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Biocontrol of Fungi Associated with Onion (*Allium cepa*) Bulb Rot during Post Harvest

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Authors' contributions

This work was carried out in collaboration among all authors. Author TEOA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PAO and ENO managed the analyses of the study. Author PMU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Post-harvest deterioration is a major problem of onions (*Allium cepa*) in Nigeria. The use of chemicals has been the main control measure. Chemicals are hazardous and environmentally unfriendly. There is therefore, a need for search for effective bio-pesticides as alternatives. A survey of fungal onions diseases was conducted in two markets in Mkpat Enin Local Government Area, Akwa Ibom State, Nigeria. Two hundred onions bulbs (100 from each market) were collected separately from the two markets. The collected bulbs were put into separate polythene bags before taken to the laboratory for macroscopic and microscopic examinations. Fungal isolates were obtained from naturally infected bulbs and their pathogenicity confirmed using Kochs' postulate. *In vivo* and *in vitro* testing based on growth inhibition were also carried out to determine the efficacy of the aqueous plant extracts. Phytochemical analysis of alcohol extracts was conducted following

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standard procedures. Results of market surveys indicated disease incidences of 26% and 23% from Akpaden and Ukam markets, respectively. Three fungal species were isolated and identified as *Botrytis allii, Fusarium oxysporum and Alternaria porri.* Pathogenicity tests confirmed these isolates to be common agents of onion bulb rot*. In vitro* testing of *Chromolaena odorata* and *Mangiferia indica* leaf extracts at 10, 20 and 30% concentrations showed inhibitory effects of both extracts with increasing concentrations on the pathogens. *C. odorata* extracts indicated significantly higher inhibition, compared to *M. indica*. *In vivo* tests of the extracts also showed a significant growth inhibition of the pathogens on onion bulb rot, compared to the control. Phytochemical screening of the extracts indicated the presence of tannins, saponins, polyphenols and flavonoids. The antifungal activities of these extracts and their availability makes them potential control agents of fungal onion rot. *C. odorata* and *M. indica* plant extracts should therefore, be further explored as alternatives bio-control agents or onions fungal rot.

Keywords: Allium cepa; fungi; leaf extracts; bio-pesticides; phytochemical screening.

1. INTRODUCTION

1.1 Background of the Study

Onions (*Allium cepa)* is one of the oldest cultivated vegetables in the family of *Liliaceae*. It is an important commercial crop grown all over the world and is consumed in various forms; as vegetables, spices and as medicine. Diseases and deterioration by pathogens constitute a menace in the production and storage of onion in Nigeria thereby, decreasing their food and market value. The onion bulbs are attacked by many fungal diseases at different growth stages which causes considerable losses in yield [1]. Also deterioration of bulbs during post-harvest, processing and marketing stages could lower the quality and export potential of the crop and result in economic losses.

It is necessary therefore, to control fungal infections in onions. However, control measures have been focused on the use of agro-chemicals. The consistent use of these chemicals can pose serious threats to the environment as well as being toxic to animals including man. In addition, these chemicals can result in build-up resistance to the pathogens. These factors have led to a search in the direction of biological control and integrated pest management [2]. Consequently, the present study was aimed at controlling the growth of fungal pathogens using two plant extracts, i.e. *Chromolaena odorata and Mangifera indica.*

2. MATERIALS AND METHODS

2.1 Materials

All the materials used in this study were obtained from Akwa Ibom and Cross River States, Nigeria.

2.2 Study Area and Sampling

The sample area comprises two markets (Ukam and Ikot Akpaden) in Mkpat Enin LGA of Akwa Ibom state. One Hundred *Allium cepa* bulbs were collected at random from each of the two markets. The collected samples were placed in separate sterile polythene bags and transported to the Botany laboratory, Akwa Ibom State University for further investigations.

2.3 Physical Examination of Samples

Samples were physically examined following the methods of [3] to determine the presence of infection on the bulbs. Bulbs were considered infected if the showed rot symptoms. Disease incidence was also determined using the formula:

$$\text{Disease incidence (\%)} = \frac{Number\ of\ infected\ fruits}{Number\ of\ fruits\ examined} \times \frac{10\ 0}{1}$$

2.4 Isolation and Identification of Fungi

Cut sections of the diseased onions were surface sterilized with 70% sodium hypochlorite (domestic blench) solution for 1 min and rinsed quickly in 3 changes of sterile distilled water, and blotted dry on Whatman's No. 1 filter paper. These sections were then placed on potato dextrose agar (PDA) in petri dishes. One (1) section was inoculate per petri dish and three petri dishes were used. The inoculated plates were incubated at 28±1℃ until fungal growth was noticed. After 7 days, the different isolates were sub-cultured on freshly prepared PDA to obtainpure cultures. Isolated fungi were microscopically examined and identified using an Olympus optical microscope using the identification guides of the International Mycological Institute [4].

2.5 Pathogenicity Test

Pathogenicity tests of the isolates were carried out using the method of [5]. Healthy onions were washed in distilled water and surface sterilized with 70% sodium hypochlorite solution. A 5 mm diameter cork borer was used to cut holes on the bulbs (three discs per bulb) and 5mm discs cultures of the isolates (four days old) were introduced into three holes. The inoculated holes were covered with Vaseline to prevent entry of secondary pathogens. Controls were made by inoculating onions with sterile distilled water. The set ups were incubated at room temperature and monitored for rot symptom development. Organisms were re-isolated and identified to confirm pathogenicity.

2.6 Preparation of Plant Extracts

Fresh leaves of mango (*Mangifera indica*), siamweed (*Chromolaena odorata*) were harvested from the Botanical garden, Akwa Ibom State University, Ikot Akpaden. The leaf samples were rinsed under running tap water to remove dirt, oven dry for 48 h and pulverized using a mortar and pestle. Two hundred grams each of pulverize materials were homogenized in 200 ml distilled water for 10 min, centrifuge at 500 rpm for 10min and filtered through Whatman No 1 filter paper [6]. The supernatant as well as dry powder were used for investigations.

2.7 Phytochemical Analysis of Extracts

Phytochemical screening of the extracts was carried out to determine the presence of secondary metabolites following [7] protocols. Standard tests were conducted for tannins, saponins, polyphenols and flavonoids.

2.8 *In vitro A*ntifungal Assay

Five ml of each concentration (10%, 20% and 30%) were added to previously sterile PDA contained in petri dishes. These plates were inoculated with culture disk obtained with sterile 5mm cock borer. Inoculated plated were incubated at room temperature and monitored for 7 days. Controls were set up without extracts. Experiments were set up in 3 replicates and radial growth measurement carried out. The effect of extracts was determined by calculating percentage inhibition using the formula: % Inhibition = $\frac{PI-P2}{P1} \times \frac{10\ 0}{1}$where *P1* is the growth on control, *P2* growth on treatment [8].

% Inhibition = $\frac{PI-P2}{P1} \times \frac{10\ 0}{1}$

Where *P1* is the growth on control, and *P2* growth on treatment [8].

2.9 *In vivo* Antifungal Assay

In vivo antifungal assay were carried out using the modified method of [9]. Whole healthy onions were surface sterilized using 99% ethanol and rinsed with distilled water and allow to dry for 2 min. The bulbs were separately sprayed with extracts of different concentrations (10, 20, 30 and 40%) and inoculated using 5mm agar disk obtained from pure culture plates of the pathogens. Controls were set up without extracts. Treated bulbs were incubated at room temperature ($28 \pm 1℃$) for 7 days. Effect of extracts was calculated as *in vitro* antifungal assay.

2.10 Data Analysis

All experiments were set up in triplicate and differences between treatment means were analyzed using Analysis of Variance (ANOVA). Significant levels were determined at $p \geq 0.5$.

3. RESULTS

3.1 Disease Incidence

Samples from the Akpaden market recorded a percentage infection of twenty-six (26%), while Ukam market had twenty-three percent infection (23%) (Table 1).

3.2 Physical Examination of Samples

Infected *Allium cepa* showed dark brown lesions with soft tissues (Plate 1).

3.3 Identifications of Fungal Isolates

Three fungi, i.e. *Botrytis salli, Alternaria porri* and *Fusarium oxysporium* were identified in Akpaden market, while two isolates *Botrytis allii,*and *Alternaria porri* were obtained from Ukam market.

3.4 Pathogenicity Test

The results of pathogenicity test showed that all the isolated fungi from the two markets caused infection on healthy *Allium cepa*.

Table 1. Percentage infection

Name of market	No. of samples	No. infected	Percentage infected (%)
Akpaden	30	8	26
Ukam	30	7	23

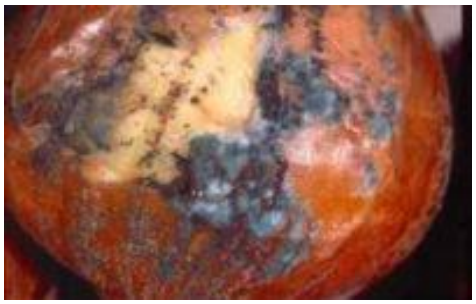

Plate 1. Infected onion bulbs

3.5 Phytochemical Screening

Apart from polyphenols that were only present in *Chromolaena odorata*, the other secondary metabolites were found in both extracts (Table 2). The four metabolites were found in higher levels in *Chromolaena odorata,* compared to *Mangifera indica* extracts.

3.6 Effect of Plant Extracts on Growth of Pathogens (*in vivo and in vitro*)

In vivo (Tables 3 and 4) and *in vitro* (Figs. 1 and 2) results showed similar trends. The two plant extracts had fungicidal properties with *chromolaena adorota* being more effective in retarding vegetative growth of the pathogens. Both extracts were less effective in controlling the mycelia growth of *Botrytis allii,* compared to the other two fungal pathogens. The level of inhibition of the three pathogens increased with increase in extract concentration.

Table 2. Qualitative analysis of plant extracts

Extracts	Secondary metabolites			
	Tannins	Saponins	Polyphenols	flavonoids
Chromolaena odorata	+++	+++	++	+++
Mangifera indica	++	+	–	+

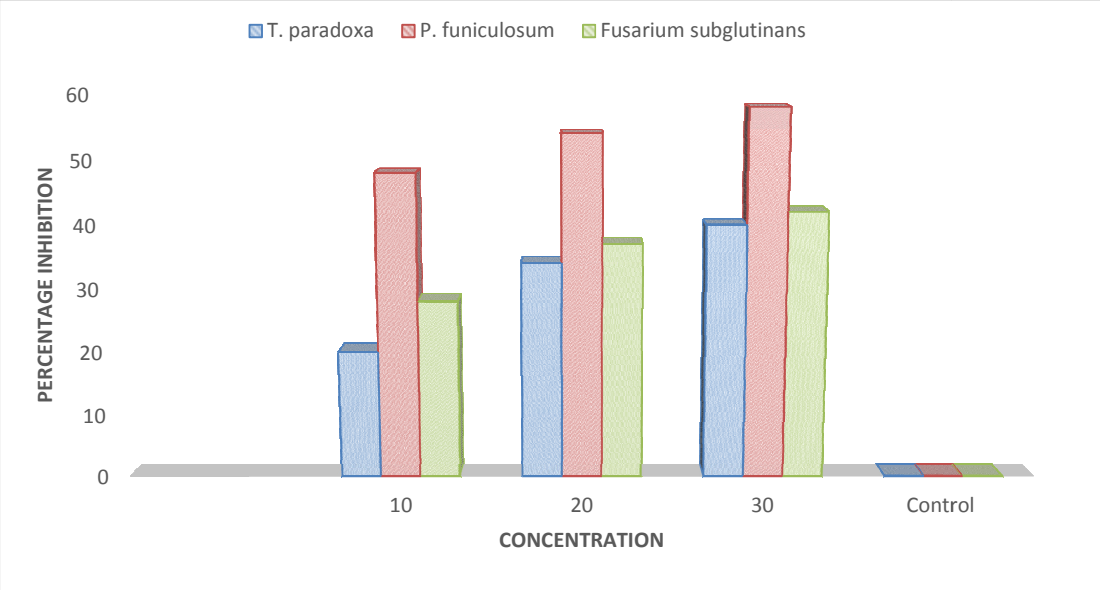

Fig. 1. Percentage growth inhibition of fungi by C*hromolaena adorota* leaf extract (*in vitro*)

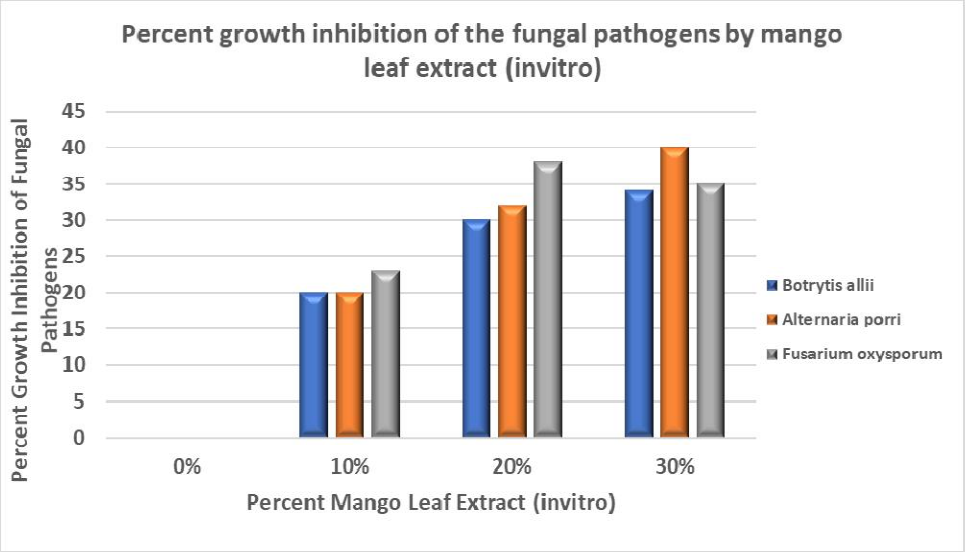

Fig. 2. Percentage growth inhibition of fungi by *Mangifera indica* leaf extract (*in vitro*)

Table 3. Percentage growth inhibition of fungal pathogens by Siam weed leaf extract (*in vivo*)

Concentration (%)	Growth inhibition (%)		
	Botrytis allii	***Alternaria porri***	***Fusarium oxysporum***
10	25.00	30.00	25.00
20	30.00	44.00	35.00
30	35.00	50.00	45.00
Control	0.00	0.00	0.00

Results are means of three replicates

Table 4. Percentage growth inhibition of the fungal pathogens by mango leaf extract (*in vivo)*

Concentration (%)	Growth inhibition (%)		
	Botrytis allii	***Alternaria porri***	***Fusarium oxysporum***
10	15.00	20.00	20.00
20	25.00	30.00	33.00
30	30.00	35.00	40.00
Control	0.00	0.00	0.00

Results are means of three replicates

4. DISCUSSION AND CONCLUSION

Akpaden and Ukam markets recorded infections of 26 and 23%, respectively. Similar high disease incidences in *Allium* species have been reported in South Western Nigeria [10,11]. These high incidences could be due to high rainfall patterns, high humidity and warm temperatures prevalent within this ecological zone. Akwa Ibom state, where the two markets in this research are located, falls within this geographical belt.

Botrytis allii, Alternaria porri, and *Fusarium oxysporum* were found to be pathogenic to *Allium cepa*. These fungi have been associated with neck rot, purple blotch and basal rot in onions [12,13]. The pathogenicity of these fungi has also been established in a number of other crop species such as yam, cassava, tomatoes, and sweet potatoes.

The two leaf extracts (*Chromolaena adorota* and *Mangifera indica*) resulted in the retardation of vegetative growth of the fungi when compared with the control. These results are similar to the reports of [13] where *Chromolaena odorata* was to be more effective in this regard. The antifungal activities of these extracts can be linked to the

presence of secondary metabolites such as saponins, tannins, flavonoids and polyphenols. The concentrations of these substances were higher in *Chromolaena odorata* than *Mangifera Indica*, which was more effective in inhibiting fungal growth of these pathogens which confirms their antifungal properties. Similar results have been reported in these and other plant extracts [14]. Leaf extracts of *Magnifera indica* and C*hromolaena adorota can be used* to manufacture bio pesticides, which are environmental friendly and with little or no toxic effects on plants, soil and humans [7]. In addition, these plant extracts can be further explored as potential medications against human dermatophyte fungi. The use of plant extracts as biopesticides is also important in the reduction of environmental pollution since there are less toxic and environmentally friendly.

DISCLAIMER

The products used for this research are commonly and predominantly products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge only. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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