VOL. 7, NO. 8, AUGUST 2012 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science
©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.
www.arpnjournals.com

INCIDENCE AND BOTANICAL CONTROL OF SEED-BORNE FUNGI OF COWPEA IN NIGER STATE, NIGERIA

Makun H. A.[1], Anjorin S. T.[2], Abidoye A. S.[1], Rufai A. R.[1] and Kabiru Y. A.[1]
[1]Department of Biochemistry, Federal University of Technology, Minna, Nigeria
[2]Department of Crop Science, Faculty of Agriculture, University of Abuja, Abuja, Nigeria
E-Mail: oyindamola35@yahoo.com

ABSTRACT

Cowpea grains marketed in Minna, Nigeria were assessed for seed-borne fungi. The fungi were isolated and identified by standard methods of culturing and sub-culturing. The isolated fungi species were *Aspergillus niger* (19.78%) and *Fusarium verticilloides* (14.85%), Mucor spp. (5.95%), *Penicillium* spp. (4.95%) and *Rhizopus* spp. (0.99%). The fungistatic efficacy of crude leaf extracts of *Azadirachta indica*, *Blumea perotitiana* and *Lippia multiflora* were assessed *in-vitro* on the predominant isolated fungi (*A. niger* and *F. verticilloides*). The percentage mycelial inhibition of the plants leaf extracts were compared with the synthetic fungicide (Apron star®) and the control for 10 days. Under 2500 and 5000ug/ml treatment, the apron star® significantly ($P \leq 0.05$) reduced the mycelial growth of *A. niger* and *F. verticilloides* by 92% and 93%, respectively (Table-3). Next to this was the inhibitory effect of lippia + blumea extracts on *A. niger* (56%) and *F. verticilloides* (32%). Under 5000ug/ml treatment, the inhibitory effect of lippia + blumea extracts on *A. niger* (65%) and on *F. verticilloides* (48.78%). Neem leaf extract treatment only was the least effective among the botanical extract tested. Though the leaf extracts used were efficacious but were not as effective as the synthetic fungicide. The crude bioextracts could be purified and formulated in order to improve its efficacy.

Keywords: cowpea, fungi, *Aspergillus niger*, *Fusarium verticilloides*, ethanolic extract, *A. indica*, *B. perotitiana*, *L. multiflora*.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp] is an important grain legume in Africa, it serves as a major source of indigenous plant protein in Nigeria, (Ndem *et al*., 2004). Its values lie in its high protein content, ability to tolerate drought and capable of fixes atmospheric nitrogen which allows it to grow and improve poor soil.

Pathogenic seed-borne fungi cause loss in yield, quality, nutritional value and viability of food and feedstuffs especially cereal crops. Fungi and their mycotoxins constitute health hazards to animals and man following consumption of contaminated grain. Many diseases in animals are known to be of fungal origin (Collinson *et al*., 1992; Makun *et al*., 2009, 2010).

Various synthetic fungicides being used for seed-borne fungi of cowpea have been reported to have some disadvantages such as toxic residue, development of resistant strains, increase in cost and toxic to mammals since some of them contain heavy metals. Hence there is need for alternatives to seed-dressing chemical fungicides in the control seed-borne fungi. Azher (2009) reported some plant diffusates to be effective against seed-borne pathogenic fungi: These include the seeds of Bittergourd (*Memoridica charanta* L.), Cardomom (*Elettaria cardamonium* Maton) and Coriander (*Coriandrum sativus* L.). Also is the fruit of Chilli (*Capsicum annum* L.), Radish (*Raphanus sativus* L.) and Brassica (*Brassica compestris* var. *rapa*. L.), others are Onion (*Allium cepa* L.) Leaves/Flax, Garlic (*Allium sativum*) Cloves, Ginger (*Zingiber officinale*), Roscoe Rhizomes and Turmeric (*Curcuma longa*) Rhizomes.

The objectives of this study is to isolate and identify fungi contaminating cowpea grains in Niger State, Nigeria, and also to assess the antifungal activities of *Azadirachta indica*, *Blumea perotitiana*, and *Lippia multiflora* at varied concentrations on prevalent fungi isolated from the cowpea seeds.

MATERIALS AND METHODS

Collection of samples

Samples of cowpea beans were obtained from 18 market stores in Minna, Niger State. Fifty marketed samples that were made up of white and brown varieties were packaged in polythene bags. They were appropriately labeled and stored in a deep freezer at -20^{o}C until required for use. Fresh leaves of *Azadirachta indica*, *Blumea perotitiana*, and *Lippia multiflora* were collected from Bosso village.

Isolation of fungi

Fungi were isolated and cultured according to the method described by Halfon-Meiri and Barkai-Golan (1990). About 10 grains of each sample were washed with successive 100ml of sterilized distilled water and were surface sterilized using 5.25% Sodium hypochlorite (NaOCl) solution (Clorose the Clorax Co. Oakland, USA). The grains were then aseptically placed in Petri-dishes containing potato dextrose agar (PDA). The dishes were incubated at 27^{o}C and examined for 4-7days.

Identification of fungi

A speck of each fungus colony was aseptically placed on a sterile slide using forceps and cello tape. The fungi on the cello tape were stained with dye (lacto phenol blue) and viewed under x40 objective lens of the microscope. The identification was accomplished using a

©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.

fungi catalogue in the Microbiology Department in F.U.T, Minna.

Sub-culturing of fungi isolated

The pure culture of different isolate (identified fungi) were aseptically sub-cultured in PDA slant and incubated at room temperature in the refrigerator for further use. Two fungi species, *A. niger* and *F. verticilloides* collected from the stock culture were used for the study.

Preparation of Ethanolic extract on plant leaves

Healthy leaf samples of Neem, Blumea and Lippia were collected separately, rinsed and air-dried. The fresh leaves were crushed using mortal and pestle, and further blended into homogenous powder using an electric blender (National MX 391 N) to enhance penetration of extracting solvent and facilitate the release of the active constituents. Each powdery sample was stored in well-labeled clean containers until required for used.

The reflux apparatus was used for the extraction procedures. Fifty gram (50g) of each powdered material was packed into the round bottom flask. The solvent (ethanol) and the plant material were placed in the flasks. The process continued for about three hours to ensure complete extraction of the leaf constituents. The set up was placed on the heating mantle until it became volatized. The extracts were cooled, and filtered to sample bottle and kept for further chemical test.

Medium preparation

In preparing the PDA medium two hundred grams of peeled Irish potatoes was weighed accurately into a beaker and boiled for about 30 mins to 1h with distilled water. The thick solution obtained was filtered and the filtrate collected into 1 L measuring cylinder, 20g each of the glucose and 0.5g chloramphenicol (antibiotic) were added to mixture in order to inhibit bacteria growth and contamination. The PDA solution was made up to one litre with distilled water and boiled to dissolve the added materials. It was sterilized by autoclaving at 121^{o}C for 15 mins. It was further mixed thoroughly before pouring into Petri dishes and after its solidification, 10 cowpea seeds were inoculated on the solidified media. Some of the medium were poured into slant bottles, kept in slant positions and allowed to solidify. This was later used for sub-culturing the fungi isolates from the culture grown on media inoculated with cowpea grains.

In-vitro test

The *in-vitro* test was carried out in PDA medium to determine the inhibitory effect of leaf extract on the growth of the fungi isolate. To every sterile 200 ml PDA, 19 ml of each leaf extract was added and Apon star (a synthetic metalaxyl + carboxin + furathiocarb fungicide) was used as a standard. The solution in each Petri-dish was gently swirled and allowed to solidify. With the aid of a sterile needle, the centre of each agar Petri plate was inoculated with the speck of pure culture harvested from the margin of actively growing nine days old culture of the two fungi. The agar plate with the test pathogenic fungi were incubated at 25^{o}C for 14 days. Each treatment (extract) including the apron star solution (1g/10ml) was replicated three times with CRD arrangements. The following treatment were used: Neem, Blumea, Lippia and the combined treatments were Blumea + Neem, Blumea + Lippia, Neem + Lippia, the synthetic chemical (apron star) and the control (without extract or synthetic fungicide).

Data collection and analysis

Measurement of mycelial growth was carried out at 5th and 10th day after inoculation (DAI), respectively. Data obtained from mycelial growth inhibition were subjected to analysis of variance (ANOVA) using SPPS version 16. The Duncan Multiple Range Test (DMRT) was used to ascertain the significance between the different treatment means at 5% level of probability.

Fungi toxicity of the treatments was determined as percentage inhibition of fungi growth using the following formula;

$$Fp = f_1\text{-}f_2 \times 100/f_1$$

Where

Fp = Percentage inhibition of fungi growth
F_1 = Fungi growth in control Petri dish
F_2 = Fungal growth in treated Petri dish with extract/apron star

RESULTS

The occurrence of fungi isolated from cowpea grain samples collected from different market places in 18 districts in Niger State, Nigeria (Table-1). One hundred and one fungi colonies were identified from 54 cultured cowpea samples. The fungi genera identified contaminating the cowpeas in order of decreasing prevalence were *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus* spp. (Table-1) while the percentage incidence of the species of the cowpea seed-borne fungi were *A. niger* (14.85%), *F. verticilloides* (13.86%) and the least was *Rhizopus* (0.99%). *A. niger* recorded the highest incidence followed by *Fusarium* spp. and *Mucor* species (Table-2).

The efficacy of the four leaf extracts against the major isolated fungi of cowpea grains are shown in Tables 3 and 4. The ethanolic leaf extract of *L. multiflora*, *B. perotitiana* and *A. indica* exhibited varying levels of inhibition on mycelia growth of *A. niger* and *F. verticilloides* when compared with the control. The synthetic fungicide at 2, 500ug/ml, and 5000ug/ml concentration recorded a minimum of 92% growth inhibition on the fungi.

At the both concentrations of the leaf extracts used in this study, *L. multiflora* combined with blumea or neem was observed to have the highest inhibitory effects on the two fungi. However the synthetic fungicide gave the highest percentage inhibition at the same levels of

©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.

concentration. *A. niger* mycelial growth was found to be more inhibited by the leaf extracts than the *F. verticilloides*.

Table-1. Incidence of fungi genera in cowpea grains marketed in Minna, Niger State, Nigeria.

Fungi	Occurrence	% occurrence
Aspergillus spp.	66	65.35
Fusarium spp	23	22.77
Mucor spp.	6	5.94
Penicillium spp.	5	4.95
Rhizopus spp.	1	0.99
Total	**101**	**100**

None of the leaf extracts completely suppressed the mycelial growth of *A. niger* and *F. verticilloides* between 5 and 10DAI. The mycelial growth of *A. niger* applied with 2500 ug /ml apron star® increased by 0.25 mm between 5 - 10DAI while at 5000ug/ml, it only increased by 0.05mm. The mycelial growth of *F. verticilloides* applied with 2500ug or 5000 ug/ml apron star® increased by 0.10mm only between 5 and 10 DAI.

Under 2500 and 5000ug/ml treatment, the apron star® significantly ($P \leq 0.05$) reduced the mycelial growth of *A. niger* and *F. verticilloides* by 92% and 93% respectively (Table-3). Next to this was the inhibitory effect of lippia + blumea extracts on *A. niger* (56%) and *F. verticilloides* (32%).

Under 5000ug/ml treatment, the inhibitory effect of lippia + blumea extracts on *A. niger* was 65% and on *F. verticilloides* was 48.78%. (Table-4) This effect was not significantly ($P \geq 0.05$) different from the inhibitory effect of other leaf extract treatment on *A. niger*. Neem leaf extract treatment only was the least effective among the botanicals.

Table-2. Incidence of species of fungi cowpea grains marketed in Niger State, Nigeria.

Fungus	Agaie	Beji	Bosso	Chanchaga	Forindoki	Garatu	Guadabi	Gwada	Gwari	Kuta	Kwankuti	Lapai	Maikunkele	Mobile	New Market	Paiko	Tunga	Tungamallam	Frequency
Aspergillus flavus				10															10
Aspergillus fumigatus				7		1				5								2	15
Aspergillus glaucus	1		1				1		3						4			1	11
Aspergilus niger		3		1			4				2	1	1		1	3	2		17
Fusarium verticilloides	1		1		1	2	2		1			1	1	1	1	1	2		15
Fusarium oxysporum		1	1		1	1	1	1						1			1		8
Mucor spp.			1								1		1	1		1	1		6
Penicillium spp.							1							4					5
Rhizopus spp.													1						1
Alternaria cassiae	3	1	1			1	1		2	2		2		1	1				13
Total incidence	5	5	5	18	2	5	10	1	6	7	3	4	4	8	7	5	6	3	101
% incidence	4.95	4.95	4.95	17.82	1.98	4.95	9.90	0.99	5.94	6.93	2.97	3.96	3.96	7.92	6.93	4.95	5.94	2.97	

Table-3. Mycelial growth inhibition of *A. niger* and *F. verticilloides* applied with ethanolic leaf extract and apron star at 2500ug/ml.

	Mycelium growth (cm)					
Treatment	***A. niger***			***F. verticilloides***		
	5DAI	**10DAI**	**% Reduction**	**5DAI**	**10DAI**	**% Reduction**
Neem	1.50	3.90	33.33 c	3.15	3.50	18.90c
Blumea	1.0	3.00	50.62 b	2.80	3.40	24.39b
Lippia	0.9	2.91	53.00b	3.00	4.10	13.41c
Blumea +Neem	1.50	2.50	50.62b	2.50	3.00	32.92b
Blumea + Lippia	0.76	2.80	56.00b	2.64	3.10	30.00 b
Lippia + Neem	1.10	2.71	52.96 b	2.50	3.10	31.70b
Apron star	0.20	0.45	92.00a	0.28	0.38	92.00a
Control	3.60	4.50	0.00 d	3.70	4.50	0.00 d

Means on the same column with same letter are not significant different (P> 0.05) by DMRT

©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.

Table-4. Mycelial growth inhibition of *A. niger* and *F. verticilloides* applied with ethanolic leaf extract and apron star at 5000ug/ml.

Treatment	Mycelium growth (cm)					
	A. niger			F. verticilloides		
	5DAI	10DAI	% Reduction	5DA	10DAI	% Reduction
Neem	1.15	3.00	48.64b	2.30	3.28	20.73c
Blumea	1.00	2.70	54.32b	1.50	3.85	34.76b
Lippia	0.759	2.41	61.00b	2.50	4.00	31.95b
Blumea +Neem	1.25	2.00	59.88b	1.00	3.20	42.00b
Blumeas + Lippia	0.75	2.09	65.00b	1.26	3.50	48.78b
Lippia+ Neem	1.00	2.08	62.00b	1.52	3.40	40.00b
Apron star	0.25	0.30	93.20 a	0.28	0.38	92.00a
Control	3.61	0.29	0.00c	3.68	4.40	0 d

Means on a column with the same letter(s) are not significantly (P>0.05) by DMRT

DISCUSSIONS AND CONCLUSIONS

The survey carried out in this study revealed that *A. niger* and *F. verticilloides* are the most frequent isolates. Microorganisms especially fungi are known to be the major cause of market and field losses of crops (Okoli *et al.*, 1989; Onifade, 2000). The incidence of fungi on other grains such as sorghum and millet have been reported by Leslie *et al*. (1992), Marassas *et al*. (1995), and Brandyopadyay *et al*., (2005) to have included *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., and *Mucor* spp. Toxigenic moulds and aflatoxin B1 has been detected from kernels, soybean oil, garri, yam flour, ginger, cowpea, maize, millet, rice, sorghum, cotton seed, and groundnut and melon seed crops from Nigeria (Bankole and Adebayo, 2003; Makun *et al*., 2010). The factors influencing the development of seed-borne fungi include the moisture content of the seeds or grains, prevailing temperature, storage period and degree of seed invasion with the pathogen. Others are level of host genetic resistance, activities of insects and mites and amount of foreign materials in the seeds lot (Miller and Trenholen, 1994).

Mehrotra and Aggarwal (2005) reported that most pathogenic seed-borne hyphae progressively ramify through the protoplast cells as the cell membranes are disrupted. *A. flavus* metabolites could bring about rapid softening and necrosis of tissues. It was in addition stated that the possible mechanism of systemic fungicide include inactivation of the enzymes and toxins of the pathogen, selective accumulation of the fungicide due to greater permeability of the fungus cell wall and through damage to the membranes ofvthe fungal hyphae and inhibition of structurs, such as appresoria, cushion formation and emergence of germ tubes.

Several plant extracts have been reported to be efficient in the preservation of plant and animal products (Earnsworth, 1990) and in the treatment of various human diseases (Pamplona, 2001; Amadioha, 2000; Onifade, 2000). Nair and Arora, (1996) reported that neem kernel oil exhibited fungicidal action through the inhibition of growth of fungi.

From the findings of this study, *A. indica*, *B. perotitiaanea* and *L. multiflora*, ethanolic leaf extracts could be explored in the protection of crop seeds against pathogenic fungi. *L. multiflora* singly or in combination Blumea extract was shown to be more fungistatic than other treatment in this study. It was also observed that the effect was dose-dependent. Since it was indicated that the extracts have inhibitory effects on the growth of *A. niger* and *F. verticilloides*, their utilization as bio-control agents is imperative. The crude leaf extracts could be purified and formulated for commercial purposes in order to improve its efficacy.

REFERENCES

Amadioha A.C. 2000. Fungitoxic effect of some leaf extracts against *Rhizopus oryzae* causing tuber rot of potato. Archive of Phytopathology and plant protection. 33: 499-507.

Azher M. 2009. Seed Mycoflora of Shisham (Dalbergia Sissoo Roxb.) and their integrated management. PhD Thesis, Plant Pathology Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan. pp. 71-72.

Bandyopadhyay R, David R Butter, Arun Chandrashekar, R Kennka Raddy and shiishail S. Navi. 2000. Biology epidemology and management of sorghum grain mold in technical and institutional options for sorghum grain management. In: Chandrashekar, A., Bandyopadhyay, R., and Hall, J (Eds.). Proceeding of an international consultation, 18-19 May, 2000, ICRISAT, Patancheru, India. Patancherus 502, 324, Andra predesh, India: international crops research institution for the semi-arid tropics.

©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.

Bankole S.A. and Adebayo A. 2003. Review: Mycotoxins in food in West Africa: current situation and possibilities of controlling it. African Journal of Biotechnology. 2(9): 253-263.

Collinson E., Ohaeri G. Wadul-mian Mn kama I, Negbenbor C and Igene I. 1992. Fungi association with stored unprocessed cowpea and groundnut varieties in Borno state, Nigeria. 36(4): 338-345.

Earnsworth N.R. 1990. Bioactive compounds from plants. Ciba F/foundations symposium, No. 154. Jhon Willey and sons. pp. 2-7.

Halfon-Meiri A. and Barkai-Golan. 1990. Mycoflora involved in seed germ discolouration of popcorn and effect on seed quality. Mycopathologia. 110: 37-41.

Leslie J.F., Zeller K.A., Lamprecht S.C., Rheeder J.P. and Marasa W.F.O. 2005. Toxicity, pathogen and differentiation of five species of Fusarium from sorghum and millet. Phytopathology. 95: 275-283.

Makun H.A., Gbodi T.A, Akanya H.O, Salako A.E and Ogbadu G.H. 2009. Health implications of toxigenic fungi found in two Nigerian staples: guinea corn and rice. Afr. J. Food Sci. 3: 250-256.

Makun H.A, Gbodi T.A, Akanya H.O, Salako A.E, Ogbadu G.H and Tifin U.I. 2010. Acute toxicity and total fumonisin content of culture material of *Fusarium verticillioides* (Sacc.) Nirenberg (CABI-IMI392668) isolated from rice in Nigeria. Agric. Biol. J. N. Am. 1: 103-112.

Marassas W. F., Nelson P. E. and Toussoum K. 1995. Toxigenic *Fusarium* spp. identification and mycotoxicology. Pennsylvania State University Press, University Park.

Mehrotra R.S. and Aggarwal A. 2005. Plant Pathology. Tata McGraw-Hill Publishing Company. New Delhi, India. 2nd Edition. pp. 228-288.

Miller J.D. and H.L. Trenholen. 1994. Mycotoxins in Grain Compounds. p. 541. Eagan Press USA.

Nair N. and Arora R. 1996. Efficacy of leaf extracts of some plants on conidial germination of powdery fungi in vivo. Proc. Indian National Science Congress Society. 8: 17.

Ndem N.U.A. and F.A. Sowemimo (Eds.). 2004. Major legumes and oil seeds of Nigeria: Principles of Production and Utilization, iii. pp. 66-95.

Okoli C.A.N and Erinle I.D. 1989. Factor Responsible for Market losses of tomato fruit in zairia area of Nigeria. Journal of Horticulture Science. 64: 69-71.

Onifade A. K. 2000. Antifungi effect of *Azadirachia indica* A. Juss extraction collectotrichum lidemuthianum. Global Journal of pure and applied science. 6: 425-428.

Pamplona, R. M. D. 2001. Encyclopaedia of medicineal plants, Vol. 2, Edication and healthy library. Marpa Artes Grafices Alfjarin Zaragaza, Spain. p. 34.