SMU
Sikkim Manipal University
Manipal
INSPIRED BY LIFE
SMU Medical Journal
ISSN : 2349 – 1604 (Volume – 1, No. 2, July 2014) Research Article

Antifungal Effects of Crude Extracts of *Moringa oleifera* on Aspergillus niger v. tieghem Associated with Post Harvest Rot of Onion Bulb.

Arowora K.A and Adetunji C.O*
Department of Microbiology and Biotechnology, Nigerian Stored Product Research Institute, Km 3 Asa dam road, P.M.B. 1489, Ilorin, Kwara state, Nigeria.

*Corresponding author
E-mail :charliguitar@yahoo.com
Telephone:+2348039120079

Manuscript received : 07.05.2014
Manuscript accepted: 17.06.2014

Abstract

This studies was carried out to isolate, identify and establish the pathogenicity of fungi associated with post-harvest rot diseases of onion bulb, a crop which is highly perishable and seasonal in Nigeria, and to determine the effect of various concentrations of crude extracts from the stem and leaves of *Moriga olifera* *in vitro* on the causative agents . Fungi associated with onion bulb rots were isolated on Potato Dextrose Agar medium and their pathogenicity was established by testing for their ability to induce rot in healthy onion bulbs. Ethanol extracts of leaves and stems of *Moriga olifera* were then screened for the potential to control a strain of *Aspergillus niger* v. Tieghem which had the highest frequency of occurrence on onion bulbs. Plate and broth assays were used at 12.5%, 25%, 50% and 75% concentrations

of extract in Potato Dextrose Agar and Potato Dextrose Broth respectively. The stem extracts inhibited the growth of the fungus at all concentrations tested. A progressive inhibition with increasing concentration of the stem extract was observed on both plates and broth cultures. On the other hand, lower concentrations (12.5%, 25%, 50%.) of leaf extract did not inhibit fungal growth on agar plates. Some level of inhibition was however observed at 75% concentration of leave extract. The reality of using antifungal plants to control most especially from *moringa olifera* is therefore highly recommended as bio protective agent on onion rot which serves as good option to chemical control.

Keywords*: *Aspergillus niger v. Tieghem, biocontrol, onions, postharvest disease, ***Moringa olifera.***

Introduction

The onion (*Allium cepa* L.) is an important vegetable crop in Nigeria. It is an important part of the diet in most homes and is of gross economic value to farmers. After harvest, onions are usually stored for one to five months to ensure a continual supply during seasons when fresh produce is not available. Bulb rots are a common cause of onion loss during storage. According to Dongondaji *et al*. (2005), storage rots reduce the quantity and quality of onion and these affect the market value. Black mould of onions is caused by the fungus *Aspergillus niger van Tieghem*. It is a high-temperature fungus, with an optimum temperature range for growth of 28-34°C.

Warmth and moisture favour development of the disease (Maude *et al*., 1984). Although the disease can occasionally be seen in the field at harvest, black mould is primarily a postharvest disorder and can cause extensive losses in storage under tropical conditions (Thamizharasi & Narasimham 1992).

Post-harvest diseases account to about 50 % losses in fruits stored in poor storage conditions especially under high humidity. They are posing a major problem to the agriculture industry (Agrios, 2005). Synthetic fungicides, such as, thiabendazole, imazalil and sodium ortho-phenyl phonate (Poppe *et al*., 2003) has been used traditionally to control the postharvest diseases, but their excessive use complemented with high costs, residues in plants, and development of resistance, has left a negative effect on human health and the environment (Paster and

Bullerman, 1988, Bull *et al*., 1997). Consumers dislike the use of chemical preservatives in their food, and with some there is an associated public health risk. This has increased the pressure for these chemicals to be removed from food and for the adoption of more 'natural' means of preservation. While there is a variety of approaches to using natural preservatives, the most often adopted approach to date has been to use biocontrol.

The use of biocontrol agents (Janisiewicz and Korsten, 2002), irradiation and other physical treatments (Nigro *et al*., 2002), natural antimicrobial substances (Ippolito and Nigro, 2003). A promising alternative to fungicides for managing postharvest diseases of fruits in a wide range of crops is plant extracts.

Plant extracts are eco friendly, accessible to rural dwellers, cost effective and non or less phytotoxic. Plant extracts have been successfully used to control a number of plant diseases (Okigbo, 2009).

Moringa oleifera is considered one of the world's most useful trees, as almost every part of the tree can be used for food or has some other beneficiary property. Moringa is a special food for the tropics, because the tree is in full leaf at the end of scarce (Iwu, 1993). It is available all year round. Almost all parts are used as food and forage for livestock (Ram, 1994). The part (leaves, fruits, flowers and immature pods) are edible and form part of traditional diet in many countries of the tropics and subtropics (Odee, 1998).

This research is therefore focused on the efficacy of *Moringa oleifera* plant extracts, through ethanol extraction on post harvest rot of onion bulb.

Materials and Methods

Collection of plant materials

Fresh leaves of *Moringa oleifera* were collected from the compound of Nigeria Stored Product Research Institute, Ilorin, Kwara State, Nigeria. The plants was identified at the Herbarium unit of the Department of Plant Biology, University of Ilorin. The plants were dried in the sun until the moisture content was reduced to a level of 10 %. The plant was then pounded in a mortar, and further ground into a fine powder of about 80 mesh using a clean electric blender and stored at 37^{o}C in polythene bags until use.

Preparation of Plant Materials and Plant Extracts

The plant leaves was sundried to constant weight and ground into small pieces in a

mortar and pulverized into powder with a blender. The prepared plant leaves was stored in polyethylene bag at 28± 2°C. Ethanol extractions were carried out as described by Adetunji *et al*., 2011. Fifty grams of the finely ground powder was introduce into conical flask and 200ml of absolute ethanol was added to the conical flask containing grounded *Moringa oleifera*. After 48hrs, the extract was decanted and passed through a muslin cloth and later filtered with a Whatman No.1 filter paper (110mm). The filtrate obtained was evaporated to dryness at 45°C, and the residue obtained were reconstituted in 95% ethanol as stock concentration of 250mg/ml.

Isolation and identification of rot inducing fungi

To isolate the pathogens responsible for the rots on the affected onion bulbs, the bulbs were stripped of their outer dry scales and surface sterilized in 1% Sodium hypochlorite solution for 60 sec.(Dimka and Onuegbu, 2010) These were then rinsed in three successive changes of sterile distilled water and blotted dry with sterile filter paper. Small segments of tissues ($3mm^3$) from the margins of rotted lesions were cut out with a sterile scalpel and plated on potato dex-trose agar in 90mm Petri – dishes. The plates were incubated at room temperature (28 ± 3℃) for 7 days. Developing fungal colonies were sub – cultured continuously on fresh PDA plates to obtain pure cultures of the isolates. Fungal isolates were identified based on cultural and morphological characteristics (Barnett and Hunter, 1998).

Pathogenicity test

Pathogenicity of the isolated fungi was established by testing for their ability to induce rot in healthy onion bulbs. The bulbs were stripped of their outer scales. The inner tissue was swabbed with cotton wool soaked in 1% Mercuric chloride and then washed twice in running tap water. Holes were dug in the bulbs by using 5mm diameter cork- borer and the plug was pulled and exchanged with 3mm diameter mycelial disc of each of the isolated fungi by placing it at the bottom of the hole to compensate for the thickness of the mycelial disc introduced into the hole. The plug was care-fully placed and the wounded area sealed with Vaseline to prevent extraneous infection. Inoculated bulbs were incubated for 4 weeks at 30℃. Three replications were prepared for each treatment. Control consisted of sterilized 3 mm PDA disc placed in the holes of the healthy bulbs. Inoculated onion bulbs were subsequently observed for rot development. The degree of Pathogenicity of each fungus was determined by measuring the extent of rot (mm) on the infected bulbs.

Plate assay

The leaf extracts of *Moringa oleifera* was incorporated into Potatoes Dextrose Agar at the following concentrations: 12.5%, 25%, 50% and 75%. The plates were allowed to solidify, inoculated with mycelia discs 9mm disc per plate) of *Aspergillus niger v. Tieghem* and incubated at 28 ± 2 ^{o}C for 7days inside a laboratory incubator (Model no DNP-9022A made by Gulfex Medical and Scientific England) .The biocontrol activities were assessed using the radial growth of the pathogen.

Broth assay

Crude extract of *Moringa oleifera* leaf was incorporated into Potato Dextrose Broth at 12.5%, 25%, 50% and 75% concentrations in conical flasks. The flasks were then inoculated with mycelial discs (four 5mm discs per flask) of *Aspergillus niger v. Tieghem* and incubated at 28 ± 2 ^{o}C for 7days. Mycelia were then harvested and the growth of pathogen estimated by the mycelial dry weight method.

Results

The most frequently occurring fungi from the onion rot samples has shown to be *Aspergillus niger* v. *Tieghem, Aspegillus flavus, Aspergillus fumigates, Rhizopus stolonifer, Sclerotium sp, Alternaria sp, Collectotrichum sp, Fusarium oxysporium* in the following increasing order as shown in Table 1.

The pathogenicity test revealed that *Aspegillus flavus v. Tieghem* shown the highest level of pathogenicity while *Sclerotium sp.* shown the lowest level of pathogenicity among all the isolate inoculated into the onion bulb with the following diameter 75mm and 6mm respectively(Table 2).

Effect different concentrations of *Moringa olifera* leaves extract on growth of *Aspergillus niger v. Tieghem* in Potato Dextrose Broth varies from 0%,12.5%,25%,50% and75% (Table 3). The 0% concentration had the highest when the weight of the dry mycelia was determine with 20.34g while the 75% concentration had the lowest when the weight of the dry mycelia was determine with 0.98g.

Table 1: Frequency of occurrence of fungi isolated from rotting of onion bulb.

Name of Isolate	% frequency of occurrence
Fusarium oxysporium	3.41
Collectotrichum sp	5.68
Sclerotium sp	11.36
Alternaria sp	5.68
Aspergillus niger v. *Tieghem*	34.09
Aspegillus flavus	17.05
Aspergillus fumigatus,	11.36
Rhizopus stolonifer	11.36

Table 2: Pathogenicity of the isolated fungi on onion bulbs.

Name of Isolate	Diameter of rot (mm)
Fusarium oxyporium	23
Collectotrichum	12
Sclerotium	6
Alternaria	8
Aspergillus niger	40
Aspegillus flavus v. Tieghem	75
Aspergillus fumigates,	32
Rizopus stolonifer	34

Table 3: Effect of varying concentrations of *Moringa olifera* leaves extract on growth of *Aspergillus niger v. Tieghem* in Potato Dextrose Agar.

S/N	Concentration	Inhibition
1	0%	+
2	12.5%	+
3	25%	+
4	50%	+
5	75%	–

+ Growth –No growth

Table 4: Effect of varying concentrations of *Moringa olifera* leaves extract on growth of *Aspergillus niger v. Tieghem* in Potato Dextrose Broth.

Concentration of extract	**Mycelia dry weight(g)**
0%	20.34
12.5%	16.22
25%	14.54
50%	5.65
75%	0.98

Discussion

Adverse effects of chemical pesticides on environment and human health are burning issues

and there is a need to search for new fungicides with improved performance as well as ecofriendly

in nature. Hence, some extracts of ethnomedicinally important higher plant species were tested for their antifungal activity. Such plant products would be biodegradable and safe to human health (Mohanlall and Odhav, 2006). Antifungal effectiveness of some tropical plants extracts in controlling several plant pathogens has been reported by several workers (Okigbo and Emoghene, 2004; Tewari and Nayak, 1991, Amadioha, 2000; Okigbo and Nmeka, 2005; Amadioha and Obi, 1999; Okigbo and Ikediugwu, 2000).

Shehu and Muhamad (2011) made a similar observation on occurrence of *A. niger* on onions in Sokoto, Nigeria. The plate assays showed that the leaf extract of *Moringa olifera* inhibited the growth of *A .niger v. Tieghem* at all concentrations tested while leaf extracts at low concentrations showed no appreciable inhibition of growth Table 4. The 75% leaf extract however had some inhibitory effect (Table 1). A progressive inhibition was observed with increasing concentration of the extract.

Conclusion

This investigation has shown that the sterilized extracts of *Mornga olifera* exhibited fungi toxicity against inoculums production of *Aspergillus niger van Tieghem,* causing serious onion rot in green house, field at harvest, in transit and market conditions. The highest concentrations, of the leaves extract inhibited radial growth and sclerotial formation of *Aspergillus niger van Tieghem* ,it may be interesting to use them under field conditions for integrated management of plant pathogenic fungi . On the whole ,*Moringa olifera* leave extracts have the potential for use in controlling black mould of onion bulb. The extract of the test plant can serve as alternative to chemical control because of lack of residual effect and can be applied cheaply.

References

1.Agrios, G.N.(2005). *Plant Pathology*, Academic Press, New York.

2.Amadioha, A. C and Obi, V.I (1999). Control of anthracnoss disease of cowpea by *Cymbopogon citratus* and *Ocimum gratissimum*. Acta Phytopathol. Entomol. Hungeria. 34 (92): 85 -89.

3.Amadioha, A.C. (2000). Fungitoxic effect of some leaf extracts against *Rhizopus oryzae* causing tuber rot of potato. Arch. Phytioathol. PFlan., 0; 1 – 9.

4.Bull, C.T, Stack, J.P. and Smilanick, J.L (1997). *Pseudomonas syringae* strains ESC-10 and ESC-11 survive in wound on citrus and control green and blue molds of citrus. Biol. Contr., 8: 81-88.

5.Dogondaji, S.D., Baba, K.M, Muhammad, I. & Magaji, M.D. (2005): Evaluation of onion storage losses and implication for food security in Sokoto Metropolis. Bulletin of Science Association of Nigeria., 26: 10 – 14.

6.Ippolito A & Nigro F (2003). Natural antimicrobials in postharvest storage of fresh fruit and vegetables. S. Roller (ed.), Natural Antimicrobials for the Minimal Processing of Foods. CRC Press, Boca Raton, DC, USA.

7.Iwu, M. (1993). Handbook of African Medical Plants. CRC press Florida, pp: 256-257.

8.Okigbo, R.N & Emoghene, A.O (2004). Antifungal activity of leaf extract of some plant species on *Mycosphaerella fijiensis* morelet, the causal organism of black sigatoka disease of banana *(Musa acuminata*). KMITL sci. J., 4: 20 – 31.

9.Odee, D. (1998). Forest biotechnology research in dry lands of Kenya. The development of *Moringa* species. Dryland Biodiversity., 2: 7-12.

10.Ram, J. (1994). Moringa: A Highly Nutritious Vegetable Tree. Tropical Rural and Island. A Toll Development, Experimental Station (TRIADES) Technical Bulletin No. 2.

11.Maude, R.B., Taylor, J.D., Munasinge, H.L., Bambridge, J.M. & Spencer, A. (1984). Storage rots of onions. National Vegetable Research Station, Annual Report 1984. Wellesbourne, Warwick. Pp. 64-66.

12.Thamizharasi, V. & Narasimham, P. (1992). Growth of *Aspergillus niger* on onion bulbs and its control by heat and sulphur dioxide treatments. Trop. Sci., 33: 45-55.

13.Janisiewicz, W.J. & Korsten, L. (2002). Biological control of postharvest diseases of fruits. Annu. Rev. Phytopathol., 40: 411-441.

14.Mohanlall, V. & Odhav, B. (2006). Biocontrol of aflatoxins B**1**, B**2**, G**1**, G**2** and fumonisin B**1** with 6,7-dimethoxycoumarin, a phytoalwxin from *Citrus sinensis*. J. Food Protect., 69: 2224-2229.

15.Nigro, F. Ippolito, A. Lattanzio, V. Di Venere, D. & Salerno, M. (2002). Effect of ultraviolet-C light on postharvest decay of strawberry. J. Plant Pathol., 82: 29-37.

16.Okigbo, R.N. & Ikediugwu, F.E.O. (2000). Studies on biological control of post harvest rot of yam (*Dioscorea* spp) with *Trichoderma viride*. J. Phytopathol., 148: 351 – 355.

17.Okigbo, R.N & Emoghene, A.O (2004). Antifungal activity of leaf extract of some plant species on *Mycosphaerella fijiensis* morelet, the causal organism of black sigatoka disease of banana *(Musa acuminata*). KMITL sci. J., 4: 20 – 31.

18.Okigbo, R.N. & Nmeka, I.A. (2005). Control of yam tubers with leaf extract of *Xylopia aethiopica* and *Zingber Officinale* . Afr. J. Biotechnol., 4 (8): 804 – 807.

19.Okigbo, R.N. (2009). Variation in phytochemical properties of selected fungicidal aqueous extract of some plant leaves in Kogi State, Nigeria. A*merican-Eurasian* J. of Sust. Agric., 3 (3): 407-409.

20.Paster, N. & Bullerman, L. B. (1988). Mould spoilage and mycotoxin formation in grains as controlled by physical means. *International Journal of Food Microbiology.*, 7, 257−265.

21.Poppe, L. Vanhoutte, S. & Höfte, M. (2003). Modes of action of *Pantoea agglomerans* CPA-2, an antagonist of postharvest pathogens on fruits. Eur. J. Plant Pathol., 109: 963-973.

22.Shehu K. & Muhammad V. (2011) .Fungi Associated with Storage Rots of Onion Bulbs in Sokoto, Nigeria. International Journal of modern Botany., 1(1):1-3.

23.Tewari, S.N & Nayak, K .(1999). Activity of four plant leaf extracts against three fungal pathogens of rice. Trop. Agric. (Trinidad)., 68: 373 – 375.

Authors Column

Dr. C.O. Adetunji **is a senior research scientist at the Department of Microbiology and Biotechnology of Nigerian Stored Products Research Institute, Ilorin, Nigeria. He has published many articles in peer reviewed reputable journals both at national and international levels. He is affiliated with many scientific societies.**

Dr. Adetunji received scientific awards like TWAS, CSIR etc. Presently he is involved in the screening of medicinal plant bioproducts and their antimicrobial effects. He has formulated various edible coatings containing antimicrobials compounds that can extend the shelf life of fruits and vegetables.