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CONTROL OF POSTHARVEST YAM (*Discorea rotundata*) ROT CAUSED BY FUNGI USING SAPROPHYTIC YEAST

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ABSTRACT

The potential of saprophytic yeasts (*Cryptococcus neoformans, Rhodoturula rubra* and *Toruiopsis candida*) isolated from the apparently healthy fruits of lime fruits, orange and tomatoes for the control of postharvest yam *(Discorea rotundata*) rot caused by fungi was investigated. Treatments comprising three isolated pathogenic fungi (*Aspergillus niger, Aspergillus flavus* and *Penicillium notatum*) from rotten yams, each paired was set up in dual culture with one of the potential antagonists (saprophytic yeasts). The extent of rot reduction was evaluated by calculating the mean percentage (%) inhibition of the pathogens by antagonists. Pathogenicity test showed that *A. niger, A. flavus* and *P. notatum* induced rot in healthy yam tubers after fourteen days of inoculation at room temperature (28±2^{o}C) with *A. niger* being the most virulent. The growth reduction or inhibition of *A. niger, A. flavus* and *P. notatum* was highest and significantly different ($P<0.05$) in *C. neoformans* (51.1, 51.7 and 50.0) and least in *R. rubra* (5.6, 4.9 and 6.4) respectively. The three biological antagonists could be used to control the rot of postharvest yam tubers. However, the field efficacies still need to be further investigated.

Key words: Saprophytic yeast, Pathogenic, Antagonist, Postharvest, *Discorea rotundata*

INTRODUCTION

Yam (*Discorea rotundata*) a monocotyledonous plant, belongs to the family Discoreaceae. It is one of the highly rated and common food crops of the tropical world. FAO, (2002) and Okigbo (2002) estimated that the production of yams was around million tons per year. The greater part of the world yam production (90%) was derived from West Africa and Nigeria alone accounts for three-quarters of the world total output of yam (Kay, 1987; Okigbo, 2002; Ezeibekwe and Ibe 2010). The edible varieties of yam are important food crops and serve as sources of carbohydrate for millions of people in both the tropical and subtropical countries (Okigbo and Ikediugwu, 2000). Yam tubers are rich in minerals such as calcium, phosphorus, iron and vitamins such as riboflavin, thiamine, vitamin B and C (Okigbo and Ogbonaya, 2006). Yam can be eaten by cooking in various ways such as boiling, roasting and frying. Postharvest handling and storage of yams are the essential aspect of economic development in Nigeria. Losses in yam in storage mostly due to rot are attributed to pathological problems of yam tubers brought about by bacteria, fungi and nematodes (Akarado and Hahn, 1995). The losses were estimated to be 10-15% in the first three months of storage in yam barn and 50% -56% after six months (Sangoyemi, 2004). The principal microorganisms associated with yam rot include *Botryodiplodia theobromae* Pat, *Fusarium oxysporum* schledit, *Penicillium oxalicum* Currie and Thom, *Aspergillus niger* Van Tiegh, *Aspergillus termarii* Kita, *Handersonula rotuloidea*, *Macrophomina phaseoli, Rhizopus nodosus*, *Fusarium monoliform, Penicillium notatum*, *Aspergillus flavus*, *Penicillium scleretoligenum* (Okigbo and Ikediugwu, 2000; Okigbo, 2005). Microorganism that causes rot did so at a high relative humidity and temperature of 25-39^{o}C (Adeniji, 1991) and some are more aggressive at a high temperature of 35^{o}C. Yams rots usually start in the soil and progress in storage, which occur when infected tubers do not yet have any sign of external symptoms (Okigbo and Ogbonnaya, 2005). The use of synthetic chemicals such as borax, captan, thiabendazole, benomyl, bleach (sodium hypochlorite) has been found to significantly reduce storage rot in yam (Okigbo, 2005) but have the additional potential disadvantages of accumulation in the ecosystem and induction of pesticide resistance in pathogens (Okigbo and Ikediugwu, 2000). There is also the problem of lack of expertise in the safe handling of pesticides among most of the farmers. Biological control may therefore be better alternative to chemical fungicide. Kuhn and Hargreaves, (1997) reported that substance found to be fungicidal *in vitro* in almost cases kill the fungus *in vivo*. The use of microorganisms such as *Trichoderma viride* Perses Gray and *Bacillus subtilis* for biocontrol of plant pathogens have been reported by Okigbo and Ikediugwu (2000); Okigbo (2002). Biological conttrol has the advantage of non repeated periodic application as in the case of chemical fungicides. It thus potentially better suited for use, particularly in developing economics (Okigbo and Ikediugwu, 2000). The objectives of this study were to isolate and identify fungi associated with rotten yam and found an alternative way of controlling postharvest yam fungal disease. Therefore, saprophytic yeast (*Crytococcus neoformans*, *Toruiopsis candida* and *Rhodoturula rubra*) which have no carcinogenic effects and environmentally safe were used to control the postharvest yam rot.

MATERIAL AND METHOD

Sources of Yam Tubers

A total of 100 rotten and 20 healthy yam tubers were collected from 25 farmers' yam barns in Lapai Local Government Area of Niger State, Nigeria. The collected samples were taken to the Biological Sciences laboratory at Ibrahim Badamasi Babangida University, Lapai for further studies. Ten fresh and healthy Tomato, orange and lime fruits each were collected from Lapai main market.

Isolation and Identification of Associated Pathogen

The collected rotten yam tubers were rinsed in sterilized distilled water and surface sterilized 10 with 70% ethanol. Each was cut open and 3 pieces of the infected tissues were removed from the point of advancement of rot using 3mm diameter cork borer and inoculated on the solidified Potatoes Dextrose Agar (PDA) in Petri dish. Three replicates of each were made. The inoculated plates were incubated at room temperature (28 ± 2^{o}C) for 48 hours and observations were made daily for fungi growth. Sub-cultures were made to obtained pure culture of the isolates. The isolated pathogens were identified using their morphological features and Fungi Families of the world mycological monographs by Samson and Hoekstra (1988) Stock cultures was prepared using slants of PDA in McCartney bottle and stored in a refrigerator at 5^{o}C for further used (Ezeibekwe and Ibe, 2010).

Isolation Purification and Identification of Saprophtic Yeast

Saprophytic yeasts were isolated from the surface of fresh apparently healthy tomato, orange and lime fruits collected from Lapai main market. Each was placed in a 500ml beaker containing 200ml sterilized distilled water (SDW) placed on a rotary shaker at 100rpm for 12 hrs. 0.1ml of suspension was taken from the beaker, spread on a Potato Dextrose Agar (PDA) plates and incubated at ambient temperature (28 ± 2^{o}C) for 24hours for yeast colonies to develop. Sub – cultures were made to obtain pure isolates. The isolates were identified to species level by physiological and morphological standard methods by Kreger-Van-Raij (1984). Pure isolates were kept on slants and put in refrigerator at 10. 5^{o}C for further uses.

Pathogenicity Test

Ten healthy yam tubers were washed under running tap water to removed soil (dirt). Surface sterilization was made by dipping each yam tuber in to 10% concentration of sodium hypochlorite for 2 minutes and rinsed twice in sterile distilled water (SDW). The tubers were placed on sterile paper towels to dry for 20minutes. Cylindrical holes were drilled at the proximal and distal ends of the yam tubers using a sterile 10mm cork borer. Discs of five days old pathogen cultures were plug in the holes created in the yam tubers and the disc of the tuber in the cork borer was placed back. Then vaseline was applied on the point of inoculation and incubated at room temperature. Three replications of each were made. After 72hrs, the yams were cut transversely and the observation of the symptoms produced was recorded and the pathogens were re – isolated (Agrios, 2005).

In vitro Inhibition of Fungi Isolates by Saprophytic Yeast

A modified dual culture technique after Ferreira *et al*., (1991); Adebola and Amadi, 2012 was adopted. A 2-dayold Yeast isolate was streaked on one side of PDA in a 9cm Petri dishes and each pathogen was inoculated at 5cm away at the opposite side and incubated at 28±2^{o}C. Three replicates of each were made. Control was prepared by inoculating only the pathogen on PDA without being challenged. Percentage inhibition of fungal growth was calculated.

Experimental Design

Randomized complete block design (RCBD) was used. Analysis of variance (ANOVA) was done and New Duncan Multiple range test was used to separate the means.

RESULTS

Isolation of Fungi Pathogen from Rotten Yam Tubers

A total of six (6) fungi from three general (Table 1) were isolated from rotten yam tissues These fungi were *Aspergillus niger, Aspergillus flavus, Penicilium notatum, P. trysogenum, Trichophyton soudanense* and *Trichophyton rubra*. The most frequently occurred fungi species was *A. niger*, with 46.88% occurrence, while the least were *T. rubra and T. Soudanses* with 1.56% occurrence each. The pathogenicity test showed that *A. niger, A. flavus* and *P.notatum* induced rot in healthy yam tubers after 14 days of inoculation. *A. niger* was more virulent followed by *A. flavus* and lastly *P. notatum* (Figures 1A and B).

Isolation, Purification and Identification of Saprophytic Yeast

Three potential antagonistic saprophytic yeasts (*Cryptococcus neoformance*, *Rhodoturula rubra*, and *Toruiopsis candida*) were isolated from the surface of fresh healthy fruits of tomato, orange and lime fruit (Table 2). The result revealed that out of these three yeasts isolated from tomato, *C. neoformans* has the highest percentage of occurrence (41.2%) and was significantly different (P<0.05) from *R. rubra* and *T. candida* with the same percentage of occurrence (29.4%). However, in lime fruits *T. candida* has the highest percentage of occurrence of 42.1% and was significantly different (P<0.05) from other two. Only *R. rubra* and *T. candida* were isolated from orange with 52.4% and 47.6% occurrence respectively and were significantly different (P<0.05). The results in Table 3 show the antagonistic activities of *Cryptococcus neoformans* and *Toruiopsis candida* on the pathogens. The inhibitory effects of *T. candida* had caused reduction in radial growth of *A. flavus* by 45.1% and 43.3% while that of *P. notatum* was reduced by 43.4%. *R. rubra* which also showed a slight inhibitory effect on the radial growth of *A. niger, A. flavus* by 5.6% and 4.9% while in *P. notatum* the radial growth was slightly reduced by 6.4% (Table 3). *C. neoformans* significantly reduced the radial growth of *A. flavus, A. niger* and *P. notatum* by 51.1%, 51.1% and 50.% respectively(Table4). *C. neoformans* was highly effective on the growth of pathogens. It reduced the growth of *A. niger* and *A. flavus* from 5.6 to 51.1% and 4.9 to 51.7% respectively, while in *P. notatum* ranged from 6.4 to 50.0% (Table3, 4 ,5 and 6). This shows a significant difference (P<0.05) among the three biological antagonists *C. neoformans, T. candida* and *R. rubra* used in controlling the pathogens. The mean incidence of growth of *A. niger, A. flavus* and *P. notatum* when paired with biological antagonists *C. neoformans, T. candida* and *R. rubra* differs from each other significantly (P<0.05) on the tested treatments. Reduction of their growth indicates a decrease in the radial growth of pathogenic fungi. In both tests, *C. neoformans* reduced the growth of the pathogens in the treatments (Table 6) significantly (P<0.05) more than the other biological antagonists.

DISCUSSION

Cryptococcus neoformans, Rhodoturula rubra and *Toruiopsis candida* used in this study, were isolated from apparently healthy lime fruit, orange and tomato. The natural occurrence of different yeast on the surface of agricultural products was previously reported by Sugar and Spotts(1999). The microorganisms found to be associated with the rot yams (*Discorea rotundata*) in this study were *Aspergillus niger, Aspergillus flavus* and *Penicilium notatum*. These organisms have been reported previously to be associated with postharvest rot by Okigo, 2002 and 2005; Okigo and Ogbonnaya, 2006. The pathogencity test established that the fungi inoculated in the yam tubers caused rot. This could be probably due to ability of the pathogen to ultilize the nutrient of the yam as a substrate for growth and development. This was similar to the report of Mehrotra *et al*., (1996). Who revealed that the *Cryptococcus neoformans, Rhodoturula rubra* and *Toruiopsis candida* were used to control rot of the pathogens of *D. rotundata* and produced a significant inhibition on the growth of the pathogens. This might be probably due to the products of their metabolism secreted, that inhibited their growth on the growth medium. The toxic activity of some microorganisms like *Bacillus subtilis, Trichoderma viride* in controlling pathogens of yam has been reported by Okigbo and Ikediugwu, (2000). Similar previous reports revealed that some other biological control measures like plants extracts have been used to control pathogens of yam (Akueshi *et al.,* 2002; Sangoyomi, 2004; Okigbo and Ogbonnaya, 2006).

The visual clear zone between the pathogens and antagonists revealed that fungitoxic compounds might be produced by *T. candida*, *C. neoformans* and *R. rubra* since they were able to suppress the growth of pathogens tested. Results also showed that the ascending order of the width of clear zone of inhibition followed the same order of recorded efficacy of yeast isolates as fungal linear growth inhibitor. This observation could be attributed to the product of metabolism by different yeast isolates which diffused in to growth medium to various extents relatively to each divers isolate. In this regard, several investigators recorded the efficacy of yeast as an inhibitor agent against pathogenic fungi (HeDan *et al*., 2003; Guozheng *et al*., 2004; Ozgur *et al*., 2005).

Several works have been reported on the use of bacterial to inhibit the growth of pathogens of yam tubers, which were used to control postharvest fungal rot of yams. Similarly, *C. neoformans, R. rubra T. candida* were used in this study to control pathogenic fungi that causes rot in yam tuber. However, there was an inhibition of the pathogenic fungi when paired with the biological antagonists (*C. neoformans, R. rubra* and *T. candida)* which was attributed to the displacement of pathogenic fungi on the tested treatments by causing a reduction in the growth of pathogen observed, showing that the three biological antagonists were effective in the treated tests. In line with this, several researchers recorded the efficacy of yeast as an inhibitor agent against pathogenic fungi (Mehrotra *et al*., 1996; and Ozgur *et al*., 2005).And therefore, suggested that some of the biological control in an operation were effective and was observed that *C. neoformans, R. rubra* and *T. candida* acted by producing antifungal substance or produced metabolites which diffused in to the growth medium faster than naturally occurring surface pathogens.The findings in this study revealed that *C. neoformans, T. candida* and *R. rubra* have potentials to control rot in postharvest yams. This can provide alternative ways in reducing rot in yams reduced will reduced the risk associated with chemical fungicides use to control rot in yam tubers. These biological antagonists are being less expensive and environmental friendly have advantage over chemical (synthetic) fungicides.

CONCLUSION

The naturally occurring saprophytic yeasts *(Cryptococcus neoformans, Toruiopsis candida* and *Rhodoturula rubra*) isolated from the surfaces of fresh fruits of Orange, Tomato and Lime fruit acted as biocontrol agents and inhibited the growth of pathogenic fungi (*A. niger, A. flavus* and *penicillium notatum*)in this study. It was observed *Cryptococcus neoformans* have the highest inhibitory effect followed by *Toruiopsis candida* and *Rhodoturula rubra*. Therefore, this study recommends the field trial on the use of *Cryptococcus neoformans, Toruiopsis candida* and *Rhodoturula rubra* for controlling of postharvest yam fungi diseases.

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Table 1: Percentage occurrence of fungi isolated from rotten yam tuber

Fungi isolated	Percentage occurrence%
Aspergillus niger	46.88[a]
Aspergillus flavus	23.44[b]
Penicillium notatum	18.75[c]
Penicillium trysogenum	7.81[d]
Trichophyton rubra	1.56[e]
Trichophyton soudanense	1.56[e]

The percentage occurrence of each pathogens isolated differs significantly at (P<0.05).

Table 2: Frequency of occurrence of saprophytic yeast isolated from different fruits surfaces

Tested fruits	Yeast isolate	Frequency (%)
	Cryptococcus neoformans	41.2[b]
Tomato	*Rhodoturula rubra*	29.4[a]
	Toruiopsis candida	29.4[a]
Lime fruits	*Cryptococcus neoformans*	31.6[b]
	Rhodoturula rubra	26.3[a]
	Toruiopsis candida	42.1[c]
Orange	*Rhodoturula rubra*	52.4[b]
	Toruiopsis candida	47.6[b]

Percentage in column followed by different letters differs significantly at (p<0.05

Table 3: In vitro antagonistic effect of *C. neoformans, T. candida and R. rubra on A. niger, A. flavus,* and *P. notatum* in dual Culture Techniques.

Saprophytic yeast	*RGP (mm)			*PIP			*ZIP (mm)		
	A. Niger	*A. flavus*	*P. notatum*	*A. niger*	*A. fllavus*	*P. notatum*	*A. niger*	*A. flavus*	*P. notatum*
C. neoformans	44.0[a]	42.0[a]	40.0[a]	51.1c	51.7[c]	50.0[c]	15.0[c]	16.0[c]	14.0[c]
T. candida	51.0[b]	48.0[b]	45.1[b]	43.3[b]	43.4[b]	43.6[b]	10.0[b]	10.1[b]	10.3[b]
R. rubra	85.0[c]	84.0[c]	74.9[c]	5.6[a]	4.9[a]	6.4[a]	2.0a	1.7[a]	3.0[a]
Control	90.0[d]	87.0[d]	80.0[d]						

Mean of three replicates, Mean in a Colum followed by different letters differ significantly (P<0.05).

Keys: *RGP=Mean radial growth of pathogens (mm). *PIP= Mean percentage (%) inhibition of pathogen. *ZIP= Mean zone of inbition of pathogens (mm).

A B

Figure 1: Pathogenecity test showing rotten of yam tuber caused by **A** *A. niger*. **B** *A. flavus*