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Original Research Article

Antifungal Potential of Neem Seeds and Onion Bulb Extracts on Anthracnose Disease of Sweet Pepper

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Abstract

Sweet pepper (Capsicum annuum L.) production is seriously restricted by anthracnose disease caused by Colletotrichum spp., leading to significant yield losses globally. Dependence on synthetic fungicides has raised serious concerns over fungal resistance, environmental contamination, and human health risks, necessitating ecofriendly alternatives. This study investigated the antifungal potential of aqueous extracts from neem (Azadirachta indica) seeds and onion (Allium cepa L.) bulbs against anthracnose in sweet pepper under controlled pot conditions. The design employed weekly foliar applications of 200 ml/m² neem seed extract, onion bulb extract, or untreated control on anthracnose-inoculated plants. Parameters assessed were leaf number, plant height, fruit yield, disease severity and agronomic efficiency index. Both extracts significantly improved vegetative growth, increased fruit yield, and reduced disease incidence compared to the control. Neem seed extract exhibited slightly better performance in promoting leaf retention and plant height. Strong positive correlations were observed between growth traits and yield, while disease severity showed significant negative correlations with productivity. The findings reveal that neem seed and onion bulb extracts effectively suppress anthracnose, improve plant performance, and offer promising eco-friendly alternatives to synthetic fungicides for sustainable sweet pepper cultivation.

Keywords: Sweet pepper, Anthracnose, Neem seed extract, Onion bulb extract, Antifungal efficacy.

1.0 INTRODUCTION

Food security is an important tool for life sustenance in the world (Aigbedion *et al*., 2022). Sweet pepper (*Capsicum annuum L.*) is one of the most nutritionally and economically important solanaceous vegetables grown worldwide. Its value comes from the high amounts of ascorbic acid, carotenoids, and phenolic antioxidants that are essential for human nutrition and health (Kim *et al*., 2019; Olatunji and Afolayan, 2020). Sweet pepper global production has increased rapidly due to yearly demand for both fresh and processed varieties. However, its sustainable cultivation and preservation are consistently hindered by a spectrum of phytopathogenic fungi that attack roots, stems, leaves and fruits throughout the growing season and storage (Moss *et al*., 2021; Sanoubar *et al*., 2022).

Fungal disease is the most common disease of sweet pepper (*Capsicum annuum L.*) in tropical and subtropical regions. The production loss can reach as high as 100% when adequate control measures are not applied in mild to warm climates and rainy seasons during cultivation and storage (Abubakar *et al*., 2024). Anthracnose disease is caused by Colletotrichum spp., the most common anthracnose pathogen attacking sweet peppers (Sudirga *et al*., 2019).

Conventional management of anthracnose diseases in sweet pepper has over the years relied on the use of synthetic fungicides such as chlorothalonil, azoxystrobin, and metalaxyl (Ristaino *et al*., 2020). However, constant application of these fungicides has led to significant resistance in the population of the fungi (Hu *et al*., 2021; Avenot *et al*., 2022). Also, there are growing concerns about environmental pollution and consumer health. Pesticide pollution of the environment also endangers human and animal life where pesticide residues accumulate over time in agricultural products and in water that exceeds permissible residue limits (Nicolopoulou-Stamati *et al*., 2016; Sudirga *et al*., 2019; Sharma *et al*., 2023).

These challenges have led researchers to search for alternatives to controlling anthracnose diseases on sweet pepper by utilising plants that are eco-friendly, biodegradable, and not harmful to consumers (Cesare *et al*., 2021; Raveau *et al*., 2022). The utilisation of plant-derived compounds with fungicidal properties offers a promising alternative to synthetic fungicides. Various plant species are rich sources of biologically active phytochemicals like flavonoids, alkaloids, phenolics, terpenoids, and tannins, which have a strong broad spectrum of antifungal activities (Almadiy *et al*., 2022; Ali *et al*., 2024). Neem (*Azadirachta indica*) leaf extracts, which are rich in azadirachtin, nimbin, and salannin compounds, have demonstrated strong antifungal effects against various phytopathogenic fungi via disruption of plasma membrane, inhibition of ergosterol production, and induction of oxidative stress in fungal cells (Bhat *et al*., 2020; Ngegba *et al*., 2022; Singh *et al*., 2023). Similarly, onion (*Allium cepa L.*) peel and bulb extracts contain high amounts of quercetin, kaempferol, and organosulphur compounds such as allicin and allyl sulphides that have significant fungistatic and fungicidal properties through membrane disruption, blocking efflux pumps, and interfering with fungal mitochondrial functions (Ye *et al*., 2019; Benkeblia and Lanzotti, 2021; Mnayer *et al*., 2022; Suleria *et al*., 2023).

Despite the research on the bioactivity of neem leaf and onion bulb extracts on fungi, there remains a research gap on the efficacy of the studied plant extracts on specific fungal pathogens affecting sweet pepper. The current study is designed to evaluate the antifungal potential of neem seed and onion bulb extracts on anthracnose fungal disease of sweet pepper.

2.0 MATERIALS AND METHOD

2.1 Study Area

The research was carried out in the Research and Demonstration Farm of the Federal Polytechnic, Nekede, in the Owerri West Local Government Area of Imo State, located in Southeastern Nigeria at Latitudes 5° 21′ and 5° 27′ N and Longitudes 7° 02′ and 7° 15′ E, and is characterised by two predominant seasons, wet (April to October) and dry (November to March of the following year), like most tropical rainforest agro-ecological zones in Nigeria. However, the area's rainforest vegetation density has been drastically altered and modified into a derived savannah by human activities such as bush burning, agriculture and construction work, contributing to the gully erosion problem that is prominent in the area (Ufot, 2012). Based on meteorological data, the relative humidity and temperature of the area are 80 ± 10% and 28 ± 8°C, respectively, all year round, with 2000 ± 500 mm mean annual rainfall (NIMET, 2017).

2.2 Soil Physiology

Soils of Nekede, southeastern Nigeria, are derived majorly from coastal plain sands parent materials (Osujieke *et al*., 2017) and are predominantly ultisols (Enyioko *et al.*, 2019), characterised by low activity clays, low nutrient reserves, low moisture retention, nutrient imbalance, high acidity, and a high tendency to crust, compact and erode but are generally porous (Ufot *et al.*, 2016).

2.3 Source of Planting Materials

The Hope variety of green sweet pepper seeds for planting as a test crop was sourced from KUCH-99 Agriculture and Seed Limited, Owerri, Imo State.

2.4 Disease Inoculation

The sweet pepper seeds were inoculated with anthracnose mycelial suspension, ensuring consistent and reproducible disease development.

2.4.1 Preparation of Anthracnose Mycelial Suspension

A pure culture of the anthracnose pathogen was obtained from an infected sweet pepper plant, incubated and cultivated in a little potato dextrose agar (PDA), then allowed to fully develop before the mycelium was carefully harvested from the PDA using a sterile technique that ensured the purity and viability of the mycelial strands.

2.4.2 Inoculation of Sweet Pepper Seeds

A sterile needle was used to create small incisions on the surface of the sweet pepper seeds at a depth sufficient for the mycelial suspension to enter. The mycelial suspension was carefully applied using a sterile dropper to the prepared inoculation points on the sweet pepper seeds, ensuring uniform coverage and proper penetration of the mycelium, and then the inoculated seeds were incubated to allow successful infection.

2.5 Plant Collection and Preparation

The neem and onion extracts were prepared following these general procedures.

2.5.1 Neem Seeds Collection and Extraction

Matured neem seeds were collected from neem trees planted at the Botanical Garden of Federal Polytechnic Nekede, Owerri, Imo State, Nigeria. The neem seeds were identified at the Biology/Microbiology Department of the same institution by a botanist. In preparation for aqueous extraction, the seeds were rinsed in clean water and dried at room temperature (25℃ to 5℃) for two weeks. The seeds were ground into a fine powder using a mortar and pestle. 100 grams of dried powdered neem seed was soaked in 1000 ml of distilled water, and the mixture was allowed to stand for 24 hours. The extract was then squeezed and filtered with filter paper. The extract was then evaporated using a rotary vacuum evaporator. The extract was stored in an amber bottle for further experimental works.

2.5.2 Onion Extract

Fresh onion bulbs were bought from the market and transported back to the laboratory. The onion bulbs were carefully rinsed with water. The peeled, fresh, healthy onions were cut into smaller pieces and ground into a smooth paste. One hundred grams (100 g) of the ground onion bulb were also soaked in 1000 ml of distilled water, and the mixture was allowed to stand for 24 hours. The extract was then squeezed and filtered with filter paper. The extract was then evaporated using a rotary vacuum evaporator. The extract was stored in an amber bottle for further experimental works.

2.6 Planting and Planting Materials

Perforated plastic pots of 25 cm top diameter and 30 cm depth were filled with air-dried soil incorporated (thoroughly mixed) with poultry manure and left to incubate for about two weeks. The pots were then watered till the soil surfaces were covered with water. A polythene sheet was then used to cover the surface of the watered soil and for drainage of the excess water till field capacity, before the anthracnose-inoculated seeds were sown at 3 seeds per pot and later thinned to 1 plant stand per pot.

2.7 Experimental Design and Research Operation

A field pot experiment was simulated, using a Completely Randomised Design (CRD) with two (2) botanical fungicides (200 ml/m² of neem extract and 200 ml/m² of onion extract) and a control, replicated three (3) times. The treatments were designated randomly to the pots and sprayed at an interval of one (1) week after germination until final harvest.

2.8 Cultural Practices of Sweet Pepper

Each pot was placed in an area that receives abundant sunlight, and the soil was kept consistently moist but not waterlogged. Weeds were manually removed at 1-week intervals till maturity and harvest. Mulch was placed around the plant after emergence to maintain a consistent soil temperature and conserve moisture. The early flowers produced were pinched off to encourage stronger root and stem growth. Stakes were used to support the plant during fruit development to prevent toppling over due to the weight of the fruit. Harvesting was carried out 9 weeks after sowing, when it was observed that the fruit were of the desired size and colour.

2.9 Data Collection

Data on plant growth (number of leaves per plant and plant height), plant yield (number and yield of fruits per plant), disease progression rate, disease severity assessment (percentage of infected leaves and fruits per plant) and agronomic efficiency index of neem and onion extracts were collected as follows:

2.9.1 Number of Leaf per Plant

The number of sweet pepper leaves per plant from each pot was counted at growth stages of 3, 6 and 9 weeks after sowing (WAS) and at harvest, and their respective means were calculated and recorded for each growth stage.

2.9.2 Plant Height (cm)

The height (cm) of the sweet pepper plants from each pot was measured from the base of the plant to the tip of the main shoot at growth stages of 3, 6 and 9 WAS and at harvest, and their respective means were calculated and recorded for each growth stage.

2.9.3 Number of Fruits per Plant

The number of sweet pepper fruits per plant harvested from each pot was counted, and their respective means were calculated and recorded.

2.9.4 Fruit Yield per Plant

The sweet pepper fruits harvested per plant were weighed on a physical balance and recorded as fruit yield (kg) per plant.

However, total fruit yield (t/ha) was determined as
TFY (t/ha) = fruit yield (kg) per plant x 204
204 was derived from the surface area of the pot and the conversion of units from kg/m² to t/ha.

2.9.5 Disease Severity Assessment

2.9.5.1 Percentage of Infected Leaves and Fruits per Plant

The number of infected sweet pepper leaves per plant at harvest and fruits from each pot were counted, and their respective percentages were calculated.

2.9.6 Agronomic Efficiency Index

The agronomic efficiency index (AEI) of neem and onion extracts was assessed as

$$AEI = \frac{(\text{Data from treatment pot} - \text{Data from control})}{\text{Data from control}}$$

The data used were the number of unaffected leaves and fruits and the fruit yield of sweet pepper.

2.10 Data Analysis

All data collected were subjected to Pearson correlation analysis and analysis of variance (ANOVA). Statistical significance was considered for $p < 0.05$, which was statistically significant.

3.0 RESULTS AND DISCUSSIONS

The present study evaluated the antifungal potential of neem seed and onion bulb extracts against anthracnose disease of sweet pepper. The experimental results of the present investigation, as presented in Tables 1-6, revealed that neem seed and onion bulb extracts significantly influence the plant's growth, fruit yield, and quality compared to untreated sweet pepper.

3.1 Effects of Neem Seed and Onion Bulb Extracts on leaf production of sweet pepper

The experimental results for the effects of neem seed and onion bulb extracts on the number of sweet pepper leaf productions are presented in Table 1. The results revealed that sweet pepper leaf production increased progressively with plant age across all treatments. However, sweet pepper plants treated with neem seed and onion bulb extracts constantly produced more leaves than the control sample, especially after growth stages. The absence of significant changes in leaf production observed at early growth stages suggests that fungal infection is yet to apply a strong suppressive effect during vegetative development. The result revealed that fungal infection on the untreated plants matured, accounting for low leaf retention. Leaf number plays a major role during photosynthetic activity and indicates plant health. Fungal pathogens are known to damage leaf tissues, disrupt chlorophyll synthesis, and increase senescence, thereby decreasing assimilate production (Moss *et al*., 2021). The increased leaf counts observed in treated plants indicate that both applied extracts efficiently reduced foliar infection, allowing sustained leaf formation and function. Comparable improvements in sweet pepper leaf production following neem seed and onion bulb extract application have been reported in pepper and tomato, respectively, where reduced disease severity resulted in improved vegetative growth (Chohan *et al*., 2022; Sanoubar *et al*., 2022). The slight improvement of neem extract on sweet pepper leaf production can be attributed to the presence of bioactive limonoids, such as azadirachtin and nimbin, which have been found to inhibit fungal growth and protect leaf tissues from infection (Bhat *et al*., 2020; Singh *et al*., 2023).

Table 1: Neem seed and onion bulb extracts effects on number of sweet pepper leaf

Treatment	Number of Leaf			
	3 WAS	6 WAS	9 WAS	At Harvest
Control	6.48	17.91	39.18	62.54
Onion Extract	6.75	18.13	48.36	89.03
Neem Extract	7.31	18.16	49.52	92.02
LSD (0.05)	**NS**	**NS**	**3.77**	**6.38**

WAS = Weeks after Sowing

3.2 Effects of the Plant Extracts on Plant Height

The experimental data on the effect of neem seed and onion bulb extracts on plant height, as shown in Table 2, revealed that extract-treated plants exhibited higher vertical growth than the control plant throughout the growth period. The difference in plant height between the treated and untreated sweet pepper plants was noticeable at early growth stages, and it became more prominent as the plants approached maturity. The observed result indicates that disease inhibition by the two applied extracts occurred early enough to positively affect stem elongation and overall plant structure. Studies have shown that fungal diseases such as anthracnose reduce plant height by interfering with nutrient uptake and hormonal balance, especially auxins and gibberellins responsible for cell elongation (Al-Sadi *et al*., 2021). The increased plant height noticed in neem seed and onion bulb treated plants indicates that these extracts eased pathogen-induced stress, allowing normal physiological growth processes to progress. Similar results have been reported in literature on capsicum and other solanaceous crops treated with plant-based fungicides, where improved plant height was attributed to reduced pathogen colonisation and improved nutrient utilisation (Gupta *et al*', 2021; Ngegba *et al*., 2022).

Table 2: Neem seed and onion bulb extracts effects on height of sweet pepper

Treatment	Plant Height (cm)			
	3 WAS	6 WAS	9 WAS	At Harvest
Control	11.90	18.80	31.01	39.67
Onion Extract	14.70	25.23	37.30	41.78
Neem Extract	15.09	25.61	37.78	43.07
LSD (0.05)	**2.09**	**3.10**	**3.08**	**2.07**

WAS = Weeks after Sowing

3.3 Effects of Neem Seed and Onion Bulb Extracts on Sweet Pepper Yield

The result displayed in Table 3 shows that neem seed and onion bulb extract application on sweet pepper resulted in higher fruit yield compared with the control plant. Studies have shown that plant yield improvement is a critical confirmation of effective disease control, as fungal infections always lead to flower drop, fruit abortion, and fruit rot, leading to substantial yield losses (Arah *et al*., 2019). The high fruit production observed in treated plants suggests that disease inhibition during flowering and fruiting stages improved fruit set and decreased premature fruit loss. Neem-based products have been extensively reported to retard reproductive organs by inhibiting pathogen growth and maintaining cellular character (Singh *et al*., 2023). Onion extracts, rich in flavonoids and organosulphur compounds, have also been shown to suppress fungal enzymes responsible for tissue maceration, thereby protecting developing fruits (Ye *et al*., 2019; Benkeblia and Lanzotti, 2021). The increase in total fruit yield further confirms the agronomic importance of the treatments. These findings agree with previous studies that reported yield improvements in pepper following plant fungicide application, specifically under high disease conditions (Sanoubar *et al*., 2022; Cesare *et al*., 2021).

Table 3: Neem seed and onion bulb extracts effects on yield of sweet pepper

Treatment	Number of Fruits per Plant	Fruit Yield per Plant (kg)	Total Fruit Yield (t/ha)
Control	2.66	0.19	38.82
Onion Extract	4.85	0.25	51.33
Neem Extract	4.27	0.24	49.21
LSD (0.05)	**1.69**	**0.29**	**4.10**

3.4 Effects of Neem Seed and Onion Bulb Extracts on Severity of Infected Sweet Pepper Plant

The effect of plant extracts applied to sweet pepper plants on disease severity, as presented in Table 4, clearly reveals that neem seed and onion bulb extracts significantly reduced infection levels on both leaves and fruits. On the other hand, the untreated plant exhibited remarkably high disease incidence, reflecting unchecked pathogen development. The reductions in disease manifestation in treated sweet pepper plants indicate that the extracts inhibited fungal growth and spread. Investigations into neem bioactive compounds have been reported to disrupt fungal cell membranes and inhibit spore germination, while onion-derived organosulphur compounds interfere with enzyme activity and mitochondrial respiration in fungal cells (Bhat *et al*., 2020; Mnayer *et al*., 2022; Suleria *et al*., 2023).

Table 4: Neem seed and onion bulb extracts effects on severity of infected sweet pepper plant

Treatment	Infected Leaves (%)	Infected Fruits (%)
Control	33.90	35.58
Onion Extract	12.57	14.60
Neem Extract	13.46	13.33
LSD (0.05)	**5.50**	**5.60**

3.5 Agronomic Efficiency of the Neem Seed and Onion Bulb Extracts

The agronomic efficiency index values tabulated in Table 5 revealed that both studies' extracts produced significant benefits over the control sweet pepper plants in terms of growth, yield, and fruit quality. Favourable efficiency values demonstrated that the plant treatments were not only suppressing fungal infections but also improving crop performance. Higher efficiency values for fruit production suggest that disease reduction had a direct impact on productivity efficiency. Similar results in the agronomical efficiency index after plant extract application have been reported by researchers on vegetable crops, supporting their role as effective components of sustainable crop management systems (Gupta *et al*., 2021; Raveau *et al*., 2022).

Table 5: Agronomic Efficiency Index of 200 ml/m^2 of Neem and Onion Extracts for Growth and Fruit Yield of Uninfected Sweet Pepper

Treatment	AEI for Number of Leaves	AEI for Number of Fruits	AEI for Fruit Yield
Onion Extract	0.88	1.42	0.32
Neem Extract	0.93	1.16	0.27

AEI = Agronomic Efficiency Index

3.6 Correlation among Plant Growth, Fruit Yield, and Disease Severity

The correlation relationship value displayed in Table 6 indicates a strong positive correlation among vegetative growth traits, yield parameters, and total fruit yield. The results revealed that improved plant growth directly indicated higher productivity. Contrastingly, fungal infection severity variables displayed strong negative relationships with plant growth and fruit yield. These results highlight the important role of disease suppression in examining sweet pepper performance. Increased disease severity was observed to cause decreased leaf counts, stunted growth, and lower fruit yield. Similarly, negative relationships between disease severity and fruit yield have been widely documented for pepper and other horticultural crops (Moss *et al*., 2021; Al-Sadi *et al*., 2021). Finally, the correlation analysis result confirms that neem seed and onion bulb extracts improved sweet pepper productivity, especially through effective disease control, proving their potential as eco-friendly alternatives for synthetic fungicides.

Table 6: Correlation Matrix of Response of Sweet Pepper to Neem and Onion Extracts

	NL	PH	NF	FYP	TFY	%InL	%InF
NL	1						
PH	0.95**	1					
NF	0.93**	0.79**	1				
FYP	0.99**	0.97**	0.90**	1			
TFY	0.96**	0.85**	0.99**	0.94**	1		
%InL	-0.99**	-0.91**	-0.97**	-0.97**	-0.99**	1	
%InF	-0.99**	-0.94**	-0.95**	-0.99**	-0.97**	0.99**	1

** = Significant at $P < 0.05$; NL = Number of Leaves; PH = Plant Height; NF = Number of Fruits; FYP = Fruit Yield per Plant; TFY = Total Fruit Yield; InL = Infected Leaves; InF = Infected Fruits; MF = Marketable Fruits

4.0 CONCLUSION

The study established that neem seed and onion bulb extracts are efficient eco-friendly alternatives for controlling fungal diseases in sweet pepper. Both plant extracts extensively reduced disease severity, enhanced vegetative growth (leaf number and plant height), and improved yield and fruit quality compared to untreated controls. Neem extract demonstrated slightly better efficacy, likely due to its rich composition of antifungal compounds such as azadirachtin and nimbin. The positive correlations between disease suppression, growth traits, and yield validate that effective disease management is essential to achieving high productivity in sweet pepper cultivation.

Conflict of Interest: The authors declare no conflict of interest.

Ethics Declaration: Not applicable.

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