International Journal of Tropical Agriculture and Food Systems, 2(1): 130-134, 2008
www.tapasinstitute.org/journals/ijotafs

EFFECTS OF *LIPPIA MULTIFLORA* LEAF EXTRACT AND ASPERGILLUS *FLAVUS* ON GERMINATION AND VIGOUR INDICES OF *SORGHUM BICOLOR* [L] (MOENCH)

S. T. Anjorin[1], H. A. Makun[2], H. E. Ihenacho[2]
[1]Department of Crop Production, Federal University of Technology, Minna, Nigeria
[2]Department of Biochemistry, Federal University of Technology, Minna, Nigeria
Corresponding author: S. T. Anjorin; E-mail: oyindamola35@yahoo.com

ABSTRACT: *This study was therefore designed to identify seed-borne fungi of sorghum in Minna, Niger State, Nigeria and investigate the effects of Lippia leaf extracts on the germination of sorghum seeds treated with A. flavus mycelia and its metabolites. The prominent isolated fungi species were Rhizopus nigricans (23.53%), Aspergillus flavus (17.65%), A. niger (17.65%), Sporisorium sorghi (11.76%) and Neurospora sitophillus (5.88%). The A. flavus metabolites were extracted with dichloromethane and phosphoric acid (90:10 v/v). Powdered Lippia leaves were extracted with ethanol for 72 h. A set of ten sorghum seeds in three replicates were subjected to each treatment and left for 24 h before been set-up in the germination chamber. At 9 days after sowing (9 DAS), the seeds treated with combined A. flavus mycelia and their metabolites had the least germination percentage (30%), the highest rot-index (3.0) and the least germination vigor (0.99). The seeds applied with only Lippia leaf extract had the highest percentage germination of 100%, the zero rot-index and highest seedlings vigour index (5.4). Though Lippia leaf extract application on seeds reduced adverse effect of A. flavus and its metabolites on sorghum germination and promotes vigor indices, further investigation on their field trial and formulation into botanical fungicide are imperative.*

Keywords: *sorghum, aspergillus, lippia leaf extract, fungicide*

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) Family Gramineae is quantitatively the world's 5th largest most important cereal grain after wheat, maize, rice and barley (FAO, 2000). The main use of sorghum is for livestock feeds and food for human. The nutritive value is similar to maize, though have more protein and fat than maize but lower in vitamin A (Coetzee, 1995).

Fungal pathogens associated with sorghum seeds have been reported to limit production of the crop in Nigeria and worldwide. Common seed-borne fungi of sorghum include *Aspergillus flavus, Fusarium moniliforme, Claviceps sorghi* and *Biploris sorghicola* and are implicated in spoilage of seeds, reduction of seedling vigor and pre and post-emergence mortality (Mehrotra and Aggarwal, 2005). Secondary metabolites produced by these seed-borne fungi constitute health hazards to animal and man following consumption of contaminated grains (Gbodi *et al.,* 1988). Seed-borne fungi on sorghum have been controlled with various antifungal synthetic chemicals but marred with myriads of problems. This includes high cost, possibility of high toxic residue, which is unfriendly to non-target organism and the environment as well as development of resistant strain (Salako, 2004).

Lippia multiflora (L) Modenke Family Verbanaceae is commonly called lemon-scented verbena. It is a woody perennial aromatic pubescent shrub that grows up to 3 m high, has bluish green leaves, white or lilac fragrant flowers. It is found in a wide ecological range throughout Nigeria. The plant is propagated through seeds and stem cuttings and produces mature fruits in January. The leaf which is aromatic and thyme-scented is the most valued part of the plant. Currently there is the need to investigate alternative controls of seed-borne fungi that are easily affordable and environment-safe,

This study was therefore designed to identify seed-borne fungi of sorghum in Minna, Niger State, Nigeria and investigate the effects of *Lippia* leaf extracts on the germination of sorghum seeds treated with *A. flavus* mycelia and its metabolites.

MATERIALS AND METHODS

Sorghum seeds samples were obtained from Bosso, Tunga and Central Market, Minna, Niger state (Lat. 04^0 00′ and 07^0 30′ N of the Equator and Long. 11^0 30′ and 04^0 00′ E of the Greenwich

Meridian) during the wet season of September, 2007. The labeled samples were stored in a refrigerator at a mean of $4^{O}C$. Healthy *Lippia multiflora* leaves were collected from Paiko, KM 20, along Suleja road, Minna.

Seed-borne fungi isolation and identification: The seeds samples from each market were aseptically placed in Petri dishes containing an autoclaved Sabouraud Dextrose Agar (SDA), incubated in lamina hood at $28^{O}C$ and examined from 5-9 days. To identify the fungi, mycelia speck from each colony were aseptically placed on a slide, stained with lactophenol blue, covered with slips and viewed under x 40 objective lens of the microscope. The identification was accomplished using fungi catalogue in the Microbiology Department of Federal University of Technology, Minna, Nigeria and also by comparing with the existing culture maintained on sorghum seeds in the Biochemistry Departmental repository already identified by International Mycological Institute, Egham, U.K. *Aspergillus flavus* isolates were aseptically sub-cultured in other to obtain its pure culture.

***Lippia multiflora* leaf extraction:** The shade-dried leaves were crushed with mortar and pestle and blended into homogenous powder using and electric blender (National M x 319 M). Three hundred grams (300 g) of the powder was cold extracted with 900 ml of ethanol for 72 h in a conical flask. The extracts was sieved into a beaker using a cheese cloth and evaporated to dryness using Heildulph rotary evaporator at $55^{O}C$. The stock extract was kept in a beaker in a refrigerator at $4^{O}C$ until it was used.

Extraction of the Fungus metabolites: Five hundred grams of certified fungi-free sorghum seeds (Samsorg-3) obtained from National Cereal Research Institute Badeggi, Nigeria was measured into eight Buchuner flasks and added with 200 ml of distilled water mixed thoroughly and left overnight for moisture equilibration (Gbodi, 1986). This was sterilized by autoclaving at $121^{O}C$ and cooled. Fifteen grams of dissolved biomass of pure culture of *A. flavus* was inoculated into the seed samples under aseptic condition and left in the inoculation hood for 21 days to allow for massive growth of the fungus and the synthesis of the secondary metabolites*.*

The extraction of mycotoxins was gone by adding 500 ml dicloro methane (84.93g/mol) and 50 ml of 1M phosphoric acid with the maize seeds in the Buchuner flask. It was left to stand for 30 minutes and blended thoroughly. The mixture was then separated using suction pump filtering process. The extracted metabolite was concentrated in a beaker on a heating water bath at $55^{O}C$. As the solvent distilled off, the residue was placed in vial and kept in a refrigerator at $25^{O}C$ until used for treatment of sorghum seeds.

Seed germination experiment: Confirmed fungi-free sorghum seeds were used for the experiment. This was through screening of the seeds and disinfestations with sodium hypochlorate. Ten treated seeds were sown per plate. The eight treatments (Table 2) were placed in a completely randomized design (CRD) with three replicates in a germination chamber in the laboratory of the Crop Production Department. Data collected were germination % at 5, 7 and 9 days after sowing (DAS), length of hypocotyls, length of roots, root indices and seedling vigor index. The vigor index was calculated by modified Randahawa (1985) method given by the formula:

S. V. = (HL + RL). %G

Where S.V = Seedling Vigor Index

HL = Hypocotyl length

RL = Root length

% G = % germinated

Data analysis: The seedling vigor index was subjected to Analysis of Variance and their means separated with Duncan Multiple Range Test (DMRT).

RESULTS

Table 1 shows the incidence and percentage occurrence of fungi colonies isolated from sorghum seed samples from three markets in Minna, Niger State. Seed samples from Central markets had the highest incidence of fungi species (41.18%), while the least was from Tunga market (23.53%). *Aspergillus* spp. had the highest percentage occurrence (41.18%), while *Neurospora* spp. had the least (5.88%). Table 2 shows the effects of the various treatments on germination, hypocotyls and root length. It was observed that the adverse effect of *Aspergillus* mycelia was less than that of its metabolite contamination.

Table 1: Incidence of Fungi in Sorghum Seeds Samples Collected From Three Markets in Minna, Niger State

Fungus	Incidence			Total Incidence	Rel. frequency (specie)	Rel. frequency (genera)
	Bosso market	Central market	Tunga market			
Aspergillus flavus	2/6	-	1/4	3/17	17.65	41.18
Aspergillus niger	1/6	2/7	-	3/17	17.65	
Aspergillus terreius	-	1/7	-	1/17	5.88	
Fusarium tricintum	-	1/7	2/4	3/17	17.65	17.65
Neurospora sitophillus	1/6	-	-	1/17	5.88	5.88
Rhizopus nigricans	1/6	2/7	1/4	4/17	23.53	23.53
Sporisorium sorghi	1/6	1/7	-	2/17	11.76	11.76
Total incidence (%)	6(35.29)	7(41.18)	4(23.53)	17	100	100

At 9 DAS, the germination percentage of the *Aspergillus* infected-seeds applied with *Lippia* extracts increased by 30 %. Also seeds applied with *Lippia* extract alone had 100% germination just like the seeds in the control plate applied with the distilled water. The highest hypocotyl and root lengths (3.8 cm and 1.6 cm) were also observed under *Lippia* treatment. This indicated that *Lippia* application was not phytotoxic. The germination percentage of the seeds applied with the metabolites combined with *Lippia* extracts also increased by 20% over the seeds applied with metabolite alone.

Table 2: Effects of *Aspergillus flavus,* its metabolites and *Lippia* leaf extract on germination of sorghum seeds, their hypocotyl and root length

Treatment	% germination			Hypocotyl length (cm)			Root length (cm)		
	5 DAS	7DAS	9DAS	5DAS	7DAS	9DAS	5DAS	7DAS	9DAS
Asp.* mycelia	20	30	40	2.0	2.5	3.0	0.7	0.9	1.1
Asp. met.**	10	20	30	2.0	2.4	2.9	0.6	1.0	1.2
Asp. mycelia +Asp. met.	10	20	30	1.4	1.8	2.1	0.5	0.8	1.2
Asp. mycelia+ Lippia extract	40	60	70	2.3	2.7	3.1	0.9	1.0	1.1
Asp. met. + Lippia extract	30	40	50	2.1	2.4	3.1	0.8	1.1	1.2
Asp. mycelia +Asp. met.+ Lippia extract	30	40	60	1.9	2.2	2.8	0.8	1.0	1.3
Lippia extract	40	70	100	2.9	3.2	3.8	0.9	1.3	1.6
Control (distilled water)	50	80	100	2.8	3.3	3.7	0.9	1.25	1.5

**Aspergillus flavus; ** metabolite*

On the 9 DAS, seeds applied with fungi metabolites alone or combined with *Aspergillus* mycelia had the highest seedling mortality (30%) while the seeds applied with neem extract alone had no seedling mortality. At 5 and 7 DAS, the seeds applied with *Aspergillus* mycelia only had the highest rot-indices. This was closely followed by the seeds treated with the combined *Aspergillus* and its metabolites. This indicated that more rottenness might result from mycelia infection than from its metabolites.

The rot-index of seeds applied with *Lippia* extract or distilled water was zero. On the 9 DAS, the seeds applied with *Aspergillus* mycelia and combined *Aspergillus* and its metabolites had the

highest and the same rot index of 3.0. This might be due to synergist interaction of the pathogenic *Aspergillus* mycelia and toxic metabolite.
Table 3 showed the seedling vigor of seeds treated with *A. flavus,* its metabolites and *Lippia* extracts. At 5 DAS, the highest vigor index (1.86) was observed from the seeds in the control plate and was significantly higher than the seeds applied with *Lippia* extract (1.48). On the 7 DAS and 9 DAS, those seeds treated with *Lippia* leaf extracts, showed the highest vigor index. The least vigor index was from those treated with *A. flavus* mycelia and its metabolites.

Table 3: Effects of *A. flavus,* its metabolites and neem leaf extract on % seedling vigor rot and vigor indices

Treatment	%Seedling mortality		Rot-index			Vigour index (x 100)		
	7 DAS	9 DAS	5 DAS	7 DAS	9 DAS	5 DAS	7 DAS	9 DAS
*Asp.** mycelia	10	20	2.5	2.5	2.5	0.50^{d} *	1.32^{e}	2.05^{d}
Asp. met.**	20	30	2.0	2.0	2.0	0.26^{e}	1.08^{f}	1.64^{e}
Asp. mycelia +*Asp.* met.	30	30	2.0	2.5	3.0	0.19^{e}	0.52^{g}	0.99^{f}
Asp. mycelia+ *Lippia* extract	20	20	1.0	1.0	1.5	1.28^{b}	2.22^{c}	2.94^{b}
Asp. met. + *Lippia* extract	20	20	1.0	1.0	1.3	1.16^{bc}	1.75^{d}	2.58^{bc}
Asp. mycelia +*Asp.* met.+ *Lippia* extract	10	20	1.0	1.0	1.3	0.81^{c}	1.60^{de}	2.46^{c}
Lippia extract	0	0	0	0	0	1.48^{b}	3.15^{b}	5.40^{a}
Control (distilled water)	0	10	0	0	1.0	1.86^{a}	3.92^{a}	5.20^{a}

*Column means followed by common letter(s) are not significantly different P ≥ 0.05 by DMRT ** *A. flavus; *** metabolite*

DISCUSSION

The identified moulds on sorghum seeds are in line with the report of Marassas *et al.* (1995) that *Aspergillus, Fusarium and Penicillium* species are considered to be the most significant toxigenic moulds of cereal crops. 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).
It has been found in this study that *A. flavus* singly or in combination with its metabolites reduces germination percentage and seedling vigor of sorghum seeds. 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. This might be due to presence of phytotoxic oxalic acid present in the metabolites that is capape of causing necrosis (Hussain and Janardhanan, 1976).
The increase in germination of the mycelia-inoculated seeds applied with the *Lippia* extracts might be due to fungitoxicity of the extract or their stimulatory effects that enhance germination of seeds. *Lippia* extracts might be antidotal to *Lippia* metabolites thus reducing its toxicity. Mwangi, *et al.,* (1991) reported *Lippia* water extract to be locally used as an insect repellant against maize weevil and also on aphids and mites and found out that the total phenolic extract contains several bioactive constituents such as carvacrol, linalool, flavonoids, pulegone 1, 2 epoxide, and saponins glycosides, which have antifungal property.

CONCLUSION

Most of the sorghum seeds sold in Minna markets were internally borne with moulds. Seeds or grains for sale should be inspected periodically and certified to be mould free by an appropriate agency. The presence of *A. flavus* singly or in combination with its metabolites in maize seeds reduced germination percentage and seedling vigor. The adverse effect of *A. flavus* mycelia was less severe on germination than its metabolites. It was further observed that *Lippia* ethanolic extract applied to sorghum seeds infected with the fungus or its metabolites witnessed improved germination percentage and seedling vigor. *Lippia* leaf is easily renewable than other parts and than synthetic fungicides and their extracts proved to be fungitoxic to *A. flavus.* Further investigation on the mode of production of mycotoxin by *Aspergillus* spp*.* in its host and mode of action of the extract on the fungus and the aflatoxin on germination and growth of cereal crops on the field are necessary.

ACKNOWLEDGEMENTS

We wish to express our profound gratitude to Mal. I. Kudu, Department of Microbiology and Mrs. F. Fadipe, Department of Chemistry of Federal University of Technology, Minna, Nigeria for their technical assistance.

REFERENCES

Anastasiah, N., Ngigi, P. and Ndalut, P. K. (2001). Evaluation of natural products as possible alternatives to methyl bromide in soil fumigations. Department of Chemistry, Moi University. Eldoret, Kenya. mba,org*/altmet00/69ndalut.pdf.*

Coetzee, J. J. (1995). Sorghum: A traditional crop in Africa. Africa Crop info. Vegetable and Ornamental Plant Institute and the Grain Crops Institute, Agricultural Research Council, Pretoria.

Christensen, C. M. and Kaufnaiann, P. (1974). Microflora. In: Christensen, C. M. (ed.); *Storage of cereal grains and their products.* 2^{nd}. St. Paul Minn. Pp: 549.

FAO (2000). National year book production. Food and Agricultural Organization, Rome, Italy.

Gbodi, T. A. (1988). The mycoflora and mycotoxins found in maize and cotton seed in Plateau State of Nigeria. PhD Thesis, Ahmadu Bello University, Zaria, Nigeria.

Hussain, A. and Janardhanan, K. K. (1976). Role of toxins in plant diseases. In: P. K. K. Nair (Ed.); *Glimpses in plant research,* Vikas, New Delhi. Pp: 148-186.

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,* Second Edition. Tata McGraw-Hill Publishing Company, New Delhi.

Miller, J. D. and Trenholen, H. L. (1994). Mycotoxins in grain compounds. Eagan Press. USA

Moss, M. O. and Smith, J. E. (1995). *Mycotoxins: Formulation, analysis and significance.* John Willey and Sons, Chichester, Britain.

Mwangi, J., Addae Mensah, W. and Muriuki, L. K. (1991). Essential oils of *Lippia* species in Kenya. Maize weevil (*Sitophilus zeamais*) repelling and larvicidal activity. *Int. Journal of Crude Drug Research,* 6: 342 - 356.

Randahawa, H. S. Sharma, H. L., Kaur, J. and Dahiwal, A. S. (1985). Effect of fungicide on seed germination and seed mycoflora of wheat under different storage conditions. *Pesticides,* 2: 36-38.

Salako, E. A. (2002). Plant protection for the resource-poor farmers. A keynote Address at Nigerian Society for Plant Protection, 30th Annual Conference, Sept. 1st – 4th, 2002, Abeokuta, Nigeria.