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Full Length Research

Phytotoxic effect of *Carica papaya* extract on seed-borne fungus of African Yam Bean (*Sphenostylis stenocarpa Hochst ex. A. Rich. Harms)* seeds

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ABSTRACT: Extracts of many higher plants have been reported to exhibit antifungal properties under laboratory experiments. This study was therefore carried out to investigate the effect of *Carica papaya* plant extracts on the African Yam Bean seed-borne pathogen. The seed health test of African Yam Bean was carried out using the blotter paper method. The experiment was conducted at the Plant Pathology Laboratory of the Department of Crop Science and Horticulture, Nnamdi Azikiwe University, Awka. Test of plant extract for inhibition of radial growth of seed-borne pathogen of African yam bean was studied under *in vitro* experiment at 0%, 50%, 75% and 100% concentrations with 0% as the control. The design was a Completely Randomized Design (CRD) with three replications. The test plant extracts of different concentrations were added into Petri dishes containing molten-sterilized Sabouraud Dextrose Agar (SDA) and swayed gently on the laboratory bench to allow an even mixture These were allowed to gel. Then nine millimeter discs of a seven-day pure culture of *Aspergillus flavus* were aseptically placed on the centre of the Petri dishes containing the SDA-extract mixture. Records on the radial inhibition effect of the test plant extract were kept for further analysis. Data on radial inhibition of fungus were subjected to Analysis of Variance (ANOVA). Results showed that *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus terreus* were isolated from incubated African Yam Bean seeds. *Carica papaya* leaf extract used was very effective and the higher the concentrations of extract, the more effective in the inhibition of radial growth of the test fungus. It could therefore be recommended that farmers should always conduct viability tests on procured seeds before planting. Farmers should rather use plant extract such as the one used in this experiment in controlling seed-borne fungal pathogens of African Yam Bean seeds than synthetic fungicides.

Keywords: African Yam Bean, *Carica papaya,* fungal organism, *in-vitro,* radial growth inhibition, seed health.

INTRODUCTION

African Yam Bean (AYB), *Sphenostylis stenocarpa*, belongs to the *leguminaceae* family. It originated in Ethiopia (Busson, 2001), but both wild and cultivated types now occur in tropical Africa as far north as Egypt and also throughout West Africa from Guinea to Southern Africa. It is cultivated in Nigeria mainly for seed and also grown for tubers in Cote d'Ivoire, Ghana, Togo, Cameroon, Gabon and the Democratic Republic of Congo (Utter, 2007). The African Yam Bean (*S. stenocarpa*) is a climbing legume that grows to a height of over 3 m and is adapted to low land tropical conditions. It is one of the lesser-known legumes (Ikhajiagbe *et al.,* 2007a, b; Apata and Ologhobo,1990). Several authors over the years have reported the proximate composition of African Yam Bean with varying values over the years, with carbohydrate (49.88–63.51%) and protein (19.53–29.53%) being the major nutrient components of African Yam Bean (*S. stenocarpa*), while other components such as ash (1.86–5.35%), fat (1.39–7.53%) and fibre (2.47–9.57%) are present in relatively small amounts (Adeyeye *et al*., 1994).

African yam bean (*S. stenocarpa*) is a hard-to-cook under exploited leguminous plant grown extensively in Western Africa (Enujiugha *et al.*, 2012; Uchegbu, 2015). For efficient growth of this crop, there is a need for a seed viability test. Seed health is a measure of the freedom of seeds from pathogens. The presence or absence of seed-borne pathogens can be confirmed through the use of seed health testing (Agarwal and Sinclair, 1997). The term "seed health test", means detecting the presence or absence of insect infestation and seed-borne diseases caused by fungi, bacteria and viruses. The test used depends on the type of organism being sought after and the purpose of the test, i.e. as phytosanitary measures when seeds are exported (ISTA, 2015). Seed health test also involves the visual examination of seeds externally or internally, macro or microscopically for the presence of pathogens as well as incubating seeds on agar or moist blotter papers and identifying the pathogens microscopically (Warham *et al*., 1990). In seed health tests for seed-borne fungi pathogens, the blotter test is no doubt one of the most important methods available (limonard, 1966). Blotter tests are similar to germination tests in such a way that seeds are placed on moistened layers of blotter and incubated under conditions that promote fungal growth. The demand for the biological control of pathogens using plant extracts containing secondary metabolites has been a common practice for thousands of years (Chávez-Quintal *et al*., 2011; Hewajulige and Dhekney, 2016). Papaya (*Carica papaya L.*) belongs to the family *Caricaceae* and is the most economically important species of the genus *Carica*. (Hewajulige and Dhekney, 2016). Papaya is native to tropical America, and seeds of papaya were taken from the Caribbean to Malacca or the Philippines, then to India. Subsequently, papaya was introduced as a plantation crop in Australia, Hawaii, Sri Lanka, and other tropical and subtropical countries in the world (Hewajulige and Dhekney, 2016). The papaya plant, including fruit, leaf, seed, bark, latex, and their active components plays a major role in the management of disease progression. *Carica papaya* leaf contains active components such as alkaloids, glycosides, tannins, saponins, and flavonoids, which are responsible for its medicinal activity. Therefore, the objective of this study is to investigate the effect of *Carica papaya* plant extracts on the African Yam Bean seed-borne pathogen.

MATERIALS AND METHODS

Germination test and seed health test

The experiment was conducted at the Plant Pathology Laboratory of the Department of Crop Science and Horticulture, Nnamdi Azikiwe University, Awka. A germination test was carried out to investigate the rates at which the test samples germinate while a seed health test was carried out to determine the seed-borne pathogen in the test sample. The percentage germination of African Yam Bean seed was determined by this formula:

$$\text{Percen. Germination} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} x\ 100$$

The percentage infection of African yam bean seed was determined by this formula:

$$\text{Percentage Infection} = \frac{\text{Number of infected seeds}}{\text{Total number of seeds}} x\ 100$$

Seed health test

Seed health test for seed-borne fungi was carried out following the rules of the International Seed Testing Association (ISTA, 2001). The standard blotter method was used in this experiment. In the blotter paper method, 12 seeds each were randomly taken from a total of 204 seeds. These seeds were sterilized with a mixture of 10 ml of ethanol and 90 ml of water (10% ethanol) for 3 minutes and rinsed twice with sterile distilled water to effectively remove surface contamination without affecting the percentage germination of the African Yam Bean seeds (Pernezny *et al.*, 2002).

Twelve seeds of the African Yam Beans were plated in each 9 cm Petri dish containing three layers of Whatman filter paper wetted with distilled sterile water. Seeds were arranged according to the International Seed Testing Association (ISTA, 2001). Seventeen Petri dishes were used for the sample. The twelve seeds of the African Yam Bean were arranged in a circular form in the Petri dishes, seven seeds in the outermost circle, four seeds in the middle circle and 1 seed in the innermost circle (Plates 1 and 2). More water was added to rewet the paper after the initial wetting because low moisture was noticed. The Petri dishes were sealed up using masking tape and labelled properly. The plates were placed on the laboratory working bench which was first sterilized with methylated spirit to ensure an aseptic condition. Germination and infection counts were observed for seven days from the second day after plating. Germination and infection counts were recorded every day until the seventh day. Fungal growth was identified on the plates using the mycelia colour and hyphal growth.

Culturing of fungi from infected seeds

Seeds infected with fungi were isolated from the plated seeds and transferred into Sabouraud Dextrose Agar (SDA) medium (Plate 3). The organisms were incubated in triplicate plates at 28°C ±2 for seven days. Sub-culturing of isolated fungi was done using SDA media to obtain pure cultures, which were kept properly for further investigation.

Plate 1. Plated seeds of AYB on day 1.

Plate 2. Germinated seeds.

Plate 3. Infected seeds of African yam bean.

Microscopic identification of isolated fungi.

Temporary slides of isolated fungi were made by placing a small portion of the mycelia of each fungus taken from the actively growing parts on the sterile glass slides having some drops of lactophenol in cotton blue and the slides were covered with a slip. The prepared slides were viewed under a compound microscope model (Olympus-XN50). Identification of the fungi was based on the culture growth patterns, colour of mycelia and microscopic examination of the vegetative and reproductive structures. Micrographs of the organisms were also taken and recorded.

***In vitro* effect of different concentrations of *Carica papaya* plant leaf extract**

Different concentrates of the agar extract mixture were dispensed into 9 cm Petri dishes and allowed to gel, then inoculated centrally with 0.9 cm diameter mycelial discs obtained from seven days pure culture of *Aspergillus flavus* with a sterilized cork-borer and placed at the centre of each Petri dish. Two perpendicular lines passing through the centre of the Petri dish were marked thinly at the base of the Petri dish to serve as a reference point for measuring growth. All plates were placed on a laboratory bench and at a room temp of 28 ± 2°C. Radial growths along each line were measured at exactly 24 hours intervals with a meter rule to determine the radial growth inhibition. The radial growth inhibitions in each plate were measured for 7 days. Each treatment was replicated three times. Percentage radial inhibition was determined according to Sundar *et al* (1995) thus:

$\text{Percentage radial inhibition} = \frac{dc-dt}{dc} \, x \, 100$ Amadioha (2003).

Where dc = control, dt = treatment.

Experimental design/Data analysis

The experiment was laid out in a Completely Randomized Design (CRD) and replicated three times. Data collected were subjected to Analysis of Variance (ANOVA) and means were separated using Least Significance Difference (LSD) at a 5% probability level. GENSTAt 10.3 released version was used for all the statistical analysis

RESULTS

Germination and infection percentage of plated African yam bean (*Sphenostylis stenocarpa*)

The germination and infection percentages of *Sphenostylis stenocarpa* were calculated for seven days.

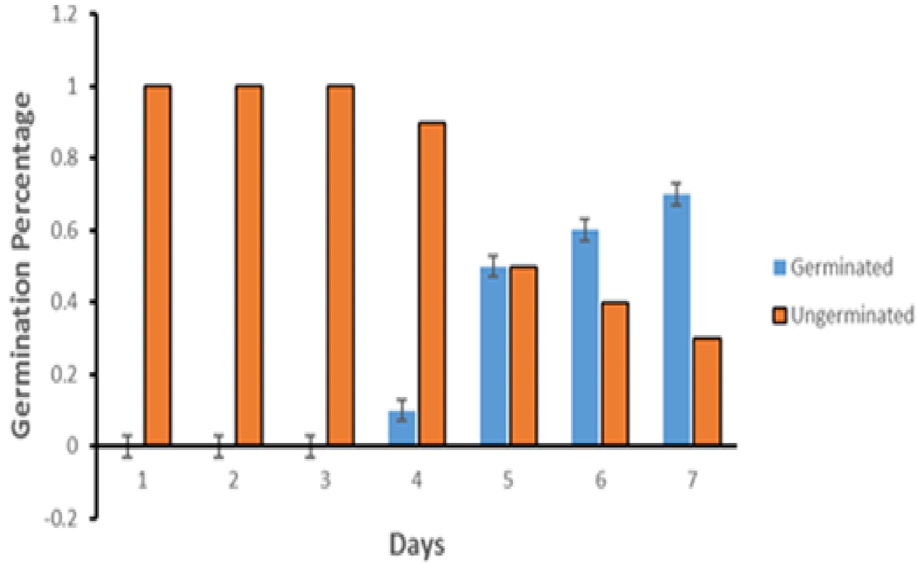

Figure 1. Percentage of African Yam bean for seven (7) days.

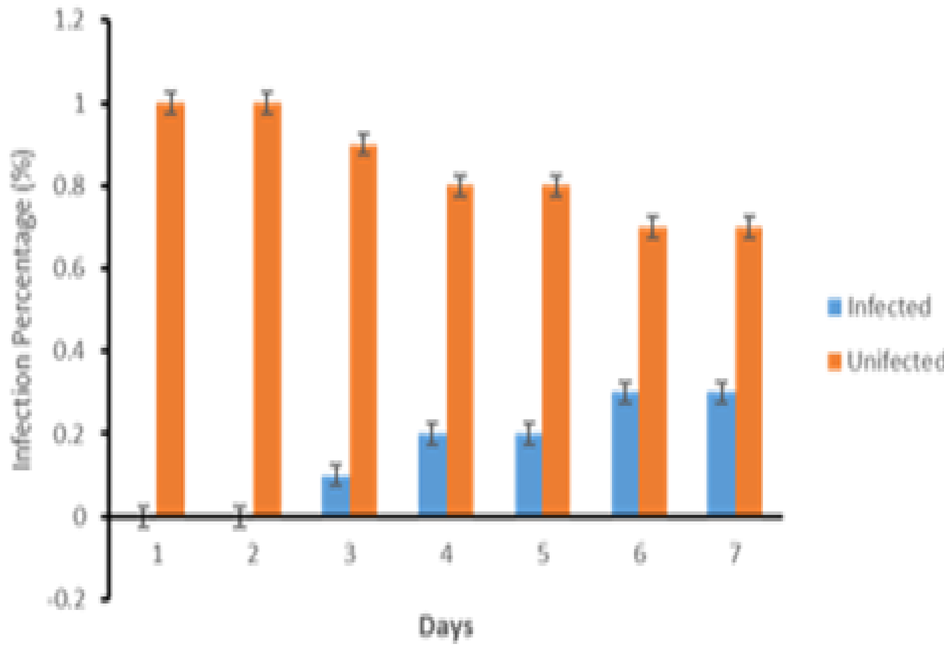

Figure 2. Percentage of African Yam Bean for seven (7) days.

Seventeen Petri dishes were used, with each containing twelve seeds. The highest germination percentage 92% was recorded in replicate 4 on the 6th and 7th day, followed by 83% in replicate 7 on the 6th and 7th day. The highest infection percentage of 50% was recorded in replicate 7 on the 6th and 7th day. From day 1 to 3, no germination was seen; therefore, there was 0% germination in the 17 Petri dishes throughout that period (Figures 1 and 2).

Fungi isolation and identification

The result of the isolation and identification of seed-borne fungi showed that *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus terreus* were implicated (Plates 4 to 9).

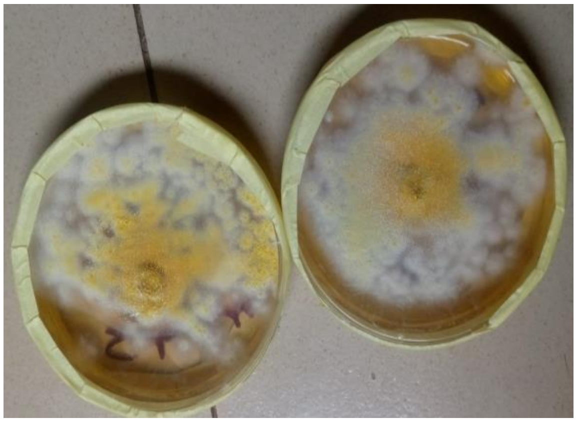

Plate 4. Pure culture of *Aspergillus niger*

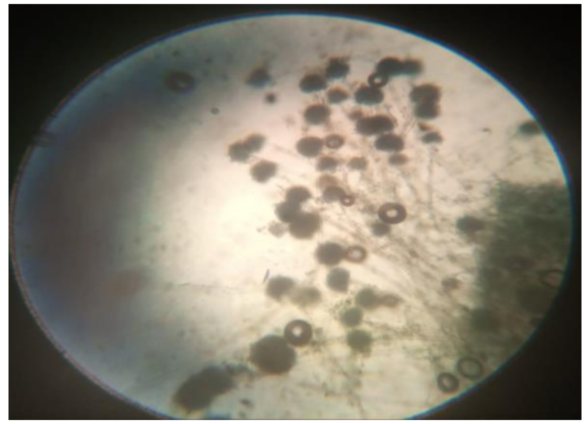

Plate 5. Micrograph of *Aspergillus niger.*

Plate 6. Pure culture of *Aspergillus flavus*

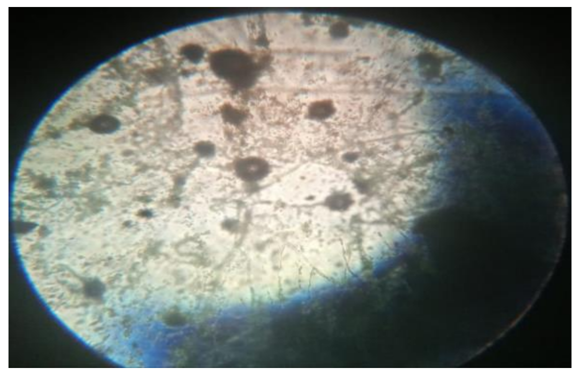

Plate 7. Micrograph of *Aspergillus flavus.*

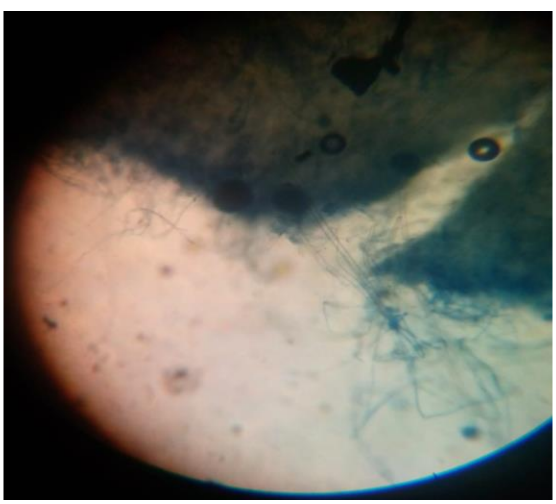

Plate 8. Pure culture of *Aspergillus terreus.*

Plate 9. Micrograph of *Aspergillus terreus.*

Effect of plant extract concentration on the percentage inhibition on the radial growth of *Aspergillus flavus*

The result of the phytotoxic effect of *Carica papaya* leaf extract showed that there was a significant inhibitory effect of the extract on the radial growth of *Aspergillus flavus* under culture. There was a significant difference among the effects of the various concentrations in inhibiting the growth of the test organism under culture. On day 2, *in vitro* treatment at 100% concentration produced the highest

Table 1 Effect of Varying concentrations of *Carica papaya* plant extracts on the percentage inhibition of growth of *Aspergillus flavus* from day 1 to 7 by Soxhlet extractions 7

Con (%)	Concentration and percentage inhibition of *Aspergillus flavus* in culture						
	Day1	Day2	Day3	Day4	Day5	Day6	Day7
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50.00	0.00	62.3	57.67	78.33	78.67	67.70	34.70
75.00	0.00	75.0	73.67	85.00	84.00	82.0	72.00
100.00	0.00	86.7	83.67	90.67	88.00	88.00	88.00
Grand Mean	0.00	56.0	53.75	63.50	62,67	59.40	48.80
$LSD_{0.05}$	0.00	8.78	6.32	3.72	3.95	10.11	18.14

(86.7%) inhibition rate followed by 75.0% concentration which produced a 75% inhibition rate while the least inhibition (62.3%) rate was observed in 50% concentration *in vitro* treatment level. All three treatments performed better than the control. This trend was observed from day 3 to 7 (Table 1).

DISCUSSION

The result of the germination test showed that the germination of African Yam Bean is not instant; it takes 3 or 4 days before germination starts. Seed treatment is the safest and cheapest way to control seed-borne fungal diseases and to prevent bio-deterioration of grains (Chandler 2005; Bagga and Sharma 2006). The efficiency of four seed health testing techniques namely the blotter, deep-freezing blotter, ragdoll and agar plate methods in detecting seed-borne fungi of African Yam Bean, *Sphenostylis stenocarpa* seeds was evaluated and the blotter method was found to be the most suitable testing technique for detecting *Aspergilus flavus, A. niger, A. terreus.* Ora *et al.* (2011) reported that the seed health test in seed companies as well as those working in research and quarantine departments are carried out using the blotter paper method. About ninety per cent of the food crops grown worldwide and the plant germplasm being distributed between and within countries are propagated by true seeds (Neergaard, 1979). He also stated that many plant pathogens (bacteria, fungi, nematodes and viruses) affecting the food crop and plant germplasm are seed-borne and seed-transmitted buttressing the economic importance of seed health tests. The result also agrees with Kumar *et al*. (2020) who reported that the conventional methods are widely used for the detection of seed-borne fungal phytopathogen presently, although they are relatively difficult to operate, require expert technicians and are time-consuming.

Isolation of seed-borne fungi

The result of isolation and identification of seed-borne fungi from *Sphenostylis sternocarpa* showed that *Aspergillus* species were implicated which included: *Aspergillus niger, Aspergillus flavus* and *Aspergillus teureus.* This is similar to the results of Iwuagwu *et al.* (2019) and Iwuagwu *et al.* (2022a), who isolated *Aspergillus niger* from seeds of *Vigna subterranean* and *Citrullus lanatus* respectively.

Phytotoxic effect of *Carica papaya* plant leaf extract on radial growth of *Aspergillus flavus*

The result of the effects of C*arica papaya* leaf extract on inhibition of radial growth of *Aspergillus flavus* isolated from *Sphenostylis sternocarpa* showed that 50% had the lowest fungal radial growth inhibition compared to C*arica papaya* leaf extract concentration of 75% and 100%. This corroborates with Olubode *et al.* (2018), who reported an exhibition of antifungal properties of *Carica papaya* leaf extract against *Aspergillus niger* and *Aspergillus flavus* under laboratory trials. This is also in line with the works of Aliero and Afolayan (2006), who also reported the antimicrobial activity of *Solanum tomentosum.* Parekh *et al.* (2006) and Iwuagwu *et al.* (2022b) have also reported the efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity against seed-borne pathogenic fungi. It also corroborates with the findings of Moumni *et al*. (2023), who asserted that research on seed treatment has increased considerably in the past decade, along with the search for green technologies to control seed-borne diseases.

Conclusion

From the experiment it was observed that all organisms isolated were *Aspergillus* species, therefore, it could be said that pathogenic fungi were the major cause of seed deterioration of African Yam Bean. It could also be inferred that seeds deterioration caused by *Aspergillus spp*, leads to poor visibility and loss of seedling vigour. The *Carica papaya* extract used was very effective in inhibiting the

Aspergillus flavus under culture. It was also observed that the higher the concentrations of the extract, the more the effectiveness in inhibition of radial growth of the fungus.

Recommendations

Having seen that the African Yam Bean loses viability when infected, it could therefore be recommended that farmers should always source their seeds from certified seed outlets. Also, farmers should always conduct viability tests before sowing to ensure adequate plant establishment in the field. Farmers should rather use plant extracts such as was tested in this research to control fungal diseases than using synthetic protective chemicals which are detrimental to human health and the environment.

CONFLICT OF INTEREST

The authors declared that no competing interest exists.

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