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Antimicrobial activities of *Carica papaya* and *Nicotiana tabacum* leaf extracts against fungal isolates of stored local rice in Abeokuta, Ogun State, Nigeria

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ABSTRACT: Ofada" is a type of local unpolished brown rice grown mostly in Ogun State, Southwest Nigeria. The fungal attack poses a major threat to its storage. The efficacy of ethanoic and distilled water extracts (crude extracts) of Carica papaya and Nicotiana tabacum leaves were tested on the isolated fungal species using the pour plate method. Antimicrobial activities of N. tabacum and C. papaya leaf extracts on fungal isolates were evaluated using the agar well diffusion method. One-way analysis of variance was used in this study to compare antimicrobial activities (dependent variable) with respect to extract concentrations (factor). Results revealed a total of seven (7) fungal species, from four different genera- Aspergillus spp, Trichothecium spp, Rhizopus spp, and Fusarium spp. The ethanol extract of C. Papaya leaf has the ability to inhibit the growth of T. roseum, A. niger, and A. flavus better than the control antibiotics. Similarly, ethanol extract of N.tabacum also compares well with the control antibiotics in the antifungal activity against the isolated test fungal species (T. roseum, A. niger and A. flavus). Also, the crude extract of both C. papaya and N. tabacum showed high antifungal activity against T. roseum, A. niger and A. flavus. Based on the findings of this study, both the ethanolic and water extracts of C. papaya and N. tabacum leaves could be used as a natural fungicide in the storage of local Ofada rice.

Keywords: Local Ofada rice, antifungal, Carica papaya, Nicotiana tabacum, storage

Introduction

Rice is a common staple food in Africa; a rich and cheap source of carbohydrate to both man and animal. It has served as a major staple, cushioning the effect of under-nutrition and severe hunger among many Nigerian households as it is commonly eaten in many localities. It is processed into different forms. The preferred forms among the Yorubas include Jollof, fried rice and white rice. It is also milled into flour and then cooked in boiling water and turned into a thick paste called Tuwo in the Northern part of Nigeria (Ologbon *et al*., 2012). One of the most popular indigenous rice varieties in Nigeria is Ofada rice. The original Ofada rice is short grain robust rice believed to be OS6 and

ITA150 varieties (Adekoyeni *et al*., 2018). The rice is processed traditionally by parboiling method that involves three stages of treatment (soaking, parboiling, and drying). This rice is especially relished due to its characteristics flavour that develops during soaking as a result of the fermentation activities of some microorganisms (Adeniran *et al*., 2012).

Storage of rice is done locally in bamboo-laid ceilings and barns, baskets, and sacks with prevailing high temperature and relative humidity that encourage colonization by microbes. The presence of microbes in stored grains may lead to various forms of deterioration, visible microbes, seed discolouration, musty odours, reduction in acceptability or outright rejection of the grain for consumption and decreased nutritive value(Somorin and Bankole, 2010).

Fungi infection not only results in a reduction in crop yield and quality with significant economic losses but also contamination of grains with poisonous fungal secondary metabolites called mycotoxins. Mycotoxins have been detected in human foods and livestock feeds in Nigeria. Most often these mycotoxins are detected in deleterious levels compounded by synergistic interactions. The ingestion of toxin contaminated grains by animals and human beings has enormous public health significance because they are capable of causing diseases in man and animals (Atanda *et al*.,2013). The involvement of fungi and their toxins in causing diseases to man and animals was dated back when the Dead Sea Scrolls were written but the impact was not obvious until the Middle Ages when ergot alkaloids poisoning broke out in Europe (Atanda *et al*., 2013).

Over the years, control of pathogenic organisms in foods has drawn considerable attention with the use of industrial chemicals such as propionic acid and ammonia in the storage of grains against microbial attack (Frazier and Westhoff, 1998). These chemicals have shown to be effective in preventing microbial growth. However, when they are concentrated on the grains they have been found to have a residual effect such as; inducing chemical poisoning, environmental toxicity, and development of resistance by microbes to the chemical agent(Bankole and Somorin, 2010; Joseph *et al*., 2017). Therefore, many research investigations have demonstrated the antimicrobial efficacy of several constituents of some higher plants. Extracts possess antifungal, antiviral, and antibacterial properties (Joshi *et al*., 2020). This has therefore led to the use of some tropical plants that have shown high antimicrobial activities and since they are natural products which are edible, there is little or no fear of poisoning even at very high concentrations (Jabeen, 2011; Joseph *et al*., 2017; Bankole and Somorin, 2010). Such plants include Carica papaya and Nicotiana tabacum(Tewari *et al*., 2014; Sharma, 2015; Okorondu *et al*., 2015). This present study, therefore, aimed at evaluating the antifungal effect of crude and ethanolic extracts of Carica papaya and Nicotiana tabacum against fungal isolates of stored local Ofada rice in Abeokuta, Ogun state Nigeria.

Materials and Methods

Sample collection

Rice sample: Stored rice (Ofada Rice) samples were purchased from different points at the Kuto market, Abeokuta, Ogun State, Nigeria.

Plant samples: Collected fresh leaves of Carica papaya and Nicotina tabacum were properly washed under running tap water and rinsed with sterile distilled water. The samples were dried at low temperature (600C) until all moisture removed(constant weight) and milled into powder by using a vegetable blender. The samples were stored in an airtight container for further use.

Extract preparation

Ethanol extract: Different grams of the milled leaves were weighed (2.5g, 5g, and 10g) into McCartney bottles and filled with 10ml of 50% ethanol to obtain the following concentration: 25mg/ml, 50mg/ml, 100mg/ml and allowed to soak overnight. These were decanted and the fluid centrifuged at 2000rpm for 10 minutes.

Water(Crude) extract: The milled leaves were weighed (2.5g, 5g, and 10g) into a conical flask and filled with a sterile distilled water to obtain the following concentrations: 25mg/ml, 50mg/ml, 100mg/ml. A magnetic probe was used to stir the concentration and allowed to soak overnight. These were decanted and the fluid centrifuged at 2000rpm for 10 minutes.

Microbial evaluation

Media preparation: Potato Dextrose Agar used was prepared in accordance with the manufacturer's instruction. It was then sterilized in an autoclave at 121°C and 100kPa (15psi) for 15 minutes. The medium was allowed to cool to about 45°C and poured into Petri-dishes aseptically and allowed to set.

Serial dilution: Ten (10) grains of rice were weighed in a test tube,10ml of distilled water added, and shaken for 3 minutes to get a stock solution. One ml (1ml) of the stock was pipetted into 9ml of distilled water in a test tube to make a serial dilution of 10-1. This was repeated for other concentrations**.**

Fungal isolation: Pour plate method was used for the isolation of fungi for each sample of serial dilution used. 1ml of each dilution was pipetted aseptically into a sterile petri dish and Potato Dextrose Agar (15-20ml) poured on it. The Petri dishes were gently rocked for thorough mixing. The medium was allowed to set and incubated in an inverted position at 25°C for 24- 72hours. After which pure colonies on the plate were sub-cultured onto fresh Potato Dextrose Agar slant by stabbings to obtain pure cultures.

Identification of isolates: The identification was based essentially on morphology/cultural and biochemical reactions. Fungal genera were determined through morphological criteria using identification keys such as the description of mycelia and of asexual reproduction forms (Domsch *et al*., 1980). Further identification was carried out according to Kreger-venrij (1984) by pseudo-mycelium formation and pattern of sugar fermentation (Glucose, lactose, Sucrose, maltose, and fructose).

Determination of Antimicrobial Activity

Extracts solution, 1 ml each was dispensed into a molten agar and poured into Petri dishes. The agar was then allowed to solidify. The plates were then incubated at 280C for three days. The zones of inhibition of each extract on the isolates were measured at a 24hrs interval for three days.

Statistical analysis

Data collected were analyzed using the Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp, 2011). Randomized design (one-way analysis of variance) was used in this study and results were presented as Mean±Standard deviation. $P < 0.05$ was considered to be statistically significant. Post hoc tests was done using the Student-Newman-Keuls (SNK). $P < 0.05$ was considered to be statistically significant

Results

Fungal isolates

From the stored local Ofada rice samples used for this study, a total of seven (7) fungal species were isolated. The fungal species include: Rhizopus spp, Aspergillus flavus, Aspergillus niger, Fusarium spp, Penicillium spp, Aspergillus nidulans, and Trichothecium roseum.Three most occurring fungal species found in the local rice samples were selected for antimicrobial studies.

Antimicrobial activities

Ethanoic extracts

Carica papaya: The antimicrobial activity of ethanol extract of C. papaya against Trichothecium roseum was significantly higher ($p < 0.05$) at 100% extract concentration (Table 1). This was

observed to reduce with a reduction in the extract concentration. The control antibiotics, however, had the least antimicrobial activity against T. roseum. Similarly, antimicrobial activity of ethanol extract of C. papaya against Aspergillus niger was highest at 100% extract concentration and this was not significantly different ($p > 0.05$) from that of the control antibiotics. On the other hand, the antimicrobial activity of ethanol extract of C. papaya against Aspergillus flavus was highest at 25% extract concentration.

Nicotina tabacum: Antimicrobial activity of ethanol extract of N. tabacum against T. roseum was also significantly higher ($p < 0.05$) at 100% extract concentration (Table 2). This was the lowest with the control antibiotics. Similarly, the antimicrobial activity of ethanol extract of N. tabacum against A. niger and A. flavus were highest at 100% extract concentration. These were however lower than the antimicrobial activity recorded against these organisms using the control antibiotics.

Crude extracts

The antimicrobial activities of the crude extracts of C. papaya and N. tabacum against the three test organisms followed a similar trend (Figure 1). The antimicrobial activities of the two plant extracts were not significantly different ($p > 0.05$) against T. roseum, A. niger, and A. flavus.

Antimicrobial activities comparison of extracts

Crude extracts of both C. papaya and N. tabacum had the highest ($p < 0.05$) antimicrobial activities against T. roseum (Table 3). Antimicrobial activity against T. roseum was lowest with the control antibiotics. Similarly, the crude extracts of both C. papaya and N. tabacum were highest in antimicrobial activities against A. niger and A. flavus and this was not different from those of the control antibiotics.

Table 1: Antimicrobial activity of ethanoic extracts of *Carica papaya* against mycelial growth of *Trichothecium roseum, Aspergillus niger,* and *Aspergillus flavus*

	Zone on Inhibition (mm)		
Conc.	*Trichothecium roseum*	*Aspergillus niger*	*Aspergilllus flavus*
25%	21.00 ± 2.10^{b}	20.50 ± 1.85^{b}	45.00 ± 4.00^{a}
50%	22.50 ± 1.05^{b}	21.50 ± 1.15^{b}	23.00 ± 2.750^{d}
100%	26.50 ± 2.00^{a}	25.50 ± 2.51^{a}	29.00 ± 2.80^{c}
Control	17.33 ± 2.50^{c}	25.50 ± 2.70^{a}	40.00 ± 3.55^{b}

^{abcde}Mean values (±Standard deviation) in the same column having the same alphabet are not significantly different at $p < 0.05$; Control = Fluconazole

Table 2: Antimicrobial activity of ethanoic extracts of *Nicotina tabacum* against mycelial growth of *Trichothecium roseum, Aspergillus niger,* and *Aspergillus flavus*

	Zone on Inhibition (mm)		
Conc.	*Trichothecium roseum*	*Aspergillus niger*	*Aspergilllus flavus*
25%	21.00 ± 1.50^{c}	19.00 ± 1.70^{c}	18.00 ± 1.50^{d}
50%	26.00 ± 2.00^{b}	16.00 ± 1.15^{d}	27.50 ± 2.00^{c}
100%	30.00 ± 2.30^{a}	23.50 ± 0.50^{b}	47.50 ± 3.50^{b}
Control	18.00 ± 1.05^{d}	45.00 ± 2.50^{a}	45.00 ± 3.05^{a}

Mean values (±Standard deviation) in the same column having the same alphabet are not significantly different at $p < 0.05$; Control = Fluconazole

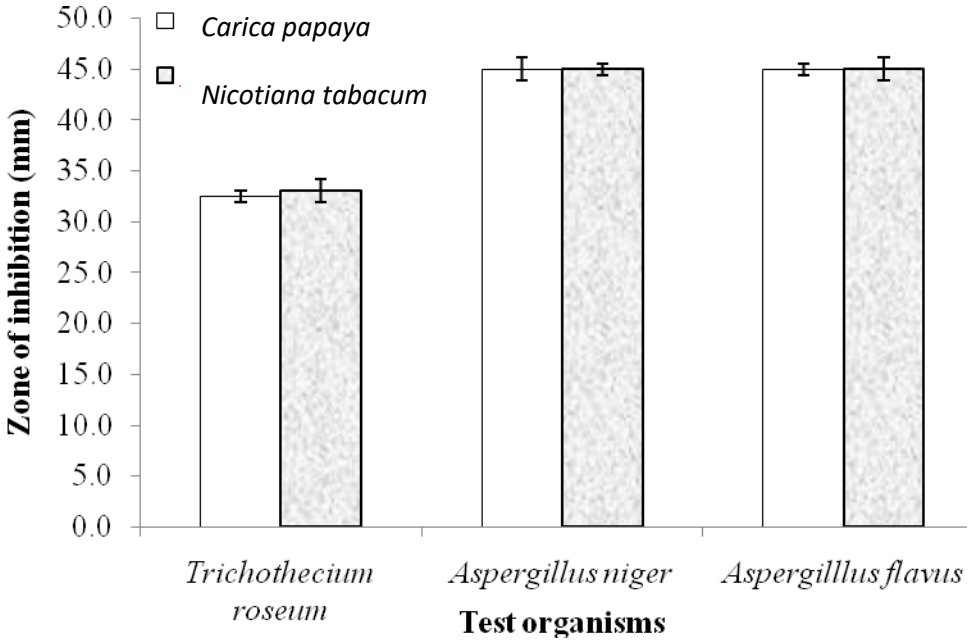

Figure 1: Antimicrobial activity of crude extracts of *Carica papaya* and *Nicotiana tabacum* against mycelial growth of *Trichothecium roseum, Aspergillus niger,* and *Aspergillus flavus* ($p > 0.05$)

Table 3: Comparison of antimicrobial activities of the different extracts of *Carica papaya* and *Nicotiana tabacum* against mycelial growth of *Trichothecium roseum, Aspergillus niger*, and *Aspergillus flavus*

	Zone on Inhibition (mm)		
Extracts	*T. roseum*	*A. niger*	*A. flavus*
Ethanolic *C. papaya*	26.50±2.50^{c}	25.50±2.05^{b}	28.00±1.50^{c}
Ethanolic *N. roseum*	30.00±3.00^{b}	23.50±1.50^{b}	23.50±1.05^{b}
Crude *C. papaya*	32.50±3.05^{a}	45.00±3.05^{a}	45.00±3.05^{a}
Crude *N. roseum*	33.00±3.50^{a}	45.00±3.00^{a}	45.00±3.00^{a}
Control	18.00±1.50^{d}	45.00±3.00^{a}	45.00±3.00^{a}

Mean values (±Standard deviation) in the same column having the same alphabet are not significantly different at $p < 0.05$; Control = Fluconazole

Discussion

This study has shown that stored local ofada rice of major market in Abeokuta contains a diversity of fungal species such as Rhizopus spp, Aspergillus flavus, Aspergillus niger, Fusarium spp, Penicillium spp, Aspergillus nidulans, Trichothecium roseum. Aspergillus species have been shown to be pathogenic in nature, causing Aspergillosis (Szalewski *et al*.,2018) and may lead to a variety of allergic reactions and life-threatening systemic infections in humans (Paulussen *et al*., 2016). Such infections are typically caused by Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus niger, and Aspergillus terreus, among other species. These microorganisms are also the culprit for the production of carcinogenic aflatoxins in grain storages, contaminating crops, and economically straining the production process (Szalewski *et al*.,2018). High levels of mycotoxins producing fungi were found in grains during storage time and at the mechanical cleaning point. Penicillium, Aspergillus, Alternaria and Fusarium species abound in grains (Doolotkeldieva, 2010). Similarly, Trichothecium roseum produces a wide range of secondary metabolites including mycotoxins such as roseotoxin and trichothecenes (Batt and Tortorello, 2014).

Thus, the presence of these fungal isolates on stored local ofada rice for consumption as recorded in this study may be dangerous and pose a great threat both to the marketability of this rice and the health of the consumers.

However, the results of this study show that the ethanol extract of C. papaya has the ability to inhibit the growth of T. roseum, A. niger, and A. flavus better than the control antibiotics. Several bioactive phytochemical constituents have been identified in the leaves of C. papaya. Such include flavonoids, terpenoids, saponins, phenol, sterols, and tannins (Tewari *et al*., 2014; Vijayakumar *et al*., 2015). The bioactive roles of these phytochemicals in plant extracts have been documented. Flavonoids are polyphenolic compounds found in plants that are beneficial for human health and play an important role in minimizing the effects of various diseases (Mishra *et al*., 2013). They are also known to possess antimicrobial and anti-inflammatory, antifungal, antioxidants, and potent metal chelators (Hayat *et al*., 2018).

Alkaloids exhibit significant biological activities and are among the most important active components in natural herbs some of which have been developed into chemotherapeutic drugs (Lu *et al*., 2012)

Tannins are secondary metabolites of plants of high molecular weight that bind to proteins, carbohydrates, gelatins, and alkaloids, and are classified as active antimicrobial compounds (Prakash and Hosetti, 2010). These phenolic compounds have been reported to possess considerable antimicrobial properties, which was attributed to their redox properties (Molan and Faraj, 2010; Zongo *et al*., 2011). Thus, Prakash and Hosetti (2010) attributed the antimicrobial properties of plants to the presence of these secondary metabolites. Hence, the antimicrobial activities of ethanol extract of C. papaya as recorded in this study could have been aided by the array of bioactive constituents it contained.

Similarly, ethanol extract of N. tabacum also compares well with the control antibiotics in the antifungal activity against the three test fungal species (T. roseum, A. niger, and A. flavus) used in this study. Previous studies have also shown that the ethanol extract of N. tabacum is an important antimicrobial agent against common pathogens (Okorondu *et al*.,2015; Jehan and Mohammad, 2012). This antimicrobial activity of N. tabacum was attributed to the bioactive ingredients they contain. According to Okorondu *et al*., (2015), Nicotiana tabacum extract contains an alkaloid, tannin, saponins, flavonoids, cyanogenic glycosides, and mineral elements. Also, the crude extract of both C. papaya and N. tabacum also showed high antifungal activity against T. roseum, A. niger, and A. flavus. The antimicrobial potential of the extracts of C. papaya and N. tabacum leaves are therefore needed to be greatly exploited especially in the preservation of local rice in storage.

Conclusion

Based on the findings of this study, 100% ethanolic extracts of N. tabacum leaves could be used as natural fungicides in the storage of local Ofada rice. This would go a long way to improve its market value and increases shelve life.

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