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In-vitro Integrated Management of *Alternaria* porri Affecting *Allium cepa* (Onion)

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Abstract

Onion (Allium cepa) is a globally cultivated crop vulnerable to fungal diseases, notably those caused by Alternaria porri. This fungal pathogen significantly reduces yield, particularly during seed production. This study aimed to identify Alternaria porri affecting onions and to evaluate integrated management strategies involving synthetic fungicides, botanical extracts, and biological control agents. The research, conducted at Federal University Dutse, Nigeria, involved morphological and pathogenic identification of Alternaria porri., followed by phytochemical screening of Azadirachta indica (neem), and in vitro evaluation of antifungal efficacy using neem extracts, Trichoderma harzianum, and synthetic fungicides. Morphological and molecular analyses confirmed the presence of pathogenic A. alternata porri Phytochemical analysis of neem extract revealed multiple antimicrobial compounds, notably flavonoids and alkaloids. Synthetic fungicides showed the highest inhibition against Alternaria spp., followed closely by neem extract, while T. harzianum displayed strong antagonistic effects at higher conidial concentrations. Results support the effectiveness of integrating chemical, botanical, and biological methods to control Alternaria-induced onion diseases. The study recommends a sustainable approach that reduces reliance on chemical fungicides, mitigates environmental impact, and enhances disease control efficacy through integrated pest management (IPM).

Keywords: Alterneria porri, Trichoderma harzianum Azadiracter indica biological control purple blight

INTRODUCTION

The onion (*Allium cepa*), a globally important vegetable, is a staple crop valued for its distinctive flavor, aroma, and numerous health-promoting properties (Meena *et al.,* 2024). It belongs to the *Allium* genus, which comprises over 1,000 morphologically diverse species adapted to a wide range of environmental conditions (Kumar et al., 2024). Although inherently biennial or perennial, onions are commonly cultivated as annuals for commercial production.

Alternaria species, particularly *Aporri* are major fungal pathogens of onions, with severe impacts observed during seed production, where infections can result in near-complete yield loss (Rai *et al.,* 2025; Zhang & Hassan, 2025).

This study aims to characterize *Alternaria* spp. affecting onions through both morphological and molecular methods, and to evaluate the efficacy of integrated management strategies, including chemical, botanical, and biological control options.

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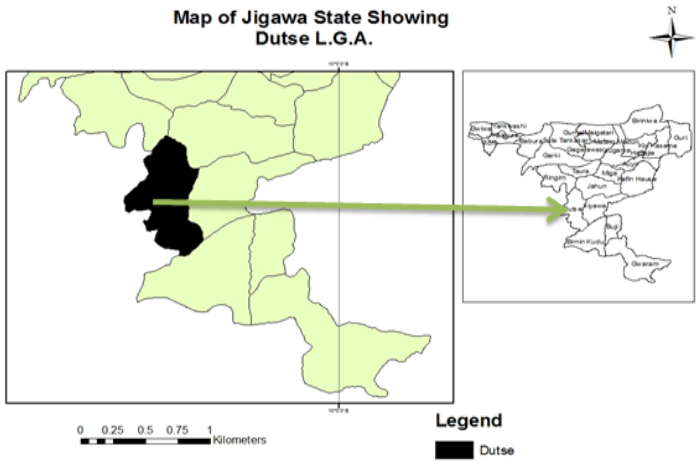
Many studies show that trichomes serve as physical barrier against biotic stressors such as insects, herbivores, fungal infections, and even parasitic plants (Peiffer et al., 2009; Runyon *et al.*, 2010; Tian et al., 2012).

Thus, the present study was conducted to understand the pathogen causing purple blotch disease through morphological identification.
Over time, numerous fungal pathogens have developed resistance to the continuous application of synthetic fungicides. This resistance results in a significant increase in the population of these fungal pathogens. To mitigate the associated risks linked to synthetic fungicides, farmers are actively seeking alternative products for controlling fungal pathogens.

Trichoderma species exhibit robust antifungal properties, and extracts from *Azadiracta Indica* also demonstrate efficacy against fungal pathogens. Consequently, these alternatives have the potential to substitute the currently employed hazardous synthetic products, providing a viable treatment option for fungal pathogens affecting plant growth, including Onion plants. The chosen Trichoderma species are not only biodegradable and locally reliable but also more cost-effective compared to conventional fungicides. This makes them a suitable choice for experimentation in addressing the challenges associated with fungal pathogen control.

Materials and Methods

Study Site
Dutse located at latitude 11°46'39" North, and longitude 9°20'3" East with a population of 290,320. Federal University Dutse is in the ancient town of Dutse the capital of Jigawa State Nigeria, with an estimated population of 153,000 (2009), the geographical coordinates are 11042'04" N9°20'311" E/11.70111°N9.34194E longite.



The research was divided into three phases:

Morphological Screening: Infected onion leaves were surface-sterilized, cultured on PDA, and incubated for seven days at 20–23°C. Colony morphology was compared with literature (Cifferi, 1930).

Pathogenicity Test: Following Rabia Kalsoom et al. (2023), healthy onion plants were inoculated with isolated Alternaria spp., and symptom development (leaf spot, necrosis, wilting) was monitored within 10–60 days post-inoculation.

Management Strategies: These included the use of:
Botanical Extracts: Neem (*Azadirachta indica*) leaves were cold macerated (Irobi & Daramola, 1993), filtered, and dried for phytochemical analysis and antifungal evaluation.

Phytochemical Tests: Qualitative analysis was performed to detect flavonoids, tannins, saponins, glycosides, alkaloids, balsams, and anthraquinones using standard methods (Harbone, 1973, 1998; El-Leyi et al., 1994; Trease & Evans, 1978).
Biological Control: *Trichoderma harzianum* was isolated from soil through serial dilution and cultured on PDA (Hajieghrari *et al.,* 2010).

Pathogenicity Test
The pathogenicity was conducted according to (Rabia Kalsoom *et al*., 2023). The Isolate was collected for pathogenicity test, *Alternaria porri* was inoculated to the healthy Onion and observation was made for the development of the disease symptom, this includes yellowish of leaf, the symptoms are expected to start appearing between 10-60 days after inoculations.

Preparation of Plant Extracts
The samples were collected biscuit tree locally named known as Darbejiya in Hausa (*Azairacta Indica*) and store in an airtight container prior to phytochemical analysis. Plant extracts were beprepared by cold maceration methods; twenty-five (25) grams of the powdered samples were soaked in 100ml of distilled water in a flask and allowed to soak for 72hrs. The mixtures obtained were passed through Whatman's No 1filter paper and evaporated using rotary evaporator and further air dried. The resulting extracts were used for phytochemical analysis (Irobi and Daramola, 1993).

Phytochemical Screening
Test for Flavonoid
Plant extracts (3mls) and 1ml of 10% NaOH were mixed in a test tube. A yellow-coloured appearance indicated the presence of flavonoid compounds (El- Leyi *et al.,* 1994; Harbone, 1998).

Test for Tannins
Five percent (5%) ferric chloride solution was added to 3mls of the extract and the colour produced was note. Condensed tannins usually give a black, green color; hydrolysable tannins usually give dark green colour (Harbone, 1998; Trease and Evans 1978).

Test for Saponins
Five milliters (5ml) of the aqueous extracts were placed in a test tube and mix with equal volume of distilled water and shaked vigorously. Froth (small bubles) appearance that lasts for several minutes indicates the presence of saponins (Harbone, 1998).

Cardiotoni Glycosides
Two and a half milliters (2.5ml) of fifty percent (50%) H_2SO_4 was added to 5ml of extract in a test tube. The mixture will be heated in boiling water for 15 minutes, cooled and neutralized with 10% NaOH. Fehling solution (5 ml) will be added and the mixture and boiled. A brick-red precipitate was observed which indicated the presence of glycoside (Harbone, 1973).

Test for Alkaloids
Two milliters (2ml) of the extracts were stirred with 2ml of hydrochloric acid. 1ml was treated with few drops of Wagners reagent and second 1ml portion was treated similarly with

Mayer's reagent. Turbidity or precipitation with either of these reagents was taken as the preliminary evidence for the presence of alkaloids (Harbone, 1973).

Test for Balsams
Extract was mixed with equal volume of 90% ethanol. 2 drops of alcoholic ferric chloride solution were added to the mixture. A dark green colour indicated the presence of balsams (El- Leyi *et al.,* 1994).

Test for Anthraquines
A quantity (10cm3) of benzene was added to 5g of plant extracts, shaken and 5ml of 10% ammonical (lower) phase indicated the presence of anthraquines (Trease and Eva, 1989).
Isolation and Identification of *Trichorderma species.*

Morphological characterization
The infected onion leaves were collected based on symptoms and microscopic studies from different areas of Dutse, Jigawa State. The leaves collected from the infected umbel showing symptoms of the disease were extracted and the surface sterilized with 0.1% sodium hypochlorite solution for 1-2 min followed by 2-3 successive washing in sterilized distilled water. The sterilized leaves were aseptically placed on solidified Potatoes Dextrose Agar (PDA) medium and incubated at 20−23^{O}C for 7 days. The morphological characteristics of *Alternaria Species* were observed for growth pattern and colony characteristics such as color of the colony, colony texture, shape, margin, pigmentation and sporulationand compared with authentic literature (Cifferi, 1930)

In Vitro Assays:
In-vitro effect of *Trichodermaharzianum*on *Alternaria spp* was carried out following the method described by (Hajieghrari et al. 2010).

The in vitro antagonistic effect of *Trichoderma harzianum* against the pathogen was performed on PDA supplemented with antibiotics (streptomycin) on Petri dishes by the method of (Hajieghrari et al. 2015). *Trichoderma harzianum* and pathogens were grown on the same PDA media. Trichoderma was seeded on PDA media in the middle of a Petri dish. Pure cultures of *Alternaria porri* were scratched on the right and left sides of the Trichoderma Harzianum with ± 3cm. PDA inoculated with pure culture of the pathogens only was used as control. Inoculated plates were incubated at room temperature and monitored regularly for 7 days.
The percentage inhibition of mycelia of the pathogen was calculated by using the formula (Nisa ea al., 2011). Formula for estimation is given under.
PGI= C-T/Tx100
Where:
PGI = Percentage growth inhibition level on pathogen
C= Average colony growth of fungal colonies obtained from control plates
T= Average colony growth of fungal colonies obtained from treated plates
IN vitro ethanolic leaf extract effect of *Azadiracta indica* extract on *Alternaria spp* carried out following the method described by Bauer *et al.* (1966)

The agar well diffusion method was used as described by Garba et al., 2018. All the tested *Alternaria spp* used was subcultured in PDA at 25^{o}C and incubated for 24hr. About 15ml of sterile PDA agar was dispensed in a Petri dish and allowed to solidify. A swabbing method using a sterile swab stick was applied to inoculate the tested organism on the agar plate. 6mm diameter well was bored in the agar with sterile micro pipette tip and filled with 0.1ml of extract (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml) of *Alternaria* and leaf extract of *Azadiracta*

indica using micro pipette and allowed to stand for 1hrs for the diffusion to take place and then incubated at 25ºC for 24hrs. Equal concentration of distilled water fungicide was used as control At the end of incubation, zone of inhibition that developed was measured with transparent ruler in millimeter (mm) and average zone of inhibition was calculated.
PGI= C-T/Tx100
Where:
PGI = Percentage growth inhibition level on pathogen
C= Average colony growth of fungal colonies obtained from control plates
T= Average colony growth of fungal colonies obtained from treated plates
In vitro and in vivo effects of synthetic fungicides on *Alternaria spp* was carried out following the method adopted by (Hajieghrari et al. 2010).

The agar well diffusion method was used as described by Garba *et al*., 2018. All the test *Alternaria spp* used was sub-cultured in PDA at 25ºC and incubated for 24hr. About 15ml of sterile PDA was dispensed in a Petri dish and allowed to solidify. A swabbing method using a sterile swab stick was applied to inoculate the tested organism on the agar plate. 6mm diameter well was bored in the agar with sterile micro pipette tip and filled with 0.1ml of extract (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml) of *Alternaria* and synthetic fungicide using micro pipette and was allowed to stand for 1hrs for the diffusion to take place and then incubated at 25ºC for 24hrs. Equal concentration of Synthetic fungicide was used as control. At the end of incubation, the zone of inhibition that developed was measured with transparent ruler in millimeter (mm) and average zone of inhibition was calculated.

RESULTS

Phytochemical Screening of *Azadirachta indica*

The phytochemical analysis of *Azadirachta indica* (neem) extract confirmed the presence of multiple bioactive compounds such as saponins, flavonoids, anthraquinones, cardiotonic glycosides, alkaloids, terpenoids, and steroids. The absence of tannins was also noted. These compounds are known to exhibit broad-spectrum antimicrobial properties, especially flavonoids and alkaloids, which disrupt microbial cell membranes and interfere with their metabolism.
The presence of these phytochemicals supports the use of neem in fungal disease management. Though qualitative, the strong presence of antimicrobial constituents aligns with neem's historical application as a botanical pesticide.

Table 1. Result Phytochemical screening

S/N	Bioactive Compound	Result
1	Saponins	Positive
2	Tannins	Nagetive
3	Flavonoids	positive
4	Anthraquinin	Positive
5	Cardiotonicglycoside	positive
6	Alkaloids	positive
7	Terpenoids	positive
8	Steroids	Positive

Antifungal Efficacy of Synthetic chemicals at Different Concentrations Against *Alternaria porri*

The antifungal assay using synthetic fungicides showed the **highest inhibitory effect** against *Alternaria* spp., with inhibition zones reaching **31.00 ± 2.31 mm** at 250 mg/ml. Efficacy consistently declined with decreasing concentrations, demonstrating dose-dependence. At the lowest concentration of 50 mg/ml, the inhibition was minimal (**3.66 ± 1.53 mm**), and the control (0 mg/ml) showed no inhibition.

This confirms the **potency and quick action** of synthetic fungicides in suppressing fungal growth. However, reliance on synthetic fungicides poses risks, including environmental pollution and the development of fungicide-resistant *Alternaria* strains.

Table 2: Antifungal Efficacy of Synthetic chemicals at Different Concentrations Against *Alternaria porri*

S\N	Concentration	Mean	SD
1	250	31	2.31
2	200	22	2.52
3	150	18.66	1.53
4	100	15.33	2.52
5	50	3.66	1.53
6	control	0	0
7	pValue	5.25E-12	

Antifungal Efficacy of *Azadracta indica* at Different Concentrations Against *Alternaria porri*

The ethanolic extract of *Azadirachta indica* showed promising antifungal activity, though less potent than synthetic fungicides. The inhibition zone at 250 mg/ml was **28.60 ± 6.00 mm**, nearly comparable to the highest dose of synthetic fungicide. The effect was concentration-dependent, decreasing to **5.67 ± 2.05 mm** at 50 mg/ml. This gradual decline indicates a cumulative effect of the bioactive compounds, which may take longer to act than synthetic chemicals.

Despite requiring higher concentrations to achieve similar inhibitory effects, neem extract offers a **biodegradable, eco-friendly, and non-toxic alternative**, making it highly valuable in integrated management systems.

Table 3: Antifungal Efficacy of *Azadracta indica* at Different Concentrations Against *Alternaria porri*

S\N	Concentration	Mean	SD
1	250	28.6	6
2	200	23.33	3.68
3	150	17	0.82
4	100	12.67	1.7
5	50	5.67	2.05
6	Control	0.00±0.00	
7	Pvalue	6.39E-10	

Antagonistic Activity of *Trichoderma harzianum*

Trichoderma harzianum demonstrated **strong antagonistic properties** against *A. alternata* and *A. solani*. The inhibition increased significantly with higher concentration. At 10^9 conidia/mL, growth inhibition reached **24.33 ± 3.33 mm**, while the lowest concentration (10^5 conidia/mL) showed **1.33 ± 1.89 mm** inhibition. The control exhibited no antifungal effect.

This confirms *T. harzianum*'s **mycoparasitic ability and competition for nutrients and space**, which disrupts the growth of pathogenic fungi. Unlike chemical treatments, *Trichoderma* offers a sustainable, eco-friendly approach with negligible side effects on the environment or non-target organisms.

Table 4: Antifungal Efficacy of *Trichoderma harzianum* at Different Concentrations Against *Alternaria porri*

S\N	Conc.(conidial suspension) **conidial/ml**	Mean	SD
1	109	24.33	3.33
2	108	15.67	2.49
3	107	9	0.82
4	106	5.33	1.25
5	105	1.33	1.89
6	Control	0.00±0.00	0
7	Pvalue	5.47E-11	

DISCUSSION

This study systematically evaluated the integrated management of *Alternaria* porri. infecting onions (*Allium cepa*) through morphological and molecular identification, followed by *in vitro* involving synthetic fungicides, *Azadirachta indica* (neem) extract, and *Trichoderma harzianum*.
Morphological and Molecular Identification: The presence of *A.* porri was confirmed, which are known to cause characteristic purple blight symptoms such as elongated leaf spots and yellow halos in leafeds in onion crops. These results align with recent reports by Lawal *et al.* (2025), who identified similar *Alternaria* species in Nigerian onion fields using both morphological keys and ITS-based molecular sequencing.

Synthetic Fungicides: The tested synthetic fungicides exhibited strong antifungal activity, showing significant inhibition of *Alternaria* porri. at even low concentrations. Despite their effectiveness, their prolonged use poses risks of environmental pollution and fungicide resistance, as emphasized by Okafor *et al.* (2024).

Azadirachta indica Extract: Neem leaf extract showed promising antifungal efficacy, especially at higher concentrations. Its bioactivity is attributed to phytochemicals such as azadirachtin, flavonoids, and saponins. The findings are supported by Musa *et al.* (2025), who reported that neem extracts suppressed *Alternaria alternata* growth by over 60% in vitro and recommended its integration into eco-friendly plant disease management strategies.
Trichoderma harzianum: This biocontrol fungus showed potent antagonistic effects against *Alternaria* spp., especially at higher conidial concentrations. Its antagonism is likely due to mechanisms such as mycoparasitism, enzyme secretion, and nutrient competition. This supports the findings by Ibrahim and Yusuf (2025), who demonstrated significant disease suppression in onion seedlings treated with *T. harzianum* under both greenhouse and field conditions.

CONCLUSION

The integrated use of chemical (synthetic fungicides), botanical (*A. indica*), and biological (*T. harzianum*) treatments provides a comprehensive and sustainable strategy for managing *Alternaria* infections in onions. While synthetic fungicides remain the most immediately effective, their ecological drawbacks warrant cautious application. The incorporation of neem extracts and *T. harzianum* can reduce dependency on chemicals, offering a more environmentally benign approach. Future research should focus on optimizing combination treatments, conducting field-scale validations, and monitoring pathogen resistance to enhance practical adoption among farmers.

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