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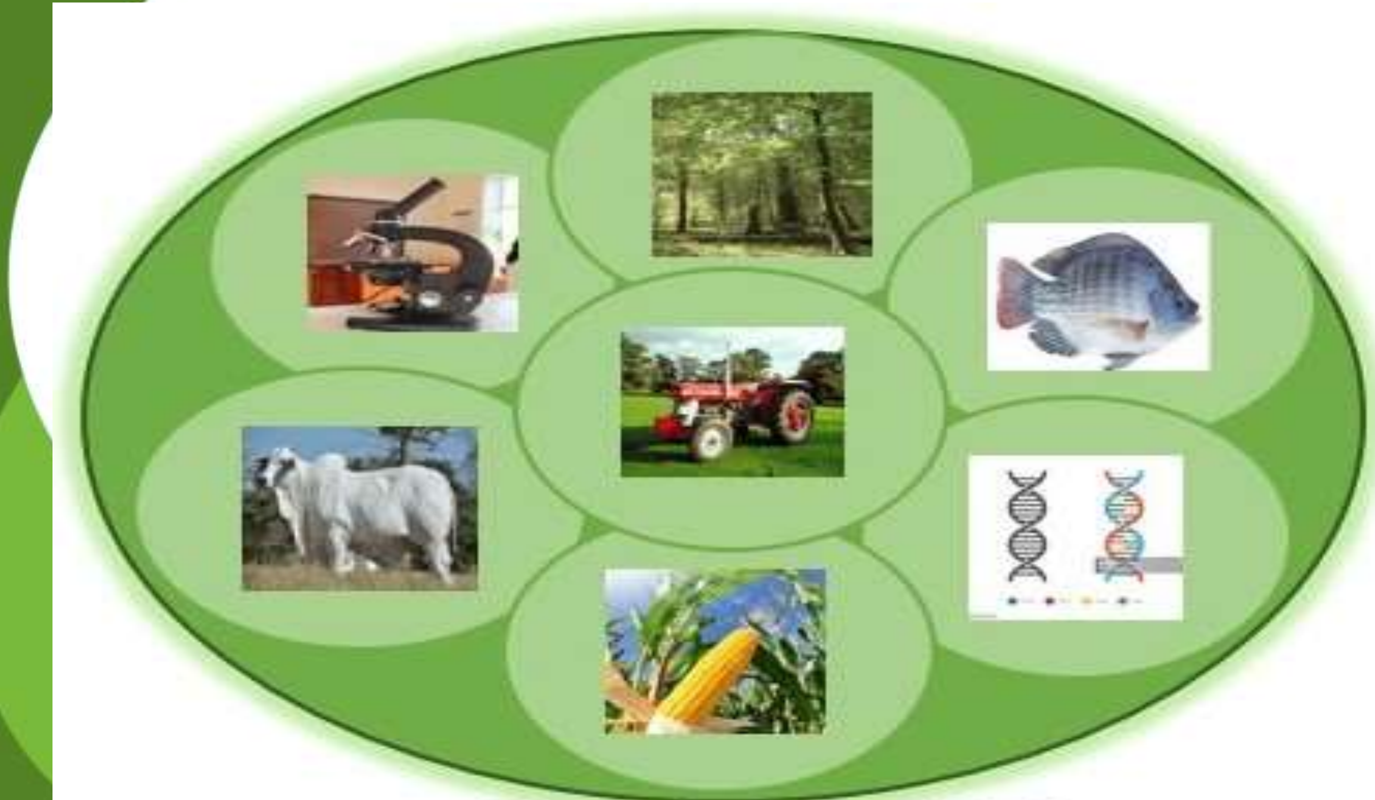

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The Kebbi Journal of Agriculture and Natural Sciences has the sole aim of providing an intellectual platform and ideas for scholars, by promoting interdisciplinary studies related to agriculture and natural science through publishing the latest scientific research findings that are of direct policy implications and beneficial to the research community. Consequently, the journal covers all aspects of Crop Science, Animal Science, Agricultural Economics, Agricultural Extension and Rural Development, Food Science, Fisheries and Aquaculture, Biotechnology, Soil Science and Agricultural Engineering, Forestry and Environment, Wildlife, Agricultural Education, Agro-allied Industries as well as all Natural Science researches related to Agriculture.

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IN-VITRO EVALUATION OF FUNGICIDAL EFFECT OF NEEM SEED OIL AGAINST *Alternaria porri* (Ellis) Cif., CAUSING PURPLE BLOTCH DISEASE OF ONION

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ABSTRACT

Purple blotch of onion, caused by *Alternaria porri* is a serious foliar disease of onion in Nigeria that reduces yield and bulb quality. Neem seed oil contains a complex mixture of compounds with well-documented insecticidal and antimicrobial properties. This research assessed the *in-vitro* fungicidal activity of neem seed oil against *Alternaria porri* using the poisoned-food technique. Neem seed oil was tested at 3 ml (T1), 5 ml (T2), 7 ml (T3), and 10 ml (T4) in potato dextrose agar (PDA). Completely Randomized Design (CRD) was used and the treatments were repeated six times. Radial mycelial growth was measured and percent inhibition relative to control was calculated. Data were subjected to Analysis of Variance (ANOVA) and significant means were separated using Least Significant Difference at $P \leq 0.05$. Results revealed that all the levels of neem seed oil used inhibited the mycelial growth of *A. porri* but with significant variation. There was no significant difference among the treatments 48 hours after inoculation. The mean colony diameter of treatment T1 and T2 (1.67 cm and 1.65 cm), respectively was statistically similar with the control (1.70 cm). After 96 and 120 hours of inoculation, there was significant ($P \leq 0.05$) difference among the treatments. Treatment T4 (10 ml) significantly reduced growth and sporulation of *A. porri* and had lowest mean colony diameter and highest percent growth inhibition (2.06 cm and 43.51%), respectively. Treatment T4 (10 ml) significantly reduced growth and sporulation of *A. porri* throughout the experiment. Complete inhibition was not observed at high volume. These findings revealed that neem seed oil has strong *in-vitro* antifungal activity against *A. porri* that caused onion purple blotch disease. Further research that will evaluate neem seed oil as a component of integrated management for purple blotch disease in Nigerian onion production under screen-house and field conditions is recommended.

Keywords**:** *Alternaria porri*, activity, fungicidal, *in-vitro*, neem seed oil, onion,

Introduction

Onion (*Allium cepa* L.) is one of the most commonly cultivated vegetable crops and important source of nutrition and income to farmers in Northern Nigeria. It belongs to the genus *Allium* and family *Alliaceae*. It is commonly known as “Queen of kitchen” or Princess of vegetables. Globally, onion is commonly grown in almost every part of the world where its cultivation can be directly from the seeds, bulbs or sets. Onion is cultivated as an annual crop but classified as biennial crop. It is one of the most consumed crops globally. Asia produced 67.8% of the total onion produced globally, while Africa produced 13.9% (FAOSTAT, 2023).

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According to FAOSTAT (2023), India is the largest producer of onion globally, producing over 30 million tons in 2023. Nigeria produces over 1.6 million tons of onion in 2023 (FAOSTAT, 2023). Onion production in Nigeria is mostly in the northern part of the country particularly Kano, Sokoto, Kebbi, Kaduna, Borno, Jigawa, Plateau, and Bauchi States.

Onion like any other vegetable crops is being affected by fungal diseases. Among the fungal diseases, purple blotch disease of onion caused by *Alternaria porri* is most damaging foliar diseases of onion worldwide; it cause lesions on leaves and necks, reducing photosynthetic area and marketable yield. The disease poses serious threat to onion production in Nigeria. Yield loss of up to 50 to 100% as a result of purple blotch disease has been reported (Tiwari *et al.*, 2022). Chemical fungicides are effective but have drawbacks including cost, environmental contamination, resistance development and limited availability for smallholder farmers (Rani *et al*, 2009). Botanical pesticides such as neem (*Azadirachta indica*) derivatives are attractive alternatives because they are biodegradable, locally available and show multiple modes of action against insect pests and pathogens (Rani *et al*, 2009).

Globally, many researchers are trying to find the solution to the harmful side effect of synthetic chemicals by developing integrated approach that involve use of botanical fungicides and biological agents (Kilani-Morakchi *et al*., 2021). Neem seed oil contains a complex mixture of compounds (e.g., azadirachtin, nimbin, salannin and fatty acids) with well-documented insecticidal and antimicrobial properties (Kilani-Morakchi *et al*., 2021; Su *et al*., 2023).

Several studies have demonstrated antifungal activity of neem extracts against plant pathogenic fungi, but information specific to *A. porri* and onion in Nigerian context is limited. The present study therefore assessed the in-vitro fungicidal efficacy of neem seed oil against *A. porri* isolated from infected onions in Aliero, Kebbi State, Nigeria using the poisoned-food technique and standard laboratory procedures.

Materials and Methods

Study Area

The experiment was conducted under laboratory conditions at Biotechnology Center of East and West African Virus Epidemiology, Abdullahi Fodio University of Science and Technology, Aliero (WAVE-AFUSTA). The center is located within the premises of Faculty of Agriculture, Abdullahi Fodio University of Science and Technology, Aliero (AFUSTA).

Source of inoculum

Inoculum of the confirmed *Alternaria porri*, the causal organism of purple blotch disease of onion was sourced from Department of Crop Protection, Faculty of Agriculture; Institute for Agricultural Research, Ahmadu Bello University, Zaria. The inoculum was cultured for multiplication before inoculation using potato dextrose agar (PDA) in Microbiology Laboratory of Abdullahi Fodio University of Science and Technology, Aliero (AFUSTA) before used for the experiment.

Preparation of Medium

Petri dishes and other glassware were washed with non-ionic soapy solution, rinsed in three changes of tap water and sterilized at 160^{o}C in hot-air oven for two hours. The culture media, Potato Dextrose Agar (PDA) was prepared following standard procedure. Two-hundred-gram (200 g) of peeled potato was boiled in one litre of water for 30 minutes, after that the supernatant was decant into sterilized breaker and stirred in twenty-gram (20 g) of agar-agar and 20 g of dextrose. This was followed by gentle heating on a hot plate, the media were pour into two litres conical flasks and sterilized in autoclave at 121.6°C for 20 minutes, PDA media were amended with 50 mg L^{-1} streptomycin to suppress bacterial growth. Twenty mill (20 ml) of PDA media were poured into 90 mm Petri dish and leave on laboratory bench to solidify (Patel and Patel, 2016).

Preparation of Neem Seed Oil and Emulsions

Crude neem seed oil (cold-pressed) was obtained from a certified local supplier and stored in amber bottles at 4^0C until use. For incorporation into PDA, neem oil emulsions were prepared by mixing the oil with sterile distilled water containing 0.1% (v/v) Tween-80 (as emulsifier) and vortexing to form a stable dispersion just prior to addition to autoclaved, cooled PDA (approximately 50^0C) (Patel and Patel, 2016).

Treatments and Experimental Design

Treatments consist of four different volumes of neem seed oil *viz*., 3 ml, 5 ml, 7 ml and 10 ml which were designated as T1, T2, T3, and T4, respectively. PDA was prepared and amended with the appropriate volume of neem oil emulsion to achieve target volume, poured into 90 mm Petri dishes (20 ml per plate) and allowed to solidify. A 5 mm mycelial disc was cut from the margin of a 7-day old *A. porri* culture and centrally placed (mycelial side down) on each plate. Plates were incubated at 25± 2^0C in the dark. PDA with 0 volume (control) of neem oil was maintained for comparison. The experiments were conducted under laboratory condition and Completely Randomized Design (CRD) was used. Treatments were repeated six times.

Data Collection

Petri dishes were labelled based on the treatments designates *viz*., A, B, C, and D. The labelled petri dishes were arranged in a Completely Randomized Design (CRD) on the laboratory bench. Radial colony diameters were measured 48 hours after inoculating the plate and there after the measurement was taken at 24 hours interval, which commenced two days after inoculation (2 DAI) until seven days after inoculation (7 DAI). Per cent inhibition of mycelial growth was calculated by using formula given by Vincent (1947) as:

$$I = \frac{C - T}{C} \times 100$$

Where:
I = percent inhibition

C = Colony diameter in control
T = diameter in treatment

Data Analysis

Data generated were subjected to analysis of variance (ANOVA) using SAS software V9.0, and where there was significant difference among the treatments, Least Significant Difference (LSD) method of means comparison was used at $P \leq 0.05$ level of significance.

Results

Effect of Neem Seed Oil on Mycelial Growth of *A. porri*

Results obtained on the effect of neem seed oil on colony diameter and percent growth inhibition are presented in Table 1, 2, and 3 below. After 48 hours of inoculating the media, there was not significant ($P \leq 0.05$) different between treatment T1 and T2 and between treatment T3 and T4. The mean colony diameter of treatment T1 and T2 (1.67 and 1.65), respectively was statistically similar with the control (1.70). The mean colony

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diameter of treatment T3 and T4 (1.46 and 1.46) differed significantly from the control (1.70). After 72 hours of inoculating the media, there was significant (P ≤ 0.05) different between treatment T3 and T4 and control. Treatment T3 and T4 recorded the lowest mean colony diameter. At both 48 and 72 hours after inoculation, both treatment T3 and T4 recorded the highest percent growth inhibition (Table 1). After 96 and 120 hours of inoculating the media, there was significant (P ≤ 0.05) different between treatment T1 and T2 and between treatment T3 and T4. Treatment T4 recorded the lowest mean colony diameter and highest percent growth inhibition (2.06 and 43.51%), respectively (Table 2).

After 144 hours of inoculating the media, there was also significant (P ≤ 0.05) different between treatment T1 and T2 and between treatment T3 and T4. Treatment T4 recorded the lowest mean colony diameter and highest percent growth inhibition (2.30 and 45.24%), respectively (Table 3). After 144 hours of inoculating the media, when the mycelial growth in the untreated plate is full, treatment T4 recorded the lowest colony diameter and highest percent growth inhibition (Plate 1 and 2).

Table 1: Effect of different volumes of neem seed oil on colony diameter and percent growth inhibition against *A. porri in vitro.*

Treatment (Neem seed oil)	**Mean Colony diameter (cm) 48 HAI**	**Percent growth inhibition (%)**	**Mean Colony diameter (cm) 72 HAI**	**Percent growth inhibition (%)**
3 ml	1.67[a]	1.76	2.46[a]	11.19
5 ml	1.65[a]	2.94	2.04[b]	26.35
7 ml	1.46[b]	14.12	1.89[c]	31.77
10 ml	1.46[b]	14.12	1.78[c]	35.74
Control	1.70[a]	0.00	2.77[a]	0.00
$LSD_{0.05}$	**0.242**		**0.417**	
CV	**2.059**		**2.059**	
Level of significance	*		*	

Means with the same letter in the column are not significantly different. LSD = least significant difference, CV = critical value of t, * = significant

Table 2: Effect of different volumes of neem seed oil on colony diameter and percent growth inhibition against *A. porri in vitro.*

Treatment (Neem seed oil)	**Mean Colony diameter (cm) 96 HAI**	**Percent growth inhibition (%)**	**Mean Colony diameter (cm) 120 HAI**	**Percent growth inhibition (%)**
3 ml	2.78[a]	16.52	3.04[a]	22.65
5 ml	2.47[c]	25.83	2.64[b]	32.82
7 ml	2.50[a]	24.92	2.63[b]	33.08
10 ml	2.06[d]	38.14	2.22[c]	43.51
Control	3.33[a]	0.00	3.93[a]	0.00
$LSD_{0.05}$	**0.52**		**0.58**	
CV	**2.059**		**2.059**	
Level of significance	*		*	

Means with the same letter in the column are not significantly different. LSD = least significant difference, CV = critical value of t, * = significant

Table 3**:** Effect of different volumes of neem seed oil on colony diameter and percent growth inhibition against *A. porri in vitro.*

Treatment (Neem seed oil)	Colony diameter (cm) 144 HAI	Percent growth inhibition (%)
3 ml	3.65[a]	13.09
5 ml	2.71[b]	35.48
7 ml	2.80[b]	33.33
10 ml	2.30[c]	45.24
Control	4.20[a]	0.00
$LSD_{0.05}$	**2.059**	
CV	**0.455**	
Level of significance	*	

Means with the same letter in the column are not significantly different. LSD = least significant difference, CV = critical value of t, * = significant

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Plate 1: control plate

Plate 2: treated plate

Discussion

Several studies have been conducted to explore the potential of essential oils as antifungal agents against plant pathogenic fungi (Ko *et al*. 2003; Oxenham *et al*. 2005). In this experiment, neem seed oil was evaluated for its fungicidal effect against *A. porri in vitro* the causal organism of purple blotch disease of onion. The fungus is a serious plant pathogenic fungus of onion. So considering the importance of essential oils as ecofriendly agents, the effect of neem seed oil was studied against *A. porri*. It is interesting to note that neem seed oil had some fungicidal effect against *A. porri*. The effect of the oil was assessed on the basis of colony diameter and percent growth inhibition. All the volumes of neem seed oil used inhibited the mycelial growth of *A. porri* but with significant variation.

The present study demonstrates that neem seed oil exhibit strong *in-vitro* antifungal activity against *Alternaria porri*, with inhibition increasing with higher volume of neem seed oil. With 10 ml, neem seed oil reduced radial growth by approximately 45%, indicating potent suppression of mycelial development. These findings agree with previous reports that neem derivatives possess antifungal properties against a variety of plant pathogenic fungi (Deshmukh *et al*., 2020; Tiwari *et al*., 2022).

The antifungal activity of neem seed oil is attributed to multiple bioactive compounds (e.g., azadirachtin, nimbin, salannin, and several fatty acid constituents) which may interfere with membrane integrity, respiration, sporulation and germination of fungal propagules (Kilani-Morakchi *et al*., 2021; Su *et al*., 2023). The volume-dependent response observed suggests that, higher volume increase the availability of active molecules that disrupt fungal physiology. Complete inactivation of the fungus *in-vitro* was not achieved in this research even at 10 ml neem seed oil, which suggests that combination of strategies (e.g., integrating neem seed oil with reduced-rate synthetic fungicides or other botanical extracts) may be required for complete control. The inhibitory effects of essential oils of neem seed, eucalyptus, *Ocimumbasilicum*, *Prosopiscineraria*, *Derrisindica* have been evaluated against plant pathogenic fungi (Rai *et al*., 1999) and Chukrasiatabularis, Meliadubia and Hyssopusofficinalis (Nagalakshmi *et al*. 2003) were evaluated against *F. oxysporum*. Antifungal properties of essential oils of three Pistaciaspecies have also been studied for *F. sambucinum* (Duru *et al*. 2003).

Treatment T4 (10 ml) recorded the lowest mean colony diameter and the highest growth inhibition in 48, 72, 96, 120, and 144 hours respectively. The present finding was supported by the reports of the previous researches (Tiwari and Srivastava, 2004; Meena 2012; Abdel-Hafez *et al.*, 2014). Neem seed oil offers environmental and socio-economic advantages: it is biodegradable, has low mammalian toxicity, and can be produced locally, making it attractive for smallholder onion farmers in the study area. While *in-vitro* assays are useful screening tools, they do not always predict field efficacy because of factors such UV degradation, wash-off by rain, and cuticular penetration on leaves. Therefore, screen house and filed evaluations are recommended to assess phytotoxicity on onion, persistence under field conditions and practical application rates. Further studies have to be done to evaluate the efficacy of neem seed oil against fungal infections, so that it importance as an alternative to synthetic fungicide can be properly assessed. The sources of this oil is available easily in the local market and if it is used in the field conditions, it may proves to be more economical and environmentally safe.

Conclusion

Neem seed oil significantly inhibits the mycelial growth of Alternaria porri in-vitro in a volume-dependent manner, with 10 ml producing 45.24% inhibition after 144 hours. These promising in-vitro results support further screen house and field trials to evaluate integration into integrated disease management programs for purple blotch disease in the study area and Nigerian onion production.

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