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RESEARCH ARTICLE

EVALUATION OF THE POTENTIAL OF *ALLIUM CEPA* L. PEEL AGAINST *CARICA PAPAYA* LINN FRUIT ROT PATHOGENS

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ABSTRACT

The harmful effect of synthetic pesticides on the environment and human health calls for sustainable plant and animal disease management. The study's objective was to evaluate the antifungal activities of red onion peel extract on papaya fruit rot fungal pathogens. Onion peel methanol extract at 5, 10, 15, 20, and 25mg/ml concentration was employed to assess the fungal inhibition. In contrast, the extract diluted with 1 mL of potato dextrose agar broth at 100, 50, 25, 20, 15, 10, and 5 mg/Ml, was utilised to assess the in-vitro minimum inhibitory concentration (MIC), in the Department of Plant Science and Biotechnology at Imo State University in Owerri, Nigeria. The identified pathogens include Rhizopusstolonifer, Fusariumoxysporium, and Aspergillusflavus. As pergillusflavus had the largest percentage frequency of occurrence (55%), while, Fusariumoxysporium displayed the lowest frequency (11%). The inhibitory effect of onion peel extract concentrations on the growth of the fungi exhibits an inverse relationship. The mancozeb treatment exhibited the lowest recorded minimum inhibitory concentration (MIC) values. The application of DMSO resulted in the highest observed minimum inhibitory concentration (MIC) values. The concentration of Onion peel extract exhibited an inverse relationship with the Minimum Inhibitory Concentration (MIC) values. The concentration of 100 mg/ml yielded the lowest minimum inhibitory concentration (MIC) value, whereas 5 mg/ml yielded the highest MIC value. The utilisation of red onion peel presents a potential non-chemical and sustainable strategy for managing plant diseases.

INTRODUCTION

Carica papaya Linn., commonly referred to as papaya or pawpaw, belongs to the family *Caricaceae* (Badillo VM, 1971). The largest group among the four genera consists of 48 species, including *Carica papaya* L. (Badillo (1971). The edible fruit has been extensively cultivated in tropical regions and is utilised in the creation of various food and beverage products such as jams, preserves, soft drinks, ice cream, cocktails, crystallised fruit, and canned syrup (Ezike *et al*., 2009). The papaya fruit possesses nutritional features that make it suitable for inclusion in a healthy diet. It is rich in essential nutrients, including dietary fibres, vitamins A, C, and B3, as well as calcium, iron, and potassium (Alara *et al*., 2022). Additionally, the presence of bioactive compounds in these plant parts may contribute to the prevention of significant ailments such as cancer (Ikram *et al*., 2015; Alara *et al*., 2022).
Papaya exhibits climacteric characteristics, indicating that its ripening process persists even after it has been harvested (Rodrigues *et al*., 2021). Multiple factors contribute to the deterioration of fruit quality after harvest. These factors encompass premature harvesting, injuries resulting from inadequate handling practices, inappropriate storage conditions, postharvest illnesses, impairment due to extreme cold or high temperatures, infestation by pests, and physiological abnormalities (Paull and Oliveira, 2020). The onion (*Allium cepa*) is a widely utilised item in Nigerian households. The onion is considered the second most significant horticultural crop, with an annual production of 47 million tonnes (FAOSTAT 2018). Numerous studies have provided evidence of the effectiveness of onion extract as a potent antifungal agent (Teixeira *et al*., 2023), as well as its anti-inflammatory and antioxidant properties (Colina-Coca *et al*., 2017, Omar *et al*., 2020). Nevertheless, the processing of onions, results in a significant quantity of waste being produced. Moreover, improper disposal of this trash might result in adverse consequences for the environment. The potential impact of chemical fungicides and pesticides on human health, recurrent emergence of disease resistance, and adverse environmental consequences areconcerns due to the exposure and subsequent deposition of pesticide residues on fruits. Presently, to mitigate the worldwide threats to plant health and achieve the objective of ensuring food security in a dynamic ecological context, there is a growing emphasis on exploring phytochemicals as a viable substitute for synthetic pesticides in the control of plant diseases (Liu *et al*., 2014).

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This heightened interest is primarily attributed to the numerous benefits associated with plant extracts, including their enhanced safety profile, facile biodegradability, environmentally sustainable nature, and minimal toxicity (Hans *et al*., 2021). The disposal of agricultural and food wastes results in the regrettable depletion of the valuable nutritional and medicinal properties contained in their bioactive compounds. Typically, these botanical byproducts are disposed of as residues in landfills. However, their utilisation as a potential source of antifungal chemicals would reintegrate them into the production cycle and align with the principles of a circular economy. Hence, an assessment was conducted to determine the efficacy of red onion peel in combating specific phytopathogenic fungi responsible for the rotting of papaya fruit.

MATERIALS AND METHODS

Study area: The research was carried out within the Owerri municipal council, in Imo State, Nigeria.Owerri is situated in the geographical coordinates of 5°29'1"N 7°1'60"E (5.4836300, 7.0332500), (GPS coordinates of Owerri-Municipal, Nigeria).Owerri lies in the humid rainforest, and the majority of the population engage in farming activities. The mycological experiment was carried out in the laboratory of the Department of Plant Science and Biotechnology,Imo State University, Owerri in the year, 2022.

Preparation of the Onion peel: Red onionspeel were procured from Ekeonunwa and Relief markets located in Owerrimunicipal council, Imo State. By the methodology outlined by Kassab-Bashi*et al*.,2014, the outer layers of the onion bulbs were detached, subjected to a thorough water rinse to eliminate any traces of sand or grit, and subsequently pulverised (500 g each). The resulting powder was then individually extracted using a Soxhlet apparatus with methanol (80%, 2 × 500 mL, 65 °C, 1 h). The initial weight of the crude extract (20 g) filtrate was measured and subsequently dissolved in 250 ml of a 20% aqueous methanol solution (v/v) to create a stock solution. This stock solution was further diluted with sterile distilled water to get the desired concentrations for various tests. The specimen was stored in containers with controlled temperature conditions of (25 ± 1) ºC in a dry environment and then utilised for the experiment.

Isolation and Identification of Papaya fruit rot fungal pathogens: A quantity of 30 *Carica papaya* fruits, were procured at the Ekeonunwa and Eke UkwuOweri markets. A total of 20 papaya fruits, comprising 10 fruits exhibiting typical rotting symptoms and 10 fruits displaying a healthy appearance, were procured and subsequently transported to the laboratory for further analysis. The fungal pathogens were isolated from rotting Papaya fruits and subsequently identified based on their morphological attributes and microscopic features (Jidda in 2017).

Pathogenicity Test: The Papaya fruit rot fungi were inoculated on papaya fruits through the use of knife-induced injuries (Chaudhary *et al.* 2020). The fungi that were isolated were subjected to an assessment of their capacity to initiate decay in Papaya fruits that were in a healthy state. The pathogenicity of each fungus on the inoculated samples was determined by measuring the length and width of rotten parts at two days intervals for ten days, by following the procedures of (Jidda, 2017).

The assessment of the antifungal activity of the extracts

The assessment of the antifungal activity of the extracts was conducted using the agar well diffusion method, as outlined by Aranda-Martinez (2016), with some modifications. The fungal pathogens were cultivated on Potato Dextrose Agar medium (OXOID, UK) at a temperature of 37ºC for 24 hours. Then the colonies were resuspended in tubes containing 5 mL of Potato Dextrose Broth, resulting in a final cell concentration of 10^6 Colony Forming Units per millilitre (CFU/mL). Every tube was calibrated to correspond to the 0.5 McFarland standard (1.5 x 108 colony-forming units per mililitre). In this experiment, wells with a diameter of 6mm were created in the agar medium. Each well was then filled with 50 µl of various plant extracts, as well as Mancozeb (1000 µg/ml), as a positive control, and DMSO (99.9%) was used as a negative control. Five different concentrations (5, 10, 15, 20, and 25mg/ml) of the extract were created. The plates were incubated at a temperature of 37 ºC for a duration of 24 hours. The antifungal activity was evaluated by determining the diameter of the inhibitory zone. All experiments were conducted in triplicate.

The formula for calculating inhibition percentage is expressed as follows: Inhibition (%) = $\frac{dc - de}{dc} \times 100$.

Let "dc" represent the diameter of the control mycelium, and "de" represent the diameter of the mycelium subjected to extracts.

The minimum inhibitory concentration (MIC): MIC refers to the lowest concentration of a substance required to impede the growth of a microorganism. The minimum inhibitory concentration (MIC) of the onion peel extract, as determined by Dhowlaghar*et al*. (2023),with some modifications was followed. The peel extract was diluted in a broth solution at various concentrations: 100 mg/mL, 50 mg/mL, 25 mg/mL, 20 mg/mL, 15 mg/mL, 10 mg/mL, and 5 mg/mL. Subsequently, 1 mL of each fungal pathogen suspension was added. The positive control, Mancozeb, was employed at a concentration of 1000 µg/ml, while the negative control consisted of DMSO at a purity of 99.9%. The tubes were subjected to incubation at a temperature of 37ºC for 24 hours. Subsequently, the fungal colonies were observedto determine the minimum inhibitory concentration (MIC) value. The experiments were conducted in triplicate and replicated three times.

Data and statistical analysis: All the collected data were subjected to an analysis of variance (ANOVA) using MINITAB 19 software package and significant differences between the means are obtained using the LSD test ($p < 0.05$).

RESULTS

Percentage frequency of occurrence of the fungal pathogens: The analysis of the percentage frequency of occurrence of the fungal pathogens revealed that *Aspergillusflavus* exhibited the highest frequency of occurrence (55%), this was followed by *Rhizopusstolonifera* (34%), while *Fusariumoxysporium*was lowest (11%) in occurrences. (Table 1).

Table 1. Percentage frequency of occurrence of the fungal pathogens

Fungal pathogens	Frequency of occurrence(%)
Aspergillusflavus	55(0.33)a
Rhizopusstolonifera	34(1.20)b
Fusariumoxysporium	11(0.25)c

Means that do not share a letter within a column in a treatement are significantly different (Turkey's HSD test (p < 0.05). Numbers in brackets are ± standard deviation.

Pathogenicity test: The experimental results indicated that both fungal suspensioninoculated wounded and unwounded fruits, exhibited signs of rotting within a period of five days after inoculation. Indications of spoilage were observed in the form of a white rot at the site of inoculation and a brownish degradation.

The extract concentration of 100 mg/mL exhibited the highest effectiveness in retarding microbial growth.

The minimum inhibitory concentration (MIC): The analysis of variance yielded statistically significant findings (p≤0.05) indicating variations in minimal inhibitory concentrations across all treatment groups. The efficacy of the mancozeb treatment was evidenced by the observation of the lowest minimum inhibitory concentration (MIC) values. Specifically, the MIC values were determined to be 0.43 mg/ml for *Aspergillusflavus*, 0.28 mg/ml for *Fusariumoxysporium*, and 0.33 mg/ml for *Rhizopusstolonifer*. In contrast, DMSO treatment exhibited the highest minimum inhibitory concentration (MIC) values, specifically 82.20 mg/ml for *Aspergillusflavus*, 90.20 for *Fusariumoxysporium*, and 89.20 for *Rhizopusstolonifer*.

Table 2. Diameter of crude onion peel extracts' inhibition zones (mm) against different fungal species

	Fungal pathogens		
	Aspergillusflavus	Fusariumoxysporium	Rhizopusstolonifer
Red onion peel extract concentrations.			
DMSO(99.9%)	0.32 (0.25)g	0.30(0.10)g	0.27(0.096)g
5%	6.30(0.25)f	5.17(0.35)f	4.63(0.3)f
10%	9.32(0.31)e	7.43 (0.50)e	8.70(0.48)e
15%	12.30(0.27)d	11.44 (0.31)d	10.8 (0.192)d
20%	15.54 (0.61)c	16.30 (0.36)c	15.43 (0.47)c
25	19.62 (0.67)b	21.53 (0.74)b	19.31(0.38)b
Mancozeb (1000 µg/ml)	24.02 (0.20)a	25.23(0.25)a	26.00(0.44)a
F- value	1236.30	1362.31	2452.07
P- value	0.003	0.001	0.002

Means that do not share a letter within a column in a treatement are significantly different (Turkey's HSD test (p < 0.05). Numbers in brackets are ± standard deviation.

Table 3. Minimum inhibitory concentration (Percentage) of crude Onions peel extracts against test fungal strains

	Fungalpathogens		
	Aspergillusflavus	*Fusariumoxysporium*	*Rhizopusstolonifer*
Onion peel extract concentrations.			
5%	83.00(3.03)b	67.03(0.896)b	74.40(0.265)b
10%	75.34(0.61)c	53.20(0.200)c	56.23(0.32)c
15%	57.03(0.25)d	43.03(0.252)d	50.43(49.50)d
20%	33.60 (0.27)e	35.30(0.31)e	33.80(0.53)e
25%	30.43 (0.67)f	33.20 (0.36)f	27.40(0.36)f
50%	15.33 (0.32)g	22.30(0.17)g	21.1(0.56)g
100%	6.34 (0.10)h	9.10(0.10)h	5.50(0.153)h
DMSO (99.9%)	82.20 (0.20)a	90.20(0.27)a	89.20(0.50)a
Mancozeb (1000 µg/ml)	0.43 (0.15)i	0.28(0.16)i	0.33(0.21)i
F-value	2803.15	18118.15	19321.29
P- value	0.001	0.002	0.000

Means that do not share a letter within a column in a treatement are significantly different (Turkey's HSD test (p < 0.05). Numbers in brackets are ± standard deviation.

The mycelial growth inhibition: The analysis of variance conducted to compare the effects of onion peel extract, synthetic antifungal drug (mancozeb), and Dimethyl sulfoxide (DMSO) on the inhibition of mycelial growth in fungal pathogens demonstrated a statistically significant difference (p≤0.05) during the duration of the study. Mancozeb exhibited the highest efficacy in suppressing the growth of the fungal pathogens, this was followed by onion peel treatment while DMSO treatments had the least inhibitory effect on the fungal pathogens, (Table 2). The statistical analysis revealed a substantial impact of varying amounts of onion peel extract on the inhibition of the fungal mycelial growth. The findings suggest that there is an inverse relationship between the concentrations of onion peel extract and the inhibition of mycelial growth of the fungal pathogens.

The concentration of Onion peel extract exhibited an inverse relationship with the Minimum Inhibitory Concentration (MIC) values,(Table 3). The concentration of 100 mg/ml exhibited the lowest minimum inhibitory concentration (MIC) value for Aspergillusflavus (6.34), *Fusariumoxysporium* (9.10), and *Rhizopusstolonifer* (5.50). Nevertheless, the concentration of 5 mg/ml exhibited the greatest MIC value for *Aspergillusflavus* (83.00), *Fusariumoxysporium* (67.03), and *Rhizopusstolonifer* (74.40) (Tables 3).

DISCUSSION

The present study has revealed a diverse range of pathogenic fungi that constitute a threat to both humans and animals.

Research suggests that the processes of harvesting, storing, packaging, and transporting fruits might result in physical damage, leading to an escalation in post-harvest losses and an elevated risk of fungal contamination. Inadequate fruit management practises and unfavourable market conditions can exacerbate the risk of contamination, particularly when coupled with substandard hygiene practises among sellers and the use of containers that are not microbiologically acceptable. The fungi species *A.flavus* and *Fusariumoxysporium* are known to produce highly toxic mycotoxins that have the potential to cause harm to both humans and animals.

Percentage frequency of occurrence of the fungal pathogens: Theresult of theisolated fungal pathogens is consistent with prior studies conducted by Baiyewu *et al*. (2007), Chukwuka *et al*. (2013), and Mailafia *et al*. (2016), which documented the identification of *A. Niger*, *F.oxysporium*, *R.avenaceum*, *R.stolonifer* and yeast in pawpaw fruit samples collected from Nigeria. Furthermore, Chaudhary *et al*. (2020) successfully identified *Aspergillusniger, Fusariumsolani, Aspergillusflavus,* and *Rhizopusmicrospora* from Papaya fruit. The findings on the frequency of occurrence of fungal were consistent with the study conducted by Mailafia *et al*. (2016), which indicated that *Aspergillusniger* had the highest occurrence (38%) in pawpaw, *Fusariumavenaceum* recorded (31%), while *Penicilliumdigitatum* and *Rhizopusstolonifer* both had the lowest frequencies of occurrence (4%).

Pathogenicity test: The results of the pathogenicity test are consistent with the findings of Chaudhary *et al*. (2020), who observed that both fully matured and immature papaya fruits, when inoculated with*Alternariaalternata, Fusariumsolani, Aspergillusniger, Aspergillusflavus,* and *Rhizopus microspore*, exhibited a water soaked condition at the wound site after three days of inoculation.

Mycelial growth inhibition: The findings on the fungal mycelial growth inhibition are consistent with the study conducted by Mounir*et al*. (2023), which demonstrated the efficacy of red onion peel extracts in inhibiting microbial growth. The inhibition of fungal mycelial growth reported in this study can be attributed to the presence of antifungal chemicals found in the extract derived from onion peels. Multiple studies have documented the presence of allicin, flavonoid, saponin, tannin, and triterpenoid compounds in red onion (Genatrika*et al*., 2020). These compounds have been found to exhibit inhibitory effects on fungal enzymes responsible for causing infections and disorders, as well as promoting fungal survival and proliferation within cells (Dewi and Warganegara, 2016). Additionally, they have been observed to interfere with the permeability of fungal cell membranes (Alfiah*et al*., 2015; Septiadi*et al*., 2013). The lack of significant variations in the susceptibility of the fungal pathogens to the extracts can be attributed to their similarities in terms of cell wall composition and plasma membrane fluidity, as discussed by Aranda-Martinez *et al*. (2016).

Minimum inhibitory concentration: The minimum inhibition concentration observed in the present study corroborates with the report by Mounir *et al*., (2023), who observed that onion peels displayed antibacterial efficacy against *Staphylococcus aureus* with MIC value of 1.9 mg/mL, while *S. epidermidis and Candida albicans* exhibited MIC value of 1.9 mg/mL, and *Pseudomonas aeruginosa*, having a MIC of 31.25 mg/mL

CONCLUSION

The findings of this study demonstrate that red onion peel possesses the potential to effectively manage the growth and spread of several plant pathogenic fungi. This has significant implications for ensuring food security, promoting environmental safety, and mitigating substantial economic losses within the agricultural industry. However, the exclusive use of red onion peel extract does not provide a definitive solution for the effective management of these fungal diseases. Therefore, it is crucial to implement comprehensive biocontrol measures in order to attain sustainable management of plant fungal diseases in Nigeria.

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