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Containing Mycotic Putridity of Pumpkin Fruits Using Derivatives of *Cucumis sativus, Annona squamosa* and *Carica papaya* Seeds

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Abstract

Healthy and rotten pumpkin fruits were randomly purchased from two different markets in Ado-Ekiti and Iworoko-Ekiti, Ekiti state. Four fungal pathogens namely: Aspergillus flavus, Phytophtora capsici, Rhizopus stolonifer and Aspergillus niger were isolated and identified from pumpkin fruits on the basis of their morphological and physiological features. The occurrence frequency of the isolated fungi from the pumpkin fruits revealed that Aspergillus niger (53.3%) was the most isolated pathogen. Crude plants derivatives were obtained from the test plants seeds using the standard extraction process; these derivatives were tested against fungal isolates. Antifungal activities of ethanolic derivatives of Cucumis sativus at 1.0, 0.8, 0.6, 0.4mg/mL restrained Aspergillus niger by 14.00, 12.00, 10.00 and 9.00 mm respectively while 0.2mg/mL curtailed Rhizopus stolonifer by 7.00 mm. The antifungal effects of ethanolic derivatives of Annona squamosa inhibited Phytophthora capsici by 24.00, 22.00, 20.00,14.00 and 18.00 mm at 1.0, 0.8, 0.6, 0.4 and 0.2 mg/mL respectively. The antifungal effects of ethanolic derivatives of Carica papaya at concentrations 1.0, 0.8, 0.6, 0.4 and 0.2 mg/mL inhibited Phytophthora capsici by 22.00, 20.00,18.00, 16.00 and 12.00 mm respectively. Cucumis sativum majorly inhibited the growth of Aspergillus niger while both Annona squamosus and Carica papaya majorly inhibited the growth of Phytophthora capsici. The results of nutrient analyses of the healthy and infected pumpkin fruits revealed that infections reduced the nutrient compositions of infected pumpkin fruits when compared with the fresh and healthy pumpkin fruits. Furthermore, the results of proximate analyses of healthy pumpkin fruit showed that they contained high moisture content of 92.24%, 5.31% of carbohydrate, 0.98% of protein, and 0.15% of fat, which revealed that tested pumpkin fruits had low fat content.

Keywords: *putridity, fungal rot, pumpkin fruit, plant derivatives, inhibition.*

INTRODUCTION

Pumpkin is in the family Cucurbitaceae, indigenous to the western hemisphere. The economically significant species being cultivated globally for food include *Cucurbita pepo* L., *Cucurbita maxima* D. and *Cucurbita moschata* D [1]. There are variations in colour, shape and weight of pumpkins [2]. In 2024, global production of pumpkin stood at 29 million tons, harvested from 2.1 million hectares. China was the largest producer of pumpkins, 7.4million tons, contributing ~25-30% of the global total, followed by India, 5.5 million tons, China: ~7.40-7.99 million tons, India: ~5.14 million tons (approx. 20% of global), Russia, USA, Turkey, Spain, Mexico, Bangladesh and Italy, accounted for 19% of global production, Ukraine and Russia: ~4% each [3,4]. According to Kassam *et al*., [5]. Cucurbita is one of the most vital vegetable crops [6]. Cultivation of pumpkins in various parts of the world is majorly for their seeds and pulp for food as purees jellies, jams, and syrups [7].

Due to pumpkin's deep yellow-orange sweetness and pleasant flavor, its flour can be used to fortify wheat and to prepare porridge [8]. Health friendly polysaccharides such as carotene and pectin, vitamins, proteins, mineral salts, terpenoids and phenolic compounds can be found in pumpkin pulp [9]. Fruits and vegetables also have therapeutic and medicinal properties [10]. *Cucurbita cultigens* cultivation as food source globally can be ascribed to its adaptability to broad climatic conditions thereby enhancing market growth and diversity [11]. Chaachouay and Zidane [12] reported that plant derivatives or their funtional principles are traditionally useful as therapies in folk medicine as global drugs consist of natural product origin at 80%. Medicinal plants possess broad immunomodulatory property as they stimulate specifically and non-specifically [13]. As a result of synthesized compounds in the secondary metabolism of the plants, various plants have been applied as antimicrobial agent [14].

Pumpkin is prone to pre-harvest attack by a number of pathogens, such as nematodes, bacteria, oomycetes, viruses and fungi. Pumpkin fruits can also be vulnerable to post-harvest infestations. Therefore, the objectives of this research are to: identify bio degradation fungal pathogens of pumpkin fruits and mitigate the effects of mycotic rot pathogens on pumpkin fruits using seeds derivatives.

MATERIALS AND METHODS

Fluted pumpkin fruits were randomly purchased from two markets in Ado and Iworoko-Ekiti. Samples included both healthy and spoilt pumpkin fruits. These samples were collected, placed in labeled cap lock bags, and immediately conveyed to the laboratory in the Department of Microbiology, Ekiti State University for processing.

Samples processing

Pumpkin fruits with disease symptoms were surface sterilized, cut into 2 mm^2 pieces and plated out on PDA already sterilized and mixed with 250 mg chloramphenicol as bactericidal medium. At room temperature, the inoculated plates were allowed to incubate for 4-5 days, this was observed for emergence of fungi, emerged fungi were thereafter sub-cultured into fresh medium. Identification of pure isolates on the condition of both micro and macro morphological features were carried out. Fungal structural features such as reproductive structures, mycelial colouration or pigmentation, absence or presence of septate, colony or hyphae and spore features were assayed according to Garcia-Rubio *et al* [15]. Developed colonies were counted and repeatedly sub-cultured on PDA plates in order to obtain pure isolates; these were thereafter stored on PDA slants in McCartney bottles for identification, characterization and further studies.

Preparation of plant derivatives

Plant derivatives were prepared from *Cucumis sativus, Annona squamosa* and *Carica Papaya* seeds. The process of extraction followed the protocol described by Zhang *et al* [16]. The seeds of test plants were washed under tap water, rinsed thrice in sterile distilled water and mopped with sterilized blotting paper. These were thereafter allowed to dry at 40°C for three weeks. The seeds were pulverized to allow easy release of the active cell contents. Fifty (50 gm) of each test plant seed powder was placed in separate sterile conical flasks and 200 mL of solvent (70% ethanol) was added to each of the test plant powder to ensure that the powder was fully submerged in the solvent, this was vigorously homogenized and the content was allowed to settle as stood on the bench at room temperature for two days though shaken at interval. The extract was poured gradually into a folded Whitman's (No.1) filter paper on sterile funnel mounted on a 500mL conical flask and the content was allowed to trickle into the flask. Sterile universal bottles were used to collect the filtrate. Concentration of the extracts was done by placing the crude extracts in the universal bottles in a rotary evaporator for 60 minutes at 50°C in order to evaporate the solvent.

Powder like matter at the bottom of the universal bottles was obtained by drying the concentrated crude derivatives in an oven at 40°C for two days. The powder was stored in the refrigerator at 4°C in marked universal bottles.

Preparation of fungal inoculum

A 5-day old fungal culture on potato dextrose agar served as a source of the fungal inoculum. Eight to ten mL of distilled water was used to flood the Petri dishes and sterile spatulas were used to scrape the conidia. In order to obtain approximately 10^5 spores/mL, adjustment of each fungus spore density was done by using spectrophotometer (A595 nm) according to Faway *et al* [17].

Assessment of crude plant derivatives on fungal growth

Evaluation of antifungal effect of the plant derivatives on four characterized pathogenic isolates from pumpkin fruits namely: *Rhizopus stolonifer, Aspergillus niger, Aspergillus flavus* and *Phytophthora capsici* were determined using the method of Abbas, *et al* [18]. Varied concentrations of the test plant crude derivatives of seeds were done by separately weighing 0.2, 0.4, 0.6, 0.8 and 1.0mg of seed powder of each of *Cucumis sativus, Annona squamosa* and *Carica papaya*, dissolved in 200mL of ethanol and thereafter in sterile distilled water. Prepare PDA media was dipensed into each Petri dish and these were set aside to solidify inside biosafety chamber. Sterile glass spreader was uniformly used to spread the inoculums on the solidified media. Thereafter, 100 µL of each extract was adjusted to the same concentration (50 mg/mL) and already soaked perforated filter papers (disc) for two hours were placed on the agar plate. The agar plates were left for 1 hour under the incubator, and later at 37°C for one daytime. The response of the fungal pathogens was determined by measuring the zone of inhibition diameter that was significantly susceptible, taken as ≥ 7 mm in diameter.

Proximate analyses of fungal pathogens infected pumpkin fruits

The proximate composition was determined according to Irabor *et al* [19].

i. determination of moisture content

The weight of both the wet and dried sample was determined by placing two grams of the sample(s) in the oven maintained at 100-103°C for 16 hours. The drying was repeatedly done till a constant weight was obtained. The moisture content was expressed in terms of loss in weight of the wet sample. % moisture content =

Weight of wet sample - Weight of dry sample x 100
Weight of wet sample

ii. Determination of ash content

Two grams (2.0g) of each of the oven-dried powder samples were carefully weighed and
placed in a known weight crucible, sparked in a muffle furnace and for 8 hours turned to ash
at 550°C.

The crucible with the ash was then taken out, allowed to cool down in a desiccator and weighed
and the ash was content expressed in term of the oven-dried weight of the powdered sample.
% Ash content = weight of ash x 100
Weight of sample

iii. Determination of protein content
Through digestion the protein nitrogen in 1g of the dried samples was converted to ammonium sulphate using concentrated H_2SO_4, $CuSO_4$ and Na_2SO_4. These mixtures were subjected to heating and the evolved ammonia was steamly distilled into boric acid solution. The nitrogen from ammonia was extracted from the titration of the collected ammonia with 0.1M HCl using Tashirus indicator (double indicator) till a purplish pink colour was derived. Calculation of crude protein was done by multiplying the value of the derived nitrogen by the factor 6.25mg.

iv. Determination of crude fibre content
Each sample ofweight two grams (2.0g) was measured into separate beakers, extraction of the samples was done using petroleum ether by stirring, settling and decanting thrice, the samples were air dried, transferred into a dried 100mL conical flask and at room temperature, 200cm^3 of 0.127M sulphuric acid solution was added. Dispersing of the sample was done by using the first 40cm^3 of the acid, for 30 minutes, this mixture was gently heated to boiling point. Filterations of the contents were done to get rid of insoluble materials, this was then awashed with distilled water, thereafter with 1% HCI, followed by twice ethanol and conclusively with diethyl ether. The oven-dried residue was then sparked in a furnace at 550°C. The fibre contents were determined by the remaining weight after sparking and defined in term of the weight of the sample before sparking.

v. Determination Fat content
Petroleum ether was used to extract fat from 10g of the samples in Soxhlet apparatus. The obtained weight of the lipid after evaporating off the petroleum ether from the extract expressed the weight of the crude fat in the sample.

vi. Determination of carbohydrate content
The content of carbohydrate of the samples were obtained as the difference in value after subtracting the values of protein, lipid, ash and fibre from the total dry matter [20].

Isolation of rot fungi from pumpkin fruit samples
The infected pumpkins were immersed in sterile water, and serial dilution was done by weighing 1g of the sample in 10mL of distilled water. The samples were allowed to soak for 10 minutes before dispensing 1mL of the diluents into another 9mL of distilled water.

The dispensing of the 1mL persisted until it got to 10^5 diluents. A 5mL syringe was used to 0.1mL out of the last diluent and poured in a Tryptic Soy Agar (TSA) triplicate plate. The entire surface of the TSA plate was smeared with inoculums suspension using sterile glass rod. Incubation of the inoculated plates at 37°C was allowed for 24 hours.
Counting of colony forming units per millilitre (CFU/mL) was observed through emerging colonies on the plates.

Pathogenicity test
Techniques described by Ling *et al* [21] was adopted in determining the pathogenicity of the pumpkin fungal isolates. The pumpkin fruits were washed in running tap to get rid of dirt. The fruits were surface sterilized using 1 % NaoCl for three minutes, rinsed in thrice in sterile distilled water and mopped dry using a sterile blotting paper. Pumpkin fruits were punched using sterile inoculating needle that contained fungal spore. Each pathogenic fungal isolate was inoculated into the healthy fruit. After exactly 24 hours, disease expression was checked. Examination and recording against expression of the inoculation of each type of fungus was done. The morphology in terms of texture of the rotten portion of the fruits was noticed and the fungi were later re-isolated from the infected samples via inoculation and compared with the initial isolates.

Data Analysis
The data obtained from the study were analyzed using the PROC ANOVA procedure of GENSTAT version 15 and significant differences among the means were compared using Fisher's protected LSD at 5% probability level. Linear regression analysis was carried out to establish any correlations among different concentrations of the essential plant extract and their overall fungicidal expressions.

RESULT AND DISCUSSION

The fungi isolated from the pumpkin fruits purchased in the market were: *Aspergillus flavus, Phytophthora capsici, Rhizopus stolonifer* and *Aspergillus niger* as shown in Table 1.

Table 1: Prevalence of fungal isolates from pumpkin fruit

Fungal pathogens	Frequency of occurrence	%
Aspergillus niger	8	53.33
Aspergillus flavus	2	13.33
Rhizopus stolonifer	3	20
Phytophthora capsici	2	13.33
Total	15	100.0

Table 2 shows the antifungal effects of aqueous derivative of *Cucumis sativus* examined against fungal isolates from rotten pumpkin fruits, it was observed that *Cucumis sativus* at all concentrations except at 0.20mg/mL, *Aspergillus niger* was the most inhibited while the least sensitive was *Aspergillus flavus*

Table 2: Antifungal activities of Cucumis sativus ethanolic derivatives against fungal isolates

Fungal Isolates	1.0 mg/mL	0.8 mg/mL	0.6 mg/mL	0.4 mg/mL	0.2 mg/mL
Aspergillus flavus	0.00^{c}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Aspergillus niger	14.0^{c}	10.0^{c}	7.00^{c}	0.00^{c}	0.00
Rhizopus stolonifer	11.0^{a}	10.0^{a}	8.00^{a}	7.0^{a}	0.00^{a}
Phytophthora capsici	12.0^{a}	12.0^{a}	9.0^{a}	7.00^{c}	0.00

Key: *Control Nil*

Values are mean ± standard error of the mean for bioassay conducted in triplicate. Means followed by the same letter(s) are not significantly different (multivariate analysis, Fisher's protected LSD at ≤ 0.05)

Table 3 shows the antifungal expression of ethanol extract of *Annona squamosa* examined against fungal isolates from rotten pumpkin fruits, it was observed that *Annona squamosa* at concentration 1.0mg/mL was most inhibitive against *Phytophthora capsici, Aspergillus niger* and *Aspergillus flavus*, while the least sensitive was *Rhizopus stolonifer* at 0.2mg/mL concentration.

Table 3: Antifungal activities of Annona squamosa ethanolic derivatives against fungal

Fungal Isolates	1.0 mg/mL	0.8 mg/mL	0.6 mg/mL	0.4 mg/mL	0.2 mg/mL
Aspergillus flavus	22.00^{a}	18.00^{ab}	16.00^{b}	10.00^{c}	9.00^{a}
Rhizopus sp.	16.00^{c}	12.00^{a}	10.00^{ab}	8.00^{c}	0.00^{a}
Aspergillus niger	23.00^{a}	18.00^{ab}	16.00^{a}	10.00^{c}	8.00^{ab}
Phytophthora capsici	24.00^{a}	22.00^{a}	20.00^{ab}	18.00^{a}	14.00^{a}

Values are mean ± standard error of the mean for bioassay conducted in triplicate. Means followed by the same letter(s) are not significantly different (multivariate analysis, Fisher's protected LSD at ≤ 0.05)

Table 4 shows the antifungal expression of ethanol derivatives of *Carica papaya* examined against fungal isolated from rotten pumpkin fruits, it was observed that *Carica papaya* at concentration 1.0 mg/mL was most inhibitive against *Phytophthora capsici*, *Aspergillus flavus* and *Aspergillus niger*, while the least sensitive was *Rhizopus stolonifer* at 0.2mg/mL concentration.

Table 4: Antifungal activities of Carica papaya ethanolic derivatives against fungal isolates

Fungal Isolates	1.0 mg/mL	0.8 mg/mL	0.6 mg/mL	0.4 mg/mL	0.2 mg/mL
Aspergillus flavus	20.00^{a}	16.00^{bc}	14.00^{c}	8.00^{ab}	7.00^{c}
Rhizopus sp.	14.00^{ab}	8.00^{ab}	12.00^{a}	6.00^{bc}	0.00^{a}
Aspergillus niger	20.00^{ab}	16.00^{ab}	14.00^{bc}	8.00^{ab}	6.00^{ab}
Phytophthora capsici	22.00^{c}	20.00^{a}	18.00^{c}	16.00^{a}	12.00^{bc}

Key: *Control Nil*

Values are mean ± standard error of the mean for bioassay conducted in triplicate. Means followed by the same letter(s) are not significantly different (multivariate analysis, Fisher's protected LSD at ≤ 0.05)

Table 5 shows that all pathogenic fungal rot isolates induced high moisture content on the pumpkin fruits with no significant difference, fat content was highest and lowest in pumpkin fruit infected with *Phytophthora capsici* (2.63%) and *Aspergillus flavus* (0.33%) respectively. *Phytophthora capsici* (1.56%) and *Aspergillus flavus* (0.67%) infected pumpkin fruits were caused highest and lowest crude protein value respectively. *Aspergillus flavus* (0.98 %) and *Rhizopus stolonifer* (0.96%) infected pumpkin exhibited high ash value, *Rhizopus stolonifer* (1.97%) and *Aspergillus flavus* (1.80%) infected pumpkin fruits resulted in high crude fiber, *Phytophthora capsici* (5.79%) and *Rhizopus stolonifer* (5.96%) infected pumpkin fruits were high in carbohydrate.

Table 5: Results of proximate analyses of fungal pathogens infected pumpkin fruits

Parameters (%)	Control	Sample codes			
		A	B	C	D
Moisture content	92.24a	92.80a	93.00a	92.60ab	92.60a
Fat	0.15a	1.15ab	0.33b	2.63b	1.13bc
Crude protein	0.98a	1.38bc	0.67c	1.56ab	1.38c
Ash	0.76a	0.53a	0.98bc	0.42a	0.96a
Crude fiber	0.56a	0.23ab	1.80bc	1.00bc	1.97a
Carbohydrate	5.31a	4.91bc	4.22a	5.79a	5.96a

Mean *Control= 16.67±37.07a A=16.83±37.25a B=16.83±37.34a C=17.33±36.92a D= 17.33±36.92a*

Key:
Control: Healthy pumpkin fruit
A = Aspergillus niger
B = Aspergillus flavus
C = Phytophthora capsici
D = Rhizopus stolonifer

DISCUSSION, CONCLUSION AND RECOMMENDATION

DISCUSSION

This study examined the antifungal activities of *Cucumis sativus*, *Annona squamosa* and *Carica papaya* seed derivatives against four isolated fungi from rotten pumpkin fruits; *Aspergillus flavus*, *Phytophtora capsici*, *Rhizopus stolonifer*, and *Aspergillus niger*. *Aspergillus niger* occurred most from the pumpkin fruits in term of frequency of occurrence, this indicated that *Aspergillus niger* could be a major fungal pathogen responsible for post-harvest degradation of pumpkin fruits, while *Rhizopus stolonifer* and *Phytophtora capsici* also posed great threats to pumpkin fruits after harvesting. The fungal pathogens isolated from pumpkin in this study had also been isolated from orange as rot pathogens in Maiduguri as reported by Ali *et al* [22]. The concentration of ethanolic derivative of *Cucumis sativus* at a 1.0 mg/mL had highest inhibition zone on *Aspergillus niger* while the least sensitive was *Aspergillus flavus* at all concentrations, indicating that

C. sativus had more potential to inhibit the growth of *Apergillus niger* in pumpkin fruits after harvesting and less effective on *Aspergillus flavus.* The ethanolic derivatives of *Annona squamosa* had the highest zone of inhibition on *Phytophtora capsici* at all concentrations, while the least sensitive was *Rhizopus stolonifer. Annona squamosa* inhibited the growth of *Phytophtora capsici* in pumpkin fruits more than the other three fungal isolates. The ethanolic derivatives of *Carica papaya* inhibited *Phytophtora capsici*, *Aspergillus flavus* and *Aspergillus niger* more than *Rhizopus stolonifer*. The occurrence of the four fungal isolates from pumpkin fruit revealed deviation of the proximate compositions of pumpkin fruit; both moisture and fat content increased, this could possibly affect dietary requirements when consumed. It was observed in this study that, the ethanolic derivatives of *C. sativus*, *A. squamosa* and *C. papaya* inhibited occurrence of the four fungal isolates and their effectiveness in inhibition varied amongst the fungal isolates. Antifungal effects of *C. papaya* was reported against dermatophytic fungus, *Microsporum canis* by Aljuhani *et al* [23], this agrees with the antimicrobial capacity of *C. papaya* in this investigation. Also, the efficacy of *C. sativus* in this study corroborated Jamilatun, *et al* [24] that reported cucumber extract fungicidal potential against *Candida albicans,* a dermatophyte. The antimicrobial capacity of *A. squamosa* from this study agreed with Irawan *et al* [25] that reported *A. squamosa* fungitoxic effects against *Staphylococcus aureus*, a bacterium causing local infections and inflammation (mastitis) of the breast tissue. This possibly indicated that different plant extracts can be synergistically applied to effectively control various post harvested plants pathogens. There are no significant differences between *Annona squamosa* and *Carica papaya* derivatives, from the statistical standpoint. *Annona squamosa* and *Carica papaya* were the most effective. Although, all the plant derivatives exhibited considerable inhibitory effects on all the fungal isolates,

CONCLUSION

Fruits and vegetables are prone to fungal attack in postharvest operations. Synthetic fungicides are the commonly used in controlling fungal pathogens of plants, but, an alternative approach like *Annona squamosa* and *Carica papaya* extracts can be adopted for pumpkin postharvest fungal diseases control, as they are safer, cheaper, easily accessible and more environment friendly than chemical fungicides.

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