CROP SCIENCE SOCIETY OF NIGERIA (CSSN)

The Role of Crop Science in the Agricultural Transformation Agenda of Nigeria

Proceedings of the

1st National Annual Conference

Held at the University of Nigeria, Nsukka

THE ANTIFUNGAL AND NEMATICIDAL POTENTIALS OF *VERNONIA AMYGDALINA* ON COWPEA *(VIGNA UNGUICULATA* (L.) WALP)

Onoja, C.U. and Fadina, O.O.
Department of Crop Protection and Environmental Biology, University of Ibadan, Oyo state, Nigeria.
*Corresponding author: clementeonoja@yahoo.com, +2347033072337

Abstract

Vernonia amygdalina is a herbaceous plant that grows in the wild and is widely cultivated as food crop in sub-Saharan Africa. The study evaluated its fungicidal and nematicidal potentials. Isolates of Macrophomina phaseolina were obtained from germinating cowpea variety IT84S-2246-4 growing on naturally infested soil at screen house in International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (7° 26¹ N, 3° 54¹ E) and then subcultured to achieve pure culture, while inoculum of the root knot nematode, Meloidogyne sp. was obtained from infested Celosia sp. plants through standard pathological and nematological procedures. Aqueous and ethanol extracts of V. amygdalina at various concentrations of 2.5% 5% and 10% were prepared and tested in-vitro against Macrophomina phaseolina and the root knot nematode Meloidogyne sp. At incubation of 27.5°C-30°C for 7-days, radial growth of the plated M. phaseolina was measured at 24-hour intervals and percentage fungitoxicity calculated, while Meloidogyne sp. egg hatch and 2nd juvenile stage mortality were observed at 24-hour intervals for a 10-day period. Fungitoxicity tests showed aqueous extracts of 5% and 10% concentration significantly inhibited mycelia growth (32.08% and 34.77%, respectively) when compared with the control. Ethanol extracts exhibited 100% fungicidal activity. Furthermore, egg hatch by Meloidogyne sp. was completely inhibited by both extracts preparation at 2.5% and 5% concentrations while 100% mortality of Meloidogyne sp. juveniles was observed on the fifth day post-treatment. Vernonia amygdalina was identified as a natural, readily available and cheap source of fungicide and nematicide. It is recommended for control of charcoal rot infection and Meloidogyne sp. infestation of cowpea.

Keywords: botanical extracts, cowpea, *Vernonia amygdalina, Macrophomina phaseolina, Meloidogyne sp.*

Introduction

Cowpea *Vigna unguiculata* is a leguminous grain grown widely in the Savannah region of the Tropics of Africa. It is an important source of qualitative nourishment to the rural and urban poor who cannot afford meat and milk products (Brader, 2002). It is estimated that cowpea supplies 40% of the daily protein requirement to most people in Nigeria (Muleba *et al.,* 1997). Although it is cultivated worldwide over 75% of the world production is obtained in Africa with Nigeria and Niger Republic constituting the largest producers (Singh *et al.*, 2002) and Nigeria as the world's largest consumer. Despite the nutritional importance of cowpea many subsistence farmers in Nigeria consider it a high-risk enterprise due to the disease and pest complexes associated with the crop (Sosanya, 2006). Among the pests and diseases affecting cowpea, fungi and nematodes are very important. About 40 species of fungi attack cowpea (Allen, 1983) and more are being discovered. One of such fungi is *Macrophomina phaseolina* (Tassi) Goid, causative pathogen of the ashy stem blight or charcoal rot disease of cowpea. Estimates of loss due to *M. phaseolina* is put at about 75% (Emechebe, 1981; Abawi and Pastor Corrales, 1989).

Nematodes are also very important sources of yield loss (Adesiyan *et al.,* 1980) with cowpea being highly susceptible to the root knot nematode *Meloidogyne sp.* (Ogunfowora, 1976; Bridge, 1981, Babatola and Omotade, 1991). *Meloidogyne incognita,* the most important of *Meloidogyne sp.* to cowpea is particularly implicated in yield reduction of 65-95% and total loss in severe cases (Olowe, 1992; Adesiyan *et al.,* 1990). It is generally accepted that these losses in fields amount to several millions of U.S. dollars annually (Sasser, 1979; Adesiyan *et al.,* 1990). Thus, with the enormous financial implications for cowpea producing economies and the looming dangers associated with the

use, misuse and overdependence on synthesized fungicides and nematicides, especially in human and environmental safety as continued dependence on these chemical substances is also seen as accelerating environmental pollution, threat to life and creation of new socio economic problems which must be avoided (Atungwu, 2006). The recent ban on many fungicides and nematicides has made the vigorous search for development of reliable, cheap, available, potent and environmentally safe options in plant protection more imperative.

Vernonia amygdalina of the Asteraceae family is a promising plant with natural pesticidal properties, it grows freely and abundantly in cropping systems all over Nigeria. Though its seeds are wind dispersed; it is commonly propagated through stem cuttings (Anon, 2000; FAO, 2000). It possesses very fast growth and can reach 2.5m height. This study therefore was set out to evaluate the potentials of *Vernonia amygdalina* on inhibition and mortality of *Macrophomina phaseolina* and *Meloidogyne sp.*

Materials and Methods

Preparation of cultures: *M.phaseolina* was cultured on potato dextrose agar (PDA) which was prepared by dissolving 39g of PDA powder in one liter of distilled water. The solution was dispensed into conical flasks of 250ml volume. The opening of the conical flasks was plugged with non-absorbent cotton wool wrapped in aluminum foil. The contents of the flasks were sterilized by autoclaving at 121^{0}C at 1.06kg/sq.cm for 15mins. After the autoclaving had been cooled and the pressure returned to zero, the flasks were removed. The agar was allowed to cool to about 45^{0}C – 50^{0}C before plating into sterile plastic petridishes of 9 cm diameter. Shortly before plating into the petridishes, lactic acid was added.

M. phaseolina isolate used for this study was isolated from germinating cowpea variety IT84S-2246-4 growing on naturally infested soil at a screen house in IITA, Ibadan, Nigeria (7^{0}26^{1}N, 3^0 54^{1}E). The isolates were sub-cultured by transferring hyphal tips from the edges of the colony to fresh plates of PDA using a cork borer of 3mm diameter from a 7- day old *M. phaseolina* culture and observed to obtain a pure culture. It was incubated at 27^{0}C.

In vitro study to test the efficacy of *V. amygdalina* extract was done using PDA. 5%, 10% and 100% concentrations of the extract was prepared by extracting the equivalent parts (5g, 10g and 100g) in 100ml of distilled water or ethanol (Olabiyi *et al.*, 1992). The concentrated extracts were made up to mark (100ml) by adding the equivalent volume of solvent and dispensed into 9cm diameter petridishes. 1ml of the extract was added to 9ml of molten PDA to prepare a corresponding 5% and 10% agar-extract concentration respectively. The plates were gently swirled to ensure even dispersion of the extract. Mycelial agar plugs were taken with a sterile cork borer of 3mm diameter from the margin of one week old culture of *M. phaseolina* and inoculated at the center of each Petri-dish. The plates were incubated at 27.5^{0}C – 30^{0}C for 7 days. Radial growth of the fungus was measured at 24 hour intervals for 7 days as the mean growth along two axes on two perpendicular lines drawn on the reverse side of the plates. The difference between the growth rate of the treated and untreated plates represented the activity of the extract in inhibiting the growth of the pathogen. Percentage fungitoxicity was expressed according to Awuah, (1989):

$$MP = \frac{M_1 - M_2}{M_1} \times 100$$

Where MP = Percentage inhibition of mycelial growth
M_1 = Mycelial growth in control plate
M_2 = Mycelial growth in extract
Liquid and agar media were sterilized by autoclaving at 121^{0}C for 15mins at 1.06kg/sq.cm. Heat labile objects used were also sterilized by dipping in ethanol and flaming over a spirit lamp until red hot.

Preparation of plant extracts and inocula: Fresh leaves of *V. amygdalina* were obtained from a parent stock in a subsistence farm at Eleyele area of Ibadan, and sun-dried until crisp. Using a blending machine, the crisp-dried leaves were ground to fine powder after which 95% ethanol was used to extract the active ingredients using the method described by Olabiyi *et al.* (1992). 100g and

10g of powdered *V. amygdalina* each was weighed into separate pear shaped flasks and 100ml of 95% ethanol added to each flask after which they were placed in a water bath and heated at 100^{0}C for 1 hour to obtain 100% and 10% concentrations ,respectively. The extract was left to cool and then filtered through Whatman's no. 1 filter paper placed in a funnel in a beaker. The stock extract was poured into McCartney bottled from which serial dilutions were obtained for 10%, 5% and 2.5% concentrations which were used as treatments in the experiments. Aqueous extracts were also obtained using the method of Olabiyi *et al.* (1992).

Galled roots of *Celosia argentea* obtained from a Fadama farm in Akobo area of Ibadan served as the source of root knot nematode, *Meloidogyne spp.* Using the method of Hussey and Barker (1973) galled roots obtained were carefully passed under gently running water to remove the soil, after which they were chopped into pieces of about 2cm using a pair of scissors and then placed in a conical flask into which 0.52% Sodium hypochlorite solution (NaOC1) was added. The mixture was vigorously shaken for 4 minutes to digest the gelatinous matrix encasing the eggs and liberate them. The content of the conical flask was poured into a stack of sieves with the largest aperture size on top collecting the root debris, the 45µm aperture size collecting the 2nd Juvenile stage (J_2) and the eggs collecting in the 25µm aperture sized mesh below. The roots were well rinsed with water before discarding and the eggs were properly rinsed with water to remove all traces of NaOC1. The eggs collected were rinsed into a one-litre beaker and made up to mark by adding distilled water. Using a magnetic stirrer, the egg suspension was thoroughly stirred to obtain a homogenous mixture, after which estimation of the population of eggs in the concentrate was carried out by taking 1ml of the concentrate using a graduated syringe and counted in a Doncaster dish, under a dissecting microscope. A tally counter was used to guarantee accuracy of counts taken. After four repeated counts, the average was taken as the number of eggs per milliliter of the concentrate. To obtain J_2 *Meloidogyne sp.*, the egg suspension obtained was incubated in the laboratory at ambient temperature (27.5^{0}C) and population estimated.
For the percentage hatch test, 20 eggs were counted into glass block and observed daily for 7days from which the percentage hatchability of the eggs was calculated.

One milliliter each of water and ethanol extracts of 10% and 5% concentration was dispensed into glass blocks containing 20 fresh eggs in 1ml of water, thus bringing the concentration to 5% and 2.5% concentration respectively. Five treatments each for the egg stage and J_2 stage were observed, totaling 10 treatments as follows:
1. Eggs + Ethanol extract of *V. amygdalina* at 5% concentration
2. Eggs + Ethanol extract of *V. amygdalina* at 2.5% concentration
3. Eggs + Water extract of *V. amygdalina* at 5% concentration
4. Eggs + Water extract of *V.amygdalina* at 2.5% concentration
5. Eggs + Water or Ethanol extract of *V. amygdalina* at 0% concentration
6. J_2 + Ethanol extract of *V.amygdalina* at 5% concentration
7. J_2 + Ethanol extract of *V.amygdalina* at 2.5% concentration
8. J_2 + Water extract of *V. amygdalina* at 5% concentration
9. J_2 + Water extract of V. amygdalina at 2.5% concentration
10. J_2 + Water or Ethanol extract of V. amygdalina at 0% concentration

Where 5 is control-1, 10 is control-2 and J_2 is second juvenile stage of *Meloidogyne sp.*
The treatments were incubated at ambient temperature (27.5^{0}C), each treatment was replicated 4 times in a completely randomized design (CRD). The glass blocks were covered with glass covers to prevent evaporation. Count of eggs was made every 24 hours for 10 days with the aid of a dissecting microscope. 1ml aliquots containing J_2, *Meloidogyne sp.* suspension containing about 20 juveniles was also dispensed into transparent glass blocks. 5 treatments for the J_2 stages were observed at 24hour intervals for 10 days and dead juveniles counted.

Count data were transformed using Log_{10} (x + 1). Data were analyzed using Analysis of variance technique and the means were separated using the Duncan's Multiple Range Test (DMRT) technique at $p<0.05$ probability level.

Results

At 5% concentration, a percentage inhibition of 32.08% was observed while the 10% concentration showed a 34.77% inhibition. Both treatments showed statistically significant differences when compared with the control plates, but statistically similar to each other. Results obtained also showed that ethanol extracts greatly inhibited mycelial growth more than aqueous extracts. In the 5% and 10% concentrations of ethanol extracts, there was a complete (100%) inhibition of mycelial growth as shown on Table 1.

At very low concentrations of 2.5% and 5%, the results obtained showed that ethanol extracts completely suppressed or inhibited egg hatch (Table 2). Aqueous extracts equally inhibited egg hatch. There was no significant difference between both concentrations in inhibiting egg hatch. The control experiment however had the highest egg hatch, thus the lowest inhibition level of 5%. There was a significant difference between the extracts and the control as 95% of eggs hatched by day 5.

In a similar pattern to egg hatch, the low concentrations of 2.5% and 5% recorded complete mortality of *Meloidogyne sp.* J_2 stages by day-5 (120 hours) as shown on table 3. Percentage mortality for all treatments was 100% with respect to the control. Statistical analysis showed no significant difference between both extract concentrations applied; however, there was significant difference in the comparison of both treatments with the control.

Discussion and Conclusion

A complete inhibition of mycelial growth by ethanol extracts showed ethanol extracts to be more potent than aqueous extracts in inhibiting the fungal pathogen. This observation suggested ethanol to be a better extracting solvent of fungicidal active components, which agreed with the work of Lale and Abdulrahaman (1999). The results obtained also suggested that the solvent (ethanol) might have inhibitory properties against *M. phaseolina*. From the results, the leaf extracts showed increased inhibition with increased concentration and maximum effectiveness in inhibition with the ethanol extracts. Some researchers have reported higher potency of ethanol in comparison with aqueous extracts (Obi 1991; Mohanpatra *et al.*, 1995; Lale an Abdulrahaman, 1999). Strong variation has been observed to exist with different solvents especially on fungi as shown in this result where ethanol extracts exhibited 100% inhibition while aqueous extracts showed 32.08% and 34.77% inhibition for 5% and 10% concentrations respectively. The observed variation in the antifungal activities of the botanical suggested the relevance of the extracting solvent on the botanical.

From the results, 2.5% and 5% concentrations of *V.amygdalina* extracts completely inhibited egg hatch of *Meloidogyne spp.* Second stage juveniles (J_2) were also completely inhibited within 24 hours, which showed a consistency between the egg hatch inhibition and J_2 mortality results. These results agreed with the work of Ajayi *et al.* (1993) who reported potency of *V. amygdalina* extracts at 25% and 50% concentrations on the root knot nematode. This study has also shown that at even lower concentrations of 2.5% and 5%, the extracts were still very potent against *Meloidogyne sp.*, suggesting a viable alternative control for the dreaded root knot nematode. This study has also shown that water and ethanol extracts could have equal nematicidal effects in disagreement with ethanol extract's superiority in extracting fungicidal properties as reported by various workers (Obi, 1991; Mohanpatra *et al.*, 1995; Adbulrahaman, 1999; Peluola, 2005). The effectiveness of the extract against the root knot nematode may be due to phytotoxicity of the active ingredients or due to the high percentage of oil present in the extract (Ajayi, 1993).

The nematicidal potential of *V. amygdalina* is a welcome development for the environment-sensitive agriculturist who can exploit its easy production and cost effectiveness.

References

Abawi, G.S. and Pastor Corrales, M.A. (1989). Charcoal not screening procedure and Virulence of Macrophomina phaseolina isolates on dry edible beans. Turialba 39; 200-207.

Adesiyan, O.A. Babalola and A.A., Idowu (1992) University of Ibadan, Nigeria, 26-29th July 1992, pp. 311-322.

Adesiyan, S.O., Cavaness. F.E., Adeniyi, M.O and Fawole, B. (1990). Nematode Pests of Tropical Crops. Heinemman Educational Books (Nigeria) Limited; Ibadan, Nigeria.

Ajayi, A.V., N. Akem, and S.O. Adesiyan (1993). Comparison of nematicidal potential of Vernonia amygdalina leaf extract and Carbofuran on the growth and yield root knot nematode of infested soybean. Afro-Asian Journal of nematology Vol. 3. No. 2 pp. 119-127.

Allen, D.J. (1983). The pathology of tropical food legumes, Disease resistance in crop improvement. Chechester UK. John Wiley and Sons.

Anonymous(2000).http://www...chemie.unibonn.de/oc/ak_br/ANALYTIC/nigeria/vermonia/vern_inf.html

Babatola, J.O. and Omotade, M.A. 1991. Chemical control of the nematode pests of cowpea, (Vigna uniguiculata (L) Walp) Crop Protection 10:131-134.

Brader. S.A. (2002). Biocontrol of brown blotch of cowpea caused by colletorichum truncatum with trichoderma viride. Crop Protection 15(7):633-636.

Bridge, J.C. (1981). Nematodes. In: Pest Control in Tropical Grain Legumes. Word, A., Mercer, S.L. & Home, V. (eds). Centre for Overseas Pest-Research, London pp 111-125.

Emechebe, A.M. (1981). Brown blotch of cowpea in northern Nigeria. Samaru Journal of Agricultural Research 1(1): 20-26.

FAO (1999). Yam Cultivation and Utilization for improved food Security. A Position Paper pp. 1-11.

Hussey, R.S., and Barker, K.R. (1973). A comparison of methods of collecting for *Meloidogyne spp.*, including a new technique. The Plant Disease Reporter, 57, 1025-1028.

Mohanpatra, S.., S.K. Sawarkar, H.P. Patnaiki, and B. Senapati (1995). Antifeedant activity of solvent extracts of neem seed kernel against Spodoptera litura F. and their persistency against sunlight through encapsulation. 154-156 pp.

Muleba, N., Ouedraogo J.T., and Tignegre J.B. (1997). Crop yield loss attributed to Striga infestations. J. *Agric.*Sci., 129; 43-48. 33

Obi, V.I. (1991). Evaluation of extracts from four locally occurring plants for their fungicidal effects on coewpea anthracnose pathogen. Collectotrichum lindemuthianum. M.Sc Thesis Department of Agric. Biology, University of Ibadan, Ibadan, Nigeria. 53pp.

Ogunfowora, A.O. (1976). Research on *Meloidogyne* species at IAR&T, Moor plantation, Ibadan. In: Proceedings of the 1st Research Planning Conference on Root-Knot Nematodes, *Meloidogyne* species: Region IV, IITA, Ibadan, 7th – 11th June, 1976.

Olabiyi, T. I., Babalola, J.O. and Oyedunmade, E.E. A. (1992). In vitro assessment of some plant extracts for their nematicida properties In: Proceedings of 1st Regional symposium of biology and control of nematode pests of food crops in Africa.

B. Fawole, O.A. Egunjobi, S.O., and Olowe, T.O. (1992). Economic importance of nematode of legume and cereal crops in Nigeria. Proceedings of the first regional symposium on the biology and control of nematodes pests of food crops in Africa 26-29 July; 1992.

Sosanya, O.S. (2006). Comparative efficacy of carbofuran and Monocrotophos for the management of nematode pests of three cowpea (Vigna unguiculata (L) Walp) cultivars. PhD thesis of the University of Agriculture, Abeokuta, Nigeria.135 pages.

Table 1: Percentage inhibition of radial growth of *M. phaseolina* by *V. mygdalina* extracts *V. amygdalina* concentration (%)

	Control	5%AE	10% AE	5% EE	10%EE
Inhibition	0	1.19	1.29	3.71	3.71
% Inhibition 100.00	0	32.08	34.77	100.00	
Overall means	3.71a	2.52b	2.42b	0c	0c
SE	0.29	0.08	0.22	0	0
CV%	15.36	5.95	18.18		

AE = Aqueous Extract, EE = Ethanol Extract, SE = Standard Error, CV = Coefficient of Variation

Treatment	Mean value
0% AE	3.71a
5% AE	2.52b
10% AE	2.42b
5% EE	0c
10% EE	0c

*All values are means of 4 replicates. * Values with different letters are significantly different at p<0.05

Table 2: Cumulative percentage effect of *V. amygdalina* on eggs of *Meloidogyne sp.*

Treatment	%conc	%egg hatch		%egg inhibition	
		DAY 1	DAY 5	DAY 1	DAY 5
Control	0	15	95	-	5
AE	2.5	5	5	90	90
AE	5	10	10	85	85
EE	2.5	0	0	95	95
EE	5	0	0	95	95

* All results are with respect to the control
AE = Aqueous extract, EE = Ethanol extract

Treatment	Mean value
0% AE	3.8a
2.5% AE	0.2b
5% AE	0.4b
2.5% EE	0c
5% EE	0c

*All values are means of 4 replicates * Values with different letters are significantly different at p<0.05

Table 3: Cumulative percentage effect of *V. amygdalina* EXTRACTS ON J_2 OF *Meloidogyne sp.*

TREATMENT	%Conc.	%MORTALITY	
		DAY 1	DAY 5
Control	0	0	30
AE	2.5	65	70
AE	5	70	70
EE	2.5	70	70
EE	5	60	70

* All results are with respect to control.

Treatment	Mean value
0% AE	1.2a
2.5% AE	2.8b
5% AE	2.8b
5% EE	2.8b
10% EE	2.8b

* Values are a mean of 4 replicates. Values with different letters are significantly different at p<0.05
J2 = Second juvenile stage of *Meloidogyne sp.*, AE = Aqueous extract, EE = Ethanol extract