African Journal of Biotechnology Vol. 7 (16), pp. 2874-2877, 18 August, 2008
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB08.398
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Comparative nematotoxicity and fungitoxicity of crude and partitioned ethanolic leaf extracts of three plant species

S. T. Anjorin[1]* and E. A. Salako[2]

[1]Department of Crop Production, Federal University of Technology, Minna, Nigeria.
[2]Faculty of Agriculture, University of Abuja, Abuja, Nigeria.

Accepted 2 July, 2008

The nematotoxicity and fungitoxicity of crude and partitioned ethanolic leaf extracts of *Azadirachta indica, Blumea perotitiana* and *Lippia multiflora* were compared *in vitro* involving *Meloidogyne* root-knot nematode and *Rhizoctonia* root-rot fungus. The experiment which involved a Complete Randomized Design (CRD) with three replicates indicated that the nematode mortality and radial growth of the mycelia of *Rhizoctonia solani* applied with ethanolic crude extracts of each of the tested plant leaves was relatively less than their respective partitioned extract. Since the purification process of natural plant products especially in developing countries is slow and cumbersome and might render pesticidal products to be ineffective, a well-prepared effective crude plant extract will be easier for the resource-poor farmers to afford and renew.

Key words: Nematotoxicity, fungitoxicity, crude extracts, partitioned extracts, plant species.

INTRODUCTION

Meloidogyne spp. is the most pathogenic species of nematode to most crops and could cause up to 64% yield reduction (Khan et al., 1996). They are numerous and adaptable to many soil ecology where they feed on roots, live and reproduce entirely within the soil or root tissue. *Rhizoctonia solani* causes root and stem rots of several young crop plants (Sinclair, 1982). Yield losses of up to 50% have been attributed to *R. solani* attack in cowpea, soyabean and rice (Akem, 1991). The association of nematode and fungus often showed additive interaction leading to more severe disease symptoms and lower yield of several crops.

The use of synthetic pesticides to control pathogens are associated with myriads of problems which include the high cost of procuring and applying them (Salako, 2002); The side or residual effects from their extensive use and their persistence lead to substantial phytotoxicity, soil and water pollution, extermination of beneficial organisms and development of pesticide resistance by many organisms (Kohli et al., 1999). The search for the new alternative fungus-nematode management strategies that are cheap, eco-friendly and environmentally safe is imperative (Rotimi and Moens, 2001). The use of several crude botanicals against pathogenic nematodes and fungi has been reported, but only few farmers or researchers have investigated the partitioned extracts. This study hereby compared the nematicidal and fungicidal efficacies of the crude and partitioned ethanolic leaf extracts of *Azadirachta indica*, *Blumea perotitiana* and *Lippia multiflora in vitro*. Potent plant-derived portion could be further purified or formulated into botanical pesticide.

MATERIALS AND METHODS

The leaves of *A. indica* (neem), *B. perotitiana* (iron weed) *and L. multiflora* (Lippia) were collected locally from Kuje and Abuja Municipal blocks of the Federal Capital Territory, Abuja and washed with clean water. The shade-dried leaves were ground into powder with an electric blender and 62.5 g of each leaf powder was cold extracted by soaking in 200 ml ethanol for 3 days. The extractable material was separated from the extraction solvent with Bochi rotary evaporator at 50°C. The jelly-like crude ethanolic extracts was concentrated with water bath and subsequently partitioned by first subjecting 20 g each of the extracts with soxhlet extraction using n-

*Corresponding author. E-mail: oyindamola35@yahoo.com.

Table 1. Percentage mortality of *Meloidogyne* spp. exposed to partitioned ethanolic leaf extracts for 24 and 48 h.

Treatment	% Mortality at 24 h exposure	% Mortality at 48 h exposure
Neem crude extract	73.38[b]	82.88[b]
n-Hexane-soluble portion	40.9[f]	43.73[e]
Ethyl acetate-soluble portion	49.6[e]	51.15[d]
n-Butanol-soluble portion	51.6[de]	55.75[d]
***Blumea* crude extract**	82.90[ab]	86.22[b]
n-Hexane-soluble portion	34.13[g]	35.95[f]
Ethyl acetate- soluble portion	43.93[f]	44.18[e]
n-Butanol-soluble portion	53.65[d]	55.03[d]
***Lippia* crude extract**	57.98[c]	61.01[c]
n-Hexane-soluble portion	39.33[f]	41.48[e]
Ethyl acetate-soluble portion	42.23[f]	43.85[e]
n-Butanol-soluble portion	48.50[e]	54.80[d]
Carbofuran	91.76[a]	99.33[a]
Control (distilled water)	1.16[h]	1.33[g]

Column means followed by the same letter(s) are not significantly different (P ≥ 0.05) by DMRT.

hexane at 50 – 60°C for 24 h. The resulting solution was concentrated in rotary vacuum evaporator to yield an oily brown mass of 1.2 g coded H-p. The defatted residual ethanolic extract mass was added with 100 ml of distilled water and agitated properly. The mixture was decanted and filtered with cheese cloth. The filtrate which is a mixture of the extract and water was poured in a separating funnel and successfully partitioned with ethyl acetate and n-butanol to yield 1.1 g golden brown ethyl acetate soluble portion coded Ee-p and a 1.8 g dark brown n-butanol soluble portion coded Be-p, respectively. The residual aqueous dark brown portion mass of 3.2 g was dried and coded Ae-p.

The duly identified preserved sample of *R. solani* inoculum obtained in the microbiology laboratory of Federal University of Technology, Minna was sub-cultured and grown on a prepared aseptic Oxoid medium in sterile Petri dishes. They were incubated at 27°C and after 72 h, fan-like radial mycelia grew from the rhizomorphs. *Meloidogyne* spp. used was multiplied on *Celosia argentea* in the garden and the inoculum was collected from the roots and rhizosphere of infected plants. In the experimental set up, the potted seeds in each replicate were treated with carbofuran i.e. Furadan® (100 kg/Ha) and Maneb + Zinc i.e. Mycotrin® (0.6 kg/100 L of water/Ha). Also a pot per replicate had 50 ml of distilled water and these served as the control. The experiment involved a Complete Randomized Design (CRD) with three replicates. Data collected was subjected to Analysis of variance (ANOVA) and treatment means were separated by Duncan's Multiple Range Test (DMRT) using SAS (1997) package.

RESULTS AND DISCUSSION

At 24 h exposure, the effect of the crude extract of *B. perotitiana on* percentage mortality of *Meloidogyne* spp. was significantly higher (P ≤ 0.05) than any other treatments, except that of carbofuran (Table 1). Among the extract treatment, n-hexane soluble portion had the least nematicidal effect but significantly higher than that of the control. At 48 h exposure the carbofuran applica-tion recorded the highest percentage mortality (99.33%). This was followed by that of *B. perotitiana* crude extract (86.22%) but was significantly less (P ≤ 0.05) than that of carbofuran. The n-hexane soluble portion of *B. perotitiana* recorded the least nematicidal effect (35.95%) but this was significantly higher (P ≤ 0.05) than that of the control (1.33%). At 4 DAI, the effect of mycotrin® treatment had the least mycelia radial growth (1.67 mm). This was followed by the radial growth of neem crude extract applied plots (11.58 g) but was significantly higher (P ≤ 0.05) than that of the mycotrin® (Table 2).

Among the leaf extracts applied, ethyl acetate soluble portion recorded the least fungistatic efficacy with a radial growth of (32.55 mm). However, it was significantly higher (P ≤ 0.05) than the radial growth in the control (37.83 mm). At 8 DAI, the trend of mycelia radial growth was similar to that of 4 DAI as it almost increased proportionately in their radial growth. At 8 DAI mycotrin®-applied fungus recorded the highest percentage reduction in the radial growth of the mycelia of *R. solani*. Among the leaf extracts used, neem crude extract had the highest radial growth reduction (70.94%). The least fungistatic treatment was the *Lippia* ethyl acetate soluble portion-applied media with11.67% radial growth reduction. This might be due to qualitative and quantitative differences in the bioactive principles present in them.

The result of *in vitro* assessment of the fungicidal and nematicidal potential of partitioned ethanolic leaf extracts of the selected plants indicated the bioactivity of group of compounds in the extracts thus could serve as basis for further purification or characterization of the active agents. The higher percentage mortality of *Blumea* crude extract than that of carbofuran at 24 h exposure might be due to the incomplete dissolution of the carbofuran granules and low concentration of the nematicidal active ingredients in the water used for the test. However, at 48 h exposure, highest percentage mortality was observed from carbofuran-applied medium probably due to the

Table 2. Radial growth of *R. solani* (mm) on partitioned ethanolic extract and mycotrin®- mended PDA at 4 and 8 DAI.

Treatment	Radial growth (mm)		% Reduction
(5000 umg/ml)	4 days after inoculation	8 days after inoculation	8 days after inoculation
Neem crude extract	11.58h	13.08g	70..94g
n-Hexane soluble portion	19.83f	24.68e	45.15e
Ethyl acetate soluble portion	24.63de	29.50d	34.44d
n- Butanol soluble portion	14.50g	16.73f	62.89f
***Blumea* crude extract**	11.83h	13.43fg	70.15c
n-Hexane soluble portion	25.68d	33.23c	26.15c
Ethyl acetate soluble portion	32.55b	31.60cd	29.78cd
n- Butanol soluble portion	14.30g	15.03fg	66.60f
***Lippia* crude extract**	12.45g	13.75fg	69.44g
n-Hexane soluble portion	22.23e	33.93c	24.60c
Ethyl acetate soluble portion	29.15c	39.75b	11.67b
n- Butanol soluble portion	14.83g	16.15fg	64.11f
Mycotrin®	1.67i	1.80h	98.22h
Control	37.83a	45.00a	0.00a

Column means followed by the same letter(s) are not significantly different ($P \geq 0.05$) by DMRT.

complete dissolution of the granules and the release of the lethal substance which inhibited the enzyme acetyl cholinesterase at the cholinergic synapses in the nematode nervous system (Singh, 2005). Rotimi and Moens, 2002 and Tang'an et al. (2003) reported that extraction methods employed and the level of concentration of an extract greatly affects the pesticidal efficacy of botanicals.

The radial growth of the mycelia of *R. solani* applied with ethanol crude extracts of each of the tested plant leaves was relatively less than their respective partitioned extract. This might be due to the synergistic relationship of bioactive ingredients in the crude extracts that was capable of exhibiting fungicidal efficacy. As soon as each crude extract is partitioned, their bioactive efficacy dwindles. Rodriguez et al. (1996) observed that the quantity of bioactive metabolites in an extract is directly proportional to the mass of the extract. Singh et al. (2001) reported that the use of plant crude extract pesticide instead of the formulations from the purified bioactive ingredients is justified since their active ingredients are normally too complex and therefore the process of simplification or isolation may lead to loss of activity.

Conclusion

The purification process of natural plant products especially in developing countries is slow and cumbersome and might render pesticidal products to be ineffective. For a local use, it seems unnecessary to further partition the crude extract in order not to break the bond of synergism in them. A well-prepared crude plant extract could be effective and will be easier for the resource-poor farmers to afford and renew.

ACKNOWLEDGEMENTS

STA recognizes the assistance of Mal. I. Kudu, Department of Microbiology and Mrs. F. Fadipe, Department of Chemistry of Federal University of Technology, Minna - Nigeria.

REFERENCES

Akem CN (1991). Cowpea disease: biology, identification, control and management. IITA Research Guide: p. 39.

Khan MR, Khan MW, Khan AA (1996). Effect of *Meloidogyne incognita* on dry weight, root gall and root nodulation of chickpea and cowpea cultivars. Test of agrochemicals and cultivars 17: 70-71.

Kohli RK, Batish DR, Singh HP, Arora V (1999). Allelopathy as a tool for pest and weed management. J. Punjab Academy of Science 1: 127-131.

Rodriguez I, Wolfender SL, Msonthi JD, Hostettmann K (1996). Quatitative and qualitative analysis of saponins from berries of cultivated *Phytolaca dodecendra by Lc/Uv and Lc/Ms.* Phytochemistry 36: 283-293

Rotimi MO, Moens M (2001). Potentials of Botanicals as alternative to synthetic nematicides. Paper presented at the 30th Annual Conference of the Nigerian Society for Plant Protection, University of Agric., Abeokuta. Sept. 1- 4, 2002.

Rotimi MO, Moens M (2002). The use of leaf extracts of some herbs in the control of *Meloidogyne incognita*. Paper presented at the 30th Annual Conference of the Nigerian Society for Plant Protection, University of Agriculture Abeokuta. Sept 1- 4, 2002

Salako EA (2002). Plant protection for the resource-poor farmers a keynote address at Nigerian Society for Plant Protection. 30th Annual conference. UNAAB. Abeokuta Sept. 1st – 4th.

SAS - Statistical Analysis Software - (1997). User's guide for personal computers Release 6.12 SAS Institute, Inc., Cary, N.C. USA. p. 956.

Sinclair JB (1982). Reaction of *Rhizoctonia solani* isolates to certain chemicals, Plant Dis. Reptr. 44: 474-477.

Singh NPI, Kohli RK, Batish DR (2001). Allelopathy in Agro-ecosystems. J. Crop Prod. (Food Products Press) an imprint of the Haworth press. Inc). 4(2): 1-44.

Singh RS (2005). The non-fumigant nematicides . *In : Plant diseases.* Eight edition. Published by Vijay Primlani for Oxford and IBH, New

Delhi, India. pp. 645-683.
Tang'an BNS, Adebitan SA, Abdul SD Ogidi JA (2002). Fungicidal activity of some plant extracts on *Cercospora sojina* Hara: the causal agent of frogeye leaf spot of soyabean. Paper presented at the 30th Annual Conference of the Nigerian Society for Plant Protection, University of Agric., Abeokuta. Sept .1- 4.