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Isolation and Physiological Studies of Yam Rotting Fungus in Makurdi Metropolis, Benue State, Nigeria

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Abstract

The study reports the isolation and physiological studies on yam rotting fungus in Makurdi metropolis, Benue State. Alternaria solani was isolated from yam tubers having not symptoms and was found to be pathogenic. Nutritional studies on utilization of fructose, starch and glucose were carried out. The results showed that Alternaria solani utilized fructose best at 0.1g/30ml medium, followed by glucose (0.81g/30ml) and starch (0.73g/30ml) in that order. Among the selected nitrogen sources supplemented, sodium nitrate was most utilized with average mycelia dry weight of 0.58g/30ml medium, followed by calcium nitrate (0.37g/30ml) while area was least utilized (0.12g/30ml) medium.

Key words: *Alternaria solani, physiology, carbon, nitrogen.*

Introduction

Yam *(Dioscorea species)* are native to the old world topics with wild species being found in both Africa and Asia (Hill and Waller, 1999).

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It is one of the most important groups of staple foods in the tropical world (Taiga, 2002). It accounts for over 50% of the total daily carbohydrate consumption of the average Nigerian population (Ene and Okoli, 1983). Edible species include: *Dioscorea rotundata* (Pour), *D. cayenesis* (Lam.), *D. alata* (L.), D. opposite (Thumb) and *D. trifida* (Lare) Onwueme, 1982).

Nigeria, the world's largest producer of yam, cultivates only six species mainly *D. rotundata* (white yam), *D. alata* (water yam), *D. esculenta* (Chinese yam), *D. bulbifera* (aerial yam) and D. *dumentorum* (cluster yam) (Adeniji, 1970). The major conters of yam production in Nigeria occur in the savana zone of the country which produces a significant proportion of the nation's output. The centres of production of yams are: Kakibia, Minna, Buga, Zaki Biam, Makurdi, Otukpo and Lafia.

In the recent times however, yam production is on the decline substantially majorly due to infestation attack by fungi and nematodes and partly due to high cost of production, low multiplication rate and storage problems, and with stiff competition that yam is receiving from cassava and other root crops; there are fears whether or not it would survive the future. Apart from serving as food, yam also serves as source of medical compounds such as steroids and alkaloids (Ene and Okoli, 1983). As one of the most efficient carbohydrate sources, yam has potential for food production as well as energy supply (Taiga, 2002).

Yams are produced in large quantities and stored for use later. Unfortunately, considerable amount of yam gets rotten during storage. Post-harvest loss of yams alone account for about 7.0 million tones out of the world's total production of 27.08 million tones (Solape *et al,* 1988). Many fungi have been identified by various workers as aetiologic agents of various yam diseases. Adebanjo and Onesirosan (1986) isolated *Colletotrichum gloesporioides* as a fungal pathogen infecting *minisettes* through infested tubers. *Botryodiplodia theobromae, Aspergllus niger, A. Tamari, Cladosporum herbarum, Cylinderocapus radicola, Gliocladium roseum, Geotrichum candidum, Gilomatrix convulata, Mucor circineloides, Fusarium monniliforme, F. Solani* were associated with yam tuber not (Ikotun, 1983). Among the yam rotting fungi associated with yam tuber as reported by

Onwueme (1982) are *Penicillum, Rhizopus, Botryodiplodia theobromea and Aspergillus Niger*. A considerable number of yam farmers have showed that fungal attack on yam occurs through injuries on the tuber as reported by Bonire, (1986). A freshly injured yam therefore rapidly loses moisture from the injured area to leave the injury dry and impervious to the hyphae of growing fungi. This work therefore, is aimed at isolating and identifying fungal organism associated with rot of yam tubers, establishing the pathogenicity of the isolate and the physiological studies of the isolate with carbon and nitrogen sources.

Materials and Methods

Collection of samples: Four varieties of yam *D. rotundata, D. alata, D. dumentorum and D. bulbifera* were collected from three different markets in Makurdi of latitude 70°44"N and longitude 80°54"E of the equator; the three areas are Wadata. Northbank and Wurukum.

Table 1: Infection indices for different levels of disease severity of yam rotting in Makurdi metropolis.

Infection Index	No of infected yams	Description
0	0	No infection
1	1-30	Very light infection
2	31-55	Moderate infection
3	56-75	Severe infection
4	>75	Very severe infection

Isolation of fungi: Infected tubers from different locations were brought into the laboratory. Disease portions were first washed with running tap water, surface sterilized with 70% alcohol. Infected portion of the tubers were cut into several 1mm disc, used sterile cork borer rinsed in three changes in sterile water, blotted dry with sterile filter paper and inoculated on PDA in 9cm diameter petridishes. The inculated plates were incubated at 25±1°C for four days, after which the growing colonies were sub cultured into fresh PDA medium.

Pathogenicity tests: Disease-free tubers were surface sterilized with absolute alcohol. Wounds were created on some of the tubbers

with sterilized knife and 5-millimeter radius cork borer by making hole, 4cm deep into the tubbers; while others were without wounds.

Physiological Studies: This was determined by dry weight measurement. Mycelia dry weight determination was carried out on mycelia growing on different carbon sources following the method of Suleiman, (2005). The following carbon sources were incorporated into basal medium; glucose, starch and fructose. The basal medium consisted of 1.0g, KCL; 0.5g. MgS04 7H20; 3.0g, Ca (NO3)2; 1.0g, K2HPO4; 0.01g, FeSO4 7H2). The appropriate weight of each carbon source (10g dissolved into 100ml distilled water) was autoclaved separately; 20ml of the basal medium were dispensed into conical flasks and autoclaved at 1.1kg/km3 at 1210C for 15 minutes metric.

Ten (10) millilitres solutions of the different sugars sterile) were aseptically added to the sterile basal medium in the flasks. Each conical flask contained 20ml sterile basal medium and 10ml sterile carbon source. The flasks were inoculated with 5mm diameter disc of test fungus of a seven day old culture growing on PDA such that the mycelia mat were uppermost and floated on the medium. Similar procedure as above was carried out with liquid medium. The appropriate weight of each nitrogen source was autoclaved separately before adding aseptically to the basal medium in the flasks. Twenty (20) g of glucose was the carbon source of the medium. The different nitrogen sources used were sodium nitrate (NaNO3), urea (NH2)2 and calcium nitrate Ca (NO3)2. Ten (10ml) solutions of the different sugars (sterile) were aseptically added to the sterile basal medium in the flasks. Each conical flask contained 20ml sterile basal medium and 10ml sterile carbon source. The flasks were inoculated with 5mm diameter disc of test fungus of a seven day old culture growing on PDA such that the mycelia mat were uppermost and floated on the medium. Three replicate flasks were used for each carbon source. The flasks were stoppered with sterile non-absorbent cotton wool and incubated for one week at 25$\pm$ 2^{0}C. Harvesting was carried out at five day intervals. The mycelia were filtered by suction through filter paper previously dried to a constant weight. Both filter paper and mycelia were then dried in

an oven at 80^{0}C to a constant weight. The weight of the mycelia was determined by subtracting the initial weight of the filter paper from the weight of mycelia and filter paper *(Go-G1)*; where G0 = initial weight of filter paper and G1 = weight of mycelia and filter paper. All results obtained were analysed using Simple Descriptive Statistics such as mean and standard error. Means were separated using analysis of variance. Least Significant Difference (LSD) was used for inferential statistical analysis while standard error was used for descriptive statistics.

Results

The isolate: From the samples collected from the various markets, Alternaria solani was isolated from diseased tubers. The results showed that Dioscorea alata was more susceptible to the disease having the highest percentage incidence, followed by D bulbfera, while D. rotundata was the least susceptible with the lowest percentage incidence (Table 2). The isolated fungus was identified based on the microscopic examinations of the spores, having a dark-green, simple rather short or elongate, typically bearing a simle or branched chain of conidia, conidia dark, typically with cross and longitudinal septa, variously shaped, obclavate to elliptical or ovoid, frequently borne acropetally in long chains; these characteristics agreed with those of Agrois, (2005), Bannette and Hunter, (1972).

Table 2: Incidence and severity of yam rotting fungus on Makurdi metropolis.

Yam varieties	Wadata		Northbank		Wurukum	
	%I	SI	%I	SI	%I	SI
Dioscorea rotundata	30	1	20	1	30	1
Dioscorea rotundata	66	3	72	3	60	3
Dioscorea dumentorum	40	2	50	2	25	1
Dioscorea bulbifra	58	3	60	3	44	2

KEY:
%1 = % Incidence
S.I = Severity Index

Pathogenicity and observation of Disease symptoms

All the wounded tubers inoculated with the conidia of the isolate showed characteristic symptoms of rot after one week of inoculation, while those without wounds did not show symptoms of rot. The tuber appears as a brown discolouration of the skin and underlying flesh of the tuber. This quickly increases in size and become sunken. As the area increases, the skin wrinkles showing typical concentric rings due to shrinking of underlying tissues. The flesh was brownish and contained cavities.

Carbon and nitrogen sources supplemented

Glucose, fructose and starch were supplemented, and growth was determined by dry weight method. The results show the effect of these carbon sources on the mycelia growth. The highest mean mycelium dry weight (0.91g) was recorded on fructose. Glucose (0.81g) ranked second and starch (0.73g) had the least mean mycelia dry weight. With LSD of 4.04g, there was a significant difference between the control and fructose at ($P\ 0.04 > 0.05$) Table 3). There was significant difference among the carbon sources incorporated ($P \leq 0.05$). The best nitrogen source for the isolate was sodium nitrate with the highest mean mycelia dry weight of 0.5g, this was closely followed by calcium nitrate with mean mycelia dry weight of 0.37g, while urea had 0.12g (Table 4). There was however no significant difference between the control and sodium nitrate, but significant difference between urea and calcium nitrate was noticed.

Table 3: Mean mycelia dry weight of Alternaria solani on different carbon sources

Carbon sources	Mean dry weight $\pm$SE (g)
Control (basal medium)	0.50 ± 0.2a
Starch	0.73 ± 0.3b
Glucose	0.81 ± 0.3c
Fructose	0.91 ± 0.3d

Mean represented by the same letter are not significantly different ($P \geq 0.05$)

Table 4: Mean mycelia dry eight of Alternaria solani on different nitrogen sources

Nitrogen sources	Mean dry weight ± SE (g)
Control (basal medium)	0.56±0.2a
Urea	0.12±0.1b
Calcium nitrate	0.37±0.1c
Sodium nitrate	0.58±0.2c

Mean represented by the same letter are not significantly different ($P \leq 0.05$).

Discussions

The work on the infection of yam rotting fungus in Makurdi metropolis indicates that Alternaria is one of the causal fungus of yam rot in Makurdi metropolis, which was further confirmed through pathogenicity test. The presence of an opening on yam tuber may always initiate an infection. Alternaria is fungal organism that not only attack many host plants, but also causes problem for the pathologist in his attempt to properly identify certain type species. The survey conducted at three different locations in the metropolis on four varieties of yam *(D. rotundata, D. alata, D. dumentorum and D. bulbifera)* showed that rot of yam by the fungus were widely distributed. The results showed that *Dioscrorea alata* was more susceptible to the disease having the highest percentage incidence, followed by *D. bulbifera,* while *D, rotundata* was the least susceptible with the lowest percentage incidence. *Alternaria* which has been widely described in various literatures has been reported as a pathogen attacking the roots, stems leaves and other plant parts, where it causes serious disease conditions to the host plant (Hill and Waller, 1999; Suleiman and Odebode, 2003; Agrios, 2005), *Alternaria termissima* was reported as being responsile for leaf-spotting disease of highbush blueberry in North Carolina; *A, alternanthera, A dianthi, A raphani* and *A. Brasicae* were reported to be responsible for a number of diseases in croton plants (Zinllngsky, 1982). *Alternaria solani* on tomatoes as well as blight disease of Zinnia (*Zinnia elegans)* caused by *A. Zinnia* were reported by Beaunmont *et al,* (1958). Apart from *Alternaria, Botryodiplodia* was reported by various workers as causal agents in post harvest rots of

yam (Sulciman and Adeyemi, 2002). Other pathogens being consistently implicated as causal agents in post harvest rots include *Penicillium sclerotigenum, P. oxycalium, Aspergillus niger, Rhizopus* sp. And *Fusarium oxysporum* (Ikotun, 1983).

The results on physiological studies obtained in the present study showed that growth was supported by all the carbon sources tested. Fructose supported the highest mycelia dry weight as against the findings of Pearson and Hall (1975). The utilization levels observed could be attributed to the fact that the host (yam) contains mainly starch. In the present study, glucose and starch were utilized with average mycelia dry weight of 0.81g/30ml media and 0.37g/30ml media respectively at the end of the fifteen days of incubation. On nitrogen utilization, it has been reported that the level as well as the nature of the nitrogen, as essential element used for both physiological as well as for structural purposes supplied are of vital importance in determining fungal development. Likewise different fungi show differential ability in utilizing different nitrogen sources (Suleiman, 2005). The results of the present study from four nitrogen sources namely, urea, C_2NO_2 and $NaNO_2$ showed that *Alternaria solani* utilized sodium nitrate better than other nitrogen sources with the highest mean mycelia dry weight of 0.58g, without any significant difference ($P\ 0.01 < 0.05$) compared with control; while calcium nitrate and urea had 0.37g and 0.12g respectively in that order.

Conclusion

The result from the study areas (Wadata, Northbank and Wurukum) shows that *Alternaria solani* was associated with yam rot in Makurdi metropolis. The results also showed that *Dioscorea alata* was more susceptible to the disease having the highest percentage incidence, followed by *D. bulbifera,* while *D. rotundata* was the least susceptible with the lowest percentage incidence.

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