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Identification and control of Fungi associated with the post-harvest rot of *Solenostemon rotundifolius* (Poir)J.K. Morton in Adamawa State of Nigeria.

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Abstract
Investigation into the fungi associated with the post-harvest rot of *Solenostemon rotundifolius* (Poir).J.K.Morton, Hausa Potato was carried out in the teaching and research laboratory of the Department of Biological sciences of the University. Completely Randomized Design (CRD) was used and the data obtained were analyzed using Analysis of Variance (ANOVA) and the means that were significant were separated using the Least Significant Difference (LSD). Four fungi *Aspergillus niger, Fusarium oxysporum, Penicillum expansum* and *Rhizopus stolonifer* were consistently isolated identified in pure cultures from the diseased tubers collected from the two markets in Yola. Mean percentage incidence showed that *A. niger* was the most prevalent with 19.69% followed by *F. oxysporum* 16.47%, *R. stolonifer* 14.38% and *P. expansum* 12.81%. The efficacy of Wood ash, saw dust and guinea corn chaff as control material against fungal rot development was used. The three plant materials reduced the rot caused by the three organisms.
Key words: *Solenostemon rotundifolius, Aspergillus niger, Fusarium oxysporum, Penicillium expansum and Rhizopus stolonifer.*

1. Introduction

Hausa potato [*Solenostemon rotundifolius* (Poir.)] J. K. Morton, (Hausa name-*Tumuku*), a root tuber and a dicotyledonous annual herb belonging to the family Labiaceae (Schippers, 2002), originated from tropical Africa (Tindall, 1983). It is currently cultivated in many African countries (Ghana and Nigeria) as a supplement in family menus (Jada *et al.*, 2007). The plant grows on well drained loamy or sandy loam soil and on ridges (Schippers, 2002), producing tubers (Blench, 1997) that contain 75% water, 1.4% protein, 0.5% fat, 21% carbohydrate, 0.1% fibre, 1% ash, 17mg calcium, 6 mg iron, 0.05 mg thiamine, 0.02 mg riboflavin, 1 mg niacin, 1 mg ascorbic acid (Grubben and Denton, 2004). They are boiled, baked, fried or roasted and eaten as snack or cooked with spices in various combinations with other foods such as beans and cook vegetables (Grubben and Denton, 2004). The most serious causes of post-harvest loss in tropical root crops are pests and diseases due to fungi (FAO,1990) and as a result of harvesting and post - harvest handling techniques (Mustapha and Yahya, 2006) with resultant 10-30% reduction in tuber quality (Muktar and Abdullahi, 2004, Kehinde and Kadiri 2006 and Basiri *et al.,* 2011). Infection of the tubers present economic loss associated with discoloration on the crops, change in flavour, thereby giving off unpleasant odour (Cockerell *et al*. 1971) and production mycotoxins harmful to human and livestock (Oguntade and Adekunle, 2010). Although the use of synthetic fungicides have been proved effective in controlling some phytopathogens, chemical control leads to environmental hazards associated with high cost and inaccessibility to indigenous farmers (Ebele, 2011). Ijato (2011) reported that plant parts, powders of plant parts, ash, aqueous extracts which are environmentally non-hazardous, locally available and can be cheaply maintained are suitable alternatives to the expensive synthetic fungicides. Mann (2012) reported the control of infectious diseases using *Anogeissus leiocarpus* and *Terminalia* avicenniodes. This paper is aimed at identification and control of the fungi associated with the post-harvest rot of *Solenostemon rotundifolius*.

2. Materials and Methods

2.1 Collection of Samples

A total of three thousand and two hundred (1200) samples of *Solenostemon rotundifolius* (both healthy and diseased) were collected from Yola town and Jimeta markets of Adamawa state. Located between latitude 9°11′ to 9°19′N and longitude 12°20′ to 12°30′E. Samples of *Solenostemon rotundifolius* tubers were collected from different selling points randomly in the markets. Both wounded and non-wounded ones were taken to the laboratory for studies. The diseased tubers were incubated in sterilized desiccators for the development of rot. Sixteen medium-sized clay pots (25x20x19cm) were purchased from Yola market for the storage of the tubers.

2.2 Collection and Preparation of plant materials

Guinea corn (*Sorghum bicolor*) chaff was purchased from Yola market, sawdust was obtained free from a carpenter at Jimeta and wood ash was obtained from domestic burnt wood of *Anogeissus leiocarpus*. Five hundred grammes of each of the plant materials in four replicates were placed into sterile polythene bags and taken to the laboratory.

2.3 Isolation and Identification of Fungi

Portion of the diseased tubers of *Solenostemon rotundifolius* was cut into small pieces into sterile Petri dishes with a sterile scissors which was flamed over and dipped inside methylated spirit (Thomas, 1979). The cut pieces were surface sterilized with 0.01% mercuric chloride for 30 seconds and rinsed in five changes of sterile distilled water and blotted dry with sterile Whatman No. 1 filter papers. Cut pieces were plated aseptically in 9 cm^2 Petri dishes containing solidified potato dextrose agar (PDA). Solidified plates were incubated at room temperature (28–30°C) for 4 days. Fungal colonies that grew from the incubated plates were similarly sub-cultured on fresh PDA plates and incubated at 28–30°C for 4 days until pure cultures were obtained.

2.4 Identification of Isolated Fungi

Microscopic examination was carried out after examining the colony characteristics (Frazier, 1978), while the morphological and cultural characteristics were observed under the microscope and compared with the structures in Alexopoulus and Mius (1986) and Snowdon (1990).

2.5 Pathogenicity Tests

Healthy *Solenostemon rotundifolius* tubers were inoculated with pure cultures of the isolates after being surface sterilized with 0.01% $HgCl_2$ for one minute and washed in five changes of sterile distilled water. A 2mm diameter cork borer was driven to a depth of about 2mm into the healthy tubers and the bored tissue removed. Then 2mm diameter disc of the culture was cut and placed in the hole and the removed tissue was put back in place. The wound was sealed with sterile vesper prepared from wax and Vaseline. The control was set up in the same manner except that sterile agar was used instead of the isolate. For each isolate four tubers of *Solenostemon rotundifolius* were inoculated and four controls were set up. The inoculated tubers were placed in desiccators and incubated at 30°C under aseptic conditions as adopted by Chimbekujwo (1994) and Basiri *et al.* (2011). Regular observations were made and isolation of any pathogenic organism was done for comparison with the original isolates. All experiments were conducted in the laboratory using Completely Randomized Design (CRD) as described by Gomez and Gomez (1984). All the experiments were replicated four times. Data collected were analyzed using analysis of variance (ANOVA), while the means that were significant were separated by least significant difference (LSD) at 1% probability level ($P<0.01$).

2.6 Assessing the efficacy of guinea corn chaff, saw dust, and wood ash in the control of Solenostemon rotundifolius tuber rot.

Eighty healthy hausa potato tubers were placed in 25 cm diameter clay pots , labeled as A,B,C and D after being surface sterilized with 0.01% $HgCl_2$ for one minute, washed in five changes of sterile distilled water and left to dry. Tubers in A were covered with guinea corn chaff, B with saw dust, C with wood ash and D served as control (untreated). Five hundred grammes of each of the treatment material were used and all the pots were arranged in a completely randomized design with four replications at room temperature. One tuber from each of the experimental pots was randomly selected and inoculated with one of the fungal isolates. Disease incidence (%) of *Solenostemon rotundifolius* was determined after four months of storage at room temperature using the formula as described by Tarr (1981).

$$\text{Disease incidence} = \frac{\text{number of diseased samples}}{\text{total number of samples examined}} \times 100$$

3. Results

The fungi isolated from diseased tubers of *Solenostemon rotundifolius* were identified as *Aspergillus niger, Fusarium oxysporum, Penicillium expansum and Rhizopus stolonifer. Aspergillus niger* was found to be responsible for about 19.69% of rots observed, followed by *Fusarium oxysporum,* 16.47%, *Rhizopus stolonifer* 14.38% and *Penicillium expansum* 12.81% (Table 1). Pathogenicity tests of the isolates revealed that *A. niger, F. oxysporum, P. expansum* and *R. stolonifer* caused rot as inoculated hausa potato tubers developed rot symptoms similar to natural diseased tubers. *A. niger* had the highest mean rot diameter of 22.35mm while *P. expansum* had the lowest 15.25mm (Table 2). The preservatives (guinea corn chaff, saw dust, and wood ash) significantly ($P<0.01$) controlled rot of the inoculated *Solenostemon rotundifolius* tubers compared to the control (Table 3). The highest disease incidence of 7.81% was recorded in the control, while the lowest incidence of 1.56% was recorded in tubers treated with wood ash, which also has remarkable reduced effects of the incidence of *Penicillium expansum,* and *Rhizopus stolonifer.* However, *Aspergillus niger* appeared to be less sensitive to the preservatives compared to the other fungal pathogens.

4. Discussion

The results of the study showed that four fungi viz; *Aspergillus niger, Fusarium oxysporum, Penicillium expansum* and *Rhizopus stolonifer,* were isolated from infected *Solenostemon rotundifolius* tubers in the study area. Out of these, *A. niger* was responsible for about 19.69% of rots observed, the least was *P. expansum* 12.81%. Muktar and Abdullahi (2004) and Umar *et al*. (2010) who studied the prevalence of *A. niger* from Irish potatoes and maize seeds respectively reported the same result. The identified isolates have been found as common post-harvest fungi (Mustapha and Yahya, 2006, Naureen *et al.*, 2009 and Ebele, 2011) and earlier reported to be responsible for rot of many root and tuber crops in Nigeria (Oduro *et al*., 1990). The significant reduction in disease incidence observed in the tubers inoculated with the fungal isolates and treated with the different preservatives may be due to inhibitory effects on the fungi which might have caused reduction in the mycelial growth of the isolates.

5. Conclusion

The findings of this study have shown that *Aspergillus niger, Fusarium oxysporum, Penicillium expansum* and *Rhizopus stolonifer* were responsible for the rot of *Solenostemon rotundifolius* tubers. Saw dust and wood ash which have fungicidal properties are good mediums for storing *Solenostemon rotundifolius* tubers and serve as alternative methods of reducing human exposure to toxins produced by pathogens such as mycotoxins, aflatoxins thus minimizing post-harvest losses.

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Table 1: Percentage Incidence of *Solenostemon rotundifolius* Tuber Rot from two Markets in Yola

Organisms isolated	**Percentage Incidence of Isolation**		
	Yola Town Market	**Jimeta Market**	**Total Isolation**
Aspergillus niger	350 (10.94%)	280 (8.75%)	19.69%
Fusarium oxysporum	275 (8.59%)	252 (7.88%)	16.47%
Penicillium expansum	210 (6.56%)	200 (6.25%)	12.81%
Rhizopus stolonifer	240 (7.50%)	220 (6.88%)	14.38%
Non- infected	573(17.91%)	600 (18.75%)	36.66 %
Totals	1648 (51.50 %)	1552 (48.51 %)	100.00%

Table: 2 Pathogenicity Test Showing rot Development of *Solenostemon rotundifolius* Tubers Incubated at 30^0C

Isolated fungi	**Mean diameter of rot (mm)**
Aspergillus niger	22.35
Fusarium oxysporum	21.50
Penicillium expansum	15.25
Rhizopus stolonifer	18.02
Control	0.00

Table 3: Mean rot percentage on Inoculated *Solenostemon rotundifolius tubers* for four months storage period.

Treatment	***Aspergillus niger***	***Fusarium oxysporum***	***Penicillium expansum***	***Rhizopus stolonifer***
Saw dust	4.14	3.43	2.19	3.51
Guinea corn chaff	5.62	4.76	3.43	4.06
Wood ash	3.17	2.26	1.56	1.87
Control	7.81	5.93	4.06	5.93
Mean	5.18	4.10	2.81	3.84
Pro. F	0.01	0.01	0.01	0.01
SE	1.53	1.28	0.80	1.26
LSD	0.87	0.80	0.63	0.79

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