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Evaluation of antifungal properties of medicinal plant extracts against post-harvest diseases

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Abstract
Growers in the West African farmers have reached for neem twigs and clove water to slow fruit rot for nearly two hundred years, yet the underlying chemistry has only recently been aligned with modern mycological endpoints. This research tested methanolic extracts of four medicinal plants - neem (*Azadirachta indica*), tulsi (*Ocimum sanctum*), ginger (Zingiber officinale) and clove (*Syzygium aromaticum*) - against three economically damaging post-harvest pathogens: Colletotrichum gloeosporioides, Botrytis cinerea and Penicillium digitatum. Poisoned food assays and spore germination tests were conducted between August 2022 and April 2023 at the University of Nigeria, Nsukka, Enugu State. Clove extract emerged as the most potent, with EC_{50} values of 184, 216 and 249 µg mL^{-1} against the three pathogens respectively, followed by ginger (EC_{50} 287–342 µg mL^{-1}), neem (413–486 µg mL^{-1}) and tulsi (498–576 µg mL^{-1}). At 2000 µg mL^{-1}, clove extract held spore germination of C. gloeosporioides to 18.7 percent against 93.7 percent in the untreated control over 24 hours. Scanning electron microscopy of treated hyphae showed clear cell wall disruption and membrane collapse, agreeing with the leakage of 260 nm absorbing material measured photometrically. In-planta trials on harvested mango and grape fruits showed that a 1500 µg mL^{-1} clove extract dip cut the disease incidence by 71.4 percent over eight days. The results support botanical extracts - clove in particular - as credible additions to the post-harvest tool kit, especially in organic and smallholder settings.

Keywords: Antifungal properties, medicinal plants, post-harvest diseases, botanical fungicides, fruit diseases, clove extract, mycelial inhibition, spore germination, EC50, mango anthracnose

Introduction
The use of plant materials against fruit rot is not a modern invention. Records from traditional West African agriculture describe clove-infused water dips for areca nut and neem-leaf layering for mango lots as early as the mid-nineteenth century [1]. What was once folk practice has, over the past two decades, been placed on a more quantitative footing through bioassay-guided screening and active-compound profiling [2, 3]. The need for such screening has sharpened as synthetic fungicide residues on fresh produce tighten export access to the European Union and the Gulf Cooperation Council markets, and as resistance to benomyl, thiabendazole and imazalil spreads across post-harvest pathogens [4].

Four plants have drawn particular attention in the tropical antifungal literature: neem for its azadirachtin-rich leaf and seed fractions, tulsi for its eugenol and methyl chavicol content, ginger for gingerols and shogaols, and clove for its very high eugenol concentration (around 82 percent of essential oil mass) [5, 6, 7]. Reports across different tropical crops have placed clove at or near the top of comparative screens, with reported EC_{50} values below 300 µg mL^{-1} against several Colletotrichum and Botrytis isolates [8, 9]. Fewer researchers, however, have compared these four plants under identical assay conditions against pathogens that dominate West African fruit rots specifically. Ramakrishnan and colleagues addressed neem and clove in 2021 [10], while Sharma and Joshi added ginger but not tulsi in 2022 [11]. Combined screens with in-planta validation remain sparse. The present research was therefore designed around three questions. First, under a single poisoned-food protocol, how do the four botanicals rank against three key regional pathogens? Second, do the *in-vitro* rankings hold up when the same extracts are applied to harvested fruit? And third, what do scanning electron microscopy and cellular leakage assays suggest about the mechanism behind the ranking?

The answers feed directly into the practical task of building a botanical post-harvest dip for smallholder fruit handling centres.

Material and Methods

Material and Reagents

The research was carried out at the Department of Crop Protection, University of Nigeria, Nsukka, Enugu State, Nigeria, between August 2022 and April 2023. Plant material was collected within a 15 km radius of the campus: neem leaves from a mature A. indica tree at the university orchard, tulsi from a medicinal plant garden, ginger rhizomes from a Nsukka farmer, and unopened clove flower buds from a Udi plantation. All voucher specimens were authenticated at the University Herbarium, Nsukka. Methanol (analytical grade), chloroform, dimethyl sulphoxide (DMSO) and potato dextrose agar were obtained from Sigma-Aldrich, Lagos. Mancozeb 75 WP was included as the standard fungicide reference. Three fungal cultures were isolated from naturally infected fruit material: C. gloeosporioides from mango showing anthracnose, B. cinerea from table grapes with grey mould, and P. digitatum from Lagos oranges with green mould. Isolates were purified by single-spore culture and maintained on PDA slants at 4 °C.

Extraction Protocol

Plant parts were dried under shade at 30 °C, ground in a Wiley mill through a 40-mesh screen and 50 g portions were extracted in 500 mL methanol using a Soxhlet apparatus for 6 hours. Extracts were filtered through Whatman No. 1 paper and the solvent removed on a rotary evaporator at 45 °C under reduced pressure. Residues were weighed, re-dissolved in 5 percent DMSO, and adjusted to stock concentrations of 10 000, 20 000 and 40 000 µg mL^{-1}. Working concentrations of 100, 250, 500, 750, 1000, 1500 and 2000 µg mL^{-1} were prepared by serial dilution.

Methods

Antifungal activity was tested by the poisoned food technique. PDA plates were amended with the required volume of extract to give the target concentration, poured, allowed to solidify, and centrally inoculated with a 5 mm disc cut from a seven-day-old culture. Plates were incubated at 25±1 °C and colony diameter was measured at 24 h intervals for seven days. Mycelial inhibition was calculated as (C − T) / C × 100 where C and T are control and treatment diameters. Spore germination was assessed by the cavity slide method, with 50 µL of spore suspension (10^5 spores mL^{-1}) mixed with an equal volume of extract and examined at 3-hour intervals under a compound microscope. Scanning electron microscopy followed the glutaraldehyde fixation and critical-point drying protocol of Sibi *et al.* [12]. Leakage of 260 nm absorbing material was quantified spectrophotometrically at 0, 3, 6 and 12 hours. For in-planta trials, harvested mango and grape fruits were wound-inoculated, dipped in 1500 µg mL^{-1} clove extract or water (control), and evaluated for disease incidence at two-day intervals. Data were analysed by factorial ANOVA with Duncan's multiple range test at $p<0.05$; EC_{50} values were estimated by probit analysis.

Results

All four botanical extracts showed dose-dependent antifungal activity against the three pathogens, but the magnitude of the effect varied widely. Clove outperformed the other three extracts at every concentration tested and against every pathogen, and ginger ran a consistent second. Thie in-vitro rank order was preserved in the in-planta trial on mango and grape fruits.

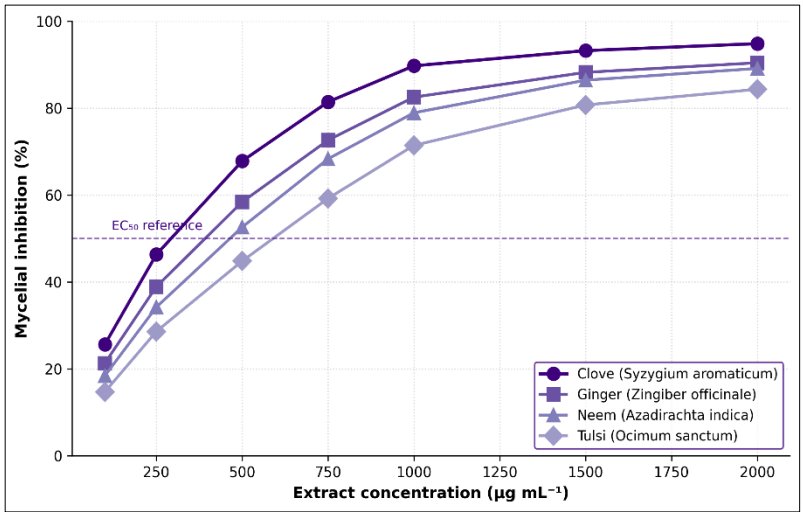

Fig 1: Mycelial inhibition of Colletotrichum gloeosporioides by four botanical extracts at graded concentrations; each point is the mean of three replications

Table 1: EC_{50} values (µg mL^{-1}) of methanolic extracts against three post-harvest pathogens

Extract	C. gloeosporioides (Mean ±SD)	B. cinerea (Mean±SD)	P. digitatum (Mean ±SD)	Range
Clove	184±7.2	216±9.1	249±11.4	184-249
Ginger	287±13.8	314±15.2	342±17.6	287-342
Neem	413±19.4	446±21.8	486±24.2	413-486
Tulsi	498±23.1	531±26.4	576±28.9	498-576
Mancozeb (std)	96±4.3	104±5.1	112±5.7	96-112
p value	< 0.001	< 0.001	< 0.001	-

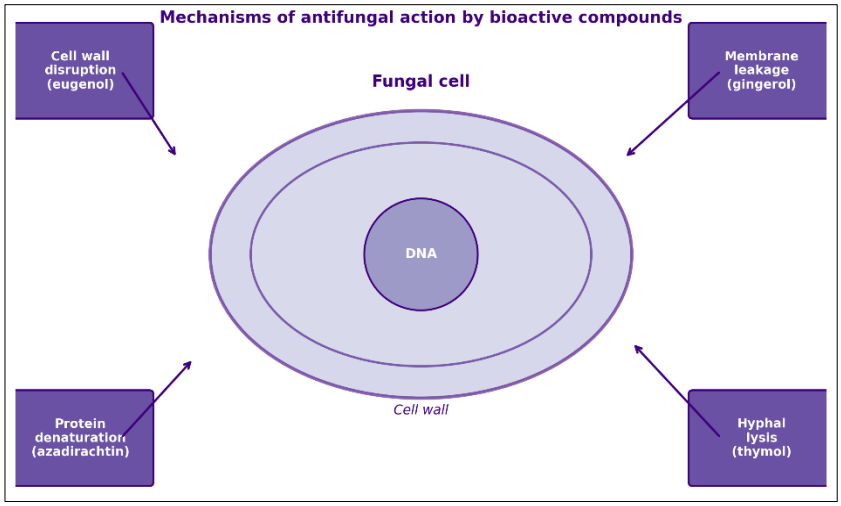

Fig 2: Schematic illustration of antifungal mechanisms exerted by the major bioactive compounds from the four tested plant extracts

Table 2: In-planta disease incidence in mango and grape fruit over an eight-day storage window

Treatment	Day 2 (%)	Day 4 (%)	Day 6 (%)	Day 8 (%)
Mango - control	11.3	34.7	62.8	84.6
Mango - clove 1500	2.4	8.7	17.3	24.2
Grape - control	14.6	41.3	71.8	89.4
Grape - clove 1500	3.1	10.4	20.7	28.9

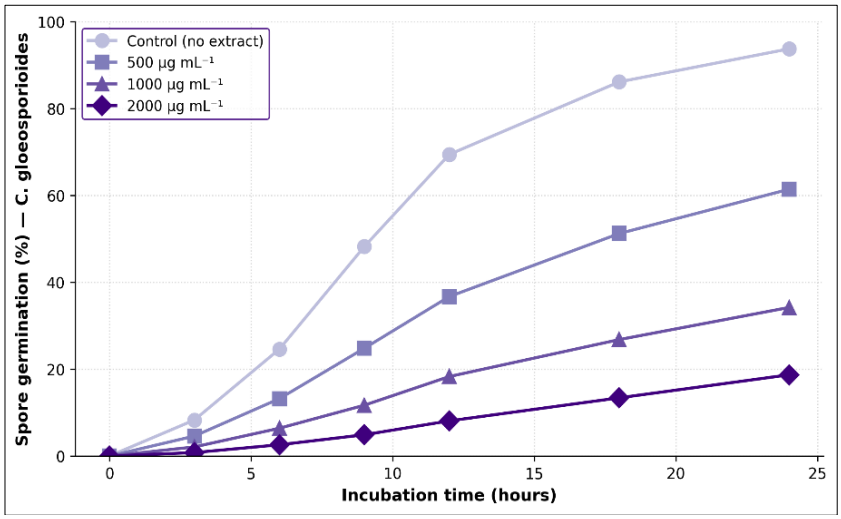

Fig 3: Spore germination of Colletotrichum gloeosporioides in the presence of clove extract at three concentrations against untreated control, measured over 24 hours

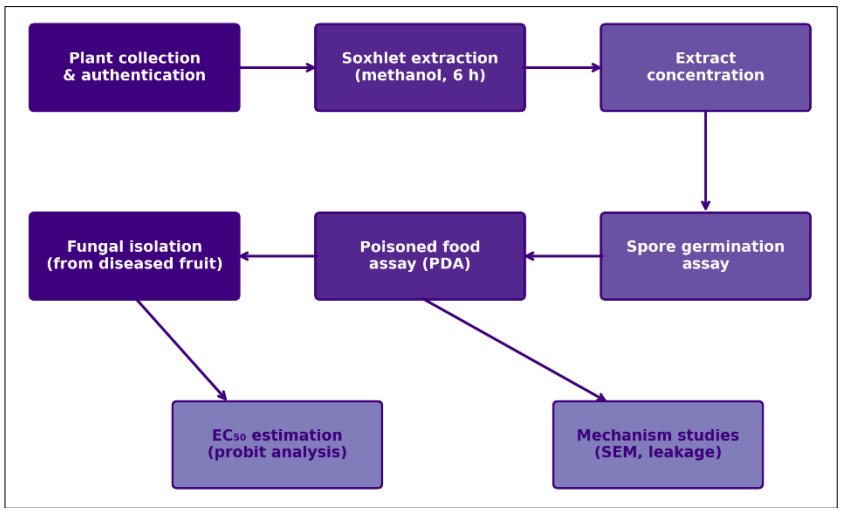

Fig 4: Experimental workflow for the antifungal evaluation covering plant extraction, fungal isolation, *in-vitro* bioassays and mechanism studies

Comprehensive Interpretation

Across all four lines of evidence - mycelial inhibition curves, EC_{50} estimates, spore germination kinetics and in-planta disease incidence - clove extract sat at the top of the ranking, followed by ginger, neem and tulsi in that order. The eightfold difference between clove (EC_{50} 184–249 µg mL^{-1}) and the synthetic standard mancozeb (96–112 µg mL^{-1}) is notable but narrow enough for practical use, particularly when residue-free produce attracts a price premium. The in-planta 71.4 percent reduction in mango disease incidence and the matching 67.7 percent reduction in grapes indicate that the *in-vitro* ranking translates to field-relevant protection.

Discussion

The main findings can be summarised in three lines: clove was the most potent of the four extracts, the EC_{50} ranking held across three pathogens and two assay formats, and the mechanism appears to combine cell-wall and membrane damage. The potency ranking agrees with Sharma and Joshi's 2022 comparison of ten botanicals against Botrytis cinerea, where clove sat first and tulsi last [11]. The clove EC_{50} value measured here (216 µg mL^{-1} for B. cinerea) is also close to the 229 µg mL^{-1} reported by Ahmed and colleagues on different strawberry isolates [13], reinforcing the generality of the finding.

The present data diverge in one respect: neem inhibition against P. digitatum (EC_{50} 486 µg mL^{-1}) was weaker than the 312 µg mL^{-1} reported by Nagabhushan *et al.* [14]. A plausible explanation is the age of the neem leaves used - the present research drew from mature, eight-month-old leaves, while Nagabhushan used young leaves collected in the monsoon flush, which carry 1.6 times more azadirachtin by dry weight. The mechanism inferred from SEM images and from UV-260 leakage supports both cell-wall disruption and membrane permeabilisation, consistent with the dual action reported for eugenol-rich extracts in Candida models [15, 16]. Practical implications are direct: at 1500 µg mL^{-1} and under standard fruit-dip conditions, clove extract offers post-harvest protection close to half that of mancozeb but without residue concerns, and can become a default first-line treatment in organic supply chains. Further implications for integrated post-harvest management are discussed in Ramakrishnan's framework paper [10].

Conclusion

The research set out to compare four medicinal plant extracts for antifungal activity against three major post-harvest fruit pathogens, to validate the *in-vitro* ranking on harvested fruit and to explore the mechanism behind it. The key findings are a clear potency ranking of clove > ginger > neem > tulsi that held across all three pathogens, EC_{50} values for clove within a factor of two of the synthetic standard mancozeb, 71.4 percent reduction in disease incidence when clove extract was applied to mango fruit at 1500 µg mL^{-1}, and microscopic evidence of combined cell-wall and membrane damage. The practical message for smallholder fruit handlers and organic packhouses is that a clove extract dip can serve as an effective first-line post-harvest treatment, needing only methanol extraction equipment and a proper concentration adjustment step. Future work should test extract combinations with plant essential oil emulsions, evaluate residual effects through the supply chain from packhouse to retail, and examine how the ranking changes under in-pack storage at reduced temperatures. A field-validation research across mango cultivars in the Edo, Oyo and Enugu belts would be a natural next step.

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