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Vitro Evaluation of the Inhibitory Effects of Neem (*Azadirachta indica*) Leaf Extract and Salicylic Acid on the Mycelial Growth of Tomato Rot Fungi and Phytochemical Analysis of Neem Leaf Extract

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ABSTRACT **Original Research Article**

This study evaluated the in vitro inhibitory effects of neem (*Azadirachta indica*) leaf extract and salicylic acid on the mycelial growth of fungi associated with tomato rot and determined the phytochemical constituents of neem leaf extract. Fungi were isolated from rotten tomato fruits, and their occurrence and pathogenicity were assessed. The antifungal activities of different concentrations of neem leaf extract and salicylic acid were evaluated using the pour plate method, while phytochemical constituents were identified through qualitative screening procedures. Four fungal species were isolated: *Colletotrichum gloeosporioides* (37.78%), *Aspergillus niger* (25.75%), *Botryoploida theobromae* (18.61%) and *Penicillium digitatum* (17.86%). The reported pathogenicity results showed that all four isolates caused decay in inoculated tomato fruits. The inhibitory effects of neem leaf extract and salicylic acid increased with concentration and incubation time. On Day 6, 30% neem leaf extract produced 78.72% inhibition, while 30 g/L salicylic acid produced 77.41% inhibition of *C. gloeosporioides*. The combination of 30% neem leaf extract and 30 g/L salicylic acid produced the highest reported inhibition of 84.15%. Phytochemical screening indicated high concentrations of alkaloids, cardiac glycosides, glycosides, flavonoids and terpenoids, while steroids, saponins, tannins, triterpenes, phytosterols and phenols were moderately present. Anthraquinones, anthocyanins, phlobatannins and amino acids were reported absent. The findings suggest that neem leaf extract and salicylic acid have potential as treatments for suppressing tomato rot fungi under in vitro conditions. Further studies are required to validate their effectiveness, safety and practical application under postharvest storage conditions.

Keywords: Neem Leaf Extract, Salicylic Acid, Tomato Rot Fungi, Mycelial Growth Inhibition, Phytochemical Screening, *Azadirachta indica.*

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Introduction

Tomato (*Solanum lycopersicum* L.), formerly known as *Lycopersicon esculentum* Mill, is an economically important vegetable widely consumed fresh and processed. It provides essential nutrients and serves as a source of income and employment for farmers and other stakeholders in the agricultural industry (Teka, 2013; Tola, 2014). However, its high moisture content makes it highly susceptible to fungal infections and rapid deterioration during storage, transportation, and marketing (Baloch et al., 2013; Haggag, 2010).

Postharvest fungal diseases are major causes of tomato losses, particularly in developing countries such as Nigeria, where inadequate storage facilities, poor handling, and transportation difficulties contribute to fruit deterioration. These losses reduce product quality, market value, and farmers' income (Mohammed & Aferwork, 2018). Although synthetic fungicides are commonly used to control fungal

infections, concerns about chemical residues, environmental pollution, human health risks, and fungal resistance have increased interest in safer, plant-based alternatives (Kim et al., 2017; Nicolopoulou et al., 2016).

Neem (*Azadirachta indica*) is a medicinal plant containing phytochemicals such as flavonoids, phenolic compounds, alkaloids, and tannins, which may possess antimicrobial properties. Previous studies have reported that Neem leaf extract can inhibit spoilage fungi and help preserve tomato quality during storage. Salicylic acid has also attracted interest for its potential to suppress plant pathogens and enhance plant defence responses. However, their inhibitory effects may vary depending on the concentrations used and the fungal species involved.

Therefore, this study evaluates the in vitro inhibitory effects of Neem leaf extract and different concentrations of salicylic acid on the mycelial growth of fungi associated with tomato rot. It also investigates the phytochemicals present in Neem leaf extract to provide information on its potential as a botanical alternative for managing postharvest fungal pathogens.

Statement of the Problem

Postharvest fungal infections cause significant deterioration of tomato fruits, resulting in economic losses for farmers, traders, and consumers. The use of synthetic fungicides to control these pathogens raises concerns about chemical residues, environmental safety, human health, and the development of fungicide resistance. Furthermore, limited access to effective and affordable postharvest disease-control technologies remains a challenge for many farmers in Nigeria. Neem leaf extract offers a potential plant-based alternative because of its phytochemical constituents, while salicylic acid may contribute to suppressing fungal growth. However, their inhibitory effects on tomato rot fungi at different concentrations require further investigation. Information on the phytochemicals present in Neem leaf extract is also important for understanding its potential antifungal activity.

This study therefore examines the in vitro effects of Neem leaf extract and different concentrations of salicylic acid on the mycelial growth of tomato rot fungi and identifies the phytochemicals present in Neem leaf extract.

Aim and Objectives

The aim was to test in Vitro Evaluation of the Inhibitory Effects of Neem (*Azadirachta indica*) Leaf Extract and Salicylic Acid on the Mycelial Growth of Tomato Rot Fungi and Phytochemical Analysis of Neem Leaf Extract

Objectives of the Study

The objectives were to:

1. Test in vitro the inhibitory effect of Neem leaf extract and salicylic acid concentration on the mycelial growth of plant pathogenic fungi associated with tomato rot.
2. Identify the phytochemicals present in the Neem leaf biofungicide.

Literature review

Tomato (*Lycopersicon esculentum* Mill.), currently classified as *Solanum lycopersicum* L., is an economically important vegetable originating from South America and widely cultivated worldwide (Abid et al., 1995; Agrios, 2005). However, its high moisture content and soft texture make it highly susceptible to postharvest fungal deterioration, resulting in reduced quality, market value, and shelf life.

Several fungi are associated with postharvest tomato rot, including *Colletotrichum gloeosporioides*, which causes anthracnose; *Lasiodiplodia theobromae*, associated with stem-end rot; *Alternaria alternata*, responsible for black rot; and species of *Aspergillus* and *Rhizopus*, which cause fruit decay (Hu et al., 2014; Islam et al., 2018; Yahia, 2005). These pathogens may infect fruits before harvest or enter through wounds during harvesting, handling, and transportation. Some remain dormant until ripening, when physiological changes favour fungal growth, leading to tissue breakdown and postharvest losses (Prusky et al., 2006).

Synthetic fungicides are commonly used to control postharvest fungal diseases. However, concerns about chemical residues, environmental pollution, human health, and fungal resistance have encouraged interest in plant-based alternatives (Ademe & Ayalew, 2013; Sivakumar et al., 2021). Neem (*Azadirachta indica* A. Juss), a member of the family Meliaceae, contains bioactive compounds with potential antimicrobial properties. Reported constituents include azadirachtin, nimbin, nimbolide, quercetin, and other secondary metabolites (Mondall et al., 2009; Oliveira et al., 2018). Onyeani et al. (2012) reported that Neem leaf extract completely inhibited the mycelial growth of *Rhizopus stolonifer*, while Ezeonu et al. (2018) demonstrated the inhibitory effects of Neem extracts against fungi associated with cocoyam rot. These findings suggest that Neem leaf extract may be useful in controlling postharvest fungal pathogens.

Materials and Methods

Experimental Location

The research was carried out in the Advanced Chemistry Laboratory of the Rev. Fr. Moses Orshio Adasu University, Makurdi, Nigeria, in 2024. Makurdi is located in North Central Nigeria along the Benue River between latitude 07° 44′ 28″ N and longitude 08° 32′ 44″ E. It is situated within the Benue Trough at the Lower Benue Valley and found in the Guinea savanna region.

Sources of Materials for the Study

Freshly harvested tomatoes, namely Roma VF, of uniform sizes and maturity and with absence of visual symptoms of

diseases and defects were obtained from the College of Agriculture Yandev farm in Gboko and taken to the Advanced Chemistry Laboratory in Makurdi.

Fresh Neem leaves (*Azadirachta indica*) were harvested from Rev. Fr. Moses Orshio Adasu University.

Synthetic fungicide, namely salicylic acid, was purchased from an agrochemical shop in Makurdi.

Preparation of Experimental Materials

Preparation of Tomato Fruits

Mature and wholesome tomato fruits of uniform sizes were selected for the experiments. The selected fruits were washed under running tap water and allowed to dry at room temperature (about 30 °C; Babiya & Dahiru, 2006) to get rid of surface contaminants and opportunistic microbes.

Preparation of Neem Leaf Extract

Neem leaf extract was prepared following procedures as described by Tunwari et al. (2019). A 500 g sample of washed and disinfected fresh Neem leaves was air-dried, chopped using sharp sterilized knives into a mortar, and a pestle was used to pound and grind it into a fine powder using a blender. Samples of 0 g, 100 g, 200 g, and 300 g of the Neem powder were dipped in 0%, 10%, 20%, and 30% (w/v), respectively, as described by Gupta and Jain (2014).

Data Collection

Storage data were collected at 2-week intervals. The parameters in the storage experiment studied include the following:

Environmental Parameters.

Temperature (°C) and relative humidity (%) in the storage room were measured three times a day (06:00, 14:00, and 20:00 hr) and three times a week throughout the storage period. These times were chosen for the even distribution of measurements. The temperature and humidity readings were taken using a digital thermo-hygrometer.

Physiological Parameters.

Postharvest weight loss, percentage tomato decay, and shelf life of tomato were evaluated during the storage period.

Postharvest Weight Loss (%).

The tomato fruits weight loss was measured using an electronic weighing scale balance and the postharvest weight loss (PWL) was determined using the formula below:

$$\text{Postharvest Weight Loss } (\%) = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

Total Soluble Solids (TSS; °Brix).

Total soluble solids were determined using a handheld refractometer and recorded in degrees Brix, AOAC (2007). A homogenous sample was prepared by blending the tomato fruits in an electric blender for 1 min and after which two drops of the juice was carefully dropped on a refractometer with the help of a plastic dropper and readings recorded directly in degrees Brix.

Titratable Acidity (TA, %).

Titratable acidity (expressed as citric acid %) content of the fruit was determined by titrating 10 ml of clear juice against 0.1 ml sodium hydroxide (NaOH), using phenolphthalein as an indicator which was added in a drop-wise manner until a pink colour was indicated after vigorous shaking (Liamngee et al., 2018b). The citric acid content of tomato fruit sample was calculated using the formula:

$$\text{Titratable Acidity } (\%) = \frac{V \times M \times F}{\text{Volume of Tomato Juice}} \times 100$$

Where V = volume of 0.1 N NaOH used, M = molarity of NaOH, and F = factor of citric acid (0.0064) (AOAC, 2007).

Ascorbic Acid/Vitamin C Content (mg/100 g).

Ascorbic acid was determined using the method described by AOAC (1990) as reported by Liamngee et al. (2018b). Indophenol blue solution was standardized using vitamin C by shaking 3.0 ml of standard vitamin C solution (0.800 mg/ml) with 0.1% indophenol blue solution in a graduated cylinder until the reaction mixture changes to a blue or purple colour. The final volume of the reaction mixture was recorded and was used to calculate the molarity of indophenol. A 3.0 ml sample was introduced into a graduated cylinder and while shaking, indophenol solution was added until the reaction mixture changed to a blue or purple colour. The final volume was recorded and the concentration of vitamin C in the sample was calculated and expressed in mg/100 g using the formula:

$$\text{Molarity of indophenol} = \frac{\text{conc.Vit C} \times \text{Volume of Vit C}}{\text{Volume of indophenol}} \text{ x } 100 \ldots\ldots$$

Lycopene Content (mg/100 g).

Lycopene was determined using a spectrophotometric method consisting of a mixture of alcohol and water in a 1:1 ratio. The amount of lycopene extracted was the difference between absorbance at wavelength λ1 = 570 nm and absorbance at wavelength λ1 = 780 nm (Liamngee et al., 2018b). Total lycopene content in the samples was recorded using the formula (AOAC, 2007):

$$\text{Lycopene} = \frac{A\lambda 1 - A\lambda 2}{m} \times 100 \left(\frac{m}{100\text{g}}\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (10)$$

Beta-Carotene (mg/100 g).

Tomato fruits were cut in small slices and ground into fine paste using an electric blender for 1 min. A 10 ml volume of the tomato juice was transferred into a beaker and 10 ml of petroleum ether was added and the resultant solution was shaken thoroughly for 1 min. The solution was filtered through Whatman filter paper and the filtrate was taken for spectrophotometric determination. Sample absorbance was measured at 451 nm and beta-carotene was calculated using the formula given by AOAC (2007):

βcarotene = A451× 19.96 $\left[\frac{mg}{100g}\right]$ …… (7)

βcarotene = A_{451}× 19.96 [mg/100g]

Where: A_{451} = absorbance at 451 nm

19.96 = extinction coefficient

Fruit Firmness (N/cm).

Firmness of tomato fruits was measured using a handheld penetrometer containing a standard probe which was allowed to penetrate into the fruit with maximum penetration force (N). The registered force at penetration of the standard probe up to a certain depth (cm) gave the textural property read as firmness (Jha et al., 2013).

Postharvest Decay (%).

The number of decayed fruits was sampled at 5-day intervals of storage and percentage decay was calculated using the formula:

$$\textit{Percentage (\%) decay} = \frac{\text{number of fruits decaying}}{\text{total number fruits/unit}} \times 100 \ldots (8)$$

Fruit Shelf Life (Days).

Fruit shelf life was calculated by counting the number of days from the beginning of storage until when above 50% of fruit samples per treatment plot or replicate had completely deteriorated (Shrestha et al., 2018).

Percentage Marketability.

Data on the marketability of tomato fruits was based on the proportion of marketable and unmarketable fruits at the time of disease assessment following the method of Alemu et al. (2014) using descriptive quality attributes such as the level of browning lesion, shrivelling, smoothness, and shininess of the fruit. Percentage of marketable fruits was calculated using the formula:

$$\%\ \text{Marketability of tomato fruits} = \frac{\text{number of marketable}}{\text{total number of fruits/unit}} \times 100 \ldots\ldots (9)$$

Experiment 1: In Vitro Efficacy of Neem Leaf Extracts on the Radial Growth Inhibition of Isolated Fungi Pathogens

The antifungal activities of the Neem leaf extracts against fungi pathogens were tested in vitro using the pour plate method described by Liamngee et al. (2015). Two milliliters each of 0%, 10%, 20%, and 30% of the respective crude aqueous plant extracts of Neem leaf was put in test tubes then 15 ml of molten PDA was added and the mixture was autoclaved at 121 °C under pressure of 15 lb for 15 min. The sterilized plant extract/PDA media was dispensed into separate sterile Petri dishes and the mixture was swirled gently on the work bench and was allowed to set. Mycelium discs measuring 5 mm was obtained from 5-day-old pure cultures and inoculated at the centre of the extract/PDA media in the Petri dishes. Each set of experiment was replicated three times. The control plates contained fresh PDA and inoculated organism only. Plates were arranged in the laboratory in a CRD in a 3 × 6 factorial. Plates were incubated at 25–30 °C until the mycelial growth of the fungal pathogen completely covers the surface of the agar medium in the control plates. The antifungal property of each concentration of plant extract was measured in relation to the mycelial growth of the pathogen on PDA. A measuring rule was used to measure the percentage of linear growth of fungal colony of the pathogen in relation to the control plates using the formula:

$$\textit{Linear Growth Reduction}\ (\%) = \frac{R1 - R2}{R1} \times 100$$

Where, R_1 = Fungi growth in control

R_2 = Fungi growth in treatment.

Experiment 2: Phytochemical Analysis of Neem Leaf Extracts

Tannin Test (Gelatin Test)

Into 2 ml each of Neem leaf extract was added 1% gelatin solution containing sodium chloride. The formation of white precipitate indicated the presence of tannins (Tamokou et al., 2017).

Test for Alkaloids (Hager's Test)

Hager's reagent (saturated picric acid solution) was added into separate beakers each containing 5 ml of aqueous extracts of Neem leaf. The presence of a yellow coloured precipitate confirmed the presence of alkaloids (Tamokou et al., 2017).

Test for Saponins (Foam Test)

Aqueous extracts of Neem leaf of 2 ml each were diluted with 5 ml of distilled water and the mixture was shaken. The formation of persistent foam that lasted for 10 min confirmed the presence of saponins (Biswas et al., 2013).

Test for Flavonoids (Shinoda Test)

Aqueous extracts of Neem leaf of 5 ml each were mixed with few fragments of magnesium ribbon and in a drop-wise manner, concentrated HCl was added. The appearance of a pink scarlet colour which appeared few minutes after, confirmed the presence of flavonoids (Yadav & Agarwala, 2011).

Test for Glycosides (Salkowski's Test)

Into 2 ml of chloroform was added 2 ml each of the respective plant extracts, followed by careful addition of 2 ml of concentrated H_2SO_4 and the mixtures were swayed gently. The appearance of a reddish-brown colour confirmed the presence of glycogen through the formation of a steroid ring (Biswas et al., 2013).

Test for Phenols (Sodium Hydroxide Test)

A 2 ml volume of the respective aqueous plant extracts was dissolved in 0.5 ml of 20% sulphuric acid solution. Followed

by the addition of few drops of aqueous sodium hydroxide solution, the mixture turned blue indicating the presence of phenols (Geetha & Geetha, 2014).

Test for Terpenoids

Crude extracts of Neem leaf of 2 ml each were dissolved in 2 ml of chloroform and evaporated to dryness. After that, 2 ml each of concentrated H_2SO_4 was added and heated for 2 min. Terpenoids were confirmed with the appearance of a grayish colour.

Test for Anthraquinones (Borntrager's Test)

A 5 ml volume of aqueous extracts of Neem leaf was put in separate dry test tubes and 5 ml of chloroform was added and shaken for 5 min. The mixtures were filtered and the filtrate shaken again with 10% ammonia solution. The appearance of a pink violet or red colour at the bottom of the test tubes indicated the presence of anthraquinones (Tamokou et al., 2017).

Test for Steroids (Salkowski's Test)

A 5 ml volume of aqueous extracts of Neem was dissolved in two separate beakers containing 2 ml of chloroform and equal volumes of concentrated sulphuric acid were added along each side of the respective test tubes. The presence of steroids was confirmed when the upper layer turned red and the bottom turned yellow with green fluorescence (Geetha & Geetha, 2014).

Triterpenes (Salkowski's Test)

Crude extracts of Neem of 5 ml each were dissolved in 5 ml of chloroform and filtered. A few drops of concentrated sulphuric acid were added into the filtrate and the mixtures were shaken and allowed to stand. The appearance of golden yellow colour confirmed the presence of triterpenes (Tamokou et al., 2017).

Data Analysis

The data recorded from the research were subjected to analysis of variance (ANOVA) using the GENSTAT Statistical Package (2015). Means were separated using Fisher's least significant difference (F-LSD) at the 5% level of probability. A *t* test was also used to separate means at the 1% and 5% levels of significance in the disease-causing potential of the fungal isolates.

Results

Four fungal species were isolated from rotten tomato fruits. *Colletotrichum gloeosporioides* was the most frequently isolated fungus, accounting for 37.78% of the 532 isolates, followed by Aspergillus niger (25.75%), *Botryoploida theobromae* (18.61%) and *Penicillium digitatum* (17.86%) (Table 1). In the pathogenicity test reported in the source, all four isolates caused decay in inoculated fruits, with 100% disease incidence, while the uninoculated controls recorded 0% incidence. *C. gloeosporioides* also produced the largest mean rot area (38.20 cm^2), indicating that it was the most prominent isolate in the reported results.

Table 1. Occurrence of fungi isolated from rotten tomato fruits

Fungal species	Number of isolates	Occurrence (%)
Colletotrichum gloeosporioides	201	37.78
Aspergillus niger	137	25.75
Botryoploida theobromae	99	18.61
Penicillium digitatum	95	17.86
Total	**532**	**100.00**

In Vitro Inhibitory Effect of Neem Leaf Extract and Salicylic Acid

The percentage inhibition of *C. gloeosporioides* increased with treatment concentration and incubation time (Table 2). By Day 6, 30% neem leaf extract produced the highest inhibition among the neem treatments (78.72%), compared with 48.62% at 20% and 47.80% at 10%; the untreated control showed no inhibition. A similar concentration-related pattern was observed for salicylic acid: 30 g/L produced 77.41% inhibition on Day 6, while 10 g/L and 20 g/L produced 48.80% and 48.70%, respectively. These results indicate that the highest tested concentrations of both treatments were the most effective in restricting mycelial growth in the reported assay.

Table 2. Main effects of neem leaf extract and salicylic acid on inhibition of C. gloeosporioides (%)

Treatment	Concentration	Day 1	Day 3	Day 6
Neem leaf extract	0%	0.00	0.00	0.00
Neem leaf extract	10%	30.20	36.77	47.80
Neem leaf extract	20%	34.20	39.85	48.62
Neem leaf extract	30%	49.92	59.45	78.72
Salicylic acid	0 g/L	0.00	0.00	0.00
Salicylic acid	10 g/L	28.31	37.32	48.80
Salicylic acid	20 g/L	31.52	39.77	48.70
Salicylic acid	30 g/L	46.64	60.13	77.41

Combined Effect of Neem Leaf Extract and Salicylic Acid

The interaction results showed that combining neem leaf extract and salicylic acid generally increased inhibition relative to the lower-dose combinations (Table 3). The strongest reported combination was 30% neem leaf extract plus 30 g/L salicylic acid, which achieved 84.15% inhibition on Day 6. The next-highest Day 6 value was 83.32% for 20% neem leaf extract plus 30 g/L salicylic acid. The combination results support the potential for the treatments to be used together; however, conclusions about synergy should be made only after an appropriate interaction analysis.

Table 3. Selected interaction effects on mycelial growth inhibition (%)

Neem extract	Salicylic acid	Day 1	Day 3	Day 6
0%	0 g/L	0.00	0.00	0.00
10%	30 g/L	39.22	63.42	75.34
20%	30 g/L	40.11	67.68	83.32
30%	20 g/L	36.72	58.21	69.34
30%	30 g/L	40.23	68.42	84.15

Phytochemical Constituents of Neem Leaf Extract

Qualitative phytochemical screening identified several constituents in the neem leaf extract (Table 4). Alkaloids, cardiac glycosides, glycosides, flavonoids and terpenoids were recorded at high levels (++). Steroids, saponins, tannins, triterpenes, phytosterols and phenols were present at moderate levels (+). Anthraquinones, anthocyanins, phlobatannins and amino acids were recorded as absent. The presence of these phytochemicals provides a basis for further investigation of neem leaf extract as a potential source of antifungal compounds; the screening results alone do not establish which constituent caused the observed inhibition.

Table 4. Qualitative phytochemical screening of neem leaf extract

Phytochemical	Test reported	Result
Alkaloids	Hager's test	++
Cardiac glycosides	Keller–Killiani test	++
Glycosides	Salkowski's test	++
Flavonoids	Shinoda test	++
Terpenoids	Not specified	++
Steroids	Salkowski's test	+
Saponins	Foam test	+
Tannins	Gelatin test	+
Triterpenes	Salkowski's test	+
Phytosterols	Not specified	+
Phenols	Sodium hydroxide test	+
Anthraquinones	Bornträger's test	−
Anthocyanins	Not specified	−
Phlobatannins	Not specified	−
Amino acids	Not specified	−

Discussion

The study identified four fungal species associated with rotten tomato fruits: *Colletotrichum gloeosporioides*, *Aspergillus niger*, *Botryoploida theobromae* and *Penicillium digitatum*. Among these, *C. gloeosporioides* was the most frequently isolated, accounting for 37.78% of the 532 isolates, followed by *A. niger* (25.75%), *B. theobromae* (18.61%) and *P. digitatum* (17.86%). These findings indicate that several fungal species were associated with tomato fruit deterioration, although their relative occurrence differed. The prominence of *C. gloeosporioides* is consistent with the literature reviewed by Hu et al. (2014), Islam et al. (2018) and Yahia (2005), which identifies various fungal pathogens as contributors to postharvest fruit decay. Differences in fungal occurrence may reflect variations in environmental conditions, fruit handling, infection opportunities and the ability of individual fungi to colonise tomato tissues. However, the present results establish the occurrence of the reported fungi in the sampled rotten fruits rather than their prevalence across all tomato-producing areas.

The pathogenicity results further indicated that all four isolates caused decay in inoculated tomato fruits, with 100% disease incidence compared with 0% in the uninoculated controls. *C. gloeosporioides* produced the largest mean rot area of 38.20 cm², followed by *A. niger* at 29.73 cm², *B. theobromae* at 18.03 cm² and *P. digitatum* at 11.27 cm². This pattern suggests that *C. gloeosporioides* was the most aggressive isolate under the reported experimental conditions.

Its prominence provides a basis for considering it in the evaluation of antifungal treatments. Nevertheless, because the presented inhibition results focus on *C. gloeosporioides*, the findings should not be interpreted as demonstrating equal treatment effectiveness against all four fungal species.

The results showed that neem (*Azadirachta indica)* leaf extract inhibited the mycelial growth of *C. gloeosporioides*, with inhibition generally increasing with concentration and incubation time. On Day 6, 10%, 20% and 30% neem leaf extract produced 47.80%, 48.62% and 78.72% inhibition, respectively. The 30% concentration was therefore the most effective neem treatment reported. The relatively small difference between the 10% and 20% concentrations, compared with the higher inhibition recorded at 30%, suggests that the response was not uniform across all concentration increments. The stronger inhibition at 30% may indicate that a higher concentration of neem constituents was required to achieve substantially greater suppression of fungal growth under the experimental conditions.

This finding supports the potential antifungal value of neem leaf extract described in the literature reviewed for this study. Mondall et al. (2009) and Oliveira et al. (2018) reported bioactive compounds in neem, while Onyeani et al. (2012) reported inhibitory activity of neem leaf extract against *Rhizopus stolonifer.* Ezeonu et al. (2018) also reported inhibitory effects of neem extracts against fungi associated with cocoyam rot. The present findings add evidence of the potential activity of neem leaf extract against *C. gloeosporioides* associated with tomato rot. However, the degree of inhibition observed in one fungal species cannot automatically be generalised to other pathogens. In addition, the present findings concern an in vitro assay and do not by themselves establish the effectiveness of neem extract in preserving tomato fruits during commercial storage. Further testing on intact fruits is necessary to determine its practical value.

Salicylic acid also inhibited the mycelial growth of *C. gloeosporioides*. On Day 6, the 10 g/L, 20 g/L and 30 g/L treatments produced 48.80%, 48.70% and 77.41% inhibition, respectively. The 30 g/L treatment produced the highest inhibition among the salicylic acid concentrations evaluated. The inhibitory response was therefore most pronounced at the highest tested concentration, while the 10 g/L and 20 g/L treatments produced similar results. This suggests that the higher concentration was more effective under the experimental conditions.

These findings support further investigation of salicylic acid as a possible component of postharvest disease management. However, the experiment does not establish the mechanism through which salicylic acid inhibited fungal growth. Its effects on fungal development, host defence and fruit quality would need to be investigated separately. Furthermore, the reported concentrations should not be adopted directly for commercial use without assessing their effects on tomato quality, treatment safety and residues.

The interaction results showed that combining neem leaf extract and salicylic acid produced substantial inhibition of *C. gloeosporioides.* The highest reported inhibition occurred with 30% neem leaf extract combined with 30 g/L salicylic acid, which produced 84.15% inhibition on Day 6. The combination of 20% neem leaf extract and 30 g/L salicylic acid produced the next-highest inhibition of 83.32%. These findings suggest that combining neem leaf extract and salicylic acid may provide greater fungal growth suppression than some of the lower-concentration treatments. The result for the 30% neem and 30 g/L salicylic acid combination was numerically higher than the reported individual-treatment values of 78.72% for neem and 77.41% for salicylic acid on Day 6. Nevertheless, a numerically higher inhibition value does not, by itself, demonstrate synergy. Establishing synergy requires an appropriate statistical interaction analysis and a defined method for comparing the observed combined effect with the expected effect of the individual treatments. The results therefore support further investigation of the combined treatments but do not conclusively establish a synergistic effect.

The qualitative phytochemical screening revealed several constituents in the neem leaf extract. Alkaloids, cardiac glycosides, glycosides, flavonoids and terpenoids were reported at high levels, while steroids, saponins, tannins, triterpenes, phytosterols and phenols were reported at moderate levels. Anthraquinones, anthocyanins, phlobatannins and amino acids were recorded as absent. The presence of these phytochemical groups provides a possible basis for investigating the antifungal activity of neem leaf extract. These findings are consistent with the literature reviewed by Mondall et al. (2009) and Oliveira et al. (2018), which describes neem as a source of biologically active secondary metabolites. The observed inhibition of fungal growth and the presence of several phytochemical groups together suggest that neem leaf extract merits further investigation as a botanical antifungal treatment.

However, qualitative screening identifies the reported presence or relative abundance of broad chemical groups; it does not quantify individual compounds or establish which constituents are responsible for fungal inhibition. The relationship between particular phytochemicals and antifungal activity would require quantitative chemical analysis, compound identification and further antifungal testing. Overall, the findings indicate that neem leaf extract and salicylic acid inhibited the mycelial growth of *C. gloeosporioides* under the reported in vitro conditions, while phytochemical screening identified several groups of compounds in neem leaf extract. These results address the study's objectives, although further investigation is required to establish the treatments' effectiveness, safety and suitability for practical postharvest application.

Conclusion

The study evaluated the in vitro inhibitory effects of neem (*Azadirachta indica*) leaf extract and salicylic acid on the mycelial growth of fungi associated with tomato rot and determined the phytochemical constituents of neem leaf extract. Four fungal species were isolated from rotten tomato fruits, with *Colletotrichum gloeosporioides* being the most frequently isolated and producing the largest mean rot area in the reported pathogenicity test.

Both neem leaf extract and salicylic acid inhibited the mycelial growth of *C. gloeosporioides.* The highest individual-treatment inhibition on Day 6 was recorded at 30% neem leaf extract (78.72%) and 30 g/L salicylic acid (77.41%). The combination of 30% neem leaf extract and 30 g/L salicylic acid produced the highest reported inhibition of 84.15%.

Phytochemical screening identified alkaloids, cardiac glycosides, glycosides, flavonoids and terpenoids at high levels, alongside moderate levels of steroids, saponins, tannins, triterpenes, phytosterols and phenols. These constituents may warrant further investigation in relation to the observed antifungal activity, although their individual contributions were not established.

The study concludes that neem leaf extract and salicylic acid have potential for inhibiting tomato rot fungi under in vitro conditions. However, their effectiveness on tomato fruits during storage, their safety at the tested concentrations and the possible benefits of combining them require further investigation.

Recommendations

Based on the findings of the study, the following recommendations are made:

1. Neem leaf extract and salicylic acid should be evaluated against all fungal species isolated from rotten tomato fruits at different concentrations to determine their effectiveness against individual pathogens and identify the most effective treatment levels.
2. The treatments should be tested on intact tomato fruits under controlled storage conditions to determine their effects on decay incidence, shelf life, marketability, colour, taste, texture, consumer acceptability and safety.
3. Further studies should investigate the combined application of neem leaf extract and salicylic acid using appropriate factorial analysis to establish whether their interaction is additive, synergistic or antagonistic.
4. Further laboratory investigations should identify and quantify the bioactive compounds present in neem leaf extract and examine their possible relationships with antifungal activity.
5. The preparation of neem leaf extract should be standardised by documenting the extraction procedure, plant material-to-solvent ratio, extraction yield, treatment preparation and storage conditions. The original experimental data and statistical analyses should also be verified to confirm the reported means, LSD values and treatment interaction effects before publication or practical application.

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