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Fungitoxic effect of ethanolic and aqueous leaf extracts of *Azadirachta indica* A. Juss, *Ocimum gratissimum and Benlate in the control of Post harvest Fungal diseases of Solanum melongena* Linn (Egg plant).

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ABSTRACT

Fungitoxic properties of ethanolic and aqueous leaf extracts of *Azadirachta indica, Ocimum gratissimum and* benlate *were* used *in vitro* for the control of post-harvest fungal diseases of *Solanum melongena,* caused by *Aspergillus niger, Rhizoctonia solani* and *Mucor ramosissimus.* Pathogenicity test confirmed them as the causal organisms of the fruit rots. The three pathogens were treated with both ethanol and aqueous leaf extracts of the two plants at concentrations ranging from 25 to 100% and benlate at concentrations between 2.5 and 10.0%. Various concentrations were added to prepared Potato Dextrose Agar (PDA) media. The pathogens were inoculated separately into the PDA media and were incubated for eight days. Fungitoxic effects of these extracts on the mycelial growth of the pathogens were significant at $P \leq 0.05$ for all treatments. The pathogens were completely inhibited at 100% concentration of both extracts and at 10% concentration of benlate. With respect to ethanolic extract; *O.gratissimum* was more efficient than *A. indica* but not with aqueous extract.

Keywords : *Fungitoxic,Azadirachita indica, Ocimum gratissimum*,Benlate, Pathogens

I. INTRODUCTION

Garden egg (*Solanum melongena* Linn) of the family Solanaceae (Obeng-Ofori *et al.,* 2007) is a kind of fruit that is very important for man; as food, supplying some major nutrients, and as a source of bioactive ingredients militating against some diseases like diabetes mellitus and liver problems. It is also used for many other purposes among which are to achieve weight control within a short period, eliminate unnecessary salts in maintaining proper functioning of the heart, reduce the sugar content level in diabetics because of its low calorie and high fibre contents and reduce blood cholesterol (Aliyu, 2006). However, the damage caused by pests and microbes often constitute a great impediment to biomass productivity of this plant. It is for example susceptible to fungal diseases caused by *Phytophthora nicotianea,* var. *parasitica* and if the fruit touches the ground, *Corticium rolfsii* will cause an infection (Obeng-Ofori *et al.,* 2007). Some pathogens that infect egg plant fruits at various stages of development and particularly after harvest include the genera such as

Phytopthora, Helminthosporium, Hemilleia, Mycosphaerella, and others (Mehrota and Aggarwal, 2003).

Extracts of plants such as *A. indica* are believed to be efficacious in the control of plant fungal diseases and have been recommended by many international organizations (Chaturvedi *et al.,* 2003 and Hasabnis and Souza, 1998 Ewekeye *et al*., 2013). Leaves of *Ocimum gratissimum* have been found to exhibit high anti-fungal activities against *Fusarium moniliforme, Aspergillus flavus*, and *Aspergillus fumigatus* (Nguefack *et al.,* 2004). Problems associated with the use of synthetic chemicals necessitated the use of antimicrobial agents of plant origin for the control of post-harvest fungal diseases of plants which is one of the reasons for this research. We hope to: 1. Isolate and identify the pathogens responsible for the post-harvest fungal diseases of garden egg. 2. Determine the frequency of occurrence for each of the fungal isolates. 3. Estimate the disease severity in the infected garden egg. 4. Determine the extent of fungal inhibition by ethanol and aqueous extracts from *A. indica* and *O. gratissimum* leaves and benlate solution.

II. MATERIALS AND METHODS

Source of Materials

The garden egg varieties (green and white) showing symptoms of rots were collected from Ogige market, Nkwo market in Ibagwa village and Ofor market in Opi village, all within Nsukka, Enugu State, Nigeria. The leaves of *Azadirachta indica* and *Ocimum gratissimum* were obtained from various locations in Nsukka. Benlate was purchased from a chemical shop in Nsukka. Water as negative control was included.

Sterilization Techniques

Potato Dextrose Agar (PDA) was aseptically sterilized in an autoclave at 103 KNM^{-2} pressure and 121°C for 15 minutes: Petri dishes, beakers, test tubes, filter papers, spatula and forceps were sterilized in a hot air oven at a temperature of 160°C for 1 hour. The wire loops were also sterilized by heating over a sprit lamp until red hot and allowed to cool. Seventy per cent alcohol was used to wipe the work tops to prevent contamination.

Preparation of Plant extracts

Plant collection

Neem leaves collected were healthy and uninfected and were washed under running tap water to remove any traces of soil particles and other dirt, and Distilled water, air dried and cut into small pieces and dried for 15 days in shade. The leaves were ground and sieved to get fine powder. The extraction process used was the method of Doherty *et al.* (2010). The extracts were reconstituted by dissolving 10 g of each extract in 20 ml of sterile distilled water and stored in a conical flask at 12°C in a refrigerator for further work.

Preparation of culture media

All culture media were prepared according to instructions by manufacturers and this includes the sterilization of all equipments.

Isolation of Fungi from S. *melongena* fruits

The isolation technique of Chiejina (2008) was used. Thin sections (2mm diameter) were cut from the periphery of diseased garden egg fruits and sterilized in 0.1% mercuric chloride for 2 minutes. These sections were rinsed in 3 changes of sterile distilled water and plated in PDA plates. The plates were incubated at room temperature ($27^0 \pm 2$°C) for 7 days. Pure cultures were obtained by several transfers of the colony growth from PDA plates to clean PDA plates aseptically.

Identification of fungal isolates

Identification of fungi was based on the growth patterns, colour of mycelia and microscopic examinations of vegetative and reproductive

structures according to (Barnett and Hunter (1999) and Alexopoulos *et al.* (2002).

Pathogenicity test

Pathogenicity test was carried out for all the isolates from the different garden egg fruits using the techniques of Okigbo *et al.* (2009). Un-inoculated garden egg fruits (control) were also set up (Okigbo and Emeka, 2010). Pure cultures were identified according to Barnett and Hunter (1999) and Alexopoulos *et al.* (2002). The symptoms were compared to those of naturally infected garden egg. Morphological characteristics of conidia and mycelia of the fungi that were re-isolated from inoculated fruits were used to prove Koch's postulates.

III. RESULTS AND DISCUSSION

Three fungi were isolated and identified as *Aspergillus niger, Rhizoctonia solani* and *Mucor ramosissimus.* Pathogenicity tests confirmed that the isolated fungi were the causal agents of the rot. These are shown in Plates 1 to 3 below.

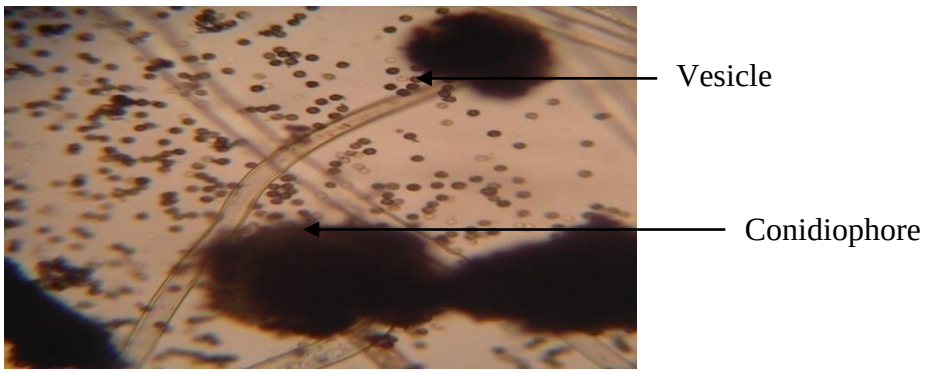


Plate 1: Photomicrograph of *Aspergillus niger*(Mg x400)

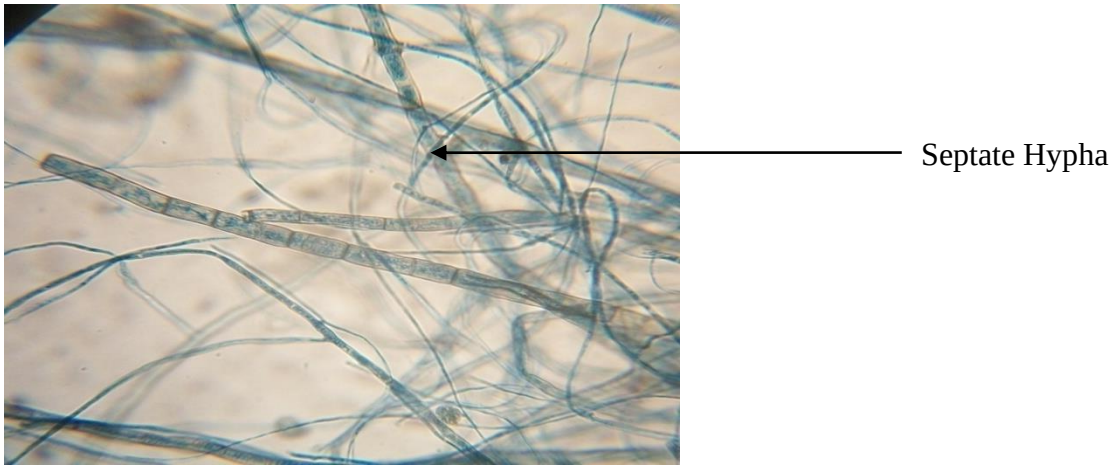
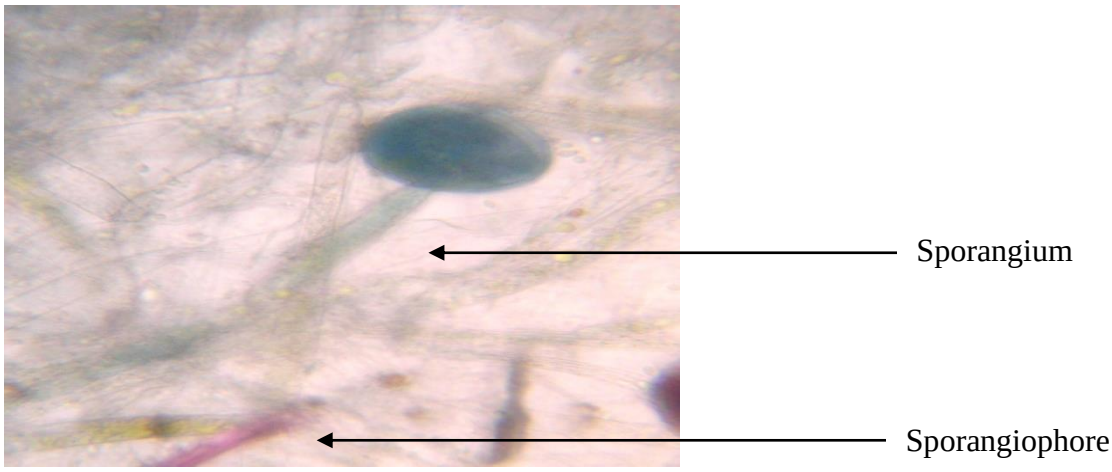


Plate 2: Photomicrograph of *Rhizoctonia solani* (Mg x400)



1) Plate 3: Photomicrograph of *Mucor ramosissimus* (Mg x 400)

Percentage frequency of fungal occurrence

Table 1 : Percentage frequency of fungal occurrence on diseased garden egg fruits

Isolates	No of isolations (%)	Frequency of occurrence
Mucor ramosissimus	11	26.82
Aspergillus niger	14	34.14
Rhizoctonia solani	16	39.02

Table 2 : Disease severity rating of the isolates in garden egg

Days	1	2	3	4	5	6	7	8	9	10
Fungal isolates	Severity ratings									
M.ramosissimus	1	2	2	2	2	2	3	3	4	4
Aspergillus niger	1	2	2	3	3	3	3	4	4	5
Rhizoctonia solani	1	2	2	3	3	3	4	4	5	5
Control	1	1	1	1	1	1	1	1	1	1

Rating scale of 1-5 (Chiejina, 2006) was used; Where: 1= No decay, 2 = Slight decay: 10 – 30%, 3 = Moderate decay: 31 – 60%, 4 = Severe decay< 61 – 90% and 5 = Complete decay: 91- 100%.

Table 3 : Interactive effects of different concentrations of ethanolic extracts on fungi

Ethanolic Extracts	Extract Concentration %	Fungal growth (mm)			Mean ± S.E.
		M. ramosissimus	*A. niger*	*R. solani*	
Control	0.00	90.00	88.66	90.00	89.55±0.45
A.indica	25.00	61.66	59.33	58.33	59.77±0.99
	50.00	40.00	45.33	40.00	41.78±1.78
	75.00	14.00	10.00	10.00	11.33±1.33
	100.00	0.00	0.00	0.00	0.00±0.00
O. gratissimum	25.00	55.66	52.66	45.33	51.22±3.07
	50.00	29.66	40.33	35.66	35.22±3.09
	75.00	14.00	8.66	9.33	10.66±1.68
	100.00	0.00	0.00	0.00	0.00±0.00
Benlate	2.5	37.33	43.33	41.00	40.55±1.75
	5.0	12.67	12.00	13.33	12.67±0.38
	7.5	0.00	0.00	0.00	0.00±0.00
	10.0	0.00	0.00	0.00	0.00±0.00

LSD ($P \leq 0.05$) = 1.70

Table 4 : Interactive effects of different concentrations of aqueous extracts on the fungi

Aqueous Extracts	Extract Concentrations %	Fungal growth (mm)			Mean ± S.E.
		M. ramosissimus	*A. niger*	*R. solani*	
Control	0.00	90.00	88.66	90.00	89.55 ± 0.45
A. indica	25.00	69.00	64.66	61.66	65.11±2.13
	50.00	55.33	49.66	49.00	51.33±2.01
	75.00	16.33	20.33	18.00	18.22±1.16
	100.00	0.00	0.00	0.00	0.00±0.00
O. gratissimum	25.00	72.66	70.00	65.66	69.44±2.04
	50.00	38.33	47.66	42.66	42.88±2.70
	75.00	20.00	17.00	16.00	17.67±1.20
	100.00	0.00	0.00	0.00	0.00±0.00
Benlate	2.5	37.33	43.33	41.00	40.55±1.75
	5.0	12.67	12.00	13.33	12.67±0.38
	7.5	0.00	0.00	0.00	0.00±0.00
	10.0	0.00	0.00	0.00	0.00±0.00

LSD ($P \leq 0.05$) = 1.18

In-vitro effects of *Azadirachta indica* extracts and benlate solutions on the pathogens at various concentrations

Figure 1: shows the effects of *A. indica* extracts and benlate solution on the pathogens at 50% and 5% concentrations, respectively. On the average ethanolic extract gave percentage inhibition on the average higher than aqueous extract while benlate solution at 5% concentration gave the highest inhibition of the three pathogens. There was a significant differnce(P = 0.025) beween the benlate solution and the plant extracts, but none between the two extracts.

Figure 2: shows the effects of *O. gratissimum* at extract at 75% concentration and benlate solution at 7.5% concentration respectively. The results showed a significant increase in the inhibitive abilities of the three solutions. However, benlate solution gave the highest inhibiion (100%) while aqueous extract gave the least (75%). There was a significant (P = 0.01) difference between the three of them.

Figure 3: showed the effects of *O. gratissimum* extract and benlate solution at 100% and 10% concentrations, respectively. Both ethanol, aqueous and benlate solutions gave 100% inhibition of the pathogens

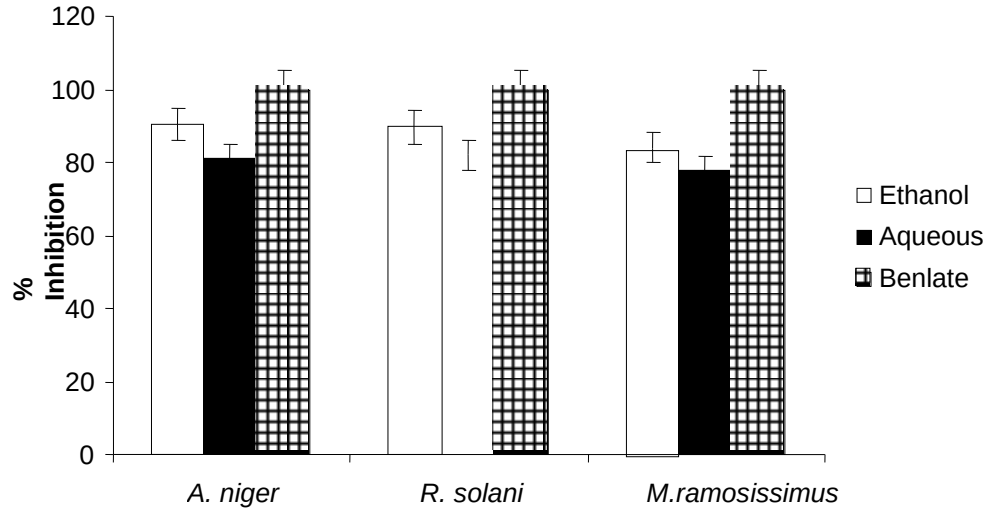


Figure1: Effect of *A. indica* extracts (50% concentration) and benlate (5%) on the pathogens

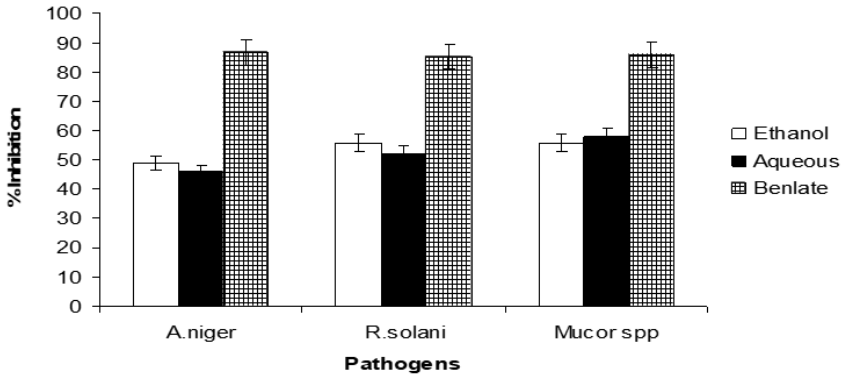


Figure2: Effect of *O. gratissimum* extracts (75% concentration) and benlate (7.5%) on the pathogens.

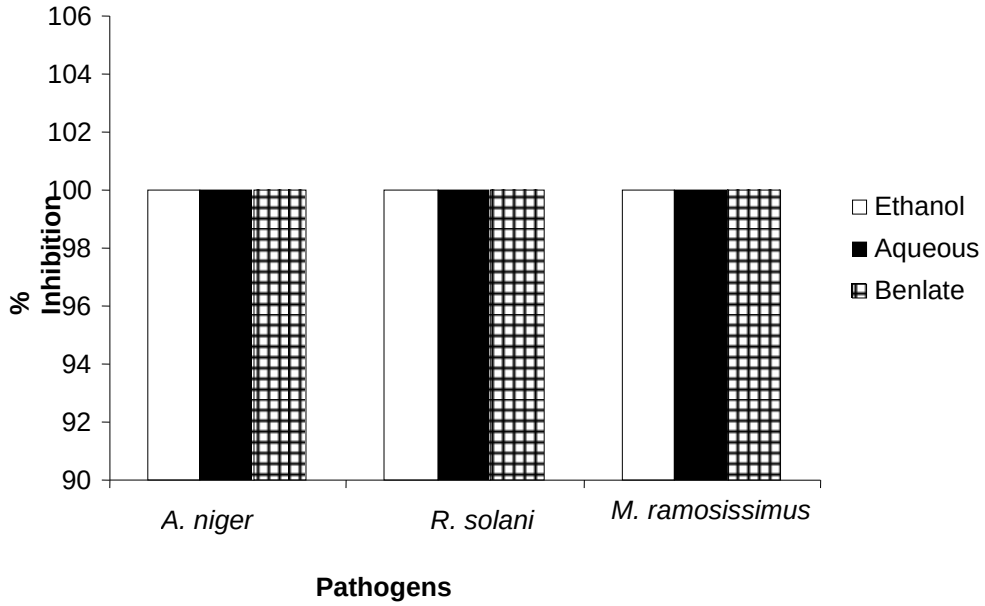


Figure3: Effect of *O. gratissimum* extracts (100% concentration) and benlate (10%) on the pathogens.

Three fungi were isolated from infected *Solanum melongena* fruits that were cultured in PDA plates and incubated at room temperature ($27^0 \pm 2^oC$) for 7 days. Pathogenicity test proved them to be the causal agents of the disease. The results of this investigation showed that garden egg fruits are prone to infections by a variety of fungal pathogens. Two of these pathogens; *Aspergillus niger* and *Mucor ramosissimus* produce numerous air-borne sessile spores that could easily land on the fruits on display in the markets and later infect the fruits. This might explain the reasons for the occurrence of these two fungi in the fruits investigated. This observation agrees with the work of Chiejina (2008) with respect to salad vegetables.

Certain environmental factors may favour the growth of these pathogens on the garden egg fruits. These include temperature; high humidity especially when fruits are wrongly packaged.

The results of the experiment on the frequency of occurrence of the pathogens have shown that *Rhizoctonia solani* had the highest frequency of occurrence and *Mucor ramosissimus* was the least frequent. Severity rating experiments also proved *Rhizoctonia solani* as the most virulent of the three isolated fungi as it gave severe decay of the fruits on the 7th day of incubation with complete deterioration on the 9th day while *Mucor ramosissimus* was the least severe. This agreed with the work of Eze and Maduewesi (1990) their work on sprouting and rotting of cocoyam in storage.

The high frequency of occurrence of these pathogens in the fruits investigated confirms their virulence in the fruits. These investigations have also shown that leaf extracts of *O. gratissimum* and *A. indica* have fungitoxic properties which conformed to Dileep *et al*. (2013), who showed the fungitoxic efficacy of *Ferula asafeotida* and *Azadirachta indica* plant extracts and ripe and unripe pericarp extracts of *Centaurea Sp., Papaver dubium* against *pythium aphanidermatum*. The fact that the leaf extracts were able to control these fungi might suggest that they possess broad spectrum fungitoxic properties. The efficacy of their fungitoxic properties is also good as that of the fungicide, benlate. The differences which occurred during the fungal growth experiment might be due to

the fact that, the crude extract of the leaves used was not purified.

These studies also proved that leaf extracts from *O. gratissimum* were more efficient in reducing fungal growth than those of *A. indica* when ethanolic extract was used but the reverse was the case with aqueous extracts. Ethanolic extracts were more effective than the aqueous extracts. This was revealed when the three fungal isolates were greatly inhibited at 75% concentration of the ethanolic extract of the leaves. With increase in concentrations of the extracts, there was corresponding increase in inhibition giving complete inhibition at 100% concentrations. This agreed with the work of Aasgana (2013), who reported the antifungal activity of crude methanolic extracts of six plants species against three economically important phyto pathogenic fungi that includes the aqueous extracts of *Zygophllum fagabo* and ethanolic extracts of *Azadirachta indica*. Jeyasakthy *et al*. (2013) also corroborated this that methanol extracts and ethyl acetate extracts of *A. indica* showed significant anti fungal activity against *Pythium aphanidermatum*. The fungicidal properties of the extracts hindered the mycelial development of the fungi by probably affecting their metabolism which may have resulted in their inability to use the substrate efficiently which is in conformity with the work of Chiejina and Ukeh (2012).The presence of these phytochemicals supports their use as anti microbial agents. Two pathogens; *Mucor ramosissimus and Aspergillus niger* isolated from garden egg fruit in this work, is in agreement with the earlier findings of Adaskaveg *et al*. (2002) who reported that species of *Mucor, Aspergillus* and *Penicillium* were common post-harvest fungi. *Rhizopus* and *Mucor* have been shown to cause mucorosis in the immune system of man, which could be a serious ailment if contacted via consumption of garden egg fruits. The toxins from these pathogens cause respiratory and ulceration diseases in human beings. *Alternaria* and *Mucor* in household dust are known to cause irritation to asthma and allergy sufferers. Thus as a corollary to the heavy crop loss caused by these pathogens, they also result in serious health problems to man. This is supported by the work of Kurup (2003) where he stated that these pathogenic fungi could cause infections or allergies in susceptible individuals.

IV. CONCLUSION

This work was able to associate the following fungi with the post-harvest rot of garden egg: *A. niger, R. solani* and *M. ramosissimus* .It also confirmed the efficiency of extracts of *A. indica* and *O. gratissimum* in inhibiting the radial growth of the fungi in PDA plates after 7 days of incubation at different concentration levels. Since plant extracts are specific, biodegradable, cheap, readily available and environmentally safer than synthetic chemicals, their use is advocated. More so, medicinal plants are not known to be phytotoxic at high concentrations but are fungitoxic.. Therefore plant extracts are recommended because of their less hazardous effects, if any.

V. REFERENCES

[1]. Aasgana, J. (2013). Antifungal activity of selected medicinal plants extracts against plant pa pathogenic fungi: Rhizoctonia solani Colletotrihum musea and Fusarium oxyporum. International Journal of Science inventions today: 2(5) 421-431.

[2]. Adaskaveg, J.E. Foster, H. and Sommer, N. F. (2002).Principles of post-harvest pathology and management of decays of edible horticultural crops. In: Kader,A. A. (ed), Post-harvest Technology of Horticultural Crops. California: University of California Publication. 163-195pp.

[3]. Agarios, G.N. (2005). Plant Pathology Academic Press. New York 922p.

[4]. Alexopoulos, C.O., Mims, C.W. and Blackwell, M. (2002). Introductory Mycology.4th ed. John Wiley and Sons Inc. Singapore. 869 pp.

[5]. Aliyu, H. M. (2006). Proximate anlaysis of some leafy vegetables. (Roselle jute and Bitter leaf). International Journal of Food Agricultural Resources, 3 (1): 112.

[6]. Barnett, H. L. and Hunter, B. B. (1999). Illustrated Genera of Imperfect Fungi (4thed.). The American Phytopathological Society. St. Paul, Minnessota, USA, pp. 218.

[7]. Chaturvedi, R., Razdan, M. K. and Bhojuwani, S.S. (2003). Production of haploids of neem (Azadirachta indica A. Juss) by anther culture. Plant Cell Report, 21: 531-537.

[8]. Chiejina, N.V. (2008). Mycoflora of some salad vegetables. Bio-Research, 6(2): 392-395.

[9]. Chiejina, N. V. and Ukeh, J. A. (2012). Antimicrobial properties and Phytochemical analysis of methanolic extracts of Afromomum melegueta and Zingiber officinale on fungal diseases of tomato fruit. Journal of Natural Sciences Research, 2 (6): 10-15.

[10]. Dileep, N., Junaid, S., Rakesh, K. N., Kekuda, P. T. R. and Nawaz, A. S. N. (2013). Antifungal activity of leaf and pericarp extract of Polyalthia longifolia against pathogens causing rhizome rot of ginger. Science Technology and Arts Research Journal 2(1): 56-59.

[11]. Ewekeye, T. S., Oke, O. A., Omenwaniri, M. O., and Durosimi, M. L. (2013). On post harvest deterioration of some fruits and vegetables in some selected markets in Lagos State, Nigeria. American Journal of Research Communication, 1(10); 2009-223.

[12]. Eze, C. S. and Maduewesi, J. N. C. (I990). Comparative efficacy of benlate, lime and plant ash treatment in controlling fresh weight loss, sprouting and rotting of cocoyams in storage. Nigerian Journal of Plant Protection, 13: 81-89.

[13]. Hasabnis, S. N. and Souza, T. F. (1998). Use of natural plant products in the control of the storage rot of Alphanso mango fruits. Journal of Maharastra Agricultural University, 12 (1): 105-106.

[14]. Jeyasakthy, R., Jeyadevan, J. P., Tavaranjit, A. C., Manoranjan, T., Srikaran, R. and Krishnapillai, N. (2013). Antifungal activity and qualitative phytochemical analysis of extracts obtained by sequential extractionof some medicinal plants in Jaffna peninsula; Archives of Applied Science Research, 2013, 5 (6): 214-221.

[15]. Kurup, V. P. (2003). Fungal allergens. Current Allergy Asthma Report 3: 416-423.

[16]. Mehrota, R. S. and Aggarwal, A. (2003). Plant Pathology (2nded.). New Delhi: Tata McGraw-Hill, 846 pp.

[17]. Nguefack, J., Leth, V., Amvam-Zollo, P. H. and Mathur, S. B. (2004). Evaluation of five essential oils from aromatic plants for controlling food spoilage and mycotoxin-producing fungi. International Journal of Food Microbiology, 94: 329-334.

[18]. Obeng-Ofori, D., Daanguar, E. Y. and Ofosu-anim, J. (2007). Vegetable and Spice Crop Production in West Africa. Ghana: The City Publishers Limited. 176 pp.

[19]. Okigbo, R.N. and Emeka, A. N. (2010). Biological control of rot-inducing fungi of water yam (Dioscora alata) with Tricoderma harzianum, Pseudomonas syringae and Pseudomonas chlororaphis. Journal of Stored Products and Postharvest Research. 1(2). 18-23.

[20]. Okigbo, R. N., Putheti, R. and Achusi, C. T. (2009). Post-harvest deterioration of cassava and it's control using extracts of Azadirachta indica and Afromomum melegueta.Journal of Chemistry, 6 (4): 1274-1280.

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