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FUTA Journal of Research in Sciences

ISSN: 2315-8239

FUTA Journal of Research in Sciences, 2014 (2): 276 - 281

ACTIVITY OF THREE MEDICINAL PLANTS ON FUNGI ISOLATED FROM STORED MAIZE SEEDS (*ZEA MAYS* (L.)

***M.N. Suleiman and O.M. Omafe**
Department of Biological Sciences, Faculty of Natural Sciences
Kogi State University, P.M.B. 1008, Anyigba, Nigeria
*Corresponding author phone: 08038956612, 08050622702, E-mail: nasirums@yahoo.com

ABSTRACT
Laboratory studies were carried out to isolate, identify and control the fungi associated with stored maize seeds (*Zea mays* L.). *Aspergillus niger* and *Penicillium digitata* were isolated from samples collected from Abocho, Egume, Anyigba and Dekina, Kogi State, Nigeria. Anti-fungal activity of three medicinal plants viz: Lemon grass (*Cymbopogon citratus* Staph.), Morinda (*Morinda lucida* L.) and castor oil (*Ricinus communis* L.) on the radial mycelial growth control and inhibition of these two fungi were evaluated at 10^0, 10^{-1}, 10^{-2} and 10^{-3} concentrations. *In vitro* application of extracts for the control showed that anti-fungal activity was more on *Penicillium digitata* than on *Aspergillus niger*, especially at higher concentrations (10^0 and 10^{-1}). These extracts on *Aspergillus niger* showed progressive retardations on the vegetative growth. The inhibitory action of the two extracts on mycelial growth increased with increase in concentrations; giving toxicity profile of $10^0 > 10^{-1} > 10^{-2}$ in that order and were significant at 0.05% on LSD value.
Keywords: Maize (*Zea mays*), fungi, plant extracts, vegetative growth, concentrations.

INTRODUCTION

Maize (*Zea mays* L.) in America and India is referred to as corn which literarily means "that which sustains life" (Akinyele and Adigun, 2006). It belongs to the family Graminaceae (IITA, 2005). Maize is the third most important food crop in the world surpassed only by two other grains, wheat and rice (Kyenpia *et al*., 2009; IITA, 2005). Maize is the largest crop in the United States of America in terms of both volume and value. The U.S grew 39% of the world corn during the fiscal year of 2010 with about 331 million metric tonnes, other countries where maize is grown extensively are China, European Union, Brazil Argentina (National corn growers Association, 2010). Maize is a widely adopted crop capable of producing during the appropriate season in almost all parts of the world where farming is done (kyenpia *et al*., 2009). In Nigeria, maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L) and rice (*Oryza sativa* L.) are the major cereal grown in the sub savanna region of Nigeria (Fatima and Abdul, 2005). Corn is a versatile grain and can be processed into a multitude of food and industrial products (Corn Refiners Association Inc., 2006).

Fungi are the major cause of plant diseases and are responsible for large scale harvest failures in crops like maize and other cereals all over the world. It was equally reported that maize cultivation in the world is limited by diseases which cause grain loss of about 11% of the total production. Uzma and Shahida, (2007) reported that maize is attacked by more than sixty diseases. During storage, several kinds of fungi can remain associated to corn seeds either causing their deterioration or simply remain viable to infect germinating seeding. The fungi genera typically found in stored grains are *Aspergillus, Penicillium, Fusarium* and some xerophytic species, several of them with capabilities of producing toxins (Castellari *et al*., 2010). Seed fungi especially species of *Aspergillus, Diplodia, Penicillium, Fusarium, Trichoderma* and a

number of phycomycetes affect the seed of all forest species (Griffin, 2010). The development of these fungi can be affected by moisture content of the product (Glorin *et al.*, 2009). Temperature, storage time and degree of fungal contamination prior to storage, insect and mite activity facilitate fungi dissemination. Previous studies by Pacin *et al.*, (2009) *i*dentified *Aspergillus* and *Fusarium mycotoxigenic* species stored grains as well as their mycotoxins and fumonisins in different concentrations (Moreno *et al*., 2009).

There is a general increase in consumption of contaminated grain with mycotoxins which causes different health problems including death (Lerda *et al*., 2005, Voss *et al*., 2007). The rank of fungi is second after insects as the cause of deterioration and loss of maize (Uzma and Shahida, 2007). *Aspergillus flavus* becomes systemic and produces aflatoxin and vivesces in seedling of maize and damage stored corn. Maize is also affected by milder pathogen (Ahmed *et al*., 2006). *Fusarium* invade more than 50% of maize grain before harvest and produce mycotoxin (Usma and Shahida 2007), while *Aspergillus flavus* is a food contaminant and capable of producing aflatoxin (Charity *et al*., 2010). The losses caused by seed fungi may occur during seed development, storage or germination. Damage may result from loss of seed viability or from seedling infection following germination (Griffin, 2010).

The control of maize disease is very important as a complementary technology to boost maize production. Tagne *et al*., (2008) reported that various approaches have been used over many decades to control maize diseases. These include breeding resistance varieties of maize, chemical treatment including seed treatment and biological control. Natural products of higher plants give a new source of antimicrobial agents with possible novel mechanism of action (Kaur and Kaur, 2010). Kirun *et al*., (2010) screened seven medicinal plants for anti fungal activity against seed borne fungi of maize seeds, these medicinal plants are *Acalypha indica* L. (leaf), *Anisomeles malaberica* (leaf), *Amaranthus spinosus* L. (leaf) *Eupotorium odoratum* L. (leaf), *Psoralea corylifolia* L. (seed), *Tribulus terrestis* L. (whole plant) and *Alternanthera pungens* (whole plant). They reported that only *P. corylifolia* showed antifungal activity whereas the others did not show any activity. Uzima and Shahida (2007), reported that neem seed powder is very good biological fungicide. Neem seed powder and sodium hypochloride has no adverse effects on germination. While Tagne *et al*., (2008) worked on essential oil and plant extract as potential substitutes to synthetic fungicides in the control of fungi and concluded that essential oil and crude powder from aromatic plants are potential treatments for the control of seed borne fungi including maize. Thus, this study was designed to isolate and identify fungi responsible for seed borne disease in stored maize in four major towns, and to a large extent, systematically screen some locally available plant extracts, as natural products for their suitability and efficacies as bio – fungicides for the control of seed borne disease of maize as a single component control measure.

MATERIALS AND METHODS

Collection of Samples

Infected seeds of maize (*Zea mays*) were collected from four locations in Dekina Local Government Area, Kogi State i.e Abocho, Egume, Anyigba and Dekina, in clean, moisture free polythene bags and brought to the laboratory for further study.

Isolation and Identification of Pathogens

Two methods of isolation were used, serial dilution and direct plating. Fifty (50) seeds of maize were kept in test tube of 50ml distilled water, shaken for five minutes to get a stock solution (10^0). One millilitre of stock solution was pipette into 9ml of distilled water in a test tube to make a serial dilution of 10^{-1}, 1ml of 10^{-1} serial dilution was pipette into 9ml of distilled water in test tube gave 10^{-2} dilution. Similar method was carried out to give final concentrations of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and the stock (10^0). While in the direct isolation, fifty (50) seeds were surface sterilized with 0.1% $HgCl_2$ for 30 sections and rinsed in distilled water. They were then spaced out in the Petri dishes containing the medium (PDA). The plates were incubated at room temperature of 25 ± 1^0C for five days according to Suleiman (2011) and fungal colonies observed were sub-cultured to get pure culture. They were identified using Barnett (1960); Alexopoulos and Mims (1988) and also based on morphological observations and characteristics.

Plant Extracts Employed

Leaves of lemon grass (*Cymbopogon citrates* Staph.), castor oil (*Ricinus communis* L.) and

Morinda (*Morinda lucida* L.) were used. The fresh and matured leaves of each of the plants were collected from their various sources, thoroughly washed in running tap water in the laboratory to remove all traces of sand and were air-dried and pounded in mortar to facilitate extraction. Cold – water extraction was obtained by infusing 2g each into a 250ml conical flask containing 20ml of distilled water. They were allowed to soak for 24 hours. The crude extract obtained from the infusion by filtration through four folds of sterile cheese cloth, corked with sterile cotton wool was exposed to U/V light for further sterilization. The filtrate obtained was the stock (10^0). One millilitre of the stock was added to 9ml of distilled water to obtain 10^{-1}, similar procedure was adopted to get the various concentrations of which represents 10^0, 10^{-1}, 10^{-2}, 10^{-3}. The filtrates obtained were poured into 250ml conical flasks, covered with cotton wool and foil. Each solidified agar in Petri dishes was pipette with 2ml of each concentration and inoculated with the pathogen and together with the control, was incubated at 27 ± 2^0C. Mean and percentage inhibition of mycelia growth determined.

RESULTS

The survey to determine disease occurrence and distribution at the four locations showed that disease incidence and frequency in storage was highest in Abocho, followed by Dekina, Anyigba and least in Egume (Table 1). Two different species of fungi isolated and identified to be associated with spoilage of stored maize were *Aspergillus niger* and *Penicillium digitata*. The two methods of isolation showed similar results. The frequency of the isolated fungal incidence on 50 seeds was generally low in Egume, while the highest incidence was recorded in Abocho. The occurrence of *Aspergillus niger* in the selected location was comparatively high compare to *Penicillium digitata* (Table 1).

The results on application of plant extracts showed that the three plant extracts had fungicidal properties, with *Morinda lucida* leaf generally more effective in retarding vegetative growth than *Cymbopogon citratus* and *Ricinus communis* leaf extracts. The effects of *M. lucida* were however more on *Penicillium digitata*. *C. citratus* and *R. communis* had almost the same effects on mycelial growth increased steadily with increase in concentration (Table 2).

Table 1: Fungal Incidence on 50 Maize Seeds

Fungi	Abocho*	Egume*	Anyigba*	Dekina*
Aspergillus niger	19	08	14	16
Penicillium digitata	17	05	13	16

***Out of 50 maize seeds**

Table 2: Effects of the Extracts on *Aspergillus niger*

Concentration	Lemon grass	Castor oil	Morinda
10^0	2.35 ± 0.21^d	3.20 ± 0.00^d	2.70 ± 0.14^d
10^{-1}	3.65 ± 0.07^c	3.45 ± 0.07^c	3.70 ± 0.14^c
10^{-2}	4.05 ± 0.07^b	4.05 ± 0.07^b	3.85 ± 0.21^{bc}
10^{-3}	4.25 ± 0.07^{ab}	3.35 ± 0.07^c	4.05 ± 0.21^b
Control	4.40 ± 0.07^a	4.40 ± 0.07^a	4.40 ± 0.07^a
KSD ($P \leq 0.05$)	0.00	0.00	0.06

Values with different superscripts in the same column are significantly different ($P \leq 0.05$) using Duncan Multiple Range Test.

Table 3: Effect of extracts on *Penicillium digitata*

Concentration	Lemon grass	Castor oil plant	Morinda
10^0	1.95 ± 0.07^c	1.95 ± 0.07^e	1.10 ± 0.14^d
10^{-1}	2.85 ± 0.07^d	2.45 ± 0.07^d	2.25 ± 0.07^c
10^{-2}	3.60 ± 0.14^c	3.45 ± 0.07^c	2.85 ± 0.07^b
10^{-3}	4.00 ± 0.00^b	3.90 ± 0.14^b	3.30 ± 0.28^b
Control	4.50 ± 0.01^a	4.50 ± 0.07^a	4.50 ± 0.01^a
KSD ($P \leq 0.05$)	0.00	0.00	0.01

Values with different superscripts in the same column are significantly different ($P \leq 0.05$) in Duncan's Multiple Range Test

The *in vitro* application of these extracts on *Penicillium digitata* showed complete inhibition in *M. lucida* extract at 10^0 concentrations, with a significant difference when compared with control. *Cymbopogon citratus* and *R. communis* extracts on *P. digitata* showed progressive retardation in vegetative growth and effectiveness in vegetative growth decreased with increase in concentrations (Table 3). The LSD values showed that there was a significant difference ($p<0.05$) between *M. lucida* and other plant extracts at various concentrations tested.

DISCUSSION

Fungi have been rated second to insect as the course of deterioration and loss of maize grain (Uzma and Shahida, 2007). *Aspergillus* and *Penicillium* have also been identified in previous studies as potentially mycotoxigenic species (Lino *et al*., 2007). The results showed that the predominantly encountered species from the infected seeds were *Aspergillus niger* and *Penicillium digitata*. These fungi are known to have strains that cause toxic metabolites (Charity and Dauda, 2010). The presence of *Aspergillus* in stored maize seeds results in deterioration, discoloration and bad odour, coupled with toxins, posses potential hazard to consumer's health; a confirmation of Lino *et al*., (2007), that aflatoxin produced by *A. niger* is found in image seeds and is hazardous to human health; similar to those of Uzma and Shahida (2007), where they reported that aflatoxin produced by *A. flavus* found in cashew nuts and is hazardous to human health.

Lino *et al*., (2007) reported the considerable importance of these fungi in the deterioration of stored maize seeds. *Aspergillus* and *Penicillium* are regarded as saprophytic and parasitic fungi, their spores are present almost everywhere in the air and are often sources of contamination, loss of grain, toxin production and also resulting in house spoilage (Dutta, 2005). In the present study, *Aspergillus niger* has an upright conidiophores, simple and terminating in a globose or elevate swelling bearing conidia (1 – celled) globose often variously colonies in mass and produced basipetally. *Penicillium digitata* composed of hyaline or brightly coloured in mass (1 – celled) mostly globose or septate with conidiophores upright.

The ability of the three leaf extracts to inhibit vegetative growth of the isolated fungi varied slightly. Results showed that the three leaf extracts were effective for reducing mycelial growth in *P. digitata* than in *A. niger*. In tables 2 and 3**,** there was only a partial variation in the level of radial mycelial growth inhibition values of the fungi on *C. citratus* and that of *R. communis* leaf extract at all the concentrations. The results showed that all the extracts at different concentration arrested the vegetative growth of the fungi for the first and second day of incubation. *M. lucida* inhibited mycelial growth of the fungi in the first three days of incubation. *Ricinus communis* showed no growth at all concentrations for the first two days on *Penicillium* and on 10^0 on *Aspergillus* for the first two days. Furthermore, *Cymbopogon citratus* showed no growth on *A. niger* at concentrations of 10^0 and 10^{-1} for the first three days while *Penicillium digitata* had no growth for the first two days at concentration of 10^0 and 10^{-1}. Plates with higher concentration showed scanty growth from the fourth to eight day indicating its antifungal tendency which was in agreement with Nwachukwu *et al*. (2001).

The mean radial growth values of the two fungi on media plates with different concentration supported the percentage inhibition noticed, which was a true reflection in the water soluble antifungal element in their respective leaves. From the result, it was observed that the aqueous extracts used for the study recorded retarded or inhibited mycelial

growth of the fungi *in vitro*. The water – soluble antifungal principles in the plants were responsible for the antifungal activities. The fact that the plants used in this study are easily available, with easy method of extraction, showed that they can be exploited in the control of maize seeds spoilage in storage.

REFERENCES

Ahmed, S., Jeffers, D., Vasal, S. K., Frederikeen, R and Magil, C. (2006). A region of maize chromosome 2 effects response to downy mildew pathogens. Theoretical and Applied Genetics 113(2): 321 – 324

Akinyele, B.O. and Adigun, A.B. (2006). Soil texture and the phenotypic expression of maize (*Zea mays* L.). Research Journal of Botany (3): 136 – 143.

Alexopoulus, C.J and Mims, C.W. (1988). Introductory Mycology 3rd Edition, Willey Eastern Limited, India 632 Pp.

Barnett, H.L. (1960). Illustrated genera of Imperfect Fungi 2nd Ed. Burgess Publishing Camp 225 Pp.

Castlellarie, C., Marcos, F.V., Mutti, J., Cardoso, L and Bartosik, R. (2010). Toxigenic fungi in Corn (maize) stored in hermetic plastic bags. National Institute of Agricultural Technologies Mardel Plata University Argentina. 115 – 297 Pp.

Charity A., Amienyo, J. and Dauda, T. (2010). Effect of relative humidity on spore determination and growth of *Aspergillus flavus*. Nigerian Journal of Botany 23(1): 1 – 6.

Corn Refiners Association Inc. report (2006) 1701 Pensylvania Avenue N.W. suite also Washington D.C., 203 – 230 Pp.

Dutta, A.C. (2005). Botany for Degree students, 7th ed. Oxford University press London 563 Pp.

Fatima, B.J. and Abdul S.D. (2005). Fungi associated with maize. Nigerian Journal of Botany 18: 81 – 86

Gtorni, P., Battilani, P. and Magan N. (2009). Effect of solute and matric potential on *in – vitro* growth and sporulation of strains from anew population of *Aspergillus flavus* in Italy. Fungi Ecology 1: 101 – 106.

International Institute for Tropical Agriculture (IITA) (2005). Annual report of IITA 3: 20 – 32

Kaur, R. and Kaur, H. (2010). The antimicrobial activity of essential oil and plant extracts of wood *Florida fruticosa*. Archives of Applied Science_Research 2(1): 302 – 309.

Kiran, B., Lalith, V and Raveesha, K.A. (2010) Screening of some medicinal plants for antifungal activity against seed borne fungi of maize seeds. Africa Journal of Basic and Applied Sciences 2 (3 & 4): 99 – 103.

Kyenpia, E.O., Namo, O.A.T., Gikyu, S.W. and Ifenkwe, O.P. (2009). A comparative study of the biochemical composition of some varieties of maize (*Zea mays* L.) grown in Nigeria. Nigeria Journal of Botany 22(1); 291 – 296

Lerda, P., Blaggi, B.M., Peralta, N., Ychari, S., Vazquez, M., and Bosio, G. (2005). Fumonisins in foods from Cordoba (Argentina), presence and genotoxicity. Food and Chemical Toxicology 43: 691 – 698.

Lino, C.M., Silva, L.J., Pena, A., Fernandez, M and Manes, J. (2007). Occurrence of fumonisisns B1 and B2 in brao, typical Portuguese maize breed. International Journal of Food Microbiology 118: 79 – 82

Moreno, C. E., Tironi, G.G., Ono, M.A., Vizoni, E., Kawamara, O., Hirooka, E.Y and Sataque Ono, E.T. (2009). Co-occurrence of mycotoxins in corn samples from the northern region of Parana state, Brazil. Journal of Food Chemistry 116: 220 – 226.

National Corn Growers Association (2010). Cepi drive Chesterfield, MO 63005 USA.

Nwachukwu, E.O. and Umechurube, C.I. (2001) Antifungal activities of some leaf extracts on seed borne fungi of African yam, beans, seed germination and seedling emergence. Journal of Applied Science and Environmental Management 5: 29 – 32.

Pacin, A.M., Boviev, E.C., Gonzazez, H.L., White Church, E.M., Martinez, E.J. and Resnik, S. (2009). Fungal and fumonisums contamination in Argentina maize (*Zea mays*). Journal of

Agriculture and Food Chemistry 57: 2778 – 2781.

Suleiman, M.N. (2010). Evaluation of two plant extracts on fungi associated with biodeterioration of cashew nuts in storage. Annals of Biological Research 1(4): 41 – 44.

Suleiman, M.N. (2011). Root rot Disease of Cowpea (*Vigna unguiculata*) and its control using plant extracts and fungicides LAP Lamber Acd. Publishing, Germany 169 Pp.

Tagne, A., Fenjio, T.P., and Sonna, C. (2008). Essential oil and plant extract as potential substitute to synthentic fungicides in control of fungi Endure International conference, New Delhi, 23 Pp.

Uzma, S. and Shahida, A (2007). The screening of seven medicinal plants for artificial activity against seed borne fungi of maize seeds. Pakistan Journal of Botany 39 (1): 285 – 292.

Vess, K.A., Smith, G.W. and Haschelh, W.M. (2007). Fumonisins: Toxicokinetics mechanism of action and toxicity. Animal Feed Science and Technology 137:299 – 325