In Vitro Management of Pre and Post-Harvest Soft Rot Fungi Associated with the Fruits of *Dalium guineense* Wild Using Extract from the Leaves of *Ocimum gratissimum* and *Cymbopogon citratus*

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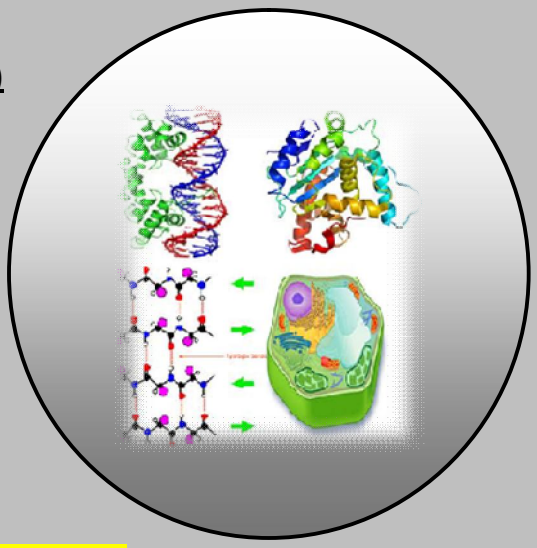

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RESEARCH PAPER

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In Vitro Management of Pre and Post-Harvest Soft Rot Fungi Associated with the Fruits of *Dalium guineense* Wild Using Extract from the Leaves of *Ocimum gratissimum* and *Cymbopogon citratus*

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ABSTRACT

This study was carried out to isolate, and identify post-harvest fungi on the fruits of Dialium guineense, and the control of the fungi using leaf extracts of Cymbopogon citratus and Ocimum gratissimum. This study is further carried out to analyse the nutrient values of the fruit. The fungi isolated from the fruits include: Aspergillus niger, Aspergillus flavus and Aspergillus japonicum. The leaf extracts of Ocimum gratissimum has a higher control effect than the leaf extracts of Cymbopogon citratus. At 600mg/ml, Aspergillus flavus was totally controlled by the use of the leaf extracts of Ocimum gratissimum and Cymbopogon citratus, while the control of Aspergillus niger, was achieved at the concentration of 700mg/ml of both plant extracts on the pulp coat, and 900mg/ml of Ocimum gratissimum and 1000mg/ml of Cymbopogon citratus on the pulp of the fruits. There are significant differences between the rate of growth of fungi on the fruits protected with leaf extract of Cymbopogon citratus and Ocimum gratissimum, therefore a dynamic growth control is achieved at 600mg/ml on the pulp coat and 800mg/ml on the pulp of the fruit using both plant extracts. The proximate analysis of the nutrient value of this fruits revealed that carbohydrate, protein, Ash, lipid, vitamin A, vitamin C and minerals. Such as calcium, phosphorus iron, sodium, zinc, magnesium, etc are contained in the fruits. However fungi control of infection could be achieved using plant extracts, washing and drying the fruits to reduce the moisture level and storing the fruit in a dry environment.

Key words: Management, Soft-rot, Post-harvest and Extract Fungi.

INTRODUCTION

Several methods used to fight against fungal pathogens include the use of chemicals and biological agents (Neergaard, 1977). Some chemicals are generally considered toxic to mammals including man. Man had tried to use an alternative way of fighting against fungi pathogens which pose less or no environmental contamination and therefore tried plant extracts (Jager, 1995). Many herbal drugs are being produced and tested for tropical and systemic fungal infections and the need for new anti-fungal is ever increasing (Khan and Jain, 2000). The wide and indiscriminate use of chemical preservatives has been the cause of the appearance of resistant micro-organisms, leading to occurrence of emerging food borne diseases, (Gibbon, 1992; Akinpelu, 2001). Plant extracts have long been speculated as vital factor to disease resistance and control against a wide range of fungi that infect crops (Mahadevan, 1982; Gerard – Ezhilan*et al,* 1994; Singh and Singh 1980; Kuruchevee*t al*, 1997; Natarajan and Lolithakumari, 1987, Tiwari, 1997). It is well established that some plants contain compounds able to inhibit the microbial growth, (Matasyoh*et al,* 2007, 2011). Plant extracts have been used successfully to control diseases in plants and tuber crops (Amadioha and Obi, 1999; Onifade, 2000). Extracts of plants have also exhibited marked effect on germination of spores (Singh *et al,* 1983; Dixit *et al,* 1983, Singh and Dwivedi, 1990). Oluma and Garaba (2004) have observed that crude extract of *Eucalyptus globulus* and *Ocimum gratissimum* reduced radial growth of *Pythium aphanidermatum* by 44.5 – 100%. Amadioha (2000) proved that *Ocimum gratissimum* leaf extract was able to control spore germination and mycelia growth of *Rhizopus oryzae.*Okigbo and Ogbonnaya, (2006), observed that the leaf extract of *Ocimum gratissimum* and *Aframomum melegueta* inhibited the spores of *Fusarium oxysporium* and *Aspergillus niger*. Narayanarrao*et al,* (1996), proved that using higher concentration of plant extracts raises the inhibition level of the extracts. Ofokansi *et al,* (2003), have proved that *Ocimum gratissimum* act as anti – bacterial agents against some bacteria. Matasyoh*et al,* (2011), showed that the essential oils from *Cymbopogon citratus,*could control the vegetative growth of five species of *Aspergillus.* Lahlou and Berrada (2001), reported that extracts of *Cymbopogon citratus* inhibits spore germination and fungal growth of *Aspergillus* species, through the respiratory suppression of aerial mycelia. Matasyoh*et al*, (2011), reported that, there are about twenty five essential oils extracted from the leaves of *Cymbopogon citratus*, which are grouped into monoterpenes and sesquiterpenes. The major components of the oils i.e. geranial, neral and geranial, extracted from the leaves of *Cymbopogon citratus* have been reported to have high antifungal activity (Lee *et al*, 2008). Some plant extracts are specific. They are biodegradable, cheap, readily available and environmental friendly, than synthetic chemicals (Dubey and Dwivedi, 1991). In general the inhibitory action of natural products on mould cells involves cytoplasm granulation, cytoplasmic membrane rupturing and inactivation and/or synthesis inhibition of intracellular and extra-cellular enzymes (Souza *et al*, 2005).

MATERIAL AND METHODS

Extraction of Active Principles for the Control of Seed-borne Fungi of *Dialium guineense*

The leaves of *Ocimum gratissmum* and *Cymbopogon citratus* were used for the controls. The leaves of the above stated plants were freshly harvested, washed separately in several changes of sterile distilled water and air dried for seven days. When dry, they were separately ground in a mortar, using pestle into fine powder. The fine powder of the plant extracts were measured into the following concentration of 1:1, 2:1, 3:1, 4:1, 5:1 mg/ml of distilled water in the flask for 24 hours. They were filtered using white cheese-cloth, and autoclaved at 121^0C for 15 minutes.

Mode of Inoculation

The fruits of *Dialium guineense* were peeled, and soaked in the different concentrations of the plant extracts, and allowed to imbibe the extracts for 10 minutes. The treated fruits were inoculated in blotter paper in 9cm Petri dishes and allowed for 7 days in an incubator before observing them. The control was not treated with any plant extracts. Each concentration was incubated in triplicates, including the control.

Determination of Fungal inhibition

The fungal inhibition or the effect of the plant extract is determined by counting the number of fruits infected by a particular species of fungus in each plate as follows= N-X. Where N = No of seed incubated in each plate.NX= the number of seeds without any growth. Then the mean percentage is calculated.

RESULTS AND DISCUSSION

At 700 mglml there was a complete inhibition of the growth of *Aspergillus niger* on the pulp coat by the extracts of lemon grass and Basil respectively, while the same effect was experienced at 900mglml in basil leaves extracts on the pulp of the fruit.At 400mg/ml of extracts of lemon grass and 300mg/ml, basil there was a complete inhibition of the growth of *Aspergillus flavus* on the pulp-coat of *Dialium guineense*, while the same effect was observed at 500mglml and 600mglml in lemon grass and Basil leaves extracts respectively on the pulp of the fruit. At 700mg|ml of extract of lemon grass and 600mglml of extract of basil leaves, there was a complete inhibition of the growth on the *Aspergillus japonicum* coat of the fruit of *Dialium guineense*, while the same effect is observed on the fruit pulp by the extract of Basil leaves at 500mglml. There is a significant different between the rate of inhibition of vegetative growth of fungi on the pulp coat and the pulp using the plant leaves extracts of both Basil leaves and the lemon grass. This may be as due to the fact that the pulp coat act as a barrier to rate the penetration and infestation of the fungi. Tables.1 and 2 showed that, *Aspergillus flavus* may be controlled on the pulp coat of the fruit of *Dialium guineense* by using basil and lemon grass at the concentration of 600mgl on the pulp coat and the pulp. Control of the growth of *Aspergillus niger* requires a higher concentration as much as 700mglml of both plant extracts on the pulp coat, and 900mglml of basil leaves and lemon grass leaves on the pulp of the fruits. Result in table1. showed that the leaf extracts of *Cymbopogon citratus* exhibited complete control over the vegetative growth of *Aspergillus flavus* on the coat and pulp of the fruit of *Dialuim guineense* at treatment concentration of 800mglml and above, whereas the vegetative growth

of *Aspergillus niger* and *Aspergillus japonicum* on the coat of the same fruit were completely inhibited by the same test plant at the concentration of 700mglml. However, there was no complete inhibition of the vegetative growth of *Aspergillus niger* and *Aspergillus japonicum* on the pulp of the same fruit by the test plant used in the study, this is contrary to the work of Matasyoh *et al,* (2011), which stated that the highest antifungal activity of essential oil was observed in *Aspergillus niger*, while the highest resistant was observed in *Aspergillus flavus*.

Table 1. Mean Percentage Inhibition of Pulp-coat Borne and Pulp-borne Fungi of Fruits of *Dialium guineense* leaf Extracts *Cymboponon citratus*.

Concentration (Mglml)	Test fungi *Aspergillus niger*		*A. flavus*		*A. japonicum*	
	Coat	Pulp	Coat	Pulp	Coat	Pulp
100	26.67±2.72	56.6±2.72	10.0±4.71	23.33±2.72	10 ±0.0	33.33±2.72
200	23.33 ±4.71	53.3 ±2.72	6.67 ±2.72	16.67±2.72	6.67±2.72	26.67±2.72
300	13.33 ±2.72	46.67±3.34	3.33 ±3.34	13.33±2.72	6.67±2.72	16.67±2.72
400	13.33 ±2.72	43.33 ±2.72	0.0 ± 0.0	6.67±2.72	6.67± 2.72	13.33±2.72
500	10.0±0.0	43.33±2.72	0.0 ±0.0	0.0 ± 0.0	3.33 ±2.72	6.67±2.72
600	6.67±2.72	40.00 ±3.34	0.0 ±0.0	0.0 ± 0.0	3.33 ±2.72	0.0 ±0.0
700	0.0±0.0	26.65±2.72	0.0 ± 0.0	0.0±0.0	0.0 ±0.0	6.67 ±5.4
800	0.0 ±0.0	23.33±3.33	0.0 ±0.0	0.0±0.0	0.0 ±0.0	0.0 ±0.0
900	0.0 ± 0.0	6.67±2.72	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0	3.3 ±2.72
Control	30±9.43	63.33±8.4	10 ± 4.71	23.33±6.09	13.37±5.44	40±16.67

Table 2. Mean Percentage Inhibitions of Pulp- coat Borne and Pulp-borne Fungi of Fruits of *Dialium guineense,* by Leaf Extracts of *Ocimum gratissimum*.

Concentration (Mglml)	Test fungi *A. niger*		*A. favus*		*A. japonicum*	
	Coat	Pulp	Coat	Pulp	Coat	Pulp
100	20.0±9.43	46.67±2.72	3.33±2.72	13.33±2.72	0.0 ±0.0	23.33±2.72
200	20.0±4.71	43.33±2.72	3.33±2.72	16.67±2.72	10.0±4.71	16.67±2.72
300	16.67±2.72	36.67±2.72	0.0±0.0	13.33±2.72	6.67 ±2.72	10.0±0.0
400	10.0±4.71	23.33±2.72	0.0±0.0	6.67±2.72	6.67 ±2.72	3.33±2.72
500	6.67±2.72	26.67±2.72	0.0± 0.0	3.33 ±2.72	10.0 ±0.0	0.0 ±0.0
600	3.33 ±2.72	20.0±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
700	0.0±0.0	13.33±4.71	0.0±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
800	0.0 ±0.0	6.7±2.72	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
900	0.0 ±0.0	0.0±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
Control	30.0±9.43	63.33±8.4	10.0±4.71	23.33±6.09	13.33±5.44	40.0±16.67

The work of Chiejina (2006) showed that the greater inhibitory effect of *Ocimum gratissImum* relative to *Cymbopogon citratus* on *Fusarium solani* and *Aspergillus niger* observed in her work was an indication that the active principle is present and potent even at the lowest concentration of 0.1%.

Table 2 showed that *Ocimum gratissimum* exhibited complete control over the vegetative growth of the fungi (*Aspergillus niger, Aspergillus flavus* and *Aspergillus japonicum*) isolated on both the coat and the pulp at the treatment concentration of 900mglml. This shows that *Ocimum gratissmum* at the treatment concentration of 900mglm could hold better promise in the effective control of deteriorating activities of these pathogens on the fruit. The use of *Cymbopogon citratus* extract at the treatment concentration of 800mglml could be recommended for the effective control of the growth of *Aspergillus flavus* and *Aspergillus japonicum* on the pulp and coat of the fruits. Amadioha (2000), showed that *Ocimum gratissimum* leaf extracts was able to control spore germination and mycelial growth of *Rhizopus oryzae*.

The fungitoxic properties of these extracts hindered the mycelial development of the fungi by probably affecting their metabolism, which may have resulted in their inability to use the substrate properly, mostly at higher concentrations. According to Amadioha and Obi (1999); Okigbo and Ajalie, (2005); and Okigbo *et al*, 2005, the active principles present in plants were influenced by many factors which may include: the age of the plant, extracting solvents, method of extraction and time of harvesting of the plant materials. Amadioha (2000), observed that inhibitors of the fungitoxic principle may be responsible for the differences observed in fungitoxic activity of the extracts.

Oluma and Garba (2004), observed that the crude extracts of *Eucalyptus globulus* and *Ocimum gratissimum* reduced radial growth of *Pythium aphanidermatum* by 44.5-100%. There were significant differences (P>0.05) between the rate of growth on the fruit protected with leaf extraction of *Cymbopogon citratus* and *Ocimum gratissimum*and the control.

CONCLUSIONS AND RECOMMENDATIONS

The use of leaf extracts of *Cymbopogon citratus* and *Ocimum gratissimum,* in preserving the fruits could arrest the growth or rate of infection of fungi on the fruits of *Dialium guineense* at the average concentration of 600mglml and elongate the shelf-life of the fruits.During harvesting, care must be taken so that the rind of the fruit is not opened, as the injury of the rind could make way for the penetration and infestation by the fungi. From the result of proximate analysis of the food value of the fruits of *Dialium guineense*, we could conclude that the fruit of *Dialium guineense*is a good source of carbohydrate and essential minerals such as calcium, potassium, phosphorus, iron, magnesium, and zinc. Others are vitamin C (ascorbic acid) and vitamin A (β-carotene) and therefore, this fruit may serve as a good source of food nutrients and food supplements.

This plant, *Dialium guineense* Willd is an eco-botanical plant, and could also be used for phytoremediation.

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