Response of Storage Fungi of Onion (*Allium cepa*) to selected Botanicals

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ABSTRACT

Onion (Allium cepa) produced bulbs are mostly lost after harvesting due to rots caused by fungi. Extracts of many plants are effective for control of fungal pathogens. So far, little information is available on the use of several plant extracts in controlling rot of onion bulbs caused by plant pathogens during storage. Therefore, this study aimed to isolate and identify rot-causing fungi of onion sold in Umuahia and their response to selected botanicals. Infected onion (White and red) n= 12, were sourced from Orie-Ugba and Ubani markets for isolation and identification using standard techniques. Also, response of Rhizopus sp, Fusarium sp and Aspergillus niger to aqueous extracts of clove and African nutmeg seeds were evaluated in-vitro and in-vivo (before and after treatment). Experiments were laid out in CRD in triplicates. Clove (Syzgium aromaticum) and African nutmeg (Monodora myristica) extracts respectively reduced Aspergillus niger (71.55, 42.96%) Fusarium sp (63.82,58.28%), and Rhizopus sp (67.79,26.06%) in-vitro. Clove applied before and after fungi inoculation respectively reduced growth of Aspergillus niger (69.64,60.71%), Rhizopus sp, (88.89,84.44%) and Fusarium sp (70.00, 58.89%) in-vivo. Growths of Aspergillus niger (69.64,69.64%), Rhizopus sp, (50.00,53.33%) and Fusarium sp (82.22,58.89%) were reduced by African nutmeg before and after treatment respectively. Extracts showed promising prospect for control of Fusarium sp, Aspergillus niger and Rhizopus sp growth in both trials and could be explored for management of post-harvest onion rots at pre and post stages of fungal infection.

INTRODUCTION

Onion (*Allium cepa*) is a round vegetable that grows underground. The bulb can be white, yellow and red; the red is the most popular one in the country and it is of great commercial value for farmers that cultivate it (Fritch *et al.*,2002). The main cause for the reduction in onion yield is the various fungal infections that infect it especially during long term storage. Despite the advances in production techniques the post-harvest loss during storage is still a major problem. Storage diseases of onions are bacterial rots, black mould (*Aspergillus niger*), Blue- green mould, Fusarium rot, neck rot, brown rot and soft rot. These storage diseases manifest rapidly depending on the condition on how the onion bulbs were planted. Different types of onion bulbs are infected in storage places. Hence the need to isolate and identity these diseases causing pathogens and proffer some management practices to reduce rots and spoilage during storage (Sang *et al*., 2018). Natural plant products have often been considered as important and alternative sources of agricultural chemicals and most have been used in the control of insect pest (Enyiukwu *et.al.*, 2016) and as bird repellent (Nisar *et.al.*, 2020). So far, little information is available on the use of several plant extracts in controlling rot of onion bulb caused by plant pathogens during storage. Therefore, the objectives of this study are: to isolate and identify fungi causing rot of onion bulbs in Umuahia, to determine the effect of identified fungal organisms on onion and the effect of selected botanicals on fungi isolated from rotted onion bulbs.

MATERIALS AND METHODS

Experimental site and Source of Onion bulbs: Multiple onion (white and red) samples were purchased from different points in Orie Ugba (5˚32N 7˚30E) and Ubani (5˚35N 7˚30E) both in Umuahia, Abia State. Each type of onion was purchased from three traders at different locations within the same market; these served as the replicates which were used for investigation in the laboratory of Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike.

Isolation of Fungi from Infected onion bulbs and Identification of Isolated Fungi: Samples of infected onion bulb were surface sterilized with 70% ethanol and then rinsed in three changes of sterile distilled water to remove surface contaminants and dried with sterile paper towel. Then isolation was carried out according to the method described by Opara and Obani (2009). Using inoculation needle, the sterilized infected samples of onion bulb were cut with sterilized surgical blade to small pieces which were inoculated on solidified PDA in the Petri-dishes and incubated at room temperature (28°C). Potato Dextrose Agar (PDA) powder 39g was prepared according to manufacturer's guide and 2 drops of lactic acid was added to the PDA prevent bacterial growth. Inoculated plates were observed 3-5 days for fungal growth and fungal colonies that grew from the inoculated plates were sub-cultured to obtain pure cultures of different isolates.

Pathogenicity test: Pathogenicity test was carried out according to Amadioha and Uchendu (2003); healthy onion bulbs were surfaced sterilized with 70% ethanol for 30 seconds and then rinsed in three changes of sterile water, dried and the inoculated with a 7-day-old fungal culture of each isolate. The point of inoculation was smeared with Vaseline to seal the inoculated portion on the onion bulb in other to prevent contamination. The control was inoculated with a disc portion of solidified potato dextrose agar medium alone and all inoculated bulb were kept in a micro humid chamber, incubated for 7 days and infected bulb that showed sign of rot were re-isolated as described above according to Koch's principle. The morphology and culture characteristics observed were compared with fungal structures in manual Snowdown (2015).

Source and Preparation of Plant Extracts: Dried seeds of *Syzgium aromaticum* (Clove) and *Monodora myristica* (Ehuru/African Nutmeg) obtained from Orie-Ugba market were washed with running tap water and then rinsed with sterile distilled water and their extracts prepared according the methods described by Amadioha (2000).

Effect of Botanicals on radial growth of isolated fungal species *in vitro*

Application of the treatments was done according to the methods of Amadioha (2000). The water extract of each plant materials, (0.2m1) each were separately introduced into petri-plates containing the solidified media (poisoned food method). The extracts were uniformly spread to form a thin film on the solidified PDA. A disc of 6mm diameter of the pure culture of each test fungus was placed at the point of intersection of the two perpendicular lines drawn at the bottom of the extract-PDA medium plate. Control plates were prepared with 0.2m1 of sterile distilled water alone without plant materials. The plates were incubated at room temperature (28°C) until the mycelium or hyphae growth of fungus in the control experiment reaches the edge of the plate. Three replicates were set up for each treatment, experiment was replicated 3 times. The radial mycelia growths were measured in each case using metric rule for both treated plates and control. Percentage growth inhibition was calculated using the formular below;

$$\text{Percentage growth inhibition (\%)} = \frac{DC-DT}{DC} \times \frac{100}{1}$$

Where; DC= Average diameter of fungal colony in control experiment plates and DT= Average diameter of fungal colony in treated plates

Effect of Treatments on Rot Development in Onion Bulb: The test of the effect of the treatment on fungal deterioration of onion (in-vivo experiment) was carried out following the method of Amadioha *et al.,* (2012). The extracts were prepared as described above. Inocula suspensions of *A. niger, Fusarium* sp and *Rhizopus* sp were prepared from fresh, mature (5-day-old) fungi cultures. Healthy onions were washed in three changes of sterile distilled water and dried with sterile paper towel. Onion bulbs were bored with 5 mm cork borer, then 5 mm disk of each test fungi from culture plate was placed in the 5mm whole made with the cork borer of allowed to stand for 30 minutes before treatment with botanical extracts and then incubated on sterile filter paper blotter. For samples treated before inoculation, onion bulbs were treated with crude botanical extracts and wrapped with sterile paper towel and allowed to stand for 30 mins before inoculated with test fungi and placed on sterile wet filter paper blotter. Both samples inoculated before and after treatment and control (triplicates each) replicates were incubated at room temperature for 14 days and percentage fungi colonization was recorded after incubation.

Disease severity was determined using a scale ranging from 0 to 5 as follows: 0-No infection/rot, 1 to 20% - Slight rot, 21 to 40% - Moderate rot, 41 to 60% -Severe rot, 61 80% -Highly rotted, 81 to 100% - complete rot

Statistical Analysis: Data collected from all the experiments were analyzed using Statistical Package and Service solutions (SPSS) version 23; means were compared and separated using least significant difference (LSD) and at 5% probability level.

RESULTS

Incidence of fungal species in onion bulbs: Figure 1 showed the percentage incidence of the seven (7) fungal species isolated from both red and white onion sourced from Orie Ugba and Ubani. The chart represents that *Aspergillus niger* had the highest occurrence for both red and white onion bulbs sourced from Orie ugba and Ubani markets except for red onion bulbs collected from Ubani. *Rhizopus* sp had the highest incidence level in red onion bulbs sourced from Ubani and also followed those collected from Orie ugba market. The least incidence level of the fungal pathogens was observed in all onion bulbs with *Fusarium* sp having the lowest incidence, followed by

Aspergillus flavus and then *Penicillium* sp. Results shows that *Rhizopus* sp should be placed as a priority and considered during storage of both red and white onion bulbs.

Pathogenicity Test: Table 1 shows the different fungi isolated from red and white onion bulbs, their symptoms and their pathogenicity test (%). Two *Fusarium* species (*F. solani* and *F. oxysporium*), *Rhizopus stolonifer*, *Botryodiplodia theobromae, Penicillium* sp and *Aspergillus* species (*A. niger A. flavus* and *A. tamarii*) were isolated from onion bulbs.

The pathogenicity test showed that the fungi were pathogenic to onion bulbs at varying percentages However, *A.* niger had high pathogenicity percentage in both white (60%), and red (70%) onion, Rhizopus sp (40%) and 20% while *Fusarium solani* had rot development of 40% and 40% in both white and red onion bulb respectively.

Effect of crude extracts of clove and ehuru on mycelial growth of fungi in-vitro: Table 4.2 and plates 4.2 - 4.3 show the effects of the crude extracts of Clove (*Syzgium aromaticum*) and Ehuru (*Monodora myristica*) percentage growth inhibition of the mycelia growth of the test fungi in culture. The botanical extracts had significant effect on the mycelia growth of the test fungi.

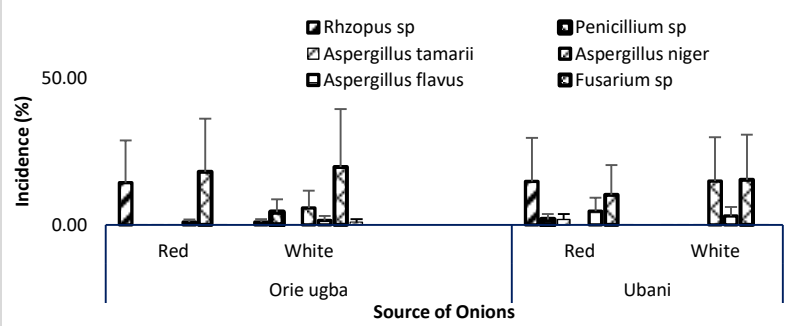

Figure 1: Percentage incidence of fungal species isolated from onion bulbs.

For *A. niger*, both Ehuru and clove had 100% growth reduction at 6 days. However, growth reduction of 52.52 to 70.84% was recorded from day 2 to day 6 for Ehuru, while 24.24 to 67.16% was recorded for clove. The difference in mycelia growth of the treated from the control was significant at ($p \leq 0.05$).

Table 1: The pathogenicity of fungi isolated from onion bulbs.

Rot Colour		Pathogenicity (%)			
	Fungi isolated	White onion	Severity index	Red Onion	Severity index
Light brown	*Botryodiplodia theobromea*	30	2	20	1
Light brown	*Rhizopus* sp	40	2	20	1
Dark brown	*Pencillium* sp	40	2	20	1
	Aspergillus tamarii	30	2	20	1
Dark brown	*Aspergillus niger*	60	3	70	4
Light brown	*Aspergillus flavus*	20	1	20	1
Brown	*Fusarium solani*	40	2	40	2
Brown	*Fusarium* oxysporium	30	1	30	2
	Control	0	0	0	0

A similar trend was observed for *Fusarium* sp mycelial growth reduction, day 1 had 100% mycelia growth reduction but reduced significantly from day 2 to day 6. For *Rhizopus* sp, Clove had 100% growth reduction in day 1-2, within a sharp decrease to 21-24% in day 3and 4, although significantly different from control (0.00%). For Ehuru, mycelia growth reduction ranged from 21.69-31.67% and were significantly different ($p \leq 0.05$) from the control.

Effect of crude extracts of clove and Ehuru **on reduction of rot development of fungi in-vivo**

In vivo effect of the crude plant extracts on the growth and sporulation of *A. niger, Rhizopus* sp, *Fusarium* sp before and after inoculation**.**

Table 2: Percentage (%) reduction of mycelia growth of fungi by crude extracts of botanicals *in-vitro*

		Growth Reduction (%) Days after treatment						
Botanical extract	**Fungi**	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**
Ehuru		100.00	69.34	70.38	66.20	52.52	70.84	70.69
Clove		100.00	67.16	63.91	48.52	45.82	24.24	65.08
Control	*Aspergillus niger*	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LSD(p=0.05)		0.54	11.72	16.98	5.09	2.68	1.23	70.01
Ehuru		100.00	79.35	80.00	35.96	54.76	56.67	
Clove		100.00	60.70	41.29	16.08	14.38	25.29	46.49
Control	*Fusarium* sp	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LSD(p=0.05)		0.77	8.86	18.13	19.41	13.09	5.06	
Ehuru		26.26	31.67	21.69	24.63			26.06
Clove	*Rhizopus* sp	100.00	100.00	28.44	26.85			63.82
Control		0.00	0.00	0.00	0.00			0.00
LSD(p=0.05)		18.85	5.40	3.62	5.20			

The effect of the plant extracts applied before and after inoculation of fungal isolates *in vivo* is presented in Table 3. The extracts of *Syzgium aromaticum* (Clove) and *Monodora myristica* (Ehuru/African Nutmeg) had a significant effect on the isolated fungi rot before and after inoculation when compared with the control. The application of Ehuru extract before treatment inhibited the *A. niger* by 69.64 and 60.71% after treatment compared to control (0.00%). However, application of Clove before and after treatment respectively, was recorded for clove while Ehuru had 50.00% and 53.33% before and after treatment respectively; with mean difference significantly different from the control (0.00%). before and after treatment respectively, was recorded for clove while Ehuru had 50.00% and 53.33% before and after treatment respectively; with mean difference significantly different from the control (0.00%). Extracts before and after inoculation of fungal pathogens inhibited the growth of *A. niger* by 69.64%compared to the control. Reduction of mycelia growth of *Rhizopus* by 88.89% and 64.44%.

For *Fusarium* sp, Ehuru reduced the fungal growth by 70.0% and 58.89% before and after treatment respectively. Clove significantly inhibited the growth of *Fusarium* sp by 82.22% before treatment and 58.89% after treatment and differed significantly from the control.

Results therefore, showed that clove seed extracts were more effective in inhibiting the growth of test fungi (64.44) when applied before inoculation of the fungal isolates than those of *Monodora myristica*. Results also showed that clove extracts were more effective in inhibiting the growth of *Rhizopus* sp and *Fusarium* sp than that of *Aspergillus niger*. Application of the plant extracts before and after inoculation of the pathogens reduced disease incidence significantly ($p \leq 0.05$) when compared with control experiment.

Table 3: Percentage (%) reduction of rot development of fungi by crude extracts of botanicals *in-vivo*

		Growth reduction (%)	
Botanical extract	**Fungi**	**Before**	**After**
Ehuru	*Aspergillus niger*	69.64	60.71
Clove		69.64	69.64
Control		0.00	0.00
LSD(p=0.05)		8.58	13.57
Ehuru	*Rhizopus* sp	50.00	53.33
Clove		88.89	64.44
Control		0.00	0.00
LSD(p=0.05)		21.00	23.66
Ehuru		70.00	58.89
Clove		82.22	58.89
Control	*Fusarium* sp	0.00	0.00
LSD(p=0.05)		8.11	7.91

DISCUSSION

The efficacy of two plant extracts were tested *in vitro* and *in vivo* against the growth of three pathogenic fungi that were isolated from stored onion bulbs. The prevalently fungal pathogens isolated were identified as *Aspergillus niger, Fusarium* sp and *Rhizopus* sp. The results of this study revealed that the fungi were majorly responsible for the post-harvest rot of red and white onion bulbs in Umuahia as evidenced by the pathogenicity tests. The pathogens (*Aspergillus niger, fusarium* sp and *Rhizopus* sp) were also reported by Shehu and Muhamed (2011) to cause storage rot of onion bulbs.

Also, Adebayo and Diyaulo (2003) reported (*Aspergillus niger* and *Rhizopus* sp) as pathogens of post-harvest rot of onion, which is in agreement with the finding of this study. This study reported that, application of botanicals before and after fungi inoculation showed antifungal activity and can be used in control of *A. flavus, Fusarium* sp and *Rhizopus* sp causing rot in of onion bulb. Some of the plant extracts of *Dioscorea dumetorum, Azadirachta indica*, etc. have been studied in Nigeria with a view to manage rot of onion bulb. Okey *et al.,* (2015) reported that ethanolic extracts of *D. dumetorum* was found to be more effective in inhibiting the growth of *Rhizopus stolonipher* and *Aspergilus niger* than *P. guajava* extracts. Enyiukwu *et.al.,* (2016) also reported that, extracts from *Azadirachta indica* and *Dennettia tripetala* should be applied as prophylactics and or before disease initiation for management of soil and thrash-borne fungal diseases of onion crop in integrated diseases management programmes in sun-Saharan Africa (SSA) for improved onion production. *Cydonia oblonga, Datura stramonium, Eucalyptus globules, Foeniculum Salix mucronate* and many other plants have been found to the effective against fungal pathogens in harvested bulbs and it has been used to control Stemphylium blight diseases of onion caused by *S. vesicarium* and *Botrytis squamosa* (Sobhy *et al*., 2013).

The differences in the fungi-toxic potentials between these plant extracts studied may be attributed to the susceptibility of each of the fungal pathogens to the different constituents of the extracts. This agreed with the results of some workers like Amienyo and Ataga (2007), who reported that plants are rich source of bioactive compounds such as tannins, terpenoids, saponins, alkaloids, flavonoids and other compounds as such have antifungal properties. Chowdhury *et al.,* (2016) reported that the plant extracts of *Allium sativum, Azadirachta indica, Citrus limon* and *Mangifera indica* showed complete growth inhibition of *Aspergillus niger* and *Fusarium* sp at 6 days and when applied before and after pathogen inoculation. This in synergy with the same test fungus used in this study. The variation might be due to selection of different plant extracts. Islam and Shamsi (2016) reported association of seven species of fungi namely *Aspergillus flavus, A. fumigatus, A niger, Fusarium* sp, *Penicllium* sp, *Rhizopus stolonipher* and *Trichoderma* sp. They also reported that bulb extracts of *Allium sativum, Azadirachta indica* and the chemical sodium showed 100% growth inhibition of the isolated fungi.

Srinivasan and Shanmugam (2006), and several others numerous authors have also reported that, the presence of these compounds in plant could be responsible for the control of fungal pathogens of plant. Nwaiwu and Imo (2019) screened *Monodora myristica* for the fungitoxicity of their essential oils against mycelia growth of three fungi species; *Aspergillus niger, Rhizopus* sp and *Aspergillus fumigatus*. The ethanolic extract of *Monodora myristica* seed possess broad spectrum antifungal activities against *Aspergillus* sp and also inhibited their mycelia growth (Ogunmoyole *et al.,* 2013). This generally confirmed that this seed is highly potent to activities of many microorganisms. *M. myristica* extracts inhibited the growth of mycelium and the formation of conidial spores and chlamydospores of *Sclerotium rolfsii*, thereby reducing the number of propagation units of this fungus in the medium (Mahesh and Satish, 2018). Ukaegbu-Obi *et al.*2015, reported that seeds extract of *M. myristica* possess some antimicrobial activities which can be employed in the development of novel therapeutic agents against the test organisms. Suwitchchayan and Kunasakdakul (2010) reported on the in vitro effects of clove in controlling crucifer pathogens. *Syzgium aromaticum* showed inhibitory effects on the pathogens. The results of the antifungal effect revealed that clove extract was indicated the minimum inhibitory concentration (MCI) of *Fusarium* sp, and *Aspergillus* sp at 1900 ppm and 2300 ppm respectively. The plant extracts differed in their potential to inhibit the growth of these fungal pathogens. This could be attributed to the difference in the bioactive compounds present in the botanicals studied. The greater efficiency of these plant extracts may be due to the phenolic substances 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 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, hereby disrupting their growth. The *in vitro* and *in vivo* effects of *Syzgium aromaticum* (clove) and *Monodora myristica* ehuru/African Nutmeg) extracts were evaluated in order to develop cheaper methods of controlling post storage rot of onion bulbs.

CONCLUSION AND RECOMMENDATIONS

The findings from this study showed *Rhizopus* sp, *Fusarium* sp and *Aspergillus niger* to be the major fungal pathogens causing rot of onion bulbs and this was confirmed through a pathogenicty test which indicated that *Aspergillus niger* had the highest rot advancement for both white and red onion bulbs. The fungicidal activity of the extracts against *Fusarium* sp, *Rhizopus* sp and *Aspergillus niger*, showed the potential of clove (*Syzgium aromaticum*) and ehuru (*Monodora myristica*) as possible natural source of fungicidal materials and the efficacy of these extracts against the rot causing fungi were tested both in vivo and in vitro; the results from the experiment showed that plant extracts considerable inhibited the mycelia growth of the fungal isolates when compared with

control. Furthermore, the crude plant extracts when applied before and after inoculation of the fungal isolates, significantly ($p \leq 0.05$) inhibited the growth on onions bulbs. Antifungal activity was confirmed in all the tested plant species, although the results showed that different plant extracts varied in their effectiveness in inhibiting the mycelia growth of different pathogens tested. The use of botanical products as alternative to chemical control of plant pathogens is possible. This approach can contribute in reducing the amount applied of fungicides and subsequently minimize its hazards to the environment and human health. These botanicals are also cheap, non- toxic, readily available and easy to use. The use of *Syzgium aromaticum* (clove) and *Monodora myristica* (ehuru/African Nutmeg) seeds should be encouraged as part of an integrated approach for the control of Onion bulb rot caused by *Rhizopus* sp, *Fusarium* sp and *Aspergillus niger*. Further investigation should be done on the chemical nature of the active components of the plants; also, further investigation can combine the plant extracts for possible synergistic effect.

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