In- vitro Effects of Crude extracts of *Jatropha curcas* on *Aspergillus niger* v. Tieghem Associated with Post harvest rot of Onion bulb.

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Abstract

In a bid to enhance storability of highly perishable and seasonal onion bulbs in Nigeria, a study was conducted to control post harvest spoilage of onions using crude extracts of *Jatropha curcas* mainly from stem and leaves. 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 *J. curcas* 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 0%, 10%, 20%. 30% and 40% 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 (10%, 20%, and 30 %) of leaf extract did not inhibit fungal growth on agar plates. Some level of inhibition was however observed at 40% concentration of leave extract. The phytochemical screening of the ethanolic extracts of Jatropha stem and leaves extracts showed that they contain high levels of saponin, tannins, phenolics, steroids and glucosides but contain little amounts of phylobatinin. The stem extracts had alkaloids in addition to other chemical constituents. It was concluded that the stem extract have the potential for use in controlling black mould of onion bulb.

Key words: *Jatropha curcas, Aspergillus niger,* crude extract, post harvest, onion bulb

Introduction

Fungi are important pathogens of fruits and vegetables particularly under tropical and sub-tropical conditions (Adebayo and Diyaolu, 2003). They are major causal agents of post harvest losses. Commonly used chemical control methods have been associated with many problems over the years. Many alternative control methods have thus been investigated. These include 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. 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. Infected bulbs may also be contaminated by mycotoxins produced by the pathogens (Muhammad *et al.*, 2004). Bulb rot is caused by a number of microorganisms. Among them, fungi are the major causal agents responsible for storage losses (Currah and Proctor, 1990; Padule *et al.*, 1996). Black mould is primarily a postharvest disorder caused by *Aspergillus niger* and can cause extensive losses in storage under tropical conditions (Thamizharasi & Narasimham, 1992). Black mould causes onion tissues to become water soaked which often induces bacterial soft rot (Agblor and Waterer, 2001). Control of the black mould of onion is therefore essential. This paper investigates the possibility of using extracts of *J. curcas* for the control of black mould of onion.

MATERIALS AND METHODS

Collection of plant materials

Fresh Stems and leaves of *Jatropha curcas* 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 plants were sundried to constant weight and ground into small pieces in a mortar and pulverized into powder with a blender. The prepared plant parts were stored in polyethylene bags at 28$\pm$ 2^{o}C. Ethanol extractions were carried out as described by Igbinosa(2009). 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 *Jatropha cacus* . 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^{o}C, and the residue obtained were reconstituted in 95% ethanol as stock concentration of 250mg/ml.

Isolation and identification of rot inducing fungi

Fungi associated with onion bulbs were isolated by direct plating method of diseased parts on Potato Dextrose Agar plates as described by Dimka and Onuegbu, 2010. Fungal isolates were identified based on cultural and morphological characteristics (Barnet and Hunter, 1998).

Pathogenecity test: Pathogenicity of the isolated fungi was established using the method described by Shehu and Muhammad (2011).

Plate assay: Stem and leave extracts were incorporated into Potatoes Dextrose Agar at the following concentrations: 0%, 10%, 20%, 30% and 40%. The plates were allowed to solidify, inoculated with mycelia discs 9mm disc per plate) of *Aspergillus niger* and incubated at 28 $\pm$ 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 extracts of Jatropha stem were incorporated into Potato Dextrose Broth at 0%,10%,20%,30% and 40% concentrations in conical flasks. The flasks were then inoculated with mycelial discs (four 5mm discs per flask) of *Aspergillus niger* and

incubated at 28 ± 2 °C for 7days. Mycelia were then harvested and the growth of pathogen estimated by the mycelial dry weight method.

Phytochemical Screening of Jatropha Extracts Phytochemical screening of stem and leave extracts of *J. curcas* for plant constituents such as alkaloids, tannins, saponins, phenolics, phlobatannis, flavonoids and glycosides was carried out using the methods described by Odebiyi and Sofowora (1978).

Results and Discussion

A variety of fungi were isolated from rotting onion bulb.These include *Colletotrichum* sp, *Alternaria* sp, *Rhizopus* sp and *Aspergillus* spp. *Aspergillus niger* occurred more frequently (34.09%) than others while *Fusarium oxyporium* had the least percent occurrence (3.41%).

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	5.6 8
Sclerotium sp	11.36
Alternaria sp	5.68
Aspergillus niger	34.09
Aspegillus flavus	17.05
Aspergillus fumigatus, Rhizopus stolonifer	11.36
	11.36

Shehu and Muhamad (2011) made a similar observation on occurrence of *A. niger* on onions in Sokoto, Nigeria. The plate assays showed that the stem extracts of *J. carcas* inhibited the growth of *A .niger* at all concentrations tested while leaf extracts at low concentrations showed no appreciable inhibition of growth. The 40% leaf extract however had some inhibitory effect (Table 1). Ogbebor and Adekunle (2008) reported that leaf extracts of Jatropha increased the diameter of *Dreschslera heveae* on agar plates. Some other workers have reported the effectiveness of *Jatropha curcas* leaf extracts in inhibiting the mycelial growth of *Colletotrichum musae.* (Thangavelu *et al.*, 2004). They stated that the extracts were able to control the anthracnose disease in three banana varieties: 'Robusta', 'Rasthali' and 'Ney Poovan'. All concentrations of stem extract tested reduced the mycelia growth of *A. niger* in Potato Dextrose Broth medium. A progressive inhibition was observed with increasing concentration of the extract (Fig. 1). According to Igbinosa *et al.* (2009), *J. curcas* stem bark has the ability to inhibit a wide range of bacteria and fungi in culture.

Table 2: Plate assay for biocontrol activities of *Jatropha carcus* extracts against *Aspergillus niger* on Potato Dextrose Agar

Concentration of extract	Leaf extract	Stem extract
0%	++++	++++
10%	++++	++
20%	++++	+
30%	+++	+
40%	+	–

+ = Level of growth of fungal pathogen

- = No appreciable growth

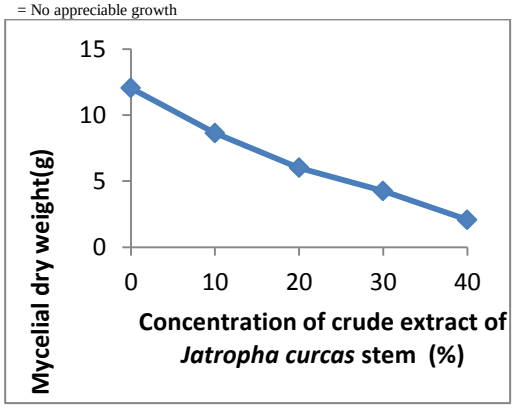

Fig. 1: Effect of varying concentrations of *Jatropha curcas* stem extract on growth of

Aspergillus niger in Potato Dextrose Broth.The ethanolic extracts of the Jatropha leaves and stems used in this study were rich in phenolic compounds; tannins, saponins and glycosides. Alkaloids were not detected in the extract of the leaf but were present in the stem. (Table 3). This probably explains the potency of stem extracts compared to the leave extracts. The presence of these phytochemicals is indicative of potential for antifungal activity (Odebiyi and sofowora, 1978).

Table 3: Phytochemical attributes of the ethanolic extract of the *J. carcus*

Samples	Leaves	Stem
Saponins	++	+++
Cardiac glycosides	++	++
Tannins	++	+++
Steroids	++	+
Alkaloids	-	+
Phlobatinins	+	+
Phenolics	++	+++

Key: - = absent; +present at low level ; ++ present at fairly high level; +++present at very high Level.

Conclusion: Jatropha stem extracts have the potential for use in controlling black mould of onion bulb.

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