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CONTROL OF SORGHUM ANTHRACNOSE CAUSED BY *Colletotrichum sublineolum* WITH *moringa oleifera* LEAF EXTRACT

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ABSTRACT

Sorghum anthracnose, caused by Colletotrichum sublineolum, is a serious disease causing substantial yield losses in Nigeria. The overdependence on chemical fungicides necessitates the need for sustainable alternatives. This study evaluated the bio-fungicidal and growth-promoting efficacy of Moringa oleifera leaf extract (MLE) on sorghum under field conditions. A randomized complete block design was used with five treatments: MLE at 10%, 20%, and 30% (v/v), a synthetic fungicide (Azoxystrobin) as a positive control, and a distilled water control. Disease incidence and severity were assessed at 15-day intervals beginning from 40 days after sowing, while growth and yield parameters were recorded at physiological maturity. Results showed that MLE application significantly reduced anthracnose incidence and severity in a concentration-dependent manner. The 30% (v/v) of MLE treatment was the most effective, showing results statistically similar to Azoxystrobin at final assessment. Furthermore, MLE at 30% significantly enhanced key growth parameters, including leaf area, stem girth, and number of leaves, and improved yield components such as panicle length and number of spikes per panicle. Consequently, grain yield from the 30% MLE treatment (2.92 ton/ha) was statistically on par with the synthetic fungicide (3.40 ton/ha). The study concluded that 30% (v/v) MLE is a potent bio-fungicide and biostimulant, offering a sustainable and effective strategy for integrated management of sorghum anthracnose and yield enhancement.

Keywords: Sorghum, Moringa oleifera leaf extract, Sorghum anthracnose, Colletotrichum sublineolum, Biostimulant

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is a gluten-free cereal and the fifth most important crop globally, serving as a staple food in many semi-arid regions of Africa and Asia. In these regions, sorghum is widely used in the production of various food products (Arouna *et al*., 2020; Xu *et al*., 2021). In Africa, sorghum is consumed both by humans and livestock (FAO, 2012), with the continent accounting for over half of global production. In 2022, Nigeria and Sudan contributed 21% of the world's sorghum output (FAOSTAT, 2022). Global production in 2023–2024 was estimated at 52.8 million tons, with Nigeria producing approximately 6.7 million tons (FAOSTAT, 2022). In Nigeria, sorghum is cultivated on about 5.4 million hectares, making it a key contributor to food security and rural livelihoods (Ajeigbe*et al*., 2018).

Beyond direct human and livestock consumption, sorghum is also utilized for energy production, as fodder, and for starch and its derivatives, with increasing interest in its potential as a biofuel source (Ferranti & Caruso, 2015; Xin *et al*., 2016; Araújo*et al*., 2017). Additionally, the functional composition of sorghum has been shown to play a crucial role in human health by reducing the risk of chronic diseases (Khalid *et al*., 2022).

Sorghum is known for its exceptional tolerance to heat stress and drought, making it one of the most resilient cereals grown under harsh environmental conditions. Its unique morphological and biochemical features, such as enhanced C4 carbon assimilation at high temperatures, confer an advantage over other cereals (Shoemaker *et al*., 2010). The plant's waxy cuticle further aids in its ability to withstand water loss, contributing to its resilience against drought and heat stress (Busta*et al*., 2021).

However, sorghum production faces significant challenges due to biotic stress, particularly fungal pathogens such as *Colletotrichum sublineolum*, which causes anthracnose. This disease affects the panicles, leaves, and stems of the plant, resulting in wilting, reduced grain development, and substantial yield losses (Gwary*et al*., 2009; Tesso *et al*., 2012). In Africa, sorghum anthracnose remains a important menace to food security, with latest studies reporting yield losses of up to 80% in susceptible cultivars under favourable conditions (Abiy *et al*., 2024). In Nigeria, the disease continues to be a primary constraint in the Northern Guinea and Sudan Savannah zones, where combined foliar and panicle infections can reduce grain yields by nearly half (Yahaya *et al*., 2022; Ajeigbe *et al*., 2021)

The reliance on synthetic chemical fungicides to control fungal diseases has raised concerns regarding their environmental impact and the development of resistance in pathogens. This has prompted interest in alternative control measures, including the use of plant-based solutions such as Moringa leaf extract (MLE). Research has demonstrated the efficacy of Moringa extracts in controlling various plant diseases, particularly fungal infections (El–Mohamedy&Aboelfetoh, 2014). Studies have also highlighted the antifungal properties of Moringa leaf extracts in managing diseases like wheat blade rust caused by *Puccinia triticina* (Afzal *et al*., 2023) and their promising potential for wheat seed protection (Yadav *et al*., 2023).

This study aims to evaluate the effects of Moringa leaf extract (MLE) on the incidence and severity of sorghum anthracnose, as well as to assess its potential impact on sorghum growth and yield under field conditions.

MATERIALS AND METHODS

Plant Materials

The plant material (*M. oleifera*) was obtained from Botanical garden of the Federal University Dutse, Nigeria. The plant was taxonomically identified and confirmed at Crop Science Department, Federal University Dutse (FUD), Dutse, Jigawa state, Nigeria.

Treatment Preparations and Applications

Fresh, young Moringa oleifera leaves were harvested from the orchard at the Faculty of Agriculture Research Farm, Federal University Dutse, Nigeria. After washing the leaves with tap water, they were dried in an oven (Model: Seradon, DHG-9053A) at 25 ± 2°C for 7 days and then ground into a fine powder using a mortar and pestle. The powdered material was weighed on an electronic digital balance (Model: Shimadzu, ATY224; Max. 220 g), labeled, and stored in a plastic bag at room temperature until needed.The prepared Moringa leaf powder was measured and mixed with sterilized distilled water. The mixture was left to soak for 72 hours in a 500 mL conical flask, with the maceration process repeated twice. After each round of maceration, the mixture was filtered through two layers of muslin. The residue was then collected via centrifugation (Multispeed Centrifuge, PK121: Italy) at 4000 rpm for 30 minutes at 20°C. The supernatant was then filtered again using Whatman® No. 1 filter paper and subjected to freeze-drying at 54°C under 010 vacuum mbar using a ModulyoD freeze dryer (Valupump VLP). The final product was stored in sterile, airtight glass bottles in a refrigerator at 4°C.The moringa leaf extract was administered as foliar sprays to the designated treatment groups. It was applied at three critical stages: the pre-flowering stage, the flowering stage, and the grain filling stage. The application method included foliar spraying to ensure even coverage across the leaf surface. Moreover, for specific treatments, soil drenching was performed by applying the extract directly to the base of the plants (Lohani *et al*., 2009)

Experimental Locations

The field experiment was carried out in 2024 during the rainy season at the Research Farm, Department of Crop Science, Federal University Dutse. Dutse (11º 70' N, 9º 34' E, and 460 m above sea level) is situated within Nigeria's Sudan savanna agro-ecological zone and receives an average annual rainfall of 743 mm, typically between early June and late September. The region's mean annual temperature is 26.5ºC. The soil in the area is classified as ferralitic (according to USDA Soil Taxonomy) and consists of clay, sand, and loam. Soil pH ranges from 5.5 to 6.5. At the experimental site, the soil has an organic carbon content of 7.68 g/kg, available potassium (K) of 0.31 cmol (+) kg^{-1}, available phosphorus (P) of 9.58 mg/kg, with 73.2% sand, 8.6% silt, and 18.2% clay (Isyaku *et al*., 2024). The experimental field had been continuously used for sorghum cultivation for many years.

Experimental Design

The sorghum variety SAMSORG-14 (KSV-8) was sourced from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Kano Office, Nigeria. The experiment was conducted with a randomized complete block design (RCBD) using five treatments and each replicated four times: Control (no Moringa leaf extract applied), MLE10% (Moringa leaf extract at 10% v/v), MLE20% (Moringa leaf extract at 20% v/v), MLE30% (Moringa leaf extract at 30% v/v), Fivestar 325 SC fungicide (Azoxystrobin 200 g/L, as a positive control). The experimental layout consisted of four blocks (replicates), each containing five treatments. Sorghum seed was planted in 3 m × 3 m experimental plots, spaced 0.8 m apart, following the recommended spacing of 75 cm between rows and 30 cm between plants. Fertilization involved a basal application of NPK 15:15:15 at 250 kg/ha, followed by urea (46% N) at 100 kg/ha as a top dressing four weeks after planting.

Isolates and Inoculum Production

The isolate of *C. sublineolum* used in this study, was isolated and identified from naturally infected sorghum leaves in Department of Crop Science Federal University Dutse Jigawa state Nigeria. Symptomatic leaves were collected and cut into small pieces, disinfested in 2% sodium hypochlorite for 2 min. Then the leaf tissue was rinsed with sterile water and placed on water ager. The petri dishes were incubated at 25±2 ℃ for 5 days. Pure cultures were obtained by monospore isolation. Conidia from single conidial colonies were transferred to casein lactose hydrolysate medium, incubated at 25-27 ℃ with indoor natural lighting for 10–15 day to allow sporulation. Conidia suspensions were prepared by flooding the culture plates mentioned above with distilled water, then filtered through a two layers of cheesecloth to remove fungal mycelium. The concentrations of suspensions were adjusted to $3x10^5$ CFU/ml following the method described by Zhang *et al*., (2020).

Inoculation and Evaluation

Sorghum plants were inoculated 30 days post-emergence. A pressurized sprayer was used to apply approximately 5 mL of *Colletotrichum sublineolum* conidial suspension per plant, directed specifically into the leaf whorls. Anthracnose infection response was evaluated at 40 days after inoculation, and the rating was based on disease response observed on inoculated leaves and a visual estimate of the percentage of infected leaf area.

Experimental Data Collection and Analysis

Disease Assessment

Disease incidence and severity were evaluated at 15-days interval from 45 to 85 days after inoculation. Incidence was calculated as follows using equation 1 as:

Disease Incidence

$$(\%)=\left(\frac{\text{Number of Diseased Plants}}{\text{Total Number of Plants}}\right)\text{x100} \quad (1)$$

Disease severity was also evaluated at 85 days after inoculation using a scale 1-9 developed by Xu et al., (2017), Thakur et al., (2007), where:1 = no symptoms; 2 = 1–5%; 3 = 6–10%; 4 = 11–20%; 5 = 21–30%; 6 = 31–40%; 7 = 41–60%; 8 = 61–75%; and 9 = more than 75%.

The Disease Severity Index (DSI) was computed as:

$$\text{DSI }(\%)=\left(\frac{\sum(n)}{\text{Nx 9}}\right)\text{x100} \quad (2)$$

Where n= individual plant severity ratings, N =total plants assessed, and 9= maximum severity score.

Growth and Yield Assessments

At physiological maturity, agronomic data were collected from the central rows of each plot to minimize border effects. Plant height (cm) was measured from the soil surface to the apex of the primary panicle. Stem diameter (mm) was determined at the third internode using a digital vernier caliper. The leaf area index (LAI) was estimated based on the individual leaf area measurements of three representative, randomly selected plants per plot; with leaf area quantified using a portable leaf area meter. All harvested plants were threshed, and the grain was cleaned and weighed. Moisture content was determined for a subsample, and grain yield was adjusted to a standard 12.5% moisture content. A separate sample of 1000 sorghum grains was counted and weighed to determine the 1000-grain weight (g).

Statistical Analysis

Data were analysed using a randomised complete block design (RCBD), ANOVA in SAS 9.4 (SAS Institute Inc.,

Cary, NC USA), Treatment means were separated by Tukey's Honestly significant difference (HSD) test at $p<0.05$.

RESULTS AND DISCUSSIONS

Effect of *Moringa oleifera* Leaf Extract Concentration on the Percentage Incidence and Severity of Sorghum Anthracnose in Field Trials

The efficacy of Moringa leaf extract (MLE) against the percentage incidence and severity of sorghum anthracnose varied significantly among treatments and over time (Table 1). Initially, at 50 days after inoculation (DAI), all MLE concentrations and the synthetic fungicide azoxystrobin significantly reduced disease incidence compared to the control (43.43%). Azoxystrobin was the most effective treatment in reducing anthracnose incidence to 15.50% compared 43.43% in the control. The effects of MLE applied at 20% and 30% were statistically similar at this stage, while MLE at 10% concentration was the least effective (29.90% disease incidence). Although, at 75 DAI, a change in effectiveness was observed, MLE at 30% was the most effective treatment, shown a lower disease incidence (13.53% incidence) produced more favourable results than azoxystrobin (18.80%) and all other MLE concentrations (10% and 20% MLE). At the final assessment, (85 DAI), the most effective treatments were azoxystrobin (22.28%), MLE 30% (27.73%), and MLE 20% (27.97%), which were not statistically different from each other. MLE 10% (37.63%) remained the least effective extract treatment, though it still provided significant suppression compared to the control (51.18%). Similarly, MLE significantly reduced final disease severity recorded. The highest concentration (MLE 30%) resulted in a severity of 14.91%, which was statistically similar to azoxystrobin (10.50%), and both were significantly lower than the control (59.29%). These results demonstrate that MLE at higher concentration (30% v/v) contains a effective antifungal effect against *C. sublineola* against sorghum anthracnose.

Table 1: Effect of *Moringa oleifera* Leaf Extract Concentration on the Percentage Incidence and Severity of Sorghum anthracnose in Field Trials

Treatments	Sorghum anthracnose incidence (%)			Disease severity (%)
	50 DAI	75 DAI	85 DAI	
MLE@10%	22.10a	25.633c	27.97c	23.06b
MLE@20%	29.90b	33.333b	37.63b	16.22c
MLE@30%	20.37c	13.5333e	27.73c	14.91c
Azoxystrobin	15.50d	18.800d	22.28c	10.50c
Control (Distilled water)	43.43a	46.6333a	51.18a	59.29a

MLE: *Moringa oleifera* leaf extract, DAI: Days after Inoculations, Values are means of four replications. Means followed by the same letter (s) within a column are not significantly different ($p \leq 0.05$) according to Tukey's Honestly significant difference (HSD) test at $p<0.05$

Growth Parameters of Sorghum as Influenced by Foliar Application of Moringa Leaf Extracts under Field Conditions

The application of *Moringa oleifera* leaf extract (MLE) significantly influenced sorghum growth parameters under field conditions, with varying effects observed different concentration levels (Table 2). Plant height was not significantly affected by the different treatments, as all treatments, including the azoxystrobin fungicide and control (distilled water), resulted in statistically similar values ($p \leq 0.05$), ranging from 174.9 cm to 197.4 cm. In contrast, the number of leaves per plant was significantly higher in plants treated with MLE at 30% (19.0) compared to all other treatments (12.3–13.4), which were statistically similar. Stem girth was significantly enhanced by the treatments application. The highest stem girth (18.66 mm) was recorded in plants treated with MLE at 30%, which was statistically significant to other treatments. Both MLE at 20% (15.69 mm) and the chemical standard Azoxystrobin (16.54 mm) resulted in statistically similar results, medium stem girth. However, both were significantly greater than the MLE 10% treatment (14.14 mm) and the control (13.26 mm). The untreated control constantly produced plants with the lowest stem girth. The effects of MLE on leaf area produced similar results as observed in the stem girth. The highest leaf area was observed with application MLE at 30% (411.56 cm^2), which was significantly higher than all other treatments. MLE at 20% (388.89 cm^2) and Azoxystrobin (387.86 cm^2) were statistically similar to each other and produced significantly larger leaf areas than MLE at 10% (320.89 cm^2). The control plants exhibited the smallest leaf area (270.20 cm^2), which was significantly lower than all treatments.

Table 2: Growth Parameters of Sorghum as Influenced by Foliar Application of Moringa Leaf Extracts Under Field Conditions

Treatments	Plant Height (cm)	Number of Leaves	Stem girth (mm)	Leaf Area (cm^2)
MLE@10%	178.241^{a}	12.443^{b}	14.1433^{c}	270.20^{d}
MLE@20%	174.940^{a}	13.320^{b}	15.6933^{b}	320.89^{c}
MLE@30%	192.493^{a}	19.026^{a}	18.6567^{a}	411.56^{a}
Azoxystrobin	184.243^{a}	13.410^{b}	16.5433^{b}	388.89^{b}
Control (Distilled water)	197.413^{a}	12.270^{b}	13.2567^{c}	387.86^{b}

MLE: *Moringa oleifera* leaf extract, DAI: Days after Inoculations, Values are means of four replications. Means followed by the same letter (s) within a column are not significantly different ($p \leq 0.05$) according to Tukey"s test

Effect of Moringa Leaf Extract on Sorghum Yield and Yield Components

The application of *Moringa oleifera* leaf extract (MLE) had a significant and concentration-dependent effect on the yield and yield components of sorghum (Table 3). The highest concentration of MLE (30%) consistently resulted in superior yield parameters. This treatment produced a number of spikes per panicle (120.8) and a grain weight (32.51 g) that were

statistically equivalent to the azoxystrobin positive control (119.3 and 32.16 g, respectively), and both were significantly higher than all other treatments. Similarly, the panicle length for MLE@30% (42.74 cm) was the greatest among all treatments, being significantly longer than that of the azoxystrobin control (40.20 cm).

The intermediate MLE concentration (20%) showed a significant improvement over the lowest concentration and the negative control for most parameters, yielding more spikes (117.7), longer panicles (38.8 cm), and heavier grain (26.86 g) than the MLE@10% and control treatments. However, these values were significantly lower than those achieved with MLE@30% or azoxystrobin. The MLE@10% treatment and the distilled water control generally resulted in the lowest values across all measured yield components, with the control consistently yielding the least. Ultimately, these component effects translated directly into final grain yield. The MLE@30% treatment produced a yield of 2.92 ton/ha, which was statistically on par with the azoxystrobin treatment (3.40 ton/ha) and significantly greater than all other treatments. Yields from the MLE@20%, MLE@10%, and control treatments were statistically similar to each other and significantly lower, ranging from 1.41 to 2.02 ton/ha.

Table 3: Effect of Moringa Leaf Extract on Sorghum Yield and Yield Components

Treatment	No. of spike/panicle	Panicle Length (cm)	Grain weight (gm)	Yield (ton/ha)
MLE@10%	101.65d	34.38c	23.44c	1.68b
MLE@20%	117.70b	38.8b	26.86b	2.02b
MLE@30%	120.79a	42.74a	32.51a	2.92a
Azoxystrobin (Positive control)	119.25a	40.20b	32.16a	3.40a
Control (Distilled water)	109.14b	33.72c	20.18d	1.41b

MLE: *Moringa oleifera* leaf extract, DAI: Days after Inoculations, Values are means of four replications. Means followed by the same letter (s) within a column are not significantly different ($p \leq 0.05$) according to Tukey"s test

Discussion

The study demonstrated that application of Moringa oleifera leaf extract (MLE) significantly suppresses anthracnose and enhanced sorghum growth and yield parameters. The efficacy was greatly concentration-dependent, with the 30% (v/v) MLE treatment emerged as a possible option to the synthetic fungicide azoxystrobin. The MLE application resulted in a significant reduction in both the percentage sorghum incidence and severity. The pattern of increasing efficacy in plant extracts contrasts with the sometimes-declining performance of synthetic fungicides and has been attributed to the induction of systemic acquired resistance (SAR) in the host plant (Mofunanya *et al*., 2023). The excellent performance of 30% MLE suggests a complementary mechanism beyond direct antifungal activity, possibly involving the upregulation of plant defense pathways, a property increasingly recognized in MLE (Agyenim-Boateng *et al*., 2021; Maqsood *et al*., 2021). Again, the direct antifungal activity of MLE is possible mediated by its rich profile of bioactive compounds. Phenolic acids, glucosinolate and flavonoids present in MLE have confirmed the ability to disrupt cell membrane and inhibit mycelial growth in a number of phytopathogenic fungi (Mouafi *et al*., 2024; Zulfiqar *et al*., 2020). In a period of increasing fungal resistance to synthetic fungicides demand for reduced pesticide residues, plant-based alternatives like MLE are gaining significant importance (Masi *et al*., 2022; Oke *et al*., 2025). Our findings were in conformity with this global shift, showing that 30% MLE produced results statistically similar to azoxystrobin, confirming its potential as an effective bio-fungicide. The positive effects of MLE transcended disease control and directly translated into enhanced yield. The MLE@30% treatment yielded the highest values for panicle length, number of spikes per panicle, and grain weight, ultimately achieving a grain yield that was statistically same with the azoxystrobin treatment. This significant boost can be attributed to the synergistic action of auxins, cytokinins and mineral nutrients in MLE, which are known to enhance nutrient absorption, nutrient translocation and photosynthetic efficiency (El-Mageed *et al*., 2021; Ali *et al*., 2023). The statistical similarity between MLE@30% and azoxystrobin in key yield components is an important finding, suggesting that the plant-based extract can effectively replace synthetic compounds for growth enhancement, aligning with the principles of sustainable crop protection (du Jardin, 2015; Rouphael & Colla, 2020). The MLE concentration-dependent response was clearly evident that higher concentration of MLE performed better than lower concentrations. It was still lower than MLE@30% even though MLE@20% generated yield components that were noticeably better than MLE@10% and the control. The efficacy of biostimulants is characterized by this traditional dose-response relationship, in which reactions frequently plateau once an ideal concentration is attained. (Sher *et al*., 2022; Yakhin *et al*., 2024). The lower MLE concentrations might not offer the strong hormonal and nutritional stimulus needed to overcome yield-limiting constraints, the observed an increase from MLE@10% to MLE@30% emphasizes the significance of optimizing application rates to produce agronomically meaningful effects.

Morphological changes were observed by the application of treatments. Interestingly, none of the treatments showed a significant difference in plant height. This implies that there was no significant change in the basic genetic and environmental factor that enhanced growth, suggesting that gibberellins-like activity is not the main mechanism of action of MLE (Mosa *et al*., 2021). On the other hand, the MLE at 30% treatment greatly increased the number of leaves and more importantly, the leaf area, High levels of growth promoting substances like ascorbate and zeatin, which promote cell division and expansion, are probably what cause this promotive impact in MLE (Aremu *et al*., 2020; Elsayed *et al*., 2020).

A clear and significant inverse relationship was observed for stem girth, with the control plants producing the thickest stems. This result was unexpected, as biostimulants are often associated with generalized vigor. One plausible explanation is that MLE induced a morpho-physiological change in resource allocation, favouring allocation in photosynthetic capacity (leaf area) over structural stem (Rouphael & Colla, 2020). This aligns with the concept of plant plasticity, where resources are allocated to optimize fitness under specific stimuli. The intermediate and statistically similar stem girths in the azoxystrobin and MLE@30% treatments further suggest that these treatments invoked a similar growth regulation response, distinct from the control. The

diminishment of the leaf number effect at higher concentrations may indicate a shift in resource allocation towards panicle development or the onset of mild allelopathic effects at elevated doses, a phenomenon documented with other potent plant extracts (du Jardin, 2020).

CONCLUSION

Based on the findings, 30% (v/v) MLE demonstrates strong and persistent antifungal activity against *C. sublineola*, performing comparably to a standard fungicide and showing potential as a sustainable bio-fungicide for sorghum. Its mode of action may involve both direct antifungal effects and induction of host defenses. Furthermore, MLE acts as a complex biostimulant, with concentration-dependent effects on growth parameters, indicating that target-oriented formulations are essential for optimizing crop outcomes. Further research should focus on mechanistic elucidation, active compound isolation, and field-scale validation.

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