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Effects of Different Plant Extracts in the Control of Yam Rot Induced by *Rhizopus stolonifer* on Stored Yam (*Dioscorea* Sp.). in Yola, Adamawa State Nigeria

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Abstract: This study on the effects of plant extracts on the control of fungal rot of stored yam (*Dioscorea* sp.) due to *Rhizopus stolonifer* was carried out in the Laboratory of the Department of Crop Production and Horticulture, Federal University of Technology Yola. The objectives of the experiment were to identify the most prevalent fungus associated with yam rots, determine the effects of different plant extracts on the growth of such fungus and to identify the most effective plant exract in the control of yam rots induced by the fungus. The treatments were plantain ash, neem seed oil, bitter leave extract palm oil and a control, which were laid in a Completely Randomized Deseign (CRD). The data collected were subjected to analysis of variance (ANOVA) and means were separated using Fisher's Least Significant Difference (LSD). The result showed that the most prevalent fungus associated with yam rots in the study area was *Rhizopus stolonifer*. The result further indicated that plantain ash gave the best control with regards to the number of spotted growth at 16 Days After Inoculation (DAI). Similarly, plantain ash also recorded the lowest growth diameter of 5.5 cm at 16 DAI followed by palm oil (5.73 cm) and bitter leave extract (5.75 cm). It was further observed that palm oil recorded the lowest weight loss of 9% followed by plantain ash and neem seed oil at 16 DAI. This study revealed that the application of plantain and palm oil particularly on bruised yam tubers could assist in prolonging the shelf life and reduced rots due to *Rhizopus stolonifer* and other related rots agents.

Key words: Plant extracts, *Rhizopus stolonifer,* yam rot, stored yam

INTRODUCTION

Yam rot is a major factor in spoilage during storage, a condition which may be soft, wet or dry (IITA, 1995). A total of 30 different fungi have been reported to be associated with the storage rots (Ikotun, 1989). *Penicillium oxalicum* is the most frequently encountered rot causing organism of yam tubers (Ikotun, 1989) followed by two species of *Aspergillus* and one of *Fusarium* (Adeniji, 1970). The most widely studied fungus associated with post-harvest yam decay appears to be *Botrydiplodia theobromea* (Noon, 1978; Morse *et al.,* 2000). Fusarium induces pinkish with yellowish border on the infected tissues (IITA, 1993). *Roellinia bunodes* and *Botryodiplodia theobramea* have been reported to cause dry black rot. The infected tubers first turned grey and the black and later become pulverulent, breaking into small dry particles (IITA, 1993). *Rhizophus* sp., *Mucor circinelliodes, Sclerotium rolfsii* and *Rhizoctonia solani* were reported by Ikotun (1989), Green *et al.* (1995), Amusa and Baiyewu (1999) to cause rapid collapse of cell walls, which turn brown and become soft and at times wet.

Microbial infection of yam tubers is rapid and severe especially in areas where high temperature and high humidity favours rapid microbial growth. Rotting is a major factor limiting the post-harvest life of yams (Osagie, 1992). Losses in yam in storage due to rot are considered heavy in Nigeria. The evaluation of rot in different parts of Nigeria revealed that the extent of rotting ranged from 0.5-18% at harvesting while storage rot ranged from 3-25% (Ikotun, 1986). Microbial rotting of yam tubers accounted for a substantial proportion of the annual losses in yam production in Nigeria. Okigbo and Ikediugwu (1999) associated the different forms of tuber rotting in the storage barn to microbial attacks that probably took place in the field and increase in storage. Fungi which are associated with storage losses are: *Botryodiplodia theobromae*, *Fusarium moniliforme*, *Penicillium sclerotigenum*, *Rosellina bunnode, Aspergillus niger*, *Hendersonula forulvidae, Macrophomina phaseoli* and *Rhizopus nodosus*. The use of chemicals has been found to be effective in reducing fungal rots of yams (Ogundana, 1981). Due to toxicity of many chemicals, biological method of control has been

preferred in some cases because it is selective with no side effects on both human beings and the environment and are relatively cheap. Resistance to biological control is rare and biological controls agents are self-propagating and self-perpetuating (Okigbo and Ikediugwu, 2005). Plant extracts have found greater acceptance and have been used successful to control disease in plants and tuber crops in agriculture for quite sometime particularly of storage crops (Okigbo and Emogheme, 2004; Okigbo and Nmeka, 2005).

There is a growing awareness on the dangers associated with the use of some chemicals for storage and preservation of farm produce. Some of these chemicals have high mammalian toxicity and are considered not safe and even on the environment. Consequently, emphasis is now being placed on the use of non-toxic and environmentally friendly chemicals particularly those of plant origins. Plant extracts have found greater acceptance in modern storage methods of agricultural produce. There are several local plant species whose extracts have proved effective in protecting yam produce before and after harvest. Formulations of extracts of neem (*Azadrictha indica*), which include neem seed oil extract, neem water extract and emulsifiable concentrate. Other plants extracts include ashes from oil palm (*Elaeis guineensis*) inflorescence, kola (*Cola natida*) and Mango (*Mangifera indica*) (Ogbeni, 1995). The advantages of these material plant include local availability, little or no toxicity to humans and simple preparation procedures. This study is therefore designed with the following objectives:

- Identify the most prevalent fungi associated with stored yam in the study area.
- To study the effects of different plant extracts on the growth of such fungi on stored yam.
- To identify the most effective plant extract in the control of yam rots.

MATERIALS AND METHODS

Two sets of experiments which involved isolation and identification of the fungal pathogen as well as its inoculation on healthy yam tubers followed by the applications of plant extracts were carried out at the Crop Production and Horticulture Laboratory of the Federal University of Technology, Yola, Adamawa State-Nigeria, between the months of March and May, 2007.

Experiment I: Isolation and identification of fungal pathogens: Infected yam tubers were procured locally from the Jimeta new market, Jimeta old market and Yola town market. Ten samples were collected from each selling point and were taken to the Laboratory for isolation and identification. The identified organism was used to infect healthy yam tubers to establish its pathogenicity.

Isolation of the fungal organism: Diseased portion of the yam tubers were cut under aseptic conditions into small bits into a sterile dish using a scissors, which was flamed over a Bunsen burner flame and dipped inside methylated spirit (Fawole and Oso, 1988). The cut diseased and sterilized bits were then placed on petri dishes containing solidified Potato Dextrose Agar (PDA). The solidified plates were incubated at room temperature (28- 32°C) for 4-5 days. Fungal colonies growth from the incubated plates were sub-cultured into fresh medium until pure culture was obtained.

Microscopic examination was used after examining the colony characteristic. A sterilize needle was used in taking a little portion of the hypae containing spores on the sterile glass slide stained with Lactophenol cotton blue and examined under the microscope, for fungal structures. The morphology and cultural characteristic observed were compared with structures in Snowdon (1990).

Pathogenicity test: Healthy yam tubers were surface sterilized with 0.1 Mercuric Chloride ($HgCl_2$) for 1 minute and washed in 5 changes of sterile distilled water. A 5 mL cork borer was driven to a depth of 4 mm into the healthy yam tubers and the bored tissue was removed. A 5 mm diameter disc from the pure culture was cut and placed in the hole and the removed tissue was replaced back. The wound was sealed with prepared candle wax according to the method of Fawole and Oso (1988). The control was set up in the same manner except that sterile agar disc was used instead of the inoculum. The inoculated yam tubers were placed in 4 replicates at room temperature (37°C), under sterile condition. The pathogen was re-isolated and identified using the same procedure described earlier.

Experiment II: Preparation and application of different plant extracts

Plantain peel ash: The plantain was prepared by using plantain peels (*Musa paradisiaca*), which was cut into smaller pieces and completely sun dried for 10 days before burning. The ash obtained was sieved through 0.25 mm wire mesh to removed any unburnt particles.

Bitter leaf extract: The bitter leaf (*Vernonia amygdalina*) extract was prepared by pounding and squeezing liquid of

the fresh bitter leaves. The pounded leaves were washed thoroughly with water and then sieved to remove particles of the leaves.

Neem seed oil: The neem (*Azadrictha indica*) seed oil was extracted by de-pulping the collected seeds. The seeds were washed and dried under a shed and later pounded in a mortar to remove the hard endocarp and chaffs and later grinded into a fine powder. The powder was poured into a bowl and pre-boiled water was added gradually to form a slurry. This procedure was repeated until a dough-like mixture was formed and the oil was then pressed out manually unto a container. The entire process was repeated until a reasonable percentage of the oil in the dough was pressed out. The oily material collected was heated to remove any trace of water.

Palm oil: The processed palm oil was locally procured from the Jimeta main market.

Preparation of inoculum from the isolate: The petri dishes containing the pure culture of the identified fungal pathogen was poured into 100 mL of distilled water to make the stock inoculum. Ten mililiter was taken from the stock inoculum and poured into 90 mL of distilled water in a sterilized container.

Sterilization and inoculation of yam tubers with fungal pathogen: The healthy yam tubers were washed in methylated spirit with distilled water to disinfested them off pathogens and other contaminants before inoculation with the identified fungal pathogens. The healthy yam tubers were surfaced sterilized and cut into 2 halves under aseptic conditions. The cut portion was injected at random in 10 spots with suspension of fungal pathogens using hypodermic syringe.

Application of different plant extracts: The cut surfaces of the inoculated yam tubers yam specimens were smeared with each of the treatments (plantain ash, neem seed oil, palm oil and bitter leaf), placed inside sterile polyethene bags and kept in 4 replications each. The design used in this aspect of the study was Complete Randomized Design (CRD) with different plant extracts including the control serving as treatments

Data collection

Number of spotted growth: The number of spotted growth was determined by monitoring tubers in each replicate for symptoms of disease at 4 days intervals by counting the number of spots on each cut surfaces of the tubers.

Percentage weight loss: The percentage weight loss of yam was obtained by subtracting the final weight of yam per replicate from initial weight. This figure was then expressed as a percentage of the initial weight thus:

$$\text{Percentage weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight of tuber}} \times 100$$

Diameter of growth of yam rots from center of inoculation: This was obtained by using rule to measure the diameter of the growth from the center of inoculation from each cut surface in centimeters.

Data analysis: The data collected was subjected to Analysis of Variance (ANOVA) based on Completely Randomized Design (CRD) as described by Gomez and Gomez (1984). Means were separated using Fishers Least Significant Difference (LSD) according to Cochran and Cox (1992).

RESULTS

Experiment 1: Isolation and identification of *Rhizopus stolonifer* from the yam tubers: Inoculation of the Potato Dextrose Agar (PDA) with the freshly infected yam tissue and subsequently incubation at 60°C for 48 h produced mixed fungal growth which was later sub-cultured. This pure culture was examined under the microscope for fungal structures and other morphological and cultural characteristics. Some whitish cottony appearance on the medium which turned black from 72 h of incubation was obtained. When the mould was further observed under the microscope (×40), some erect and unbranched sporangiosphores with the sporangium containing sporangiospores was observed. Comparing this with structures described by Snowdon (1990), the fungus was identified to be *Rhizopus stolonifer*.

Pathogenicity test: The result of the pathogenicity test earned out with a 24 h pure culture of the *Rhizopus stolonifer* with a hypodermic syringe and needle on a healthy yam tuber gave rise to soft rot. The infected tissues become soft ramified by the fungal mycelium. The causal fungi quickly ramified the tissue, which turn brown and become soft due to the rapid collapse of the cell walls. These symptoms were similar to those observed earlier on the infected tubers procured from the market. This confirmed the identify and pathogenicity of *Rhizopus stolonifer*.

Table 1: Effect of different plant extracts on the number of spotted growth induced by *Rhizopus stolonifer* at 4-10 DAI

	DAI			
Plant extracts	4	6	8	10
Plantain peel ash	4.25	5.25	6.25	7.25
Neem seed oil	12.5	15.00	21.25	24.50
Bitter leaf extract	10.00	16.00	22.00	24.75
Palm oil	17.00	20.00	23.25	26.50
Control	14.00	20.00	26.00	32.00
SE (±)	1.46	1.42	1.44	1.41
LSD	9.84	9.55	9.68	9.51

DAI = Days after inoculation

Table 2: Effect of different plant extracts on growth diameter (cm) of yam induced by *Rhizopus stolonifer* at 4-16 DAI

	DAI						
Plant extracts	4	6	8	10	12	14	16
Plantain peel ash	2.27	3.00	3.58	4.15	4.68	4.98	5.50
Neem seed oil	2.98	3.70	4.13	4.58	5.30	5.68	6.05
Bitter leaf extract	2.43	3.00	3.50	4.38	4.88	5.35	5.75
Palm oil	2.80	3.70	4.20	4.56	5.00	5.38	5.73
Control	3.20	3.80	4.48	5.25	5.70	6.10	6.48
SE (±)	0.19	0.15	0.01	0.06	0.06	0.08	0.05
LSD	1.28	0.91	0.73	0.44	0.41	0.46	0.39

DAI = Days after inoculation.

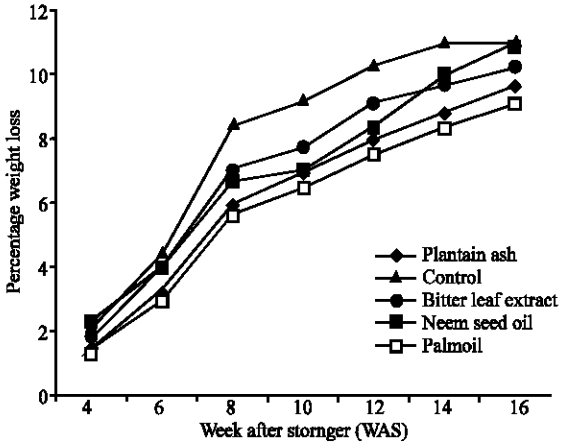

Fig. 1: Effect of different plant extracts on percentage weight loss of yan induced by *Rhizopus stolonifer*

Experiment II: Controlling of yam rot induced by *Rhizopus stolonifer* using different plant extracts: Results in Table 1 indicated that there was a gradual increase in the number of spotted growth among the different treatments with time. It was observed that the control recorded the highest growth of 32 spots at 10 DAI due to the pathogen. Furthermore it was observed that the tubers treated with plantain ash recorded the lowest number of spots at 6, 8 and 10 DAI. The number of spotted growth in these respective days were 5, 6 and 7. The result also indicated that there was significant difference at 6 DAI ($p = 0.05$) and at 8 and 10 DAI ($p = 0.01$) among the treatments.

The results in Fig. 1 showed that there were variation in percentage weight loss due to *Rhizopus stolonifer* with time and among treatments. The highest weight loss at 4 DAI was on the control. There were no significant differences observed at 8, 10 and 12 DAI among the treatments. The lowest weight loss at 16 DAI was 9% observed on tubers treated with palm oil while the mean weight loss and at 16 DAI was 10%.

The effects of different plant extracts on the growth diameter of yam rot induced by *Rhizopus stolonifer* at 4-16 DAI: The result on the growth diameter of the rot from the centre of inoculation induced by *Rhizopus stolonifer* presented in Table 2 indicated highly significant difference ($p = 0.01$) at 10, 12, 14 and 16 DAI. The highest growth of 3 cm was observed on the control at 4 DAI, though it did not differ significantly from those of plantain ash, neem seed oil, bitter leaf extract and palm oil. The lowest growth of 2.80 and 5.73 cm were observed on the tubers treated with plantain ash at 4 DAI and 16 DAI, respectively.

DISCUSSION

Yams in storage are subject to losses of up to 50% of the fresh matter due to *Rhizopus stolonifer* and other numerous fungal pathogens (Osagie, 1992). This study has revealed the presence of *Rhizopus stolonifer*, which was principally responsible for the rots observed on the yam tubers in the study area. The organism, *Rhizopus stolonifer* isolated and identified in this study corroborated with the report of Woolfe (1992) who revealed that *R. stolonifer* was the most widespread fungus responsible for sweet potato rot in the tropics. Opara (1999) also reported that *Rhizopus stolonifer* induced rot as the major post-harvest disease of yam tubers. Pathogenicity test carried out in this study revealed that *Rhizopus stolonifer* produce an infected tissue that becomes soft ramified by the fungal mycelium. Ikotun (1989) had declared that fungi caused 57-77% of all rots of yam tubers in Nigeria and about 30 different fungi were isolated. In West Indies, fungi were responsible for 80% of all yam rots. The findings in this work agreed with that of Woolfe (1992) who reported that *Rhizopus stolonifer* is a very destructive pathogen at tropical environment as well as being an important parasite of yam in storage.

The effects of plant extracts on the number of spotted growth on cut surface of yam tubers revealed that plantain ash recorded the lowest number of 7 spots due to *Rhizopus stolonifer*. The plant extract did not show any significant difference with respect to diameter of growth

on the cut surfaces. The plantain ash appeared to have significantly suppressed the expansion of diameter growth of *Rhizopus stolonifer* in a similar manner as those of palm oil, bitter leaf extracts at 16 DAI. These findings are similar to those by Okigbo (2003) and Channya (1991) in their study on yam tubers, plantain and kolanuts who observed that wood ash reduced fungal rots in yam, plantain and kolanuts. The plantain ash in this study appeared to have caused desiccation of fungal germ-tubes before they establishment of the fungus. It might have also absorbed moisture from the fungal tissue surface and exerted some chemical effects due to the minerals contained in the ash. This corroborates report by Okigbo (2003) and Channya (1991) in their study on the effects of wood ash on the growth of *Rhizopus stolonifer* on yam, plantain and kolanuts.

Yam rots apart from reducing the nutritive and market value also have significant effects on the weight of the commodity (Ikotun, 1986; Brady, 1982). Several measures have been and are still going on to reduce post-harvest losses in yam due to fungal pathogens and to ensure prolonged storage life Nwakit (1982). The effects of plant extracts on the weight loss showed that the yam tuber treated with palm oil recorded the lowest weight loss of 9% at 16 DAS followed by neem seed oil and plantain ash each of which recorded 10%. The oil might have reduced the penetration of *Rhizopus stolonifer* into the yam tissue and hence reduced damage and consequently weight loss. This is similar to the findings of Oduro *et al.* (1999) when he conducted a study on controlling yam rots using palm oil. The inhibition of fungal penetration might be due to the physical and chemical properties of the palm oil in protecting the wounded yam tubers. Although a lot of post-harvest losses due to bacteria and fungal pathogens have posed serious threats to yam production in Nigeria and all over the world, efforts are being made to reduce their attack.

CONCLUSION

This study revealed that the most prevalent fungus associated with yam rot in Yola was *Rhizopus stolonifer*. The study also found out that the best plant extract that could arrest the growth of this pathogen is plantain ash followed by palm oil. Their use could go a long way in retarding fungal growth and prolonging the storage life of the tubers thereby reducing the scarcity of the commodity that is usually experienced particularly during the off-season. However, further study on these plant extracts should be done to ascertain their chemical activitities against *Rhizopus stolonifer* and other rots agents of yams.

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