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Growth Studies of *Aspergillus niger (Van Tieghem)* inciting storage Rot of Onions and its Control using Different Concentrations of Neem (*Azadirachita indica*) parts in Umudike, Abia State

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Abstract

The study was carried out at the laboratory of Plant Health Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria to evaluate the fungitoxicity of different concentrations of crude extracts of neem plant parts against *Aspergillus niger* associated with onion storage rots. *A.niger* was isolated from infected onion bulbs on PDA media and the pathogenicity was determined by testing its ability to induce rot in healthy onion bulbs. Results showed that the A. niger treated with the crude extracts of different concentrations of neem parts significantly ($P<0.05$) inhibited the mycelia growth of the pathogen in-vitro when compared with the control (Sterile water) 3 days after incubation at 28 □ 20C. Neem leaves significantly ($P < 0.05$) gave the highest growth inhibition at 75% concentration (82%). Neem root extracts were also effective at all levels of concentrations studied while the neem stem/bark extracts gave the least growth inhibition at 75% concentration.

Keywords: Onion rot, *Aspergillus niger*, neem extracts, concentrations, *in vitro*

1. Introduction

Onion (*Alluim cepa* L.) is an edible bulbous, biennial herb of Alliaceae family (Opara & Wokocha, 2008), with about 300 species known. Onion is an important vegetable crop grown worldwide and China ranks first in total production of dry onion with 12, 438, 000 tons, India, the United States and Turkey follow with 4,900,000 tons, 3,060,000 ton and 2,200,000 tons respectively (FAO, 2004). However, global average yield of onion in 2004 has been estimated at 18.3 tons/ha, with 15.21 tonnes/ha for Africa, 1.518 tons/ha for West Africa and 1.5 tons/ha for Nigeria (FAOSTAT, 2008). The unique flavor and odour of on ions have made the man excellent food source. In Nigeria, the crop is second only to tomatoes in importance among the vegetables and it's mainly grown for its bulbs (Hussaini, *et al.,* 2000).

Onion is a valuable ingredient in human diet due to its sugars, vitamins and mineral contents (Ole *et al.,* 2004). Onions can be infected by many different diseases and pests both in the field and in storage, many of which can affect the yield of the crop (Nuray & Koycu, 2004). Post-harvest disease account for about 50% losses in fruits stored in poor

storage conditions, especially under high humidity and this pose a serious challenge to increased production (Agrios, 2005). According to Dongondaji, *et al.,* (2008) storage rots of onions caused by the black mould fungus *Aspergillus niger* is a high temperature loving fungus. It also thrives in an optimum temperature of about 28 – 340^{C} thereby reducing the quantity and quality or market value of the crop.

More than 25% of the world vegetables and other crops are contaminated with known mycotoxins produced by fungi and more than 300 fungal metabolites produced are reported to be toxic to manand animal (Galvano, *et al.,* 2001). The main toxic effects are carcinogenicity, hepetotoxicity, genototoxicity, terrotogenicity, reproductive disorder and immunosuppression (Desjardins, *et al.,* 2000). Many workers have reported the use of plant extracts in controlling the fungal pathogens causing onion rots (Nuray & Koycu, 2004 Rana *et al.,* 2007; Singh, *et al.,* 2007).

Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratories trials (Okigbo & Ogbonnaya, 2006). The objective of this present study therefore, was to evaluate growth response of *Aspergillus niger* inciting onion storage rots and its control using different concentrations of neem plant parts.

2. MATERIALS AND METHODS

Experimental Location

The experiment was conducted in the Department of Plant Health Management Laboratory of Michael Okpara University of Agriculture, Umudike, Nigeria. Both healthy and infected onion bulbs showing symptoms of rotting and discolouration were collected randomly from Nkwoegwu, Orieugba, Apumiri, Onuimo and Urbani markets in Umuahia, Abia State, Nigeria. The markets were on the average of 12km apart, the healthy and rotted onion bulbs were put in clean sample materials and brought to the laboratory for further investigation. Fresh leaves, roots and bark of neem plant (*Azadirachta indica* L.) were collected locally within Michael Okpara University of Agriculture, Umudike.

Isolation and Identification of Rot Inducing Fungi

Potato Dextrose Agar (PDA) was used as a culture medium, to isolate the pathogen responsible for the rots on the infected onion bulbs, the bulbs were stripped off their outer dry scales and surface sterilized in 1% sodium hypochlorite solution for 60 seconds (Dimka & Onuegbu, 2010), the bulbs were then rinsed in 3 successive changes of sterile distilled water and blotted dry with sterile filter paper. Small segments of tissues (3mm) from the margins of rotted lesions were cut out with a sterile scalpel and plated on potato dextrose agar media in 90mm Petri dishes. The plates were incubated at room temperature 280 – 300C 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 & Hunter, 1999). Spores were taken from the pure cultures and mounted with Lactophenol cotton blue stain on glass clean slides, teased gently in order to obtain a uniform spread and covered with coverslip. The prepared slides were mounted on the stage of a binocular light microscope for observation and identification of the fungi by spore characteristic features with the aid of an identification manual by Barnett and Hunter (1999) on illustrated genera of Imperfect Fungi.

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 off their outer scales, the inner tissue was swabbed with cotton wool soaked in 70% alcohol to inhibit bacterial growth, and a cotton wool soaked in distilled water was used to clean the bulbs so as to reduce the effect of the alcohol. A hole was dug on each healthy onion bulb sample using a 5mm cork borer, while a flame sterilized needle was used to collect 5mm mycelia

disc of a 7days old pure culture of *A. niger* and aseptically inverted into the hole and then covered with a petroleum jelly. The inoculated bulbs were carefully put into a transparent cellophane bag containing cotton wool dipped in sterile distilled water so as to maintain humidity at 28oC ± 2oC. Two replications were prepared from each treatment. Control consisted of sterilized 5mm PDA displaced in the holes of the healthy bulbs. Inoculated onion bulbs were subsequently observed for rot development. The degree of pathogenicity for each fungus was determined by measuring the extent of rot on the infected bulbs.

Preparation of Plant Extracts

Cold water was used for the extraction of plant materials. Fresh leaves, bark/stem and roots of Neem (*Azadirachta indica* L.) were collected, washed thoroughly in a clean tap water, rinsed and then air dried on the laboratory bench for 15 – 20 days at room temperature of about 270C (Amadioha, 2004). Thereafter, they were milled separately into fine powder using a milling machine. Each powder was weighed out separately in 75g, 50g, and 25g for the three extracts and put into different conical flasks, to which 100ml of sterile distilled water was added and the flasks stoppered with foils. These were allowed to stand for12 hours and then strained separately through double folds of sterile cheese cloth to obtain the filtrate of 75%, 50% and 25% of each extract Amadioha and Obi (1999). Control consisted of sterile water incorporated into the PDA.

Data Collection

Data was collected for percentage growth inhibition of the different extracts at various concentrations as well as the fungal spore density. The percentage growth inhibition was determined using the formula by Amadioha (2003, 2004) as follows;

$$\%\text{growth inhibition} = \frac{dc - dt}{Dc} \times \frac{100}{1}$$

Where dc: diameter of control and dt:

diameter of treatment

Data collected were subjected to analysis of variance (ANOVA) procedure for factorial experiment in CRD at 5% probability level (Steel & Torrie, 1997).

3. Results

The fungus *A. niger* was identified as the rot inducing organism in onion bulb in the study area. The result showed that the mycelia growth of the organism was reduced in PDA incorporated with different plant extracts when compared to sterile water (control) after 3 days of incubation at 75%, 50% and 25% concentration levels (Table1). Leaf extract of *Azadirachta indica* significantly ($p<0.05$) gave its highest growth inhibitory effect on *A. niger* at 75% concentration level (82.60%), with its least inhibitory effect at 25% concentration level (62.86) table 1. However, there was no significant difference at 50% concentration (76.28%). Root extract of *Azadirachta indica* has an effective control on *A. niger* at all concentration. However, it gave its highest inhibitory effects at 75% concentration level (79.74%), although, there was no significant difference in percentage inhibition at all concentrations after two days of incubation (Table1). While the bark extract had the least inhibitory effect compared to the leaf and root extract at all concentrations after three days of incubation (Table1).

Table 1: Effect of leaf, stem/bark and root extracts of *Azadirachta indica* L. at different concentration on the mycelia growth of *A. niger* grown on PDA and incubated for three days at room temperature (28±20C).

	Percentage inhibition (%)		
Neem Parts/ % Concentration	**Days of incubation**		
	1	**2**	**3**
Control	0.00	0.00	0.00
Leaf extract 25%	62.80	64.40	64.29
50%	72.00	76.28	71.08
75%	77.71	82.60	74.89
Stem/bark extract			
25%	60.00	68.16	7.71
50%	62.30	69.28	11.38
75%	68.30	72.36	18.7
Root extract			
25%	49.39	55.11	55.66
50%	51.51	59.30	64.71
75%	69.69	76.74	79.62
LSD (0.05)	**0.4312**	**0.4326**	**1.32**

4. DISCUSSION

Several investigators (Okereke and Wokocha, 2006) have reported the efficacy of neem extract in the control of plant diseases against the backdrop of the widespread use of synthetic pesticides. However, results from this study on the efficacy of neem in-vitro demonstrated that extracts of plants possess active ingredients which exhibit fungitoxic action, this is in harmony with reports of different workers (Amadioha, 2003, Okereke & Wokocha, 2006, Sidra & Javed, 2012, Wokocha & Uchendu, 2009). This study showed that the leaf and root extracts of *Azadirachta indica* had a better control on*A. niger* isolated from diseased onion bulbs (*Allium cepa* L.) than the stem/bark extract.

A. niger is a filamentous ascomycetes fungus that is ubiquitous in the environment and has been implicated in opportunistic infections of humans. In addition to its role as an opportunistic human pathogen, *A. niger* is economically important as a fermentation organism used for the production of citric acid (Amadioha, 1998). From the result, it was observed that the extracts used for the study recorded retardation or inhibition of mycelia growth of the fungus in-vitro. The use of plant extracts on fungi that cause rots and other diseases of plants should be encouraged as they have been found to reduce the damaging effect of fungi on plant produce and even stored produce

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