Toxin Production By Fungi Isolated From Rotten Pawpaw Fruits In Parts Of Imo And Abia States Of Nigeria

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Abstract: Culture filtrates of *B. theobromae, A. niger, A. flavus* and *R. oryzae* isolated in parts of Imo and Abia states of Nigeria contained toxic metabolites which elicited electrolyte leakages and discoloration on young leaf discs of pawpaw. Procedures for extraction of the metabolites based on solvent extraction are described. The sensitivity of the pawpaw leaf discs to the toxic metabolite preparations corresponded to the conductances and discolorations inferring their susceptibility to the pathogens in the bioassays conducted.
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Key words: Toxin, Fungi, PawPaw, Imo, Abia, Nigeria

1. INTRODUCTION

A great deal of information on toxins has been obtained from investigations of relatively serious from investigations of relatively serious plant pathogen relationships. Fungi noted for their saprophytic activity and weak parasites has received little attention. However some toxic metabolites cause disease symptoms on host plants and host plant parts in bioassay. *Aspergillus niger* have been known to produce Aflatoxins (Wogan, 1973) which induced electrolyte leakage in living plant tissues and altered cell permeability, (Kohmoto et. al; 1976). The principle of conductivity has been used in toxin detection due to the increase in electrolyte presence caused by the leakage of ions from host plant tissues attributed to the metabolite (Vidhyase Karan et. al; 1986).

In a survey of the prevalence of post-harvest fruit rots of *Asmila tribola* (pawpaw) in Imo and Abia States of Nigeria, *Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae* and *Rhizopus oryzae* were constantly isolated from affected fruits and individually produced fruit rots in inoculation exercise (Ezeibekwe, 1993).

The production of toxins by these isolates was studied using the principle of electrolyte conductivity in bioassay.

2. MATERIALS AND METHOD

Culture Medium and Extraction of Toxic Metabolite

Pawpaw 200g was washed with clean water, peeled and sliced for preparation of pawpaw broth. The slices were boiled until they become soft and then strained through cheese cloth. Dextrose 20g was added and the extract made up to one litre with distilled water. The medium (30ml) was dispensed in Erlen meyer flasks, and sterilized in the autoclave at 121^{o}C for 15 minutes.

The medium was inoculated with mycelial discs 8mm with each of *B. theobromae, R. oryzae, A. niger* and *A. flavus* isolated from infected pawpaw fruits. Potato dextrose agar discs 8mm were put into the control flasks. Each set up was in three replicates. After two weeks of incubation at 25^{o}C, the cultures were filtered with No. 1 filter paper and the filtrate stored in MacCarthney bottles at 10^{o}C.

The culture filtrate was extracted with two volumes of acetone. The acetone supernatant was decanted and the precipate left in the test tube. Any acetone left on the precipitate was removed by evaporation at 30^{o}C. The supernatant was evaporated in a water bath at 30^{o}C and the precipitate redissolved in methanol (10mls).

The supernatant and the precipitate were each diluted with 5ml of deionized water and tested fro toxin activity. The controls were similarly treated.

Test For Toxin Activity (Bioassay)

Young fully expanded leaf, 21 days old was used. The leaf was cut into small discs 1 cm by 5mm in size and rinsed in several changes of distilled water. The extract 1ml from each isolate was diluted with 50ml of deionized water and put in sterile test tubes.

A leaf disc was put in each test tube and incubated for 30 minutes at 25^{o}C on a shaker set at 100 strokes per minute. Five hours after, conductance of the ambient solution was determined with a conductivity meter.

The control and water blank were subjected to the same screening. The conductance in umhos of the blank (water) was subtracted from those of the control and the toxin infiltrated leaves to determine the increase in the electrolyte leakage induced by toxin. All tests were conducted in three replications.

Extraction Of Toxin From Infected Pawpaws F[illegible]

Healthy pawpaw fruits were inoculated with isolates of *A. niger* and *A. flavus*. The mature [illegible] fruits were surface sterilized by thoroughly scrubbing their surfaces with cotton wool soaked with [illegible] hypochlorite [illegible]ed w[illegible] ch[illegible]s of d[illegible] water and allowed to [illegible]. [illegible] healthy [illegible] were used for [illegible] pathogenicity re[illegible]ions.

Cylindrical di[illegible] p[illegible] removed [illegible] the fruits with 10mm [illegible] cork borer. T[illegible] discs of five [illegible] old [illegible] grown fungal c[illegible] were introduced into [illegible] in [illegible] fruits. [illegible] paraffin wax [illegible] applied [illegible] the points of inoculation [illegible] exclude extraneous pathogens.

Disc[illegible]non [illegible] PDA [illegible] used in the control experiment. The inoculated fruits were each enclosed in [illegible] micro[illegible]idity environment of a polythene [illegible] containing a soaked aseptic cotton wool for several days [illegible] Discs 10mm [illegible] were removed [illegible] sterile cork borers from the following zones of each inoculated and control fruits:-

Zone 1:- Completely rotted area, close to point of inoculation.

Zone 2: - Apparently healthy area, [illegible] sp[illegible]a[illegible].

Each disc was [illegible] with sterile motar washed into a test tube and made up to 10ml with deionized water. Each solution was extracted with two volumes of acetone as previously [illegible]. Bioas[illegible] were conducted on the extracts.

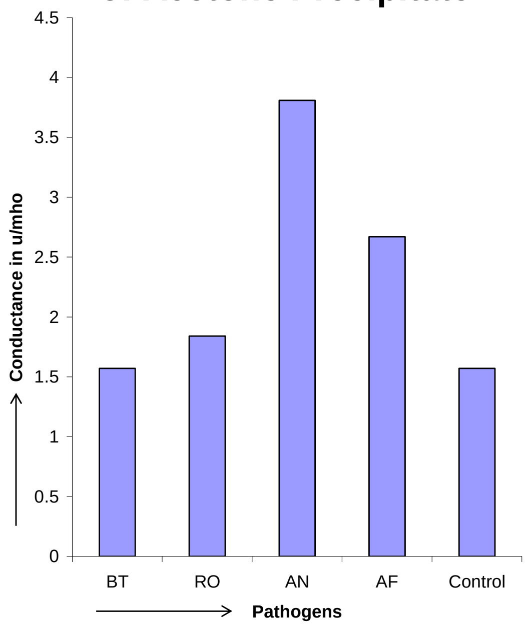
3. RESULTS

[illegible] Extracts of the culture filtrates obtained from the i[illegible]s caused electrolyte leakages in leaf disc bioas[illegible]. Conductivity determinations recorded from the e[illegible]s of *A. [illegible]* and *A. flavus* culture filtrates w[illegible]ficantly ([illegible]0.05) greater than those of *B. t[illegible]e* and *R. [illegible]e*. Figs. 1 and 2.

[illegible]e condu[illegible]y of the acetone precipitate r[illegible]om bioa[illegible] with the extracts of *A. niger* a[illegible]*vus* diffe[illegible]ignificantly (p.0.05) from the c[illegible].

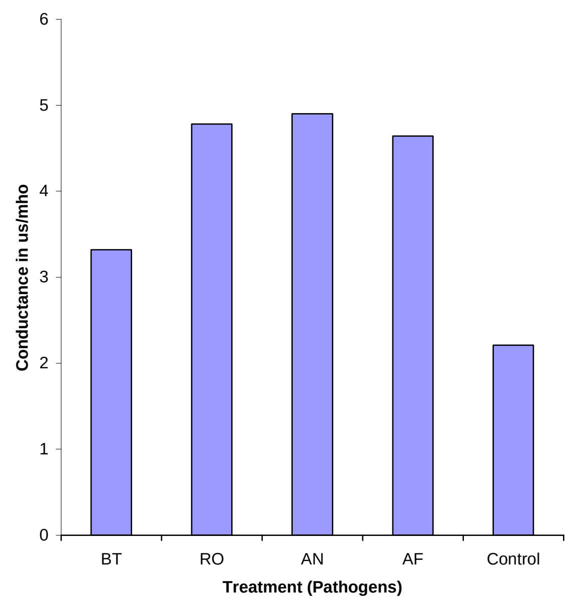
[illegible]uctivity [illegible]ings of the extracts from *B. t[illegible]e* and *R. [illegible]e* acetone precipitates did not d[illegible]ficantly f[illegible]he controls (p = 0.05). Fig. 1. Differences between conductivities of the supernatants and their controls were highly significant (p = 0.05). Figs. 1 and 2.

Extraction of toxins from infected pawpaw fruits inoculated with *A. niger* and *A. flavus* and their conductivities in leaf disc bioassays are presented in tables 1 and 2. The results in tables 1 show that the acetone supernatant was more toxic resulting in greater conductivity leakages (p = 0.05, F = 2.4) than the acetone precipitate (F = 0.25) for *A. niger*. The same trend was also observed in *A. flavus* with the acetone supernatant proving more toxic (p = 0.05, F = 1.02) than the acetone precipitate (F = 0.10) table 2.



BT. *Botryodiplodia theobromae*
RO. *Rhizopus oryzae*
AN. *Aspergillus niger*
AF. *Aspergillus flavus*

Fig. 1: Bioassay with Pawpaw Leaf Discs Conductivity of Acetone Precipitate



BT. *Botryodiplodia theobromae*
RO. *Rhizopus oryzae*
AN. *Aspergillus niger*
AF. *Aspergillus flavus*

Fig. 2: Bioassay with Pawpaw Leaf Discs Conductivity of the Acetone supernatant.

Table 1: Effect of Metabolite Extracted from Pawpaw Fruit Inoculated with *Asperillus niger* on Pawpaw Leaf Discs

Electrolyte leakage test by conductance
Anova table

Source of Variation	df	ss	ms	f	f	
Acetone Supernatant	2	0.84	0.42	2.4	0.05*	0.01**
Acetone Precipitate	2	0.56	0.28	0.23	0.05*	0.01**
Errow	4	0.70	0.175			
Total	**8**	**2.10**				

* non significant ($P < 0.05$, $V_1 = 2$, $V_2 = 4$ df)
** Highly insignificant ($P > 0.01$, $V_1 = 2$, $V_2 = 4$ df)

Table 2: Effects of Metabolite Extracted from Pawpaw Fruit Inoculated with *A. flavus* on Pawpaw Leaf Discs
Electrolyte leakage test by conductance

Amova table

Source of Variation	df	ss	ms	f	f	
Acetone Supernatant	2	0.90	0.45	1.2	0.05*	0.01**
Acetone Precipitate	2	0.11	0.55	0.10	0.05*	0.01**
Errow	4	2.20	0.55			
Total	**8**	**3.21**				

* non significant ($P < 0.05$, $V_1 = 2$, $V_2 = 4$ df)
** Highly insignificant ($P > 0.01$, $V_1 = 2$, $V_2 = 4$ df)

4. DISCUSSION

The results of the investigation on bioassays involving the four pathogens show a significant variation in their toxic effects as revealed by the electrolyte leakages, figures 1 and 2. *A. niger* and *A. flavus* generally appeared to produce more toxic effect than B. *theobromae* and *R. oryzae*. Fig. 1 and 2. The result of the bioassay work using extracts from the various zones on and around the inoculation points show observable differences in toxicity effect between replicates in both the experimental and the controls. Vidhyase Karen et. al; (1986) stated that the maximum dilutions at which visible symptoms were noticed was considered as the dilution and point of activity of the toxin and the minimum concentration required, to induce brown spot symptoms on rice by toxin extracted from *Helminthosporium oryzae* was 1.04 ug/ml when purified with chloroform and 0.63 ug/ml when purified with charcoal. In the same work they held that in electrolyte leakage bioassays, the effectiveness of each step in removing contaminating materials from the toxins whose action was seen to have increased with each step of purification the conductance of a solution is the sum of the contributions of all the ionic species present, and hence not only depend on the species for which one is analyzing. This explains part of the factors that likely affected the purity and the effect of the target metabolite phytotoxins.

The toxic metabolite extracts showed an ability of eliciting electrolyte leakages in leaf discs biossay at varying depress. The invitro extracts from the pathogens revealed significant differences in toxicity. There were no significant differences in toxicity between the zonal extractions in the invivo tests, P = 0.05.

5. CONCLUSION

The production of toxic metabolites by the fungal associates of pawpaw rots appeared to be important in the mechanism of disease spread. Although the toxic extracts need to be subjected to further purification, Aflatoxin is known to be produced by *A. flavus*.

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