocybe lacteal Cans J. Bot. 74:431-434.

Jansen, p. c. m. (1996) *Plectranthus rotundifolius* (Poiret) Sprengel: In: Flach, M and Rumawas, F. (editors). Plant resources of South East Asia. No.9. Plants yielding non-Seed carbohydrates. Backhuys Publishers, Leiden, Netherlands. pp 141-143.

Kwok, O. C. H, Plattner, R. Weisleder, D; Wicklow, D. T. (1992) a nematicidal toxin from Pleurotus ostreatus nrrl 3526. J. Chem. Ecol. 18:127-136.

Mail, W. F,. Mullin, P. G; Howard, L. H. Loeffler, K. (1996). Plant parasitic nematodes a pictorial key to genera, 2nd edition. The National Academic Press: Public pp. 56-57.

Nwauzor, E. C; Afuape, S. O, Korieocha, D. S., Ezuhike, T. O. (2005). Studies in the use of neem-leaf preparation for the control of Cylas punticollis damage of potato root tubers in potato production, in Nat ional Root Crop Research Institution, Umudike 2005, Annual Report.

Okorocha, E. O. A; Olojede, A. O. , Ogbuji, R. A. (2006) Studies on effect of different inoculum densities of root-knot nematodes on growth of Hausa potato *(solenostemon-rotundifolius)* Poir. In: National Root Crop Research Institute, Umudike 2006-Annual Report. Pp.219.

Oye Dunmade, E. E. and Fatoki, O. K. (1995). The effecacy of Nitta *Hypts suaveoleus* plant in the control of root-knot nematode *M incognita* on 3 cultivars of okra (*Abelmoschus esculentus*). pp. 137-140. In: Pocs of 13th Annual Conference of the Hort. Soc. of Nig. Hortson, University of Illorin Nigeria. March, 12-15, 1995.

Schippers, R. R. (2000). African Indigenous vegetable. An overview of the cultivated species. National Resources institute/Acp. Ev Technical Centre for Agic. and Rural Co-operation, Chathan, United Kingdom 214pp.

Tettah, J. P. and Guo, J. I. (1993). Problems of Frafra potato in Ghana, Dissertation, School of Agriculture, University of Cape Coast Ghana Journ. Linn. soc. Lond; Bot: 58:272.

Tzean, S. and J. Y. Liou, (1993). Nematophagous resupinate basidiomycetous fungi Phytopathology. 1015-1020.