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### In vitro Assessment of the Antifungal Effects of Papaya Seed Extracts on Selected Fungal Pathogens Associated with Garden Egg Plants

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Garden egg (*Solanum aethiopicum*) is a nutritionally important vegetable widely cultivated in Africa, yet its production is significantly hampered by fungal pathogens that infect wounds and other plant tissues. This study evaluated the in vitro antifungal efficacy of *Carica papaya* (papaya) seed extracts against fungal species commonly associated with garden egg infections, including *Fusarium* spp., *Aspergillus niger*, and *Rhizopus stolonifer*. Methanolic and ethanolic extracts of papaya seeds were prepared and tested using agar diffusion and biomass inhibition methods. Fungal isolates were obtained from infected garden egg samples in Kogi State, Nigeria, and identified morphologically. The methanol extract showed superior inhibitory effects on fungal radial growth compared to the ethanol extract, with minimum inhibitory concentrations (MIC<sub>50</sub>) of 35% for *Fusarium* spp. and 40% for *A. niger*, while *Rhizopus* sp. was less sensitive. Both extracts significantly suppressed fungal growth, though commercial fungicide Benlate exhibited complete inhibition. The study highlights the potential of papaya seed extracts as affordable, eco-friendly, and safe alternatives to synthetic fungicides for managing fungal diseases in garden egg cultivation, promoting sustainable agriculture and reducing reliance on chemical pesticides.

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**INTRODUCTION**

*Solanum aethiopicum*, commonly known as African eggplant, is a vital horticultural crop widely consumed across Africa due to its rich nutrient content (Chuku & Emelike, 2013). A member of the Solanaceae family, the plant contains numerous small edible seeds, although some species may taste bitter because of their alkaloid and nicotinoid content, traits linked to their relation to tobacco. The fruits are often consumed as dessert, commonly paired with groundnuts in many African regions. Both fruits and leaves of garden egg plants are commonly used in traditional dishes and are valued for their medicinal properties, largely due to their phytochemical content. They are also rich in essential nutrients, including dietary fibre, folate, ascorbic acid (vitamin C), vitamins K, B6, and B3 (niacin), pantothenic acid, as well as minerals such as potassium, iron, magnesium, manganese, phosphorus, and copper. The vegetable is extensively cultivated across Africa and was introduced to other parts of the world, such as Brazil, via the transatlantic slave trade (Chinedu *et al.*, 2011).

In Nigeria, garden egg cultivation is predominantly practised in the northern and southern regions, exhibiting a variety of fruit colours, shapes, and sizes (Chinedu *et al.*, 2011). It thrives in hot, wet climates and provides a vital dietary component, especially for low-income populations during periods when other vegetables are scarce. However, the crop is highly vulnerable to several fungal diseases, such as damping off (caused by *Pythium*, *Phytophthora*, and *Fusarium* species), root rot (*Rhizoctonia* and *Sclerotium* species), blight (*Phomopsis* species), fruit rot (*Phomopsis vexans*

and *Rhizopus stolonifer*), and wilt (*Verticillium* and *Fusarium* species (Giuliani *et al.*, 2011). These infections are commonly introduced through contaminated seeds, soil, debris, and can be spread by wind, water, animals, tools, and human activities. Entry points for fungi include natural plant openings and wounds resulting from mechanical or biological damage (Chiejina *et al.*, 2013). Significant foliar diseases include downy mildew, powdery mildew, and white blister, while soilborne diseases are primarily caused by pathogens such as *Plasmidiophora brassicae* (clubroot), *Pythium*, *Fusarium*, *Rhizoctonia*, *Sclerotinia*, and *Sclerotium* species. (Barnett & Hunter, 1998).

Garden egg is extensively cultivated in Nigeria, especially in irrigated regions. Its high demand as a food source and its economic importance to local farmers make it a key crop (Bonsu *et al.*, 2008). However, fungal pathogens significantly hinder production by damaging plant tissues, leading to economic losses. Considerable resources are spent annually on disease diagnosis and control. Conventional fungicide poses risks to human health and the environment.

**Aim**

To evaluate the in vitro antifungal efficacy of papaya seed extract against fungal pathogens infecting garden egg plants, with a goal of reducing dependence on synthetic fungicides.

**Objectives**

- To isolate and identify fungal pathogens associated with garden egg plant diseases from the field.

- To extract phytochemical compounds from papaya seeds using methanol and ethanol solvents.
- To evaluate the antifungal effects of papaya seed extracts on the isolated pathogens using in vitro agar diffusion methods.

### Purpose

The purpose of this study is to assess the in vitro antifungal activity of *Carica papaya* (papaya) seed extracts against fungal pathogens commonly linked to garden eggplant wounds. Specifically, it aims to identify the fungi responsible for infections in garden egg, prepare and analyse methanolic and ethanolic extracts of papaya seeds, and assess their inhibitory activity against pathogens such as *Fusarium* spp., *Aspergillus niger*, and *Rhizopus stolonifer*. The study seeks to determine the effectiveness and minimum inhibitory concentrations (MICs) of these extracts as alternative, plant-based antifungal agents.

### Justification

Synthetic fungicides commonly used to control plant fungal infections are costly, potentially harmful to the environment, and pose health risks to both consumers and farmers. With the increasing demand for safer and eco-friendly alternatives, this study is justified in exploring the antifungal potential of papaya seed extracts, a natural, accessible, and underutilised resource. By identifying effective, plant-derived treatments for garden egg diseases, the study supports sustainable agriculture, reduces chemical input in food production, and promotes the use of biodegradable, low-toxicity plant-based disease management strategies in vegetable farming.

## MATERIALS AND METHODS

### Sample Collection

Infected garden egg samples were collected from gardens in Kogi State, Nigeria and transported in sterile containers to the laboratory for analysis.

### Sterilisation of Equipment

Adopting Aakanchha *et al.* (2020), all glassware, including Petri dishes, flasks, containers, slides, and coverslips, was thoroughly washed with detergent, rinsed with distilled water, air-dried, and then sterilised in a hot air oven at 160 °C for one hour.

### Sample Preparation and Isolation

Infected tissue sections were surface sterilised using 70% sodium hypochlorite for 1 minute, rinsed thrice in sterile water, and swabbed. The swab was rinsed in 10 ml peptone water. Serial dilutions (up to 10<sup>-6</sup>) were performed, and 10<sup>-2</sup>, 10<sup>-4</sup>, and 10<sup>-6</sup> dilutions were pour-plated on modified Potato Dextrose Agar (PDA) containing chloramphenicol. The plates were incubated at 28 °C for five days (Musa & Buashir, 2013).

### Fungal Identification

Distinct colonies were sub-cultured on fresh PDA to obtain pure cultures, which were then identified microscopically using International Mycological Institute guides (Iwuagwu *et al.*, 2013).

### Fungi Maintenance

Identified isolates were stored on PDA slants and in 10% sterile glycerol solution for future use (Thiyam *et al.*, 2013)

### Preparation of Papaya Seed Extracts

#### Seed Processing

Larson *et al.*, (2015) method was adopted with slight modifications. Papaya seeds were thoroughly washed with distilled water, oven-dried at 45 °C until a constant weight was achieved and then ground into a fine powder. The resulting powder was stored in airtight containers.

#### Methanol and Ethanol Extracts

For both extracts, 90 g of seed powder was soaked in 900 ml of the respective solvent for two weeks with constant shaking. The solutions were filtered,

evaporated at 40°C, air-dried, and stored at 4°C for further use (Larson *et al.*, 2015).

### In Vitro Antifungal Testing

#### Mycelial Growth Inhibition

Extract concentrations of 15, 20, 30, 35, 40, 45, and 50 g/100 ml were prepared using water as a solvent. Five ml of each extract was added to Petri dishes, followed by PDA, and mixed gently. After solidification, 5 mm mycelial discs of *Fusarium spp.*, *A. niger*, and *Rhizopus spp.* were placed at the centre. Plates were incubated at room temperature. Radial growth was measured and inhibition calculated using the Enio *et al.* (2012) formula. Benomyl (Benlate) served as the chemical control.

#### Minimum Inhibitory Concentration (MIC<sub>50</sub>)

Leaf extract (LE) was selected for MIC<sub>50</sub> testing due to its broad antifungal activity. *Rhizopus stolonifer* was excluded due to a lack of sensitivity. To determine the MIC<sub>50</sub> for *Fusarium spp.* and *Colletotrichum gloeosporioides*, a 20 mg/ml stock solution of the extract was prepared in 0.5% Tween 80 and then serially diluted to concentrations of 10, 5, 2.5, 1.25, and 0.625 mg/ml. Each dilution was inoculated with fungal spore suspensions and incubated at 180 rpm and 30 ± 3°C for 5 to 10 days. Biomass was assessed by centrifuging the cultures at 10,000 rpm and 5°C for 5 minutes, followed by drying at 80°C for 12 hours. Cell population (X) was determined using a microscope with a Petit-Salumbeni counting chamber.

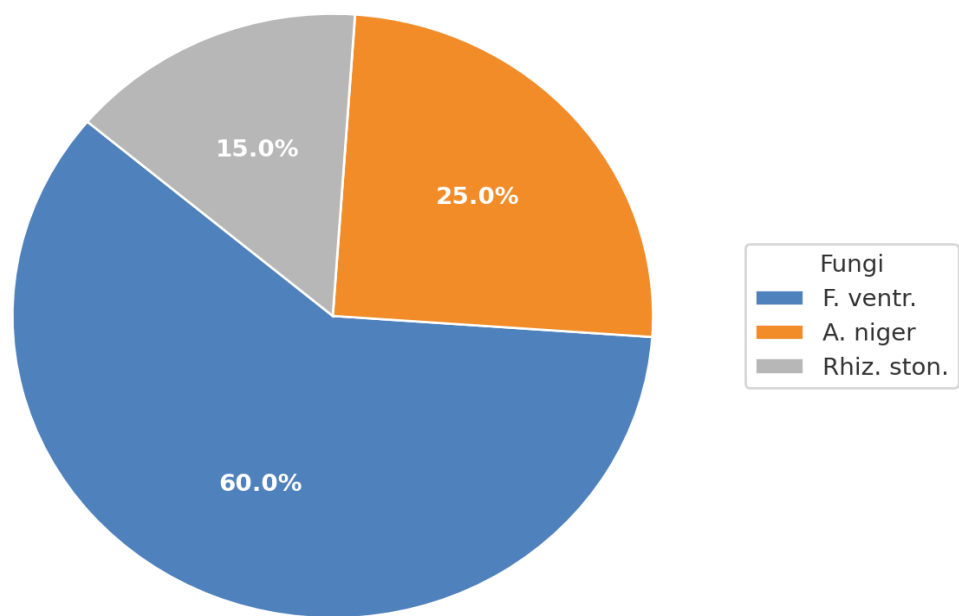
### RESULTS

**Table 1: Cultural and Morphological Characteristics of Isolated Fungi**

Characteristics	<i>Fusarium sp.</i>	<i>Aspergillus niger</i>	<i>Rhizopus stolonifer</i>
<b>Conidia</b>	Crescent or sickle-shaped, typically 3–5 septate.	Globose to subglobose, 3.5–5 µm in diameter; brown with warts, spines, or irregular ridges	Large, globular, initially white, turning black with age
<b>Texture</b>	Cottony to woolly, with dense aerial mycelium	Velvety to woolly, with dense conidiophore production	Fuzzy/fluffy, with extensive rhizoidal growth
<b>Spore Color</b>	White to cream initially, turning pink or purple with age	Initially white or yellow, becoming black as conidia mature	White cotton-like, turning black as sporangia mature
<b>Surface</b>	Smooth to slightly rough, with radial growth patterns	Smooth, with concentric rings and radial cracks on the reverse	Smooth, with radial growth and distinct zonation
<b>Zonation</b>	Absent or faint; reverse may show slight pigmentation	Deeply furrowed on the reverse, with radial cracks	Distinct zonate pattern, with concentric rings
<b>Reverse Color</b>	Cream to pale yellow	Yellow to pale yellow	Black to dark brown
<b>Mycelium Type</b>	Aerial mycelium with sporodochia; chlamydospores present in some species	Aerial mycelium with conidiophores; conidia produced in dense heads	Coenocytic hyphae with stolons and rhizoids; sporangia borne on sporangiophores
<b>Type of Reproduction</b>	Asexual (conidial and chlamydospore formation); sexual reproduction in some species	Asexual (conidial formation); sexual reproduction via cleistothecia in some species	Asexual (sporangial formation); sexual reproduction via zygospores

Characteristics	Fusarium sp.	Aspergillus niger	Rhizopus stolonifer
Septation	Septate hyphae; chlamydospores may be septate	Septate hyphae; conidiophores septate	Coenocytic hyphae; septa present only at reproductive structures
Presumptive Identification	Fusarium oxysporum or Fusarium solani complex; species identification requires molecular techniques	Aspergillus niger; identification confirmed by conidial morphology and molecular methods	Rhizopus stolonifer; identification based on sporangial and rhizoidal morphology

**Figure 1: Percent Occurrence of Fungal Isolates From Garden Egg Wounds**  
Proportion of Fungal Species



The *in vitro* assessment of *Carica papaya* seed extracts, using methanol and ethanol as solvents, was conducted to evaluate their effects on the radial growth of isolated fungal species. The antifungal agents demonstrated a highly significant impact on fungal radial growth at **P ≤ 0.01**. However, treatment with the ethanol extract at a concentration of 15g/100ml did not lead to a significant reduction in radial growth for any of the isolates at **P ≤ 0.05**.

As shown in Tables 2 and 3, the methanol extract exhibited greater efficacy in inhibiting the mycelial

growth of all fungal species compared to the ethanol extract, although its effectiveness was still inferior to that of a commercial antifungal agent. At a 35% concentration, methanol extract completely inhibited the growth of *Fusarium* species, while only slightly inhibiting the growth of *A. niger* and *Rhizopus* species. Conversely, a 40% concentration of the extract was required to inhibit the growth of both *Fusarium* and *A. niger*, with *Rhizopus* showing no inhibition at this concentration. The commercial antifungal agent Benlate entirely suppressed the



growth of all three fungal pathogens (Table 3), effectively controlling their development.

The Minimum Inhibitory Concentration (MIC) of the methanol extract was 35% for *Fusarium* sp.,

40% for *A. niger*, and 0% for *Rhizopus* sp., while the MIC of the ethanol extract was 40% for both *Fusarium* sp. and *A. niger*, and 0% for *Rhizopus* sp.

**Table 2: Effects of Methanol on the Isolated Fungi**

Extract (g/100ml)	<i>Fusarium</i> sp. (mm)	<i>A. niger</i> (mm)	<i>Rhizopus</i> sp. (mm)
15	22	27.3	32
20	18	24.7	29.4
25	16.5	20.5	29.0
30	7.4	5.7	25.3
35	0.0	3.5	25.2
40	0.0	0.0	20.5
10 (Benlate)	0.0	0.0	0.0

**Table 3: Effects of Ethanol on the Isolated Fungi**

Extract (g/100ml)	<i>Fusarium</i> sp. (mm)	<i>A. niger</i> (mm)	<i>Rhizopus</i> sp. (mm)
15	31.6	27.3	36
20	28.3	25.2	32.3
25	23.5	23.3	26.4
30	14.4	13.4	22.3
35	9.0	11.4	19.5
40	0.0	0.0	15
10 (Benlate)	0.0	0.0	0.0

## DISCUSSION

This study revealed a mixed association of fungal isolates in wounds and lesions of garden egg (*Solanum melongena*). The presence of fungi in these tissues is likely due to the ubiquitous nature of microorganisms. This finding aligns with the report by Nasiru and Dalhatu (2020), who identified several fungal species—*Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifera*, *Mucor racemosus*, and *Microsporium audouinii*—from garden eggs. These researchers also reported post-harvest mould in storage conditions involving mixed fungal species such as *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus*, and *Alternaria*.

In the present study, *Fusarium ventricosum* was the most frequently isolated fungus, which is somewhat unexpected given that *A. niger* has often been

reported as the most prevalent species in previous literature (Samuel et al., 2017). The high frequency of *Fusarium* in this case may be due to its natural occurrence in the soil, with potential transmission to plants via rain splash. As shown in Figure 1, *Fusarium* dominated the mixed fungal cultures responsible for the disease in garden egg, at least in this study. This supports the conclusion that *Fusarium* spp. are major fungal agents involved in *Fusarium* wilt of garden egg (*S. melongena*). Consequently, the hypothesis that *Aspergillus* predominates in such environments is rejected. This finding agrees with Bello et al. (2015), who studied *Fusarium* wilt of garden egg in Imawa Village, Kura Local Government, Kano State, Nigeria.

The significant inhibitory effect of antifungal agents on fungal isolates in this study indicates that all

tested agents could suppress the growth of the three fungal species *in vitro*. This observation agrees with the findings of Giovanni *et al.* (2019), who reported complete inhibition of fungal isolates by methanol extracts of *Carica papaya* seeds. It also agrees with the work of Assefa *et al.* (2011) that fungicides such as benomyl, carbendazim, carboxin, maneb, methoxymethyl mercury chloride, prochloraz, tebuconazole, and thiram were effective in controlling Fusarium basal rot (*Fusarium oxysporum* f. sp. *cepae*) in shallots when applied as fungicidal bulb treatments.

Although ethanol extracts of *C. papaya* seeds did not completely inhibit fungal isolates at lower concentrations, they still exhibited significant antifungal activity. This supports previous findings by Afolabi and Kehinde (2019), who documented the effectiveness of *C. papaya* in controlling seed-borne fungi in agricultural crops. The observed negative and significant correlation between fungal radial growth and inhibition confirms the suppressive action of the antifungal agents. Furthermore, the positive and significant correlation among the inhibition rates of the three fungal isolates suggests broad-spectrum activity of the extracts. This implies that suppression of one fungal species is likely accompanied by the inhibition of others. Karavaev *et al.* (2018) similarly reported a correlation between the use of plant extracts for fungal suppression and their protective action on wheat seedlings.

## CONCLUSION

This study demonstrates that both methanolic and ethanolic extracts of *Carica papaya* seeds possess the ability to inhibit fungal pathogens associated with infections in garden egg plants. These extracts are cost-effective, non-toxic, biodegradable, and generally recognised as safe (GRAS), making them viable alternatives to synthetic fungicides for managing seed-borne fungi and other plant pathogens. Although the specific antifungal compounds in *C. papaya* seed extracts are not fully characterised, flavonoids, alkaloids, and terpenes

are believed to play key roles in their antifungal action by interacting with fungal membrane components. Therefore, both methanol and ethanol extracts of *C. papaya* seeds are promising sources of bioactive secondary metabolites with potent antifungal properties.

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