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Identification and Morpho-Cultural Characterization of *Colletotrichum sublineolum* Causal Agent of Sorghum Anthracnose and Its Management with Extracts of *Azadiracta indica* in Dutsin-Ma, Nigeria

[1]Gwa, V. I.* and [2]Adam, S.

Author's Affiliation
[1,2]Department of Agronomy, Faculty of Agriculture, Federal University of Lafia, PMB 146, Nasarawa State, Nigeria

***Corresponding Author:**
Gwa, V.I.,
Department of Crop Protection, Faculty of Agriculture, Federal University Dutsin-Ma, PMB 5001, Katsina State, Nigeria
E- mail: gwavictor9@gmail.com
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Abstract
This study investigated the isolation, identification, characterization, and control of *Colletotrichum sublineolum*, the fungus responsible for sorghum anthracnose, using neem (*Azadirachta indica*) extracts (seed, leaf, and bark) combined with methanol at concentrations of 0%, 10%, 20%, and 30%. Infected sorghum leaves were collected from local farms, and the fungus was isolated from five fields, resulting in five distinct isolates: DTMCOE, DTMHYG, DTMDRW, DTMSRB, and DTMSMQ. DTMSRB had the highest frequency of occurrence (12), followed by DTMDRW (8), while DTMSMQ was least frequent (4). Pathogenicity testing on detached sorghum leaves revealed that DTMSRB and DTMHYG isolates were highly virulent, producing severe infection symptoms. DTMDRW and DTMCOE isolates caused moderate infection, while DTMSMQ exhibited only slight symptoms. Isolates were further characterized on Potato Dextrose Agar (PDA) based on physical traits such as colony elevation, margin, texture, color, and conidia shape. Most isolates showed hyaline, smooth-walled, falcate conidia without septa and smooth colony margins, though a few had undulated margins. Morphological diversity among isolates indicated the possible presence of several *C. sublineolum* sub-species in the study area. *In vitro* evaluation showed that all neem-methanol extracts effectively controlled fungal growth, with neem bark extract having the most significant impact in reducing the radial growth of *C. sublineolum* compared to leaf and seed extracts. Variations in concentrations (especially higher levels) improved the antifungal efficacy of the neem extracts. The study suggests that neem-based treatments could be a viable approach for managing *C. sublineolum* and recommends formulating neem-methanol extracts for practical use in sorghum anthracnose control.

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INTRODUCTION

Sorghum (Sorghum bicolor L. Moench) is a major cereal crop in sub-Saharan Africa, including Nigeria, where it is a staple food for millions of people. It is also significant for its use in animal feed and industrial applications. Sorghum is one of the most important food crops in Nigeria due to its adaptability to arid and semi-arid conditions (Adeoti et al., 2020). It is widely grown in the northern regions, where it contributes significantly to food

security. The crop's resilience to drought and its suitability for cultivation in marginal soils make it a preferred choice among smallholder farmers (Usman et al., 2021). However, sorghum production is often constrained by various diseases, with anthracnose, caused by *Colletotrichum sublineolum*, being one of the most destructive.

C. sublineolum is considered to be a very heterogeneous species and is prevalent in all sorghum growing areas of sub-Saharan Africa where it causes huge economic losses in terms of quality and quantity of grain yields, depending on the susceptibility of the sorghum cultivars, epidemics of the region and environmental conditions (Ali & Warren, 2019). In Nigeria, anthracnose is prevalent, especially in the northern regions where sorghum is extensively cultivated (Ibrahim et al., 2019). *C. sublineolum* is the only *colletotrichum* species that has been confirmed to cause foliar, stem, panicle and grain anthracnose on sorghum ((Christopher *et al*., 2016; Aragaw et al., 2023). The foliar infection occurs at any stage of plant growth and yield losses on leaves, stems, and seeds from 20 % to 80 % have been reported in susceptible cultivars and areas characterized by alternating dry and humid weather conditions combined with moderate to high temperatures (Tesso *et al*. 2012; Ali & Warren, 2019; Prom *et al*., 2019).

The disease symptoms vary and are determined by environmental factors, plant developmental stage, and crop variety (Tesso *et al*. 2012). Leaf symptoms appear as circular or elliptical to elongated lesions of dark reddish purple to tan colour. The centre of the lesion is straw colored, with reddish brown or reddish orange margins, containing black acervuli with setae (Prom *et al*. 2016). The disease also manifests as leaf blight, stem lesions, and panicle and grain infections, leading to reduced photosynthesis, poor grain fill, and significant yield losses (Mohan et al., 2020)

Characterizing *C. sublineolum* involves both morphological and cultural studies. Morphological characterization includes observing the size, shape, and color of conidia, while cultural characterization involves the study of colony morphology on different media (Oliveira et al., 2017). These techniques are essential for accurate identification and differentiation of pathogenic strains, which is crucial for effective disease management (Sarr et al., 2018).

The increasing concern over the environmental impact and health hazards associated with chemical fungicides has spurred interest in biological control methods. Chemical control method is more efficient and dependable in most instances (Gwa & Akombo, 2016). On the other hand, the synthetic chemicals are hazardous to living organisms including beneficiary plants, animals, humans, water bodies, soils micro and macro organisms, non-target specific, non-biodegradable and as well have a lengthy residual effect in the ecosystem (Lakshmeesha et al., 2013) Biological control with the use of antagonistic organisms such as *Trichoderma* species, *Pseudomonas syringae* and *Pseudomonas chlororaphis* have been extensively used by various researchers across the globe due to their characteristics such as fast competition, parasitism, plant root colonization, induced systemic resistance, plant growth promotion, production of antibiosis (Khan et al., 2018; Nwankiti & Gwa 2018; Sharma et al., 2019; Gwa & Ekefan, 2021; Gwa & Ekefan, 2024).

In recent years, the need for effective and sustainable disease management strategies has led to increased interest in the use of plant-based products such as neem for their antifungal properties (Kumar & Dutta, 2019). Neem extracts contain bioactive compounds such as azadirachtin, nimbin, and salannin, which exhibit broad-spectrum antimicrobial activity against various plant pathogens (Singh & Singh, 2020). The use of neem as a biopesticide offers a sustainable alternative to chemical fungicides, with the added benefits of being eco-friendly and safe for non-target organisms (Sharma et al., 2021). Studies have demonstrated the effectiveness of neem extracts in controlling anthracnose in crops such as mango, chili, sorghum and yam (Gwa & Nwankiti, 2017; Farooq et al., 2018; Abu et al., 2022).

Despite the proven efficacy of neem extracts in controlling various plant diseases, there is limited information on their use against *Colletotrichum sublineolum* in sorghum, particularly in Nigeria. This study seeks to fill this gap by investigating the potential of neem

extracts in managing sorghum anthracnose in Dutsin-Ma, Katsina State. The findings will contribute to the development of sustainable disease management strategies that can enhance sorghum production in the region. This study aims to identify and characterize *C. sublineolum* isolates from sorghum in Dutsin-Ma, Katsina State, Nigeria, and to evaluate the effectiveness of neem extracts in controlling the pathogen.

MATERIALS AND METHODS

Study Area

The research is conducted in Dutsin-Ma, Katsina State, Nigeria, and a region known for its significant sorghum cultivation. The area experiences a semi-arid climate with distinct wet and dry seasons, making it suitable for studying sorghum diseases and their management (Usman et al., 2021).

Sample Collection

Sorghum plant parts exhibiting symptoms of anthracnose, such as leaf blight, stem lesions, and panicle infection, were randomly collected from five fields namely: Dutsin-Ma College of Education (DTMCOE, Dutsin-Ma Hayingada (DTMHYG), Dutsin-Ma Darawa (DTMDRW), Dutsin-Ma Sototo Rima Basin (DTMSRB) and Dutsin-Ma Shema's Quarters (DTMSMQ) in Dutsin-Ma Local Government Area of Katsina State, Nigeria. The diseased plant parts, including leaves, stems, and seeds, were carefully cut and placed in sterile plastic bags for transportation to the laboratory for isolation and identification of pathogens (Ibrahim et al., 2019).

Isolation of *Colletotrichum sublineolum*

The collected samples were surface-sterilized in 5 % Sodium hypochlorite solution for1 minute (Sani & Gwa, 2018). This was followed by rinsing the sterilized tissues in three successive changes of sterile distilled water. The aseptically prepared Potato Dextrose Agar (PDA) was amended with 0.16g of powdered Streptomycin Sulphate to suppress bacteria growth. Small pieces of the infected sorghum tissue were then aseptically placed on the solidified PDA and incubated at 30° C for 7 days. Plates were regularly monitored and growth colonies were examined to determine the frequency of each of the pathogens isolated (Gwa & Lum 2024).

Determination of frequency

The frequencies of the isolated pathogens were determine by counting the number of times each isolate occurred out of the total number of isolates grown on PDA during the period of isolation from each location and were expressed as percentage of the total number of all isolates in all the locations as described in the equation below.

$$\% \, frequency \, of \, occurrence = \frac{a}{b} \times \frac{100}{1}$$

a = number of times *C. sublineolum* isolates was encountered in a location.
b = total number of times *C. sublineolum* isolates was encountered in all the locations.

Colonies that grew on the plates were examined and aseptically sub-cultured to obtain pure isolates (Oliveira et al., 2017). Stock cultures of *C. sublineolum* isolates were maintained on slant of acidified potato dextrose agar (PDA) in McCartney bottles for pathogenicity test.

Pathogenicity test of *C. sublineolum* using detached leaves

To confirm the pathogenicity of the *C. sublineolum* isolates (DTMCOE, DTMHYG, DTMDRW, DTMSRB and DTMSMQ) from the representative five locations, healthy detached sorghum leaves were used. The designated codes were given based on the locations where isolates were collected and tested. One isolate was randomly selected from stock cultures of isolates from each location and grown on PDA at 30°C for 14 days. The method of Binyam et al., (2016) was used where conidia suspension of each isolate was got by scraping from a 7 day old mycelial and suspending the culture in sterile distilled water and shaking vigorously for 90 seconds. The spores' suspensions were then filtered through a three layer cheese cloth and the spores' concentrations were adjusted to 1 × 10^6 spores/mL using haemacytometer prior to inoculation. The healthy sorghum leaves were collected from sorghum field at the University crop research field and were washed thoroughly in sterile distilled water before surface-sterilizing in 5 % Sodium hypochlorite solution for 30 seconds. Sterilized leaves were rinsed in three successive changes of sterile distilled water (Sani & Gwa, 2018). Leaves were aseptically cut and placed in Petri dishes

lined with four layers of sterilized and moisten tissue papers. The leaves were sprayed with spore suspensions of each isolate and kept in a humid chamber at 30°C for 14 days. Some of the leaves sprayed with sterile distilled water served as control. Treatments were arranged in completely randomized design (CRD) and replicated three times each. The development of anthracnose symptoms were monitored after the 14 days period of inoculation (Adeoti et al., 2020). The causal agent from the infected leaves was re-isolated and the characteristics studied and compared with the initial culture (Binyam et al., 2016).

Cultural Characterization and morphological Characterization

Cultural characterization and morphological characterization of *C. sublineolum* isolates were performed by observing the colony characteristics on PDA, including color, texture, and colony margin, colony elevation, growth rate, sporulation, and pigmentation. The conidia were examined under a microscope to measure their size, shape, and septation. These observations were compared with standard descriptions for identification (Sarr et al., 2018; Mohan et al., 2020; Aragaw et al., 2023).

Preparation of *Azadirachta indica* extracts

Seeds, barks and leaves of *A. indica* (Neem) were acquired from matured neem trees from the take-off Campus of the Federal University Dutsin-Ma. They were meticulously washed with running tap water and left to air dry. Once dried, the *A. indica* products including seeds, barks, and leaves were separately crushed into a powdery consistency using a sterilized mortar and pestle. Subsequently, 50 g of the powdered seed, bark and leaf were separately dissolved into 100 mL of sterile distilled water. Additionally, different concentrations of methanol (0 mL, 10 mL, 20 mL, and 30 mL) were separately added to the *A. indica* products (after subtracting the equivalent quantity from the products before adding methanol), resulting in concentrations of 0 %, 10 %, 20 %, and 30 % methanol, respectively. The prepared extracts were stored at 4°C for subsequent use (Gwa et al., 2019; Kumar & Dutta, 2019).

In Vitro antifungal assay

The antifungal activity of neem-methanol extracts against *C.sublineolum* was assessed using the poisoned food technique. PDA plates were amended with different concentrations of the neem-methanol (0 %, 10%, 20 %, and 30 %) extracts and inoculated with a 5 mm disc of a 5 day actively growing *C. sublineolum* culture. The plates were incubated at 30°C for 5 days, and the radial growth of the fungus was recorded (Gwa & Iliyasu, 2024).

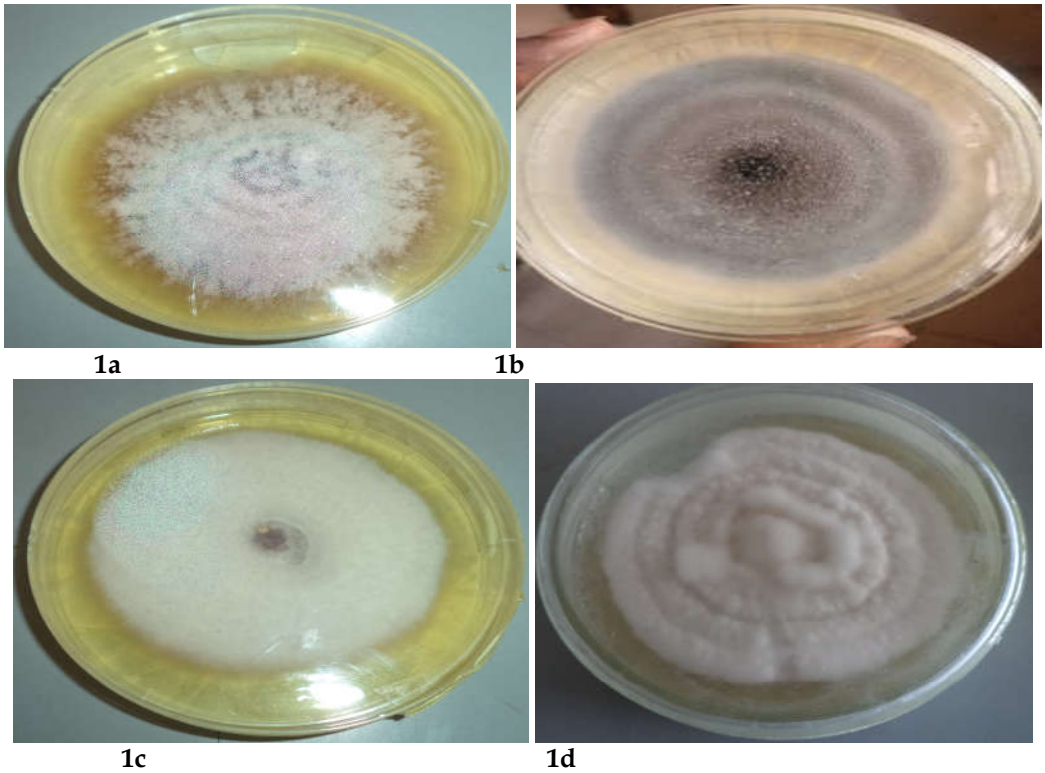
Data Analysis

The data obtained from the experiments were analyzed using statistical software. Analysis of variance (ANOVA) was performed to determine the significance of differences between treatments. Mean comparisons was carried out using the Least Significant Difference (LSD) test at a 5 % significance level (Sharma et al., 2021).

RESULTS

Isolation and Identification of *C. sublineolum*

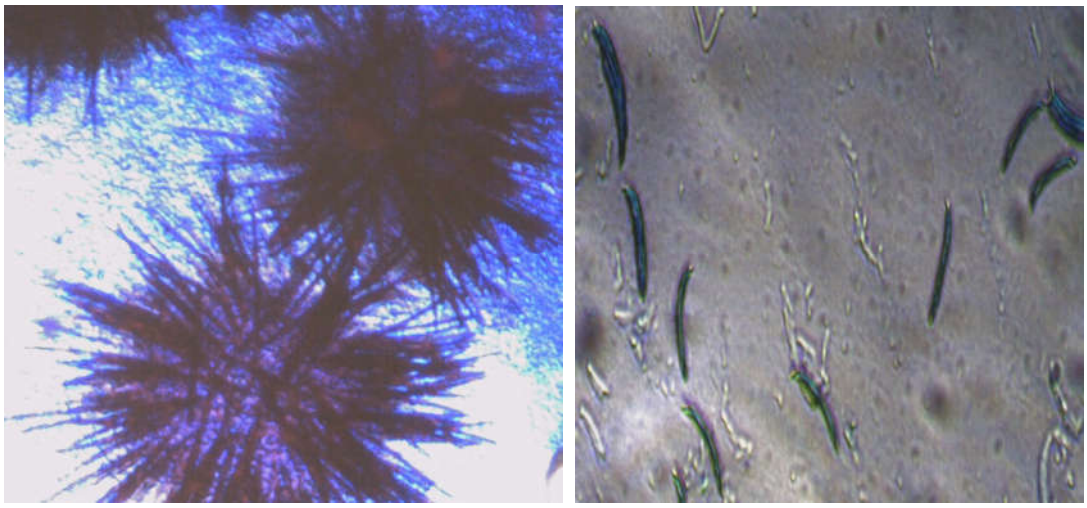
Cultural characteristics of *C. sublineolum* isolates on PDA showed variation in colony color, margin, colony elevations and textures as shown in Figure 1a, 1b, 1c and 1d. *C. sublineolum* isolates show variation in color from white, salmon white, ivory-white, rosy-brown, and plum-pink. Others observed were gray colony colors to light-gray to gray, and purple-gray to cottony-gray. Other isolates produced saddle-brown to sandy-brown, goldenrod to light-goldenrod, dim-gray, tan-brown to wheat-brown and light-yellow when viewed on the reverse side of the plates. Microscopic examination showed the length and width of size of acervulus and conidial of *C. sublineolum* as well as the shape at eyepiece lens magnification of ×10 and objective lens magnification of ×40 (mg = ×400) as presented in Figure 2a and 2b.



1a 1b

1c 1d

Figure 1: Cultural characteristics in the colonies of *C. sublineolum* isolates associated with sorghum anthracnose (a) DTMDRW, white on the upper view (b) DTMCOE, saddle-brown to sandy-brown on the upper view (C) DTMSRB, ivory-white and cottony on the upper view (d) DTMHYG, white and cottony on the upper view grown on potato dextrose agar at 25◦C after 7 days of incubation.



2a 2b

Figure 2: Acervulus with setae of DTMSRB isolate of *C. sublineolum* infecting sorghum at Dutsin-Ma (2a) and conidia of DTMSRB isolate of *C. ssublineolum* (2b)

Frequency of occurrence of *C. sublineolum* isolates

Frequencies of occurrence of *C. sublineolum* isolates on leaves of sorghum from different locations were observed after each time of isolation. Number of isolates from the leaf samples were separately counted and totaled from each of the locations. The result in Table 1 revealed that *C. sublineolum* isolates were highest (12) in Sokoto Rima Basin (SRB) and least (4) in Shema's Quarter (SMQ) with frequency of 34.29 % and 11.43 %, respectively.

Table 1: Frequency and percentage frequency of occurrence of *C. sublineolum* isolates on sorghum leaves in different location

Location	Frequency of isolates	% frequency of isolates
DRW	8	22.85
COE	5	14.29
SRB	12	34.29
HYG	6	17.14
SMQ	4	11.43
Total	35	100.00

Pathogenicity test of *C. sublineolum* with detached leaves

Healthy sorghum leaves were detached and inoculated with different isolates of *C. sublineolum.* After 14 days of incubation, the inoculated detached leaves of sorghum manifested symptoms of anthracnose disease which were similar to those symptoms that were seen under natural condition. Table 2 shows that the degree of infectivity and virulence varies with the type of isolate with severe symptoms being observed on isolates DTMSRB and DTMHYG while DTMDRW and DTMCOE produced moderate infection of the leaves with slight symptoms observed in isolate DTMSMQ. The typical symptoms of infection observed on the artificially inoculated leaves were similar with those symptoms originally produced under the natural infection. All the detached uninoculated healthy leaves did not illicit any symptom of infection on the sorghum leaves throughout the 14 days of incubation. The inoculated *C. sublineolum* isolates were re-isolated from the infected detached leaves of sorghum whereas the uninoculated (control) had no fungi for isolation. This experiment confirmed, Koch's postulates and the isolates of the five *Colletotrichum* species were scientifically confirmed to be the pathogenic causal agents of sorghum anthracnose disease. Koch's postulate was verified by identifying the re-isolated isolates using their morpho-cultural characteristics based on established literatures.

Table 2: Pathogenicity test of *C. sublineolum* on detached sorghum leaves across locations

	Level of infection on sorghum		
***C. sublineolum* isolates**	**inoculated**		**Uninoculated (Control)**
DTMDRW	**++**		**-**
DTMCOE	**++**		**-**
DTMSRB	**+++**		**-**
DTMHYG	**+++**		**-**
DTMSMQ	**+**		**-**

+ = slight infection; ++ = moderate infection; +++ = severe infection; - = no infection

Morpho-Cultural Characterization of *C. sublineolum* isolates

Result in Table 3 presents the mycelial and colony characteristics of the five isolates of *C. sublineolum* after 7 days of incubation on PDA medium, Variations in colony color of the *Colletotrichum* isolates grown on PDA were observed after the incubation period and the five isolates were classified ranging from white to cottony appearance to saddle-brown to sandy-brown. Table 3 shows all the mycelial characteristics of all the isolates were typically same as all conidia were hyaline, falcate, and smooth with no septate formation observed. All the isolates showed similar characteristics of appressoria with black glogbose-shaped and unicellular in nature after 7 days of growth on PDA medium.

Table 3: Morpho-cultural characteristics of *C. sublineolum* isolates grown on PDA and incubated

at 25°C for 7 days

Isolate ID Code	Cultural Characteristics							
	Location	Colony Elevation	Plant Part	Colony Margin (mm)	Colony texture	Colony color	Microscopic Character	Pathogen Identity
DTMDRW	DRW	Flat	Leaf	Smooth	Velvet	Top-White	Falcate, no septation and hyaline	*Colletotrichum sublineolum*
DTMCOE	COE	Flat	Leaf	Smooth	Velvet	Rosy-brown	Falcate, no septation and hyaline	*Colletotrichum sublineolum*
DTMSRB	SRB	Flat	Leaf	Smooth	Velvet	Top-White	Falcate, no septation and hyaline	*Colletotrichum sublineolum*
DTMHYG	HYG	Raised	Leaf	Undulated	Fluffy	Top-White	Falcate, no septation and hyaline	*Colletotrichum sublineolum*
DTMSMQ	SMQ	Flat	Leaf	Smooth	Velvet	Top-White	Falcate, no septation and hyaline	*Colletotrichum sublineolum*

DTMDRW, DTMCOE, DTMSRB, DTMHYG and DTMSMQ are isolates collected from Dutsin-Ma Darawa, Dutsin-Ma College of Education, Dutsin-Ma Sokoto Rima Basin, Dutsin-Ma Hayingada and Dutsin-Ma Shema's Quarters, respectively. DRW, COE, SRB, HYG and SMQ means Darawa, College of Education Sokoto Rima Basin, Hayingada and Shema's Quarters, respectively.

In vitro* efficacy of seed extract of *A. indica* and methanol against *C. sublineolum

Table 4 presents the efficacy of neem seed and methanol (0%, 10%, 20%, and 30%) extract on the radial growth of *C. sublineolum* over five days of incubation. The result revealed that the control treatment (0 %) on the 1st day showed the highest radial growth; with values of 10.000 mm while the 30 % neem-methanol produced radial growth of only 2.50 mm. The same trend continued till the 5th day where the control (0 %) produced the highest radial growth of 55.00 mm and the 30 % treatment reached the least radial growth of only 14.00 mm. There was statistical significance among the treatments throughout period of incubation.

Table 4: **Effect of concentration of neem seed extract and methanol on *C. sublineolum* after five days on inoculation**

Conc (50g seed + % methanol)	Days after inoculation and radial growth (mm) 1	2	3	4	5
0%	10.000[a]	15.00[a]	24.33[a]	36.00[a]	55.00[a]
10%	6.500[b]	8.50[b]	12.50[b]	15.50[b]	18.50[b]
20%	8.000ab	11.50[b]	14.50[b]	16.50[b]	18.50[b]
30%	2.500c	6.00c	8.00c	12.00[b]	14.00[c]
SEM	1.10	1.269	0.850	2.646	1.291

The means were separated using DMRT= Duncan multiple range test

Table 5 presents the effects of different concentrations of bark extract combined with methanol on the radial growth of *C. sublineolum* over a five day period. The control treatment (0%) exhibited the highest radial growth, starting at 10.000 mm on the 1st day while the 30 % treatment did not produce any growth (00 mm) of the pathogen. The 10 % and 20 % however produced 6.00 mm each on the 1st day of incubation. There was a progressive increase in radial growth for the other concentrations up till the 5th day except

30 % concentration which demonstrated the strongest inhibitory effect, completely inhibiting fungal growth with 00.00 mm of radial growth recorded across all days. Similarly, the neem bark extract and methanol combination, particularly at a concentration of 30 %, significantly (P≤0.05) inhibited the growth of *C. sublineolum* compared with the other concentrations.

Table 5: Effects of neem bark extract and methanol on C. sublineolum after five days on inoculation

Conc (50g bark + % methanol)	Days after inoculation and radial growth (mm)				
	1	**2**	**3**	**4**	**5**
0%	10.000a	15.000[a]	24.33[a]	39.00[a]	55.00[a]
10%	6.500[b]	9.500[b]	12.50[b]	15.50[b]	17.50b
20%	6.000[c]	8.000[c]	10.50[b]	12.50[c]	14.50c
30%	0.000d	0.000d	0.00c	0.00d	0.00d
SEM	0.391	0.514	0.707	0.577	0.612

The means were separated using DMRT= Duncan multiple range test

Table 6 presents the effects of different concentrations of neem leaves extract combined with methanol on the radial growth of *C. sublineolum* over a five day period. The control treatment (0 %) exhibited the highest radial growth, starting at 10.00 mm on the 1st day while the 30 % concentration only produced radial growth of only 2.50. Decrease in radial growths were observed across the different levels of the concentration with the control having the highest of 55.00 mm on the 5th day with the least value of 13.00 mm being observed in 30 % concentration. Statistical significance (P≤0.05) was observed across the different levels of concentrations for each day throughout the period of incubation. The result revealed that neem leaves extract and methanol, particularly at higher concentrations, significantly inhibited the growth of *C. sublineolum* more than at lower concentrations.

Table 6: Effects of Neem Leaves Extract and Methanol on Colletotrichum Sublineolus after inoculation

Conc (50 g leaves + % methanol)	Days after inoculation and radial growth (mm)				
	1	**2**	**3**	**4**	**5**
0%	10.000[a]	15.00[a]	24.33[a]	36.00[a]	55.00[a]
10%	8.500[a]	9.667[b]	15.50[b]	18.50[b]	17.50[b]
20%	6.33[b]	8.500[b]	9.50[c]	13.50[c]	15.50[c]
30%	2.500[b]	4.500[b]	8.00[c]	11.00[c]	13.00[d]
SEM	1.434	1.871	0.905	2.134	0.391

The means were separated using DMRT= Duncan multiple range test

Table 7 presents the interaction effect of mean concentrations of neem seed, bark, and leaves

extracts for the 5 days period of incubation. The results show that mean concentrations for neem seed, neem bark and neem leaves across the 5 days of incubation was statistically significant (P≤0.05) throughout the incubation period for each of the type of neem products used with the highest value being observed on the 5th day.

Table 7: Interaction effect of mean concentrations neem seed, bark and leaves extracts on *C. sublineolum* after 5 days of inoculation

Mean Conc.	Days after inoculation and radial growth (mm)					
	1	2	3	4	5	S.E
SEED	6.75[d]	10.25d	14.83c	20.00[b]	26.50[a]	1.431
BARK	5.62e	8.12d	11.83c	16.75[b]	21.62[a]	0.560
LEAVES	6.83[d]	9.42d	14.21c	19.75[b]	25.88[a]	1.347

Result presented in Table 8 shows the interaction of mean concentrations of neem seed, bark and leaves for each day of interaction. The result indicated that mean concentration of seed produced the highest radial growth of 26.50 mm after the 5th day of incubation followed closely by leaves which produced radial growth of 25.88 mm. The most potent neem product was bark which produced the least radial growth of 21.62 mm on the 5th day of incubation. Significance differences (P≤0.05) among neem products were observed only on the 3rd and 5th days of incubation.

Table 8: Variation of mean concentrations neem seed, bark, and leaves on radial growth of C. *sublineolum* after 5 days of incubation

Mean Conc.	Days after inoculation and radial growth (mm)				
	1	2	3	4	5
SEED	6.75	10.25	14.83[a]	20.00	26.50[a]
BARK	5.62	8.12	11.83[b]	16.75	21.62[b]
LEAVES	6.83	9.42	14.21[b]	19.75	25.88[a]
S. E	0.975NS	1.218NS	0.820	1.786NS	0.765

The means were separated using DMRT= Duncan multiple range test

DISCUSSION

Frequency of occureence of *C. sublineolum* isolates was more at Sokoto Rima basin and Darawa locations; this may be probably due to their closeness with water bodies (Zobe dam and Dutsin-Ma dam) which aid in the interaction of the pathogen and the host in the favorable environment. This is similar to the result of conducted by Chala et al., (2011) that asserted that environment factors could play a significant role in *C. sublineolum* isolates behaviours. Pathogenicity test symptoms observed under natural conditions in the field were found to be similar when the isolates were inoculated into the healthy detached leaves under artificial conditions. However, the tested *C. sublineolum* isolates showed some degree of variation in infectivity from severe to moderate to low infection across locations. The differences in the level of infectivity could be attributed to variation in their genetic makeup and physiological characteristics. Similar observations were found by were and Ochuodho, (2012) that variation in symptoms is from one species/strain of the *Colletotrichum* isolate to the other and varies throughout sorghum growing areas of the world.

C. sublineolum isolates collected from different locations of Dutsin-Ma showed variation in cultural and morphological characteristics. The presence of different isolates of *C. sublineolum* is probable indication that there are other pathotypes or strains of same pathogen infecting sorghum within the study

area. Resistance varieties may therefore be difficult to come by giving the different strains of the pathogen even within same location (Aragaw et al., 2023; Koima et al., 2023). This implies that variation in morphology and cultural characteristics of *C. sublineolum* could be due to location, lesion of single–conidia that comes from single-lesion culture or colony growth, conidia size or pigmentation (Koima et al., 2023)

Morphological characteristics of conidia and conidia shape vary from one *C. sublineolum* isolate infecting sorghum to another and it depends on sorghum genotype and environmental conditions as well as other possible factors (Prom et al., (2016) Also, attempt by pathogen to overcome panicle resistance to infection may result to differences in conidia shape. The *C. sublineolum* isolates obtained from different locations in Dutsin-Ma revealed variations in conidial sizes and produces falcate, oval and hyalinel shape. Report by were and Ochuodho (2012) also reported same colony may produce variations in their sizes of conidia among the leaf isolates. Moreover, the presence of many strains of *C. sublineolum* coincides with the pathogen-host evolution and differentiation theory which states that as the number of host species increases through breeding for resistant varieties of the same host, the pathogens of the same species feeding on these different hosts also change in their genetic characteristic after a given period of time (Were and Ochuodho, 2012).

The result also revealed that all the three neem products (seed, bark and leaves) at the various levels of concentrations of methanol tested were effective in inhibiting the radial growth of *C. sublineolum* in culture. Studies conducted by Gwa and Nwankiti (2017) showed the antifungal potency in neem leaf extract in controlling *Colletotrichum* species isolated from yam tubers. Accordingly, neem plant contained compounds such as azadirachtin which is responsible for fungal cell walls and membrane degradation leading to reduced growth and eventual death of the fungus. This mechanism of action is similar to what has been observed in the reduction in the radial growth of *C. sublineolum* in this study.

The study also showed that neem bark is more potent in inhibiting the radial growth of *C. sublineolum* compared with the other parts of the neem products. On the contrary, research carried out by Gupta *et al*. (2017), have shown that neem seed extracts have comparable or superior antifungal effectiveness compared to other parts of the neem plant, such as leaves and bark. In this study, neem bark extract combined with methanol demonstrated high efficacy in inhibiting the radial growth of *C. sublineolum* after 5 days of inoculation more than the other parts of the neem products. A study by Sharma *et al*. (2012) demonstrated that neem bark extracts, when combined with solvents like Methanol, enhance their antifungal activity. The results indicate a significant reduction in fungal growth compared to the other neem parts, with the bark extract demonstrating notable higher antifungal properties. Comparative studies of different neem extracts have also highlighted the efficacy of neem bark. For example, Galili & Amir (2013) found that neem bark extracts showed superior antifungal activity compared to leaves and seeds, aligning with the current study's results where bark extract demonstrated a more pronounced effect compared to other parts of the neem plant.

It has also been found that methanol is a commonly used solvent for extracting bioactive compounds from plant materials. Research by Keta *et al*. (2019) demonstrated that methanol extracts from neem leaves are particularly effective in extracting antifungal compounds, which enhance their efficacy. This supports the current study's results, where neem leaves extract combined with methanol showed significant inhibition of fungal growth. In a related development.

CONCLUSION

This study revealed that sorghum plants collected from farmers' fields across locations in Dutsin-Ma are infected with *C. sublineolum* the causal agent of anthracnose of sorghum and there are different isolates of this pathogen depending on the location they are found. Determination of frequency of occurrence of these isolates in the locations showed that the *C. sublineolum* isolates occurred most in SRB followed by DRW and the least number of occurrence of *C. sublineolum* isolates was in SMQ. Pathogenicity test on the isolates using healthy detached leaves showed that DTMSRB and DTMHYG isolate showed severe infection followed by DTMDRW and DTMCOE isolates which

exhibited moderate infection with the slight infection recorded in DTMSMQ isolates. The study also showed that *C. sublineolum* isolates identified had varying cultural and morphological characteristics when grown on Potato Dextrose Agar (PDA) for 5 days. Variation in colony growth, color, texture, conidia size, and conidia shape was largely due to location where the isolates were collected indicating that the *C. sublineolum* is a heterogeneous pathogen. The isolates predominantly showed falcate conidia, white, light-gray colony color and smooth colony margin when grown on PDA. The use of neem plant part (seed bark and leaves) extracts separately amended with methanol (0 %, 10 %, 20 %, and 30 %) in inhibiting the radial growth of *C. sublineolum* was found effective throughout the 5 days of incubation. The 30 % neem-methanol extract was more potent compare with the other concentration. However, neem bark was found to be more effective compared with neem seed and neem leaf during the period of incubation. It is therefore, recommended that neem plant parts such as seed, bark and leaf amended with methanol be formulated to manage *C. sublineolum* which is the causal agent of anthracnose of sorghum. A field trial experiment should also be conducted to ascertain the efficacy of these products under field condition.

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