Nig J. Biotech. Vol. 22 (2011) 1- 7
ISSN: 0189 17131
Available online at www.biotechsocietynigeria.org.

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Effects of Botanical Extracts on the Mycelial Growth of Seed-Borne Fungi of The African Yam Bean, *Sphenostylis stenocarpa* (Hochst ex a. Rich) Harms.

Onyeke, C.C.[1] and Ugwuoke, K.I.[2]

[1]Department of Botany, University of Nigeria, Nsukka; [2]Department of Crop Science, University of Nigeria, Nsukka

(Received 24.2.11, Accepted 17.6.11)

Abstract

The effects of crude ethanolic plant extracts of Garcinia kola seeds and Nauclea latifolia root on mycelial growth of seed-borne fungi of African yam bean at different concentrations (100 mg/ml, 60 mg/ml and 20 mg/ml) were investigated. The seed-borne fungi were Aspergillus flavus, Fusarium moniliforme, Penicillium sp. and Absidia sp. Results showed that all the plant extracts at various concentrations inhibited the mycelial growth of the seed-borne fungi. However, the inhibitory effects of the plant extracts increased with higher concentrations. The percentage inhibition of mycelial growth of fungal isolates by G. kola ranged from 57.9%-100%; 38.8%-72.6%; 31.4%-82.3% and 68.7%-100% for Absidia sp., Penicillium sp., A. flavus and F. moniliforme, respectively. Also, the percentage inhibition of growth of isolates by N. latifolia ranged from 52.3%- 89.4%; 37.1%-62.5%; 37%-93.6% and 49.7%-95.2% for the seed-borne fungi, respectively. G. kola extracts had more inhibitory activities on Absidia sp., Penicillium sp. and F. moniliforme while N. latifolia was more active against A. flavus.

Key Words*:* Extracts, Inhibition, Seed-borne fungi, African yam bean, Mycelial growth.

Correspondence: onyekechris@yahoo.com

Introduction

Seed health status determines to a very great extent the production of healthy crops. In fact, seeds are known to carry some important diseases caused by pathogens which can cause immense yield losses in fields (Al-Kassim and Monawar, 2000). African yam bean, *Sphenostylis stenocarpa* (Hochst Ex A. Rich) Harms is a crop species at a relatively early stage of domestication (Potter, 1992) and it is important that the germplasm is stored in good condition for future exploitation. *S. stenocarpa* seed is a source of cheap good quality protein for the many poverty-stricken Africans who cannot afford the exorbitant animal protein. The presence of seed-borne pathogens on seeds can be a constraint to the exploitation of the genetic resources of a crop as this will hinder its movement to countries where there are strict quarantine systems (Richardson, 1990). Seed borne fungi can cause significant reduction in seed germination, seedling emergence and seed and tuber yields of African yam bean (Umechuruba and Nwachukwu, 1994) as well as the nutritional composition of the seeds (Nwachukwu and Umechuruba, 1997).

The control of seed-borne pathogens through the application of chemicals for seed dressing has been effective in reducing seed-borne pathogens and even improved the germination ability of seeds (Nene *et al* 1969; Yorinori, 1994; Okoro, 2005). However, there is fear about the safety of their residues and the possibility of human toxicity. Hence, there is need for alternatives that are natural and with little or no safety concerns.

Nwachukwu and Umechuruba (2001) reported significant (P≤0.05) reduction in the incidence of seed-borne fungi of African yam bean using leaf extracts from *Ocimum basilicum*, *Vernonia amygdalina*, *Cymbopogon citratus*, *Azadirachta indica* and *Carica papaya*. Similarly, Akinbode and Ikotun (2008) observed significant (P≤0.05) inhibitory effect on the growth of seed-borne fungus, *Colletotrichum destructivum* on cowpea using aqueous leaf extracts of *Moringa oleifera*, *Vernonia amygdalina* and *Annona muricata*.

The present work is undertaken to ascertain the effects of crude ethanolic plant extracts on the mycelial growth of seed-borne fungi of African yam bean at different concentrations.

Materials and Methods

Plant Collection: The seeds of bitter kola, *Garcinia kola* Heckel were bought from Ogige Market in Nsukka, Enugu State while the roots of *Nauclea latifolia* (Dewild&Th. Dur) Merril were collected from the Department of Botany Garden, University of Nigeria, Nsukka, Enugu State.

Seed Source: African yam bean seeds used were harvested from an African yam bean farm in the research farm of the Department of Crop Science, University of Nigeria, Nsukka.

Preparation of Plant Materials: G. kola seeds and *N. latifolia* roots were dried to constant weights in an oven at a regulated temperature of 45^0C for some days. Each of the materials was ground to fine powder with a laboratory mill.

Extraction of Active Principles: The method of extraction of active principle was similar to that of Onyeke and Maduewesi (2006). Absolute ethanol was used as the solvent for extraction. Sixty grammes each of the powdered plant materials were soaked in 600 ml of absolute ethanol and allowed to stand for 4 days on the laboratory bench. Filteration was done using No 1 Whatman filter paper. The filterates were dispensed into plastic saucers and placed before a standing fan which evaporated the solvent and a pasty crude plant extracts were formed. The crude extracts were stored in sterile bottles and put in the refrigerator throughout the duration of the experiments.

Isolation and Identification of Seed-borne fungi: The seeds were screened for seed-borne fungi using the blotter method (ISTA, 1976). About 20 seeds randomly selected from each sample were surface sterilized in 1% sodium hypochlorite solution for 3-5 minutes and rinsed in several changes of sterile distilled water. The seeds were plated on two layers of moistened filter paper (No 1 Whatman) in sterilized Petri-dishes. The plates were incubated on laboratory benches at room temperatures for 8 days. Filter papers were moistened by aseptically dispensing sterile distilled water as the need arose. Fungal growths from points of inoculation were aseptically transferred to Petri-dishes containing potato dextrose agar (PDA) for subsequent isolation and identification. The isolated fungal pathogens were identified using some reference materials (Jurgen *et al*., 1978; Barnett and Hunter, 1987and Labbe and Garcia 2001).

Crude Extract Yield: G. kola = 11.88 g/ 60 g powdered seeds; *N. latifolia* = 4.98 g/ 60 g powdered roots.

Effects of Plant Extracts on the Mycelial Growth of Seed-borne Fungi: The plant extracts were each dissolved in 50% concentration of dimethyl sulphoxide (DMSO; $(CH_3)_2SO$) in the ratio of 1:10 (1g of crude extract dissolved in 10 ml of DMSO) to give a concentration of 100 mg/ml. Further concentrations of 60 mg/ml and 20 mg/ml were made from the stock concentration (100 mg/ml). Two millilitres each of the plant extract concentrations were aseptically dispensed into sterile Petri-dishes and 18 ml of molten streptomycin sulphate-modified PDA was immediately poured into each of the Petri-dishes containing plant extracts. The Petri-dishes were swirled on the laboratory bench to mix the extracts and the PDA thoroughly before solidification. Each of the plant extract-PDA Petri-dishes received a mycelial disc of a test fungus cut using a cork borer. The mycelial discs were placed

upside down at the centre of the medium in the Petri-dishes. Petri-dishes without extracts served as controls. Some Petri-dishes contained 8.5 mg/ml of Benlate in place of plant extracts to compare the efficacy of the plant extracts according to the method of Onyeke and Maduewesi (2006). The experiments were laid out in completely randomized design (CRD) in three replications. The diameter (mm) of the mycelial growth of each fungus in the Petri-dishes was taken after 7 days. The fungi toxicity of plant extracts was determined as a percentage inhibition of mycelial growth with the formula by Onuh *et al*. (2005): $Fp = F_1 - F_2 / F_1 \times 100$.

Where: Fp= percentage inhibition of mycelial growth; F_1 = mycelial growth in control Petri-dishes and F_2 = mycelial growth in treatment Petri-dishes.

Results

Results on the effect of ethanolic plant extracts on mycelial growth of *Absidia* sp. are shown in Table 1. Results revealed that both plant extracts produced significant ($P \leq 0.05$) levels of inhibition of mycelial growth of *Absidia* sp. at various concentrations. However, the inhibitory effect on mycelial growth by plant extracts increased with higher concentrations.

Table 1: Effects of ethanolic plant extracts on mycelial growth (mm) of *Absidia* sp.

Plant extracts	*Garcinia kola*		*Nauclea latifolia*	
Concentrations	Mycelial Growth(mm)	% inhibition	Mycelial Growth(mm)	% inhibition
100 mg/ml	0.00	100	0.716	89.4
60 mg/ml	1.37	78.3	1.93	71.4
20 mg/ml	2.65	57.9	3.22	52.3
Benlate	0.00	100	0.00	100
Control	6.30	-	6.75	-
LSD(0.05)	0.797		0.439	

The highest (100%) percentage inhibition of mycelial growth by plant extracts was produced by *G. kola* at the concentration of 100 mg/ml while the least (52.3%) percentage inhibition was recorded by *N. latifolia* at the concentration of 20 mg/ml. Generally, the percentage inhibition of mycelial growth by both extracts at 100 mg/ml compared well with benlate even though at a lower concentration. Table 2 shows the effect of plant extracts on mycelial growth of *Penicillium* sp. Results also showed that both plant extracts produced significant ($P \leq 0.05$) levels of inhibition of mycelial growth of *Penicillium sp*. at various concentrations. The highest (72.6%) percentage inhibition of mycelial growth by plant extracts was produced by *G. kola* at the concentration of 100 mg/ml while the least (37.0%) percentage inhibition of mycelial growth was recorded by *N. latifolia* at the concentration of 20 mg/ml.

Table 2: Effects of ethanolic plant extracts on mycelial growth (mm) of *Penicillium* sp.

Plant extracts	*Garcinia kola*		*Nauclea latifolia*	
Concentrations	Mycelial Growth (mm)	% inhibition	Mycelial Growth(mm)	% inhibition
100 mg/ml	2.02	72.6	2.96	62.5
60 mg/ml	3.25	55.9	3.98	49.6
20 mg/ml	4.51	38.8	4.96	37.1
Benlate	0.00	100	0.00	100
Control	7.38	-	7.89	-
LSD(0.05)	0.664	-	0.705	-

Results on the effect of plant extracts on mycelial growth of *Aspergillus* sp. is shown in Table 3. The results again showed significant (P≤0.05) levels of inhibition of mycelial growth of *Aspergillus* sp. by both extracts at various concentrations. The highest (93.6%) percentage inhibition of mycelial growth by plant extracts was produced by *N. latifolia* at the concentration of 100 mg/ml while the least (31.4%) percentage of mycelial growth was recorded by *G. kola* at the concentration of 20 mg/ml.

Table 3: Effects of ethanolic plant extracts on mycelial growth (mm) of *Aspergillus flavus*.

Plant Extracts Concentrations	*Garcinia kola* Mycelial Growth(mm)	% inhibition	*Nauclea latifolia* Mycelial Growth(mm)	% inhibition
100 mg/ml	1.52	82.3	0.489	93.6
60 mg/ml	3.70	57.0	3.20	58.4
20 mg/ml	5.90	31.4	4.85	37.0
Benlate	0.00	100	0.00	100
Control	8.60	-	7.70	-
LSD(0.05)	0.858		0.549	

Table 4 shows the effect of plant extracts on mycelial growth of *Fusarium* sp. There were significant (P≤0.05) levels of inhibition of mycelial growth of *Fusarium* sp. by both extracts at various concentrations. The highest (100%) percentage inhibition of mycelial growth by plant extracts was produced by *G. kola* at the concentration of 100 mg/ml while the least (48.7%) percentage inhibition of mycelial growth was recorded by *N. latifolia* at the concentration of 20 mg/ml. Generally, the percentage inhibition of mycelial growth by both plant extracts at 100 mg/ml compared well with benlate even at a lower concentration.

Table 4: Effects of ethanolic plant extracts on mycelial growth (mm) of *Fusarium moniliforme*.

Concentrations	*Garcinia kola* Mycelial Growth(mm)	% inhibition	*Nauclea latifolia* Mycelial Growth(mm)	% inhibition
100 mg/ml	0.00	100	0.29	95.2
60 mg/ml	0.00	100	1.62	73.0
20 mg/ml	2.11	68.7	3.02	49.7
Benlate	0.00	100	0.00	100
Control	6.75	-	6.00	-
LSD(0.05)	0.232		0.574	

Discussion

Some of fungal organisms implicated as seed borne in African yam bean are not strange as they have been well reported (Nwachukwu and Umechuruba, 1991;Du *et al*., 2001; Sultana and Ghaffar, 2009) excepting *Absidia* sp. Results have exposed the antifungal activities of the botanical extracts on the common seed borne pathogens of *S. stenocarpa.* This result is in agreement with reports of past researchers on the antimicrobial effects of certain plant based extracts (Singh *et al*., 1980; Onyeke and Maduewesi, 2006; Chiejina, 2008). Generally, *G. kola* extracts demonstrated higher antifungal properties as against that of *N. latifolia*. The seed of *G. kola* contains some bioactive substances such as biflavonoids (Iwu and Igboko, 1982), triterpenes, tannins, saponins(Braide, 1990),glycosides and alkaloids(Ebana *et al*.,1991) which could be responsible for the antimicrobial activities. Iwu (1993) also reported that root extract of *N. latifolia* has both antibacterial

and antifungal activities. The identification of *Aspergillus flavus*, *Fusarium moniliforme* and *Penicillium* sp. as seed-borne fungal organisms of African yam bean presents with a serious health concern as they are known to produce highly potent mycotoxins which are dangerous to man and animals if consumed (WHO, 1979; Krogh, 1988). Also, it has been reported that aflatoxins synthesized by *A. flavus* limit seedling elongation, inhibit chlorophyll synthesis, inhibit assorted enzymes and degranulate the endoplasmic reticulum (Mehrotra and Aggarwal, 2003). In this regard, considerable efforts should be made to keep seeds that are meant for both consumption and sowing free of seed borne fungi.

From the results of this experiment, ethanolic extracts of *N. latifolia* roots and *G. kola* seeds could be recommended for use as seed treatment option since they demonstrated high levels of fungitoxity on those commonly reported seed borne fungi.

Acknowlegdement

The authors are grateful to Mr. John Ezema of the Mycology/Plant Pathology unit of the Department of Botany, University of Nigeria, Nsukka for his input towards the identification of the fungal isolates.

References

Akinbode, O.A. and Ikotun, T. (2008).Efficacy of certain plant extracts againstseed-borne infection of *Colletotrichum destructivum* on cowpea (*Vigna unguiculata*). *African Journal of Biotechnology*, *7* (20): 3683-3685.

Al-Kassim, M.Y. and Monawar, M.N. (2001). Seed-borne fungi of some vegetable seeds in Gazan province and their control. *Saudi Journal of Biological Sciences, 7* (2): 179-184.

Barnett, H.L. and Hunter, B.B. (1987). *Illustrated genera of imperfect fungi*. MacMillian Publishing Company, New York, 218pp.

Braide,V.S.(1990).Pharmacological effects of chronic ingestion of *Garcinia kola* seeds in the rat. *Phytotherapy Research, 4*: 39-41.

Chiejina, N.V. (2008). Potentials of extracts from young leaves of *Chromolaena odorata* (Linn.) King and Robinson and *Carica papaya* L. on two fungal pathogens of carrot, *Daucus carota* L. *Plant Product Research Journal*, *12*: 27-31.

Du, P.V., Loan, L.C., Cuong, N.D., Nghiep, H.V. and Thach, N.D. (2001). Survey on seed borne fungi and its effects on grain quality of rice cultivars in the Mekong Delta. *Omonrice*, *9*:107-113.

Ebana, R.U., Madunagu, B.E., Ekpe, E.D. and Otung, I.N.(1991). Microbiological exploitation of cardiac glycosides and alkaloids from *Garcinia kola*, *Borreria ocymoides*, *Kola nitida* and *Citrus aurantifolia*. *Journal of Applied Bacteriology*, *71*(5): 398-401.

ISTA (1976). International rules for seed testing. *Seed Science and Technology, 4*: 51-57.

Iwu, M.M. and Igboko, A.O. (1982). Flavonoids of Garcinia *kola* seeds. *Journal of Natural Products*, *45*: 650-651.

Iwu, M.M. (1993). *Handbook of African medicinal plants.* C.R.C. Press, Forida.330pp.

Jurgen, K., Heinz, S. and Werner, K. (1978). *Diseases, pests and weeds in tropical crops*. John Wiley & Sons Ltd, New York. 666pp.

Krogh,P.(1988). Adverse effects of mycotoxins in human health. *In*: Proceedings of the CTA seminar. S.B. Mathur and J. Jorgensen (eds.) pp20-25.Technical Centre for Agriculture and Rural Co-operation.

Labbe, R.G. and Garcia, S. (2001). *Guide to food-borne pathogens*. John Wiley & Sons Inc., New York. 400pp.

Mehrotra, R.S. and Aggarwal, A. (2003). *Plant pathology.* Tata McGraw-Hill Publishing Company Limited, New Delhi. 846pp.

Nene, Y.L., Agarwal, V.K. and Srivastava, S.S.L. (1969). Influence of fungicide seed treatment on the emergence and nodulation of soybean. *Pesticides 3*: 26-27.

Nwachukwu, E.O. and Umechuruba, C.I. (1991). Seed-borne fungi associated with African yam bean seeds in four Eastern States of Nigeria. *Seed Research, 19*(1): 13-20.

Nwachukwu, E.O. and Umechuruba, C.I. (1997).Changes in nutritional values of yam bean seeds due to seed-borne fungi. *Global Journal of Pure and Applied Sciences, 3*(2): 141-147.

Nwachukwu, E.O. and Umechuruba, C.I. (2001). Antifungal activities of some leaf extracts on seed-borne fungi of African yam bean seeds, seed germination and seedling emergence. *Journal of Applied Sciences and Environmental Management, 5*(1): 29-32.

Okoro, J.K. (2005). Chemical control of leaf spot disease and seed infection of soybean caused by *Phomopsis sojae* in Benue State, Nigeria. *Nigerian Journal of Botany, 18*: 138-144.

Onuh, M.O., Ohazurike, N.C. and Emeribe, E.O. (2005). Efficacy of *Jatropha curcas* leaf extract in the control of brown blotch disease of cowpea (*Vigna unguiculata*(L) Walp).*Nigerian Journal of Plant Protection, 22*: 46-52.

Onyeke, C.C. and Maduewesi, J.N.C. (2006). Evaluation of some plant extracts for the control of post-harvest fungal diseases of banana (*Musa sapientum* Linn.) fruit in south-eastern Nigeria. *Nigerian Journal of Botany, 19*(1): 129-137.

Potter, D. (1992). Economic botany of *Sphenostylis* (Leguminosae). *Economic Botany* 46(3):262-275.

Richardson, M.J. (1990). An annotated list of seed borne diseases. International Seed Association, Zurich, Switzerland.320pp.

Singh, U.P., Singh, H.B. and Singh, R.B. (1980). The fungicidal effect of neem (*Azadirachta indica*) extract on some soil borne pathogens of Gram (*Cicer arietinum*). *Mycologia 72*: 1077-1093.

Sultana, N. and Ghaffar, A. (2009). Seed borne fungi associated with bottle gourd (*Lagenaria sicerania* (Mol.) Standl. *Pakistan Journal of Botany*, *41*(1): 435-442.

WHO (1979). Mycotoxin. *Environmental Health Criteria No 11*. Geneva. World Health Organization.

Yorinori, J.T. (1994). Fungal diseases. *In*: *Tropical soybean, improvement and production*. F.A.O. Rome, pp37-60.