ANTIFUNGAL ACTIVITY OF SIAM WEED (*Chromoleana odorata* (L.) AND WOODLAND TOBACCO (*Nicotiana sylvestris* (Speg &Comes) AGAINST PHYTOPATHOGENIC FUNGUS OF ONIONS (*Allium cepa* L.) BULB.

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ABSTRACT

Antifungal activities of *Nicotiana sylvestris* and *Chromoleana odorata* against phytopathogenic fungus of onions bulb were assessed at the Plant Physiology Laboratory, University of Port Harcourt Nigeria. The result of the phytochemical screening revealed the presence of tannins, saponins, flavoniods, alkaloids, phenols and glycosides in both plants but terpenoids were only found in *C. odorata*. The efficacy of the plant extracts varied at various concentrations (50, 75, 100 and 150mg/mL^{-1}). Extracts from *C. odorata* showed progressive retardations of the mycelial growth and high fungitoxic effect on the growth of *Aspergillus niger* at 75% concentration at 100 mg mL^{-1}. *N. sylvestris* extract decreased the growth of the fungi but not as effective as *C. odorata.* The aqueous extracts of *C. odorata* at 75g/100 mL concentration gave the best inhibition of 1.66 ± 0.06^{a} while *N sylvestris* at 75g/100 mL gave 5.22 ± 0.03^{b}. It is recommended that these plants be used as natural fungicides as they possess antifungal potentials.

Keywords: Antifungal Activity, *Nicotiana sylvestris, Chromoleana odorata, Allium cepa,* Phytopathogenicity

INTRODUCTION

The main post-harvest infectious agents of onions are pathogenic fungi. Onions are prone to pathogenic fungal attack due to their moisture content and nutrient composition. There are wide variety of fungal genera limiting shelf life of fruit and vegetables, (Agrios, 2004). Some of these fungi produce mycotoxins and are indirectly responsible for the release of toxins to foods and then to consumers. Post-harvest diseases account for about 50% losses in plants stored in poor storage conditions especially under high humidity (Agrios, 2005).

For example, in Nigeria local farmers are responsible for close to 98% of the farm produce. Most of these farmers are least educated and therefore in most cases cannot practice the various control measures recommended for disease control (Chiejina and Ukeh, 2013; Amadioha, 2000 and Okigbo, 2009).

The phytopathogenic fungi that cause post-harvest diseases can be controlled using synthetic fungicides; but, there are restrictions in the use of synthetic fungicide due to their harmful effect on humans and the environment (Harris *et al.*, 2001). These synthetic fungicide, are very expensive and are also not environmentally friendly. At present the use of synthetic fungicides in controlling these diseases leave negative effects on human health and the environment (Ebele, 2011; Paster and Bullerman, 1988; Sahu *et al.*, 2012; Bull *et al.*, 1997), as they also pollute the soil, water and air, due to their residual toxicity (Satish *et al.*, 1999 and Strange, 1993). This therefore, explains the need to substitute these synthetic fungicides with biological fungicides.

Since the end of the Second World War, there has been a great boom in the use of fungicides for control of fungal diseases worldwide. Lately the use of biological control to tackle agricultural pests and diseases has increased primarily due to increased pressure to reduce the use of synthetic chemicals which are toxic to humans and the environment (Vasantharaj, 2008; Carson, 1962).

In order to reduce the toxic effects of these synthetic chemicals on plants, several researches have been conducted to demonstrate that different plant part possess chemicals that have inhibitory properties against bacteria, fungi and insects (Davicino *et al.*, 2007).

Plants have pharmaceutical and antimicrobial properties (Bari *et al.*, 2010). Cowan, (1999) also reported that plants have antimicrobial properties. They produce defensive agents that protect them from microbial infection and other diseases. According to Azoro (2002), ''several plants species have been tested for antimicrobial properties but a lot of them have not yet been adequately evaluated''.

So many other studies have been published, investigating the antifungal and antibacterial activities of plant derived compounds against a variety of pathogens (Tassou, *et al*., 2000; Friedman, *et al*., 2002; Momtaz and Abdollahi, 2010; Manikandan, *et al.*, 2011; Ara, *et al.*, 2009). Antimicrobial compounds derived from plants might also inhibit bacteria through different mechanisms.

These diverse substances have been identified in plants which are believed to be the antimicrobial agent and they include; different forms of alkaloids, diterpenes, saponins, flavonoids, sterols, quinines, different forms

of other proteins as well as lipids (Sofowora, 1993). These antimicrobial agents are aromatic secondary metabolites and plants have the ability to synthesize them (Cowan, 1999). Some of them like Saponins generally protect plant against microorganisms (Umaru, *et al.,* 2006).

Most components of plant are toxic to pathogens. When these components are extracted from the plant and applied on infested crops, they are called botanical pesticides (Malkhan, *et al.,* 2012). These botanical pesticides are important because they reduce crop losses, they are eco-friendly, bio-degradable and are cheap and affordable.

Several researchers have shown that extracts of many plants may control different fungal plants pathogens.

According to Ebele, (2011), aqueous plant extracts from the leaves of *Carica papaya, Chomolaena odorata,* and *Acalyphaciliata*was tested on the growth of the pathogenic fungi rot on pawpaw fruits. The different concentrations (10, 20 and 30%) significantly reduced the mycelia growth of *Aspergillus niger in-vitro* by (26.83%), *Botryodiplodia theobromae* (31.71%) and *Fusarium solani* (39.02%) respectively.

Barnabas, (2012), researched on the efficacy of aqueous leaf extracts of *Carica papaya, Azadirachtaindica, Moringaoleifera, Cassia alata*and *Nicotiana tobaccum* in managing seed borne fungi of onion and reported that all the aqueous leaf extracts significantly ($P < 0.05$) inhibited the radial mycelia growth of the test fungi *Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Botrytis sp*and *Fusarium oxysporumin-vitro* which are the fungal affecting onion.

Allelopathic effect of leaf extract of *Azardirachtaindica* and *Chromolaena odorata* were reported by Ijato *et al.,* (2010) against post- harvest and transit rot of tomato (*Lycopersicumesculentum* L). According to Suleiman, (2011), Plant extracts was employed to control the vegetative mycelia growth from neem *(Azadirachtaindica*) and tobacco (*Nicotiana tabacum*) at 20, 30,40, 50 and 60% concentrations. *In vitro* application of extracts for the control showed that tobacco and neem (*Nicotiana tabacum* and *Azadirachtaindica*) had fungitoxic effect that controlled the mycelia growth and there was complete inhibition on all the fungi. Chiejina and Oneabi, (2007) had also reported the antifungal properties of *C. odorata* and *M. olifera* in fungal rot of *C.sativus.*

To grow the search for new antifungicidal agents from plants, *Nicotiana sylvestris* and *Chromoleana odorata* were evaluated in this study.

Onions are being affected by a large numbers of fungi which has become unfavourable leading to substantial yield loss worldwide. This has become a source of serious concern (Ijato *et al.,* 2010). This study therefore is aimed at evaluating *in vitro* antifungal activity of *Nicotiana sylvestris* and *Chromoleana odorata* extracts against phytopathogenic fungus of onions – *Aspergillus niger.*

MATERIALS AND METHODS

Collection of plant samples: The plant samples *Nicotiana sylvestris* and *Chromoleana odorata* were collected July, 2017 from Rukpokwu and Choba in Obio/Akpor Local Government Area, Rivers State. The plant samples were identified at the Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Choba Rivers State.

METHODS

Preliminary phytochemical screening: Qualitative phytochemical screening of the extracts was conducted using standard procedure as described by Harborne, (1973) to determine the presence of phytochemicals such as tannins, saponins, alkaloids, flavonoids, phenol, glycosides and terpenoids.

Isolation of fungi from diseased onions: The isolation method of Chiejina, (2008) was used. Fungal identification was confirmed with the aid of books by (Agrios, 2005; Samson, *et al.,* 2010; Ellis, *et al.,* 2007).

Pathogenicity test: The method of Okigbo and Ikediuqwu (2000), was used.

Preparation of plant samples: The leaves of *N. sylvestris* and *C. odorata* were washed and cut with knife into bits and dried at room temperature for 2 days to get rid of moisture. After this, the samples were then crushed into powder using a blender.

Extraction: Parekh and Chanda, (2006) method of extraction was moderated and used.

Aqueous extraction: The aqueous extract of the plant was prepared by adding the crushed leaves in 100 mL of distilled water. The concentration of each extract was determined by adding 50, 75, 100 and 150g/ mL^{-1} in different mL of distilled water. The mixture was left for 24 hours at room temperature and afterwards filtered using No. 1 Whatman filter paper. The extract was then concentrated by heating on water bath to 50 mL of the original volume of the extract.

Test microorganisms: *Aspergillus niger* were collected from onions based on its pathological importance.

Sources of test microorganisms: The pure cultures of *Aspergillus niger* was obtained and maintained on Potato Dextrose Agar (PDA) at Mycology/Pathology laboratory in the Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Choba Rivers State.

Determination of Antifungal Activity: The zone of inhibition of the extracts was determined using agar well diffusion method by following modified procedure of ICMSF (1998). Briefly, PDA was inoculated with the fungi by spreading the fungal

inoculums on the media. The fungi were later sub-cultured in PDA. Wells were made into the PDA using a sterile 3mm stainless steel borer. The borer was deeped into the alcohol for sterilization and then was used to make wells. The wells were then filled up with 0.2 mL of the extracts and care was taken not to allow the solution to spill on the surface of the medium. Different well-isolated colonies of the fungus were selected from a pure agar plate culture then the top of each colony was touched with a loop, and the growth was transferred PDA. Control wells containing sterile distilled water were also plated. The plates were incubated at 28°C for 24 hours and the sensitivity of the organisms to the extract was recorded by measuring the zone of inhibition. This was done by measuring the diameter of the zone of inhibition using a transparent meter rule. The effects of the extracts on the fungal pathogen was compared with that of the water control, respectively.

Statistical analysis: The results were analyzed using ANOVA. The Duncan's multiple range test significance was used to test the difference among treatments. All analyses were carried out at 5% level of significance.

RESULTS

Phytochemical Analysis: It was discovered from the result that tannin, saponins, alkaloid, flavonoid, phenol, glycoside and terpenoid were present in leaf extracts (Table 3.1).

Table 3.1: Phytochemical Screening of *Nicotiana sylvestris* and *Chromoleana odorata* Extracts.

Compounds	*Nicotiana sylvestris*	*Chromoleana odorata*
Tannins	++	++
Saponins	+++	++
Alkaloids	+	+
Flavonoids	++	+
Phenols	++	+
Glycosides	+	+
Terpenoids	+	-

+++ = Present in high concentration, ++ = present in moderate concentration, + = present in low concentration, - = absent.

Identification of pathogens: One fungal isolate was obtained from diseased onions and identified as *Aspergillus niger*as was confirmed by the pathogenicity test carried out.

Pathogenicity test: On establishment of disease condition, inoculum were taken from the infected onions and cultured. The organisms were re-isolated and identified as *Aspergillus niger*

Effects of plant extracts on radial growth of pathogens: The aqueous extracts of *Nicotiana sylvestris* and *Chromoleana odorata* where found to be fungitoxic on themycelial growth of the rot fungi. The disease development of *Aspergillus niger* was inhibited by the plant extracts. The inhibitory effects of the plant extracts did not increase as the concentrations increased. *Chromoleana odorata* extracts exhibited high inhibitory effects than*Nicotiana sylvestris* in all the concentrations (Table 3.2). The results revealed that both plant extracts produced significant ($p<0.05$) levels of inhibition of mycelial growth of *Aspergillus niger* at the various concentrations. The aqueous extracts of *C. odorata* at 75g/100 mL concentration gave the best inhibition.

Table 3.2: Antifungal Activity of Aqueous Extracts of *Nicotiana sylvestris* and *Chromoleana odorata* of the Extracts

Concentrations (g/100 mL)	Microorganisms	*Nicotiana sylvestris*	*Chromoleana odorata*	Control
50	*Aspergillus niger*	8.15 ± 0.06^{a}	1.82 ± 0.00^{c}	8.46 ± 0.06^{b}
75	*Aspergillus niger*	5.22 ± 0.03^{b}	1.66 ± 0.06^{a}	8.66 ± 0.09^{c}
100	*Aspergillus niger*	6.23 ± 0.03^{a}	5.32 ± 0.02^{b}	18.30 ± 0.14^{c}
150	*Aspergillus niger*	6.75 ± 0.07^{a}	6.65 ± 0.05^{a}	21.53 ± 0.10^{c}

DISCUSSION

From the result obtained, it is evident that *C. odorata* inhibited the growth of *Aspergillus niger* more than *Nicotiana sylvestris* (Figure 4.1). Comparing the two extracts used, the inhibition of *C. odorata* was more than that of *N. sylvestris at* 75% concentration followed by 50%, 100% and 150% concentrations while 75% concentrations, showed the lowest growth of *Aspergillus niger*.

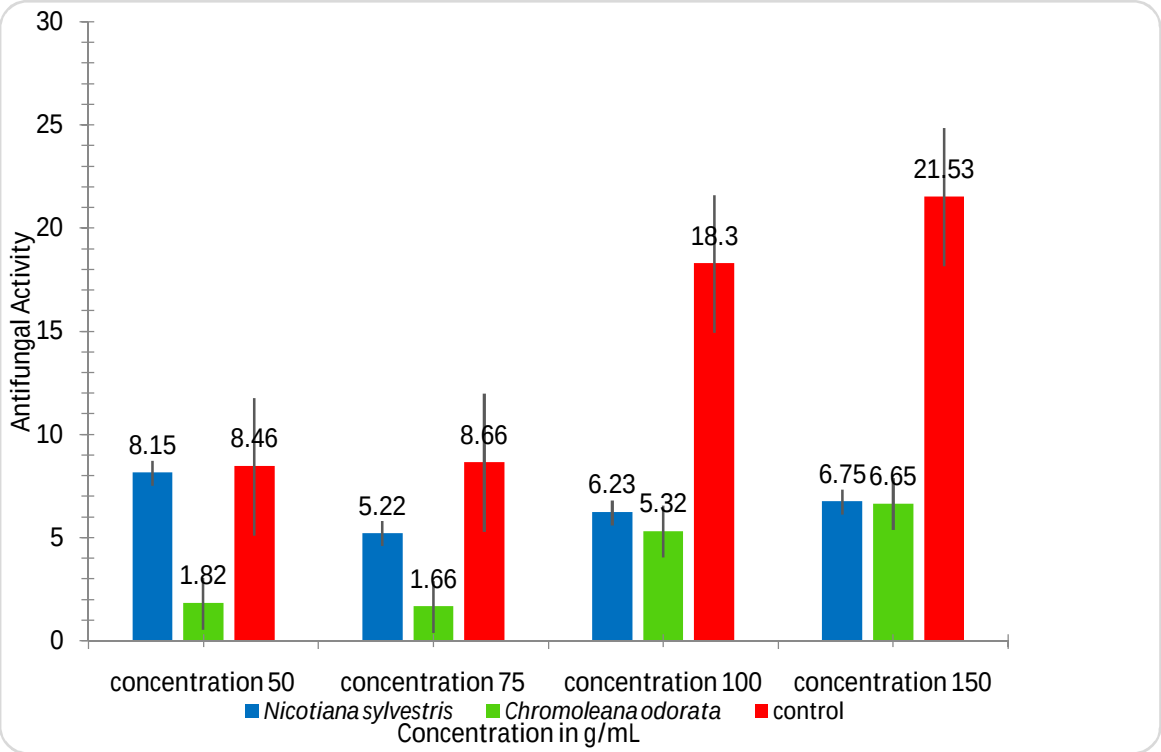

Figure 4.1: Effects of aqueous plant extracts of *Nicotiana sylvestris* and *Chromoleana odorata* on *in-vivo* treatment of *A. niger*.

Aspergillus niger can produce air-borne spores that lands on the bulb while on display in the market. This organisms, is associated with post-harvest rot of onions. The results indicated that the tested plant extracts, *Chromoleana odorata* and *Nicotiana sylvestris*; caused a significant reduction in the radial growth of the pathogens. This shows that they have fungitoxic potentials. The observed fungitoxicity of the extracts confirms the report of Ijato *et al.* (2010) who reported fungitoxic activity of *Azadirachtaindica* and *Chromoleana odorata* against *A. niger, F. oxysporum, R. stolonifer* and *G. candidum*. This result is also in agreement with the findings of Barnabas, (2012) and Suleiman, (2011) who investigated the efficacy of various leaf extracts on seed borne fungi of onion. Furthermore, the result obtained from this study is also in agreement with the findings of Davicino, *et al*., (2007), Bari, *et al.*, (2010), and Cowan, (1999) who stated that the inhibitory effects of plant extracts on mycelial growth of plant pathogenic fungi lie in their phytochemical constituents which include alkaloids, tannins, flavonoids, phenols, saponins and terpenoids.

The fact that plant extracts from *C. odorata* were used to control the rot of onions bulb makes this weed a possible substitute for synthetic fungicide and this approach to plant disease management is economically viable.

CONCLUSION

Microbial deterioration is the problem faced in the world so a lot of struggles are on to determine new antimicrobial agents from plant sources. This study revealed that these plant extracts possessed bioactive compounds such as tannins, flavonoids, phenols, saponins that act against *A niger*, which warranted their use in ethnomedicine for treatment of infectious diseases. The aqueous extract of *C. odorata* showed significantly higher inhibition than that of *Nicotiana sylvestris.*

RECOMMENDATION

This work has shown the antifungal activity of a weed (*Chromoleana odorata).* The plant has the ability to suppress the growth of other plants growing with it (allopathic properties), and can also be used as antifungal plant. Furthermore, before use in human, isolation of pure compound, toxicological study and pharmacological activity should be carried out thereafter.

The plant should also be cultivated and be used in a sustainable manner to avoid extinction. Further research should be conducted on these findings, as these data are very useful for plant protection practice, particularly for medicinal plant which demands for non-Pollutant and environmental friendly alternative methods to combat fungicides.

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