***In Vitro* and *In Vivo* Biological Evaluation of Crude Extracts of *Dioscorea dumetorum* and *Psidium guajava* Leaves in the Control of Storage Rot of Onion (*Allium cepa* L.) Bulbs**

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ABSTRACT. The antifungal activity of the ethanolic crude leaf extracts of *Dioscorea dumetorum* and *Psidium guajava* on the fungal pathogens isolated from infected onion bulbs were investigated *in vitro* and *in vivo*. The pathogens were *Aspergillus niger* and *Rhizopus stolonifer*. For the *in vitro* antifungal assay, various concentrations of the extracts ranging from 10g/200ml, 20g/200ml, 30g/200ml, 40g/200ml and 50g/200ml were separately added to PDA media. The fungal pathogens were separately inoculated into the media and incubated for seven days. For the *in vivo* assay, healthy onion bulbs were properly surface sterilized using 99% ethanol and sodium hypochlorite (bleach). The sterile onion bulbs were then sprayed with the extracts at different concentrations two hours prior to inoculation with the fungal pathogens. Each onion bulb after being treated with different concentration of the extracts of 10g/200ml, 20g/200ml, 30g/200ml, 40g/200ml and 50g/200ml were dipped into beakers containing dissolved spore of each pathogen and incubated for seven days. Results of the *in vitro* antifungal assay shows that at 10g/200ml – 50g/200ml concentration, ethanolic leaf extracts of *D. dumetorum* and *P. guajava* completely inhibited the radial growth of *A. niger* and *R. stolonifer* after seven days observation period. For the *in vivo* antifungal assay, results shows that the extracts had a significant effect ($P \leq 0.05$) on the mycelial growth of the fungal pathogens at all the different concentrations tested. Pathogen growth inhibition was most effective at 30g/200ml, 40g/200ml and 50g/200ml concentrations. The inhibitory action of the extracts increased/decreased with a corresponding increase/decrease in the concentration of the plant extracts. Ethanolic extracts of *D. dumetorum* was found to be more effective in inhibiting the growth of the fungal pathogens than *P. guajava* extracts.

INTRODUCTION

The onion (*Allium cepa* L.), also known as bulb (Fritsch and Friesen, 2002), or common onion, is one of the most widely cultivated species of the genus *Allium*. The species is probably native to South-western Asia but is now commercially grown throughout the world, chiefly in the temperate zones (Ozer and Koycu, 2004). The onion often called as “queen of kitchen” is commonly used for cooking by almost all the people. It ensures excellent taste to dishes and also exhibits a number of therapeutic properties such as antibacterial, antifungal, antihelmentic, anti-inflammatory, antiseptic and antispasmodic. Due to its enormous commercial and medicinal value, the plant is now cultivated in almost all countries of the world and consumed across the globe (Rai and Yadav, 2006).

Onion belongs to the Lily family, *Alliaceae*, however, some classification place it in the family *Liliaceae* (Fritsch and Friesen, 2002). Modern varieties of onion typically grow to a height of 15 to 45cm (6 to 18inch) with real stem very short, formed at the base of the plant in the form of a disk with adventitious root at the base, the leaves are yellowish-green and grow alternately in a flattened, fan-shaped varying from globule to ovoid to oblate up to 20cm in diameter (Brickell, 2002). The species is biennial but usually grown as annuals in the temperate climates. In Nigeria, it is mostly

cultivated during the dry season (October to April) and as such are easily propagated, transported and stored (Zohary and Hopf, 2000).

Common onions are normally available in three colour varieties ranging from yellow or brown onions (called red in some European countries), are full flavour red and are the onions of choice for everyday use. With its pungent aroma and strong flavour, it is a good all-round onion. Their varieties are grown in countries like Australia, Nigeria and they vary from cream gold and muray brown. Other varieties of onion crop also include Red onion; these are sometimes called Spanish onions, which have purplish red skin and white flesh tinged with red. This onion tends to be medium to large size and can have a mild to sweet flavour but after being stored for a short time can become quite pungent. They are often consumed raw, grilled or lightly cooked with other foods or added as colour to salads, these variety of onion plant can be stored three to four months under ideal condition, and their varieties include red shine, red wing and red emperor. Although white onions are considered to be the strongest in flavour after brown onions, their varieties vary in size, skin characteristics and flavour; they include Bianca, Gladalan white and white Spanish (Mower, 2013).

Onion is a valuable ingredient in diet due to its content of sugars, vitamins and minerals (Ole *et al.*, 2004). Most species of onion contain low amount of essential nutrient, are low in fats and have an energy value of 166KJ (40 cal) per 100g. The onion cultivars are about 89% water, 4% sugar, 1% protein, 2% fiber and 0.1% fat (Fern, 2011). Furthermore, onion contain bioactive compound such as phenol that are under basic research to determine their possible properties in human (Slimestad *et al.*, 2004). Yellow and Red onions have considerable content of anthocyanin pigments with 25 different compounds representing 10% of total flavonoid content (Yang *et al.*, 2004). As a result of the bioactive compounds contained in onion, some people suffer from allergic reaction after handling them. Some of the allergic reactions include eye irritation, bronchial asthma, blurred vision, intense itching etc. This stinging sensation can be avoided by cutting onions under running water or submerged in a basin of water (Williamson *et al.*, 1997).

Onion as an important vegetable crop is eaten all over the world. They play the role of being antioxidant and cardio-protective in nature as a result is rich in vitamin B, C, E and other trace elements (Conant, 2006). Recent report suggest that onion plays an important role in preventing cancer, diabetes and even common cold and are also reputed to increase fertility in men and milk in nursing mothers, more so to cure headache when mixed with milk and vinegar (Rai and Yadar, 2006). In addition, *Allium* Species are used as traditional medicine in countries like Europe, North Asia and Africa to treat intestinal worms, gastroenteritis, heart diseases and other ailments (Brewster, 1999) planting them closely to other vulnerable crops such as carrot discourages insect attack on those crops (Wane and Taskeen, 2011).

Onions have greater water and sugar content that gives them their sweeter-and milder-tasting but reduces their shelf life (Olsson *et al.*, 2010). Onion are better stored at room temperature, optimally in a single layer, in mesh bags in a dry cool, dark, well-ventilated location, storing them in a cool dry place with plenty of circulating air can help prolong their shelf life approximately one to five months irrespective of type (Jauron, 2009). For propagation, the onion plant are monocotyledons, they sometimes behave like the annuals, by setting seed-bolting in their first year. They grow from the base of their leaves pushing their leaves up and out from the base, by contrast, grow outward from the edges of the leaves (Savon *et al.*, 2006), may also be grown from seed or seed sets ranging from 3.5 to 4.5kg/ha when transplanted, 4 to 6kg/ha when direct seeded with spacing usually 25-40cm between rows.

Onions are adapted to moist soils where nutrients are in sufficient quantity; viability falls rapidly in hot humid conditions and this may be 0% in less than a year especially in their F₁ hybrid (Klaas and Friesen, 1998).

Fungi especially moulds are important pathogens of fruit and vegetables particularly under tropical and subtropical conditions (Adebayo and Diyaulo, 2003). The effect of storage rots includes reduction in the quantity and quality of onion which affects the market's value (Adebayo and Diyaulo, 2003). Black mould disease caused by *Aspergillus niger* Van Tieghem is a limiting factor in onion production worldwide (Ozer and Koycu, 2004). *Aspergillus niger* Van Tieghem is a

high temperature fungus, with an optimum temperature range from growth 28-34°C, are ubiquitous in occurrence, it attacks/infect bulbs of onion in field/storage, whenever they find injured tissues by producing various toxins (Srinivasan and Shanmugam, 2006). Other fungal pathogens in association with post-harvest rot of onion include Neck rot of onion caused by *Botrytis allii*, white rot and a host of other pathogens (Amienyo and Ataga, 2007).

According to Okigbo (2009) and Okigbo and Nmeka (2005), in Nigeria, for instance, about 98% of the food consumed is produced by the local farmers. Most of these farmers are not educated and therefore in most cases cannot practice the various control measures recommended for disease control. In addition to these inadequacies, most of the chemicals are not available and where they are, they are very expensive. These problems have necessitated the search for alternatives to synthetic fungicides. Among the various alternatives, natural plant products that are biodegradable, ecologically, friendly and readily available have therefore been sort by scientists worldwide. Similarly, Lee *et al.*, (2007), Verastegui *et al.*, (2008) and Santa *et al* (2010) reported that extracts obtained from many plants have recently gained popularity and scientific interest for their antifungal activities. Other research workers Amadioha and Obi (1999) Amadioha (2000) and Okigbo (2009) see the significance of fungicides of plant origin as possible means of fungal disease control in fruits and vegetables as they are easily biodegradable and non toxic to humans.

Diseases and deterioration by fungal pathogens constitute a menace in the production and storage of onion bulb thereby decreasing their food and market value. In view of this, the aim and objectives of this research work was to isolate and identify the fungal pathogens associated with storage rot of onion bulb as well as evaluate the antifungal potentials of crude extracts of *Psidium guajava* and *Dioscorea dumetorum* on fungal pathogens associated with postharvest storage rot of onion bulb both *in vitro* and *in vivo*.

MATERIALS AND METHODS

Sources of materials

Infected and uninfected onion bulbs were obtained from three different markets (Watt, Akim and Marian) in Calabar Metropolis, Cross River State, Nigeria and wrapped in sterile cellophane bags and transported to the Laboratory for further studies. *Dioscorea dumetorum* and *Psidium guajava* leaves were obtained from the Botanic Garden of the Department of Botany, University of Calabar, Calabar, Cross River state, Nigeria. The research was carried out in the Laboratory of the Department of Botany, University of Calabar, Calabar, Cross River State, Nigeria.

Isolation of fungal pathogens and morphological identification

Cut sections of the diseased onion bulbs were surface sterilized with 70% sodium hypochlorite (bleach) solution for 1min and rinsed quickly in 3 changes of sterile distilled water, blotted dry on Whatman's No.1 filter paper and placed on Potato Dextrose Agar (PDA) in Petri dishes. Four (4) sections were inoculated per Petri dish. The plates were incubated at $28 \pm 1^\circ\text{C}$ until fungal growth was noticed. After 7 days, the different isolates were subcultured on freshly prepared PDA to obtain their pure culture. Isolated fungi were microscopically (Olympus optical, Phillipines) identified as far as possible using the identification guides of the International Mycological Institute, Kew, Barnett and Hunter, (1998), Dugan, (2006).

Pathogenicity test and Koch's postulates

Pathogenicity test was carried out using the method of Amienyo and Ataga (2006). Healthy onion bulbs were washed in distilled water and surface sterilized with 70% Sodium hypochlorite solution. A 5mm diameter cork borer was used to cut disc from the bulbs (three discs per bulb) and cultures of the isolates (four days old) were introduced into bulbss and replaced with the discs. The discs portions were sealed with Vaseline to prevent entry by other pathogens. They were kept for 24-48 hours wrapped in sterilized cellophane bags. A sterile cotton wool was soaked with distilled water and dropped inside the cellophane bags to enable the organism to breath (respire). The

inoculated fruits established symptoms after 24 hours, tissue segment from the healthy and infected bulbs was excised, surface sterilized and plated into freshly prepared PDA and incubated at $28\pm 1^{\circ}\text{C}$ for four days. Pure cultures of the isolate were identified.

Preparation of plant extracts

Leaves of *Psidium guajava* and *Dioscorea dumetorum* obtained were washed with distilled water and oven dried at a temperature of 80°C for 24 hours grounded into fine powder and extracted separately using 200ml of 99% concentration of ethanol.

Susceptibility test

The extracts percentage concentrations were prepared at 10g/200ml, 20g/200ml, 30g/200ml, 40g/200ml, and 50g/200ml with ethanol as solvent.

In vitro antifungal assay

5ml of each concentration was first poured into different Petri dishes using sterile syringe. The sterile Potato Dextrose Agar (PDA) was also poured into the plates containing the solvent extracts after which the plates containing the solvent extracts were gently swirled to ensure mixing. The media was allowed to solidify and with a sterilized No.2 cork borer of 5.5mm in diameter, a disc of the matured culture was punched out and inoculated at the centre of plates and incubated at room temperature of $(28\pm 1^{\circ}\text{C})$ for 7days. As a control, the dishes were inoculated on PDA instead of extracts-agar mix. Three (3) control plates were prepared for each extract. Measurement of the mycelia radial growth was done daily for seven days (Umana *et al.*, 2014).

In vivo antifungal assay

In vivo antifungal assay of the plant extracts was carried out using the method described by Poppe *et al.*, (2003) with some modifications. Whole healthy onion bulbs were properly surfaced sterilized using 99% ethanol rinsed with distilled water and allowed to dry for 2-3minutes. The sterile bulbs were then sprayed with the extracts at different concentrations, two hours prior to inoculation with the fungal pathogens. Each bulb after being treated with different concentrations of the extracts of 10g/200ml, 20g/200ml, 30g/200ml, 40g/200ml and 50g/200ml were dipped into beaker containing dissolved spores of each pathogen. The bulbs were then allowed to dry for 2-5minutes, after which each bulb was incubated at room temperature $(28\pm 1^{\circ}\text{C})$ for twenty-eight days. The control which was also dipped in beakers containing the dissolved spores of each pathogen was not treated with any of the extracts. Growth rate were measured after one week of inoculation and was repeated after every four days for four times.

RESULTS

Identified fungal pathogens

The fungal pathogens isolated and identified as the causative agents of storage rot of onion bulbs from this study and used were *Aspergillus niger* and *Rhizopus stolonifer*.

Pathogenicity test and Koch's postulates

Result from the pathogenicity confirmed that the two fungal isolates *Aspergillus niger* and *Rhizopus stolonifer* were responsible for the storage rot of onion bulbs obtained from three major markets in Calabar Metropolis of Cross River State, Nigeria. Pathogenicity was established 24 - 48 hours after inoculation (Plate 1).

Plate 1: Infected onion bulbs $\times 400$ showing symptoms of rot and mycelia growth of *Aspergillus niger* (Black) and *Rhizopus stolonifer* (white) two days post inoculation (dpi).

***In vitro* effect of the plant extracts on the radial growth of *A. niger* and *R. stolonifer* at the different concentrations.**

The *in vitro* effect of the crude plant extracts at the different concentrations on the radial growth of the fungal isolates is presented in (Tables 1 - 4). Results showed that, at 10g/200ml, 20g/200ml, 30g/200ml, 40g/200ml and 50g/200ml *D. dumetorum* and *P. guajava* extracts completely inhibited the radial growth of *Aspergillus niger* and *Rhizopus stolonifer* at the different concentrations tested when compared with the control (Plate 2).

Plate 2: *In vitro* effect of the ethanolic crude extracts of *Dioscorea dumetorum* and *Psidium guajava* on the radial growth of *Aspergillus niger* and *Rhizopus stolonifer* after seven days of incubation.

Table 1: *In vitro* effect of ethanolic crude *Dioscorea dumetorum* leaf extract on the radial growth of *Aspergillus niger*.

Concentration	Incubation period (Days) and radial growth cm ²						
	1	2	3	4	5	6	7
10g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.60	0.80	2.40	2.90	3.20	3.80	4.20

Table 2: *In vitro* effect of ethanolic crude *D. dumetorum* leaf extract on the radial growth of *Rhizopus stolonifer* (cm).

Concentration	Incubation period (Days) and radial growth (cm ²)						
	1	2	3	4	5	6	7
10g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.90	1.80	3.90	4.20	4.50	4.50	4.50

Table 3: *In vitro* effect of ethanolic crude *Psidium guajava* leaf extract on the radial growth of *A. niger* (cm).

Concentration	Incubation period (Days) and radial growth (cm ²)						
	1	2	3	4	5	6	7
10g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.60	0.90	1.90	2.40	3.20	3.60	3.80

Table 4: *In vitro* effect of ethanolic crude *Psidium guajava* leaf extract on the radial growth of *Rhizopus stolonifer* (cm).

Concentration	Incubation period (Days) and radial growth (cm ²)						
	1	2	3	4	5	6	7
10g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50g/200ml	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Control	0.90	1.70	2.90	3.90	4.40	4.50	4.50

***In vivo* effect of the crude plant extracts on the growth and sporulation of *A. niger* and *R. stolonifer* at the different concentrations.**

The *in vivo* effect of the plant extracts at different concentrations on the radial growth of the fungal isolates is presented in (Tables 5-8). Investigations from the study showed that extracts of *Dioscorea dumetorum* and *Psidium guajava* had a significant effect on the isolated pathogens at all the concentration levels (10g/200ml, 20g/200ml, 20g/200ml, 40g/200ml and 50g/200ml) tested when compared with the control. Results (Table 5) showed that at 30g/200ml, 40g/200ml and 50g/200ml concentrations, *Dioscorea dumetorum* extract completely inhibited the radial growth of *Aspergillus niger* on the first and second day of incubation. However, at 50g/200ml concentration, extract of *D. dumetorum* completely inhibited the radial growth of *A. niger* in the first four days when compared with the control. While results (Table 6) showed that *D. dumetorum* extract completely inhibited the radial growth of *Rhizopus stolonifer* at 40g/200ml and 50g/200ml concentrations on the first and second day and at 50g/200ml on the third day of incubation respectively. Results (Table 5-8) showed that there was a corresponding increase in the antifungal activity of the plant extracts as the concentration of the extracts increased from 10g/200ml to 50g/200ml. Results (Table 7 and 8) showed that *Psidium guajava* extract completely inhibited *Aspergillus niger* at 50g/200ml concentration on the first and second day of incubation. While at 50g/200ml *Psidium guajava* extract inhibited the radial growth of *R. stolonifer* at 50g/200ml concentration only on the first day of incubation. Results therefore, showed that *Dioscorea dumetorum* leaf extracts was more effective inhibiting the growth of *Aspergillus niger* and *Rhizopus stolonifer* than those of *Psidium guajava*. Results also showed that the extracts were more effective in inhibiting the growth of *Aspergillus niger* than that of *Rhizopus stolonifer*.

Table 5: *In vivo* effect of ethanolic crude *Dioscorea dumetorum* leaf extract on the growth of *Aspergillus niger* (cm).

Concentration	Incubation period (Days) and radial growth (cm ²)						
	1	2	3	4	5	6	7
10g/200ml	3.0±2.08	4.0±3.12	8.0±0.09	10±0.13	10±0.40	13±2.16	14±0.17
20g/200ml	3.0±0.01	3.0±0.30	6.0±1.31	7.0±2.11	9.0±1.12	11±0.12	12±3.21
30g/200ml	-	2.0±1.18	4.0±1.19	6.0±0.01	6.0±2.21	8.0±0.95	9±1.138
40g/200ml	-	-	2.0±1.19	4.0±0.08	5.0±2.13	6.0±0.01	7±0.21
50g/200ml	-	-	-	-	2.0±0.01	2.0±1.12	3±0.97
Control	7.0±0.31	10.0±0.01	16.0±2.21	23.0±0.24	30.0±2.21	36.0±3.12	42.0±0.12

Note: Values are means of three replicates ± standard error

Table 6: *In vivo* effect of ethanolic crude *Dioscorea dumetorum* leaf extract on the growth of *Rhizopus stolonifer* (cm).

Concentration	Incubation period (Days) and radial growth (cm ²)						
	1	2	3	4	5	6	7
10g/200ml	5.0±0.01	7.0±0.98	9.0±2.09	13.0±0.13	14.0±2.01	17.0±0.01	19.0±0.10
20g/200ml	3.0±2.21	4.0±0.09	8.0±2.11	8.0±2.98	9.0±0.11	10.0±2.18	12.0±0.81
30g/200ml	2.0±0.91	3.0±0.04	5.0±2.19	6.0±0.81	7.0±2.11	7.0±2.98	8.0±0.15
40g/200ml	-	-	3.0±0.05	5.0±2.11	5.0±2.97	6.0±2.16	6.0±3.32
50g/200ml	-	-	-	2.0±0.05	4.0±2.13	5.0±0.19	5.0±2.12
Control	10.0±0.12	15.0±2.11	21.0±0.09	27.0±2.15	35.0±1.19	41.0±2.23	46.0±2.91

Note: Values are means of three replicates ± standard error

Table 7: *In vivo* effect of ethanolic crude *Psidium guajava* leaf extract on *Aspergillus niger*.

Concentration	Incubation period (Days) and radial growth (cm ²)						
	1	2	3	4	5	6	7
10g/200ml	4.0±2.05	5.0±0.01	7.0±0.21	7.0±2.28	10.0±0.07	14.0±0.91	20.0±0.02
20g/200ml	4.0±0.01	4.0±2.25	5.0±0.97	6.0±0.17	8.0±3.16	12.0±2.16	14.0±0.17
30g/200ml	3.0±2.10	4.0±0.02	4.0±2.28	5.0±0.29	7.0±0.67	8.0±0.12	10.0±1.41
40g/200ml	3.0±0.01	3.0±1.12	4.0±0.09	4.0±2.23	5.0±0.09	6.0±0.02	8.0±2.01
50g/200ml	-	-	3.0±0.98	4.0±0.11	4.0±0.01	4.0±2.23	6.0±0.01
Control	9.0±0.13	13.0±1.11	15.0±2.21	21.0±0.09	31.0±2.11	38.0±2.25	48.0±2.81

Note: Values are means of three replicates ± standard error

Table 8: *In vivo* effect of ethanolic crude *Psidium guajava* leaf extra on *Rhizopus stolonifer*.

Concentration	Incubation period (Days) and radial growth (cm ²)						
	1	2	3	4	5	6	7
10g/200ml	6.0±0.11	8.0±0.03	12.0±2.13	14.0±0.16	18.0±0.02	20.0±1.09	24.0±0.01
20g/200ml	5.0±0.06	4.0±0.09	8.0±0.95	13.0±2.13	16.0±0.91	16.0±2.31	19.0±0.05
30g/200ml	4.0±0.31	3.0±0.04	6.0±0.04	8.0±2.11	9.0±2.09	10.0±0.11	13.0±0.06
40g/200ml	3.0±0.01	3.0±2.11	4.0±0.07	5.0±2.09	6.0±2.04	8.0±0.91	10.0±0.01
50g/200ml	-	3.0±0.07	3.0±2.21	4.0±0.01	4.0±0.98	5.0±2.31	6.0±2.161
Control	12.0±0.01	13.0±3.25	23.0±0.91	30.0±0.31	37.0±2.12	44.0±0.03	50.0±2.05

Note: Values are means of three replicates ± standard error

DISCUSSION

The efficacy of two plant extracts was tested *in vitro* and *in vivo* against the radial growth of two pathogenic fungi associated with post harvest storage rot of onion bulb. The fungal pathogens isolated were identified as *Aspergillus niger* and *Rhizopus Stolonifer*. The results of this study revealed that the fungi were responsible for the post harvest rot of onion bulbs in Calabar Metropolis as evidenced by the pathogenicity tests. The fungal spores of these pathogens could be air-borne and therefore spread by wind and may land on susceptible host like onion bulbs, fruits and other plants. The pathogens (*A. niger* and *R. stolonifer*) were reported by Shehu and Muhamed (2011) to cause storage rot fungi of onion bulb. Also Adebayo and Diyauro (2003) reported (*A. niger* and *R. stolonifer*) as pathogens of post harvest rot of onion, which is in agreement with the finding of this study. The *in vitro* and *in vivo* inhibitory effects of *D. dumetorum* and *P. guajava* extracts on the isolated fungal pathogens are presented in (Tables 1-8). The results showed that, the extracts significantly ($P<0.05$) inhibited the radial growth of the fungal pathogens at the different concentrations tested and the rate of inhibition differed from one extract to the other. However, results, (Table 1-4) showed that in the *invitro* study, the plant extracts completely inhibited the radial growth of the fungal isolates at all the different concentration levels tested, while in the *in vivo*, increase in antifungal activity was observed with the corresponding increase in the concentrations of all the plant extracts (Table 5-8). The differences in the fungi-toxic potentials between these plant extracts may be attributed to the susceptibility of each of the fungal pathogens to the different concentration of the extracts. This agrees with the result of some workers like Amienyo and Ataga (2007), Joon *et al.*, (2001) who reported that plant are rich source of bioactive compounds such as tannins, terpenoids, saponins, alkaloids, flavonoids and other compounds as such have antifungal properties. Cushnie and Lamb (2005), Srinivasan and Shanmugam (2006) and Faridt *et al.*, (2002) also reported that, the presence of these compounds in plant could be responsible for the control of fungal pathogens of plant. The plant extracts differed significantly in their potential to inhibit the growth of these fungal pathogens. Generally, radial growth decreased with increase in each of the plant extract concentrations. The *in vitro* and *in vivo* effects of *D. dumetorum* and *P. guajava* ethanolic extracts at five different concentrations were evaluated in order to develop cheaper methods of controlling post storage rot of onion bulbs. The greater efficiency of these plants extract maybe due to the high content of the phenol substance they contain

(Amienyo and Ataga, 2007), since they are ranked as the most efficient therapeutically significant plant substances (Okuwu and Igara, 2011). This result is also consistent with the findings of Okigbo and Nmeka (2005), Santas *et al.*, (2010) who reported that plants have phenolic compounds and that their antifungal activity may be due to the action of the proteolytic enzymes which is their major component, as such have adverse effect on the protein component of fungal cells as well as disrupting their growth.

CONCLUSION

The fungal pathogens isolated and identified from this study as the causative agents of postharvest storage rot of onion bulb obtained from different markets in Calabar Metropolis of Cross River State were *Aspergillus niger* and *Rhizopus stolonifer*. Pathogenicity test confirmed that these pathogens actually caused storage rot of onion bulb. The efficacy of the crude plant extracts (*Dioscorea dumetorum* and *Psidium guajava*) against storage rot fungi was tested *in vitro* and *in vivo*. The *in vitro* results showed that the extracts significantly inhibited the mycelia growth of the fungal pathogens at all the concentrations tested when compared with the control. However, in the *in vivo* assay, the crude plant extracts significantly ($p \leq 0.05$) inhibited the mycelia growth of the fungal isolates at the higher different concentrations tested and the rate of inhibition differed from each other. The extracts showed significant effect on the growth and sporulation of *A. niger* and *R. stolonifer* both *in vitro* and *in vivo* at all the concentration levels tested and as such can be used in the control of postharvest storage rot of onion bulb.

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