PATHOGENICITY OF *CURVULARIA* SP. CAUSING LEAF BLIGHT DISEASE OF SORGHUM (*SORGHUM BIOCOLOR* (L.) MOENCH AND ITS MANAGEMENT USING EXTRACTS OF TEN DIFFERENT WILD PLANTS

Dania, Victor Ohileobo*[1] and **Adedoyin**, Anjolaoluwa Charis[1]

[1] Department of Crop Protection and Environmental Biology, University of Ibadan, PMB 001, Ibadan Nigeria
E-mail address: victorohileobo@gmail.com

Abstract
The pathogenicity of Curvularia sp. causing leaf disease blight of sorghum in Southwest Nigeria and efficacy of aqueous extracts of ten plants was evaluated for its management. Healthy sorghum plants were inoculated with an inoculum concentration adjusted to 1.25 ×10^5 conidia/ml using an hemacytometer and applied as foliar spray with hand sprayer at four weeks after sowing. The in vitro experiment was a 12 ×6 factorial in a completely randomized design with twelve treatments that were evaluated at six levels with three replications, while the screenhouse consisted of twelve treatments and three replications in a completely randomized design using a susceptible sorghum variety SORG 302. Data were collected on effect of treatments on disease incidence, severity, growth and yield parameters. Curvularia sp. was pathogenic among Aspergillus spp., Rhizopus spp., and Penicillum spp that were associated with sorghum leaf blight disease. Plants that were inoculated and treated with S. aromaticum extract had the lowest incidence of 10.31% relative to control (66.72%), while disease severity was also significantly ($p<0.05$) lower among plants that were inoculated with Curvularia sp. and treated. Sorghum plants that were inoculated with the pathogen and treated with S. aromaticum extract produced the highest grain yield of 3576.7kg/ha^{-1} which was significantly higher than other treatments. The simplicity and effectiveness of the extracts used in this study will succour to poor resource farmers who depends mostly on the use of hazardous fungicides in the control of this diseases on their farms.

Key words: Disease, Extract, Grain yield, Fungicide, Pathogen.

Received: 26.03.2020 Reviewed: 20.05.2020 Accepted: 29.05.2020

INTRODUCTION

Sorghum (*Sorghum bicolor* (L) Moench) is a major food crop that is cultivated mainly in areas that are characterized by low rainfall and of high temperature worldwide. Its growth performance is, however, lower than that of more important crops like rice and maize. It is relatively drought tolerant and can, therefore, be grown in marginal, semi-arid areas where rainfall is unreliable and the cultivation of food crops such as maize is not feasible. Sorghum is a vital crop in Africa, where it ranks second to maize as the staple grain for millions of people (USDA, 2019). Although it is mainly consumed as a grain, sorghum is also prepared into a wide variety of other food products such as porridge, bread, alcoholic beverages, and animal feed. Global production is estimated to exceed 63.5% million tonnes, with Africa accounting for 40% of this value (FAOSTAT, 2015)

Diseases of sorghum have significantly reduced the number of functional leaves and have led to global yield reduction of about 50% (Ogolla et al., 2019). Sorghum is affected by a number of pathogens including fungi, bacteria, nematodes, and viruses. According to Funnel and Pedersen (2006) fungal diseases of *C.* sorghum are the most important which are enhanced by favourable environmental conditions. *Exserohilum turcicum* has been reported as the causal organism of leaf blight of sorghum which is has been responsible for significant decline in yield of sorghum (Mohan et al. 2010; Beshir et al., 2015; Rajeshwar et al., 2014). However, the influence of anthropogenic activities and climate change have greatly influenced rapid development of more virulent pathogens and emerging sorghum diseases. Also, the responses of some newly developed lines to fungal pathogens

have not been encouraging (Deanna et al., 2013.

Curvularia lunata (Wakker) Boedijn (teleomorph *Cochliobolus lunatus* Nelson & Haasis) is one of the known genera that is responsible for causing grain mould in sorghum (Tarekegn et al., 2006; Sharma et al., 2010), leaf blight of pearl barley (Dai et al., 2019) and leaf spot disease in cotton (Shirsath et al., 2018).The major routes of grain infection by these fungi are likely to be via the floret and developing grain on the field (Navi et al., 2005). It is also possible that grain can be infected systemically from plants grown from infected grain or infected through roots or crowns, as has been observed in other grains (Al-Sadi and Deadman, 2010; Murillo-Williams and Munkvold, 2008).

The use of botanical pesticides in the control of plant diseases constitutes a natural process which helps to reduce the high dependence by peasant farmers on synthetic pesticides characterized by health and environmental hazards. The advocacy for organic farming has given an additional impetus to this eco-friendly method of disease management. The biodegradability and harmless nature of these crude plant extracts make their application a promising alternative to chemical control. The antifungal potential of various plant extracts have been reported (Javaid and Siddique, 2011; Jadon and Shah, 2012; Singh and Garampalli, 2012; Dania and Omidiora, 2019b). Foliar spray of plant extracts eliminates fungal spores during contact either in the air or on the leaf surface (Singh and Garampalli, 2012) and has also been reported to induce systemic resistance on treated plants (Guleria and Kumar, 2006; Latha et al., 2009) to pathogens.

Therefore, this study evaluated the pathogenicity of *Curvularia* sp. on sorghum and the efficacy of ten different botanicals that are readily available the in natural environment for the management of leaf blight disease.

MATERIALS AND METHODS

Source of Experimental materials

A susceptible local sorghum variety SORG 302 with red grains was obtained from an agricultural seeds store at Ibadan, southwest, Nigeria. Botanicals that were screened in this study were selected from the locally and readily available plants, based on the following requirements: occurrence of general antimicrobial toxins according to literature or traditional knowledge, presence of pre-infection defense biochemicals and easy availability in bulk throughout the year, with very little commercial value (Singh and Garampalli, 2012) (Table 1).

Table 1. List of botanicals used in the study

Common name	Family	Scientific name	Plant part used
Neem	Meliaceae	*Azadirachtha indica*	Seed
Pawpaw	Caricaceae	*Carica papaya*	Leaf
Moringa	Moringaceae	*Moringa oleifera*	Seed
Ginger	Zingiberaceae	*Zingiber officinale*	Rhizome
Garlic	Alliaceae	*Allium sativum*	Bulb
Siam weed	Asteraceae	*Chromolaena odorata*	Leaf
Clove	Myrtaceae	*Syzigium aromaticum*	Seed
Scent leaf	Lamiaceae	*Ocimum gratissimum*	Leaf
Bitter leaf	Asteraceae	*Vernonia amygdalina*	Leaf
Jatropha	Euphorbiaceae	*Jatropha curcas*	Leaf

Pathogen isolation

Leaf samples were collected from an infected field at Ogbomoso town (Lat 8.14°N, 4.24°E) Oyo State, Nigeria at the peak of the rains when environmental conditions were most favourable to the disease. Plants in the diagonal were visually examined from top to bottom for symptoms of leaf blight disease. Cut lesions of 3 mm× 3 mm dimension were surface-sterilized in 5% sodium hypochlorite for 1 minute and rinsed in three changes of sterile distilled water. These were then inoculated on dehydrated potato dextrose. agar (PDA) growth medium using 9 cm Petri dishes and incubated at 28±2°C for four days. Growing fungal colonies were further purified to obtain pure cultures using single spore technique. The Petri dishes were incubated at 28-30C in the dark for 10 days to enhance full sporulation. Isolates with similar cultural and morphological features were maintained on PDA slants and kept at 4°C in the refrigerator until when required for use. Three isolates were randomly selected for further identification. The three randomly selected isolates were cultured on fresh PDA plates at 28°C for seven days. Morphological characteristics of the mycelium, pycnidia and conidia, including shape, colour, size, and presence or absence of septation, were observed and recorded (Dai et al. (2019) using a compound microscope 400x magnification. Identification of species was carried out using a standard monograph guide for fungi (Barnett and Hunter, 1998; Watabe, 2000) and following confirmation at the Mycological Herbarium at the International Institute of Tropical Agriculture, Ibadan, Nigeria

Pathogenicity of isolates

To evaluate pathogenicity, three seeds of a susceptible sorghum variety SORG 302 were sown in experimental pots that were filled with sandy loam soil and watered to field capacity in the screenhouse. Inoculum preparation was done by maintaining a pure culture of eachfungus on potato dextrose agar (PDA) a 9-cm Petri dish that was incubated in the dark for 14 days to facilitate complete sporulation. To obtain the conidial suspension, 10 ml of sterile distilled water was added to each Petri dish, while two drops of Tween 20 detergent were added to enhance scooping of the conidia and mycelia. The conidia were filtered through double-layer cheese cloth. The plants were inoculated with an inoculum concentration that was adjusted to 1.25×10^5 conidia mL^{-1} (Dai et al., 2019) at four weeks after sowing and sprayed on test plants until run-off. Plants that were sprayed with distilled served as control. The inoculated plants were incubated at 28–30°C in a screenhouse (12 h light per day, and relative humidity (RH) > 90%) until symptoms appeared at 14–21 days after inoculation. Symptomatic plants were carefully observed and lesions cultured on PDA to re-isolate the infecting fungi in order to confirm Kock's postulates.

Preparation of aqueous extracts and *in vitro* assay

The leaves, seeds, bulbs and rhizomes were air-dried in the Pathology Laboratory at room temperature for 10 days before being pulverized into a fine powder using a Waring commercial blender, Springfield, MO blender. The pulverized samples were then packed in clearly labelled polythene bags and stored until needed. Six equal weights of 5, 10, 20, 30, 40 and 50 g of each plant powder were weighed separately, dissolved in distilled water and mixed properly. The mixture was kept overnight for the exudation of biochemical constituents. The solution was later filtered through a double layered cheese cloth. The filtered plant extracts which served as 5, 10, 20, 30, 40 and 50% stock solutions were stored at 4°C in the refrigerator and were used at full strength or diluted by the addition of distilled water, based on experimental requirements (Singh and Garampalli, 2012). The extracts were bioassayed against the test pathogen in sterilized glass Petri dishes according to Dania and Omidiora (2019b). The experiment was a 12 ×6 factorial in a completely randomized with twelve treatments that were evaluated at six levels with three replications.

Inoculated treatments impregnated with extracts were incubated at room temperature and radial mycelial growth was measured for seven consecutive days. Mycelial inhibition was determined and expressed as a percentage. The most effective concentration of each extract was selected and used in the *in vivo* trial.

Screenhouse evaluation of plant extracts for the management of leaf blight disease

At the end of the *in vitro* screening of the various extract levels, 50% plant concentration was the most effective and was therefore selected and used in the in *vivo* experiment. The treatments included two controls: a an negative control which consisted of sterile distilled water and a second control made up of Red force fungicide (50% WP) used as foliar spray at a recommended rate of 2 g/litre). Top soil used in the experiment was obtained from an uncultivated area at the crop garden of the Department of Crop Protection and Environmental Biology, University of Ibadan. Experimental pots were filled with 10 kg each of soil sterilized at 120°C for three hours. Five seeds of a susceptible local sorghum variety SORG 302 were sowed per pot and these were thinned to two seedlings per stand at a week old. Infected leaf samples were collected from a sorghum field at the peak of the rainy season in the month of June-July when environmental conditions favour epidemiology of the disease.

Inoculum concentration was prepared and adjusted to 1.25 $\times 10^5$ conidia/ml using an hemacytometer (Singh and Garampalli, 2012). The plants were inoculated with the pathogen using hand sprayer until run off at four weeks after sowing (WAS).The extracts were prepared as earlier described were applied at one week after inoculation (WAI) just before the appearance of visible symptoms with a repeated application at 6 WAI. The screenhouse was kept humid by ensuring regular watering to create conditions that encourage disease development with the daylight regime of 10–12 h at 28–30C and a relative humidity of about 70–95% (Dania and Gbadamosi, 2019c). The disease was readily identified by large cigar-shaped lesions on the leaf with reddish or purple margins and the black conidia formed by sporulation of the fungus giving the lesion an ashy gray to dark olive appearance.

The screenhouse experiment was laid out in a completely randomized design with 12 treatment combinations and three replicates. The treatments were:

T1 = *Azadirachtha indica*, T2 = *Carica papaya*, T3 = *Moringa oleifera*, T4 = *Zingiber officinale*, T5 = *Allium sativum*, T6 = *Chromolaena odorata*, T7 = *Syzygium aromaticum*, T8 = *Ocimum gratissimum*, T9 = *Vernonia amygdalina*, T10= *Jatropha curcas.* T11= *Redforse fungicide*, T12= *Control*

Disease incidence was determined as proportion of plants showing symptoms in the field. The number of plants. showing symptoms were counted and expressed as a percentage of the total number of stands per treatment (Nwanosike et al., 2015)

$$\text{Disease incidence} = = \frac{\text{Number of symptomatic plants}}{\text{Total number of assessed plants}} \times 100$$

Disease severity was evaluated on a rating scale of 1-6 using the modified method of Aliyi et al., (2018): where, 1 = No infection, 2= < 5% infection i.e. a few restricted lesions on the lower leaves 3= 5-10% i.e. several small and large lesions on many leaves 4= 11 -25%) i.e. numerous small and large lesions on many leaves 5 = 26 - 50% i.e. many enlarged and coalesced lesions on many leaves 6 = >50% i.e, several coalesced lesions, leaf showing wilting, blighting. The treatments that showed significant difference in disease incidence when compared with negative control were selected for measurement of growth parameters such as number of leaves, stem girth and plant height. Effect of the disease on yield indices including number of panicles and grain yield per hectare were also evaluated.

Data were taken at three main growth stages, vegetative (1-35 days), panicle initiation or reproductive stage (36-70 days) and grain filling (71-105 days).
Harvest index (HI) was determined according to Biya (2018):
HI (%)=SY/BY × 100
where SY = seed yield and BY = above ground dry biological yield.

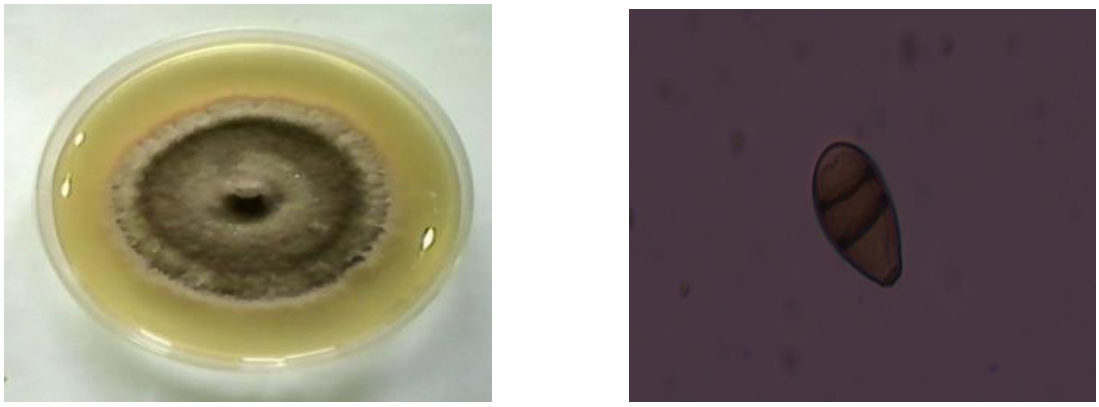
Statistical analysis

Experiments were laid out in a completely randomized design with four replicates. Data were subjected to way analysis of variance (ANOVA) using the Generalized Linear Model of SAS (2002) ver. 9.2 and means were separated with the Duncan's Multiple Range Test (DMRT) at 5% level of probability (Gomez and Gomez, 1984).

RESULTS

Results obtained from this study showed that all inoculated leaves had identical leaf blight symptoms as those observed initially on naturally infected leaves in the at 14–28 days after inoculation. Curvularia sp. was pathogenic among *Aspergillus* spp., *Rhizopus* spp., and *Penicillum* spp that were associated with sorghum leaf blight disease (Figure 1) There was no symptom in the leaves of control plants that were observed on the leaves but sprayed with distilled water. *Curvularia* produced typical blight symptoms on reinoculation (Figure 2) and were re-isolated from all the lesions. The cultures of the isolates showed identical features as the initial isolates on PDA, which confirmed Koch's postulates.

Inhibitory effect of the plant extracts on radial mycelial growth of *Curvularia* sp. Varied between 4.89-17.21% at 5% w/v concentration (Table 2). *Jatropha curcas* was the most effective with 17.21% inhibition while *Ocimum gratisimum* was least effective (4.89%. However, there was zero inhibition in the negative control which consisted of Petri dishes that were inoculated with the pathogen alone without extracts. There was complete inhibition of the in Petri dishes that were that were impregnated with Redforce fungicide and inoculated with the pathogen. There was significant difference ($p<0.05$) between the treatments and the two controls. Efficacy of the botanical increased at higher concentrations of the extracts. The best extract performance was achieved at 50% w/v with inhibitory range varying from 40.11-90.61%. *Syzygium aromaticum* was the most effective in the *in vitro* trial with 90.61% reduction of radial mycelial growth of the test pathogen, while *O. gratissimum* extract was the least effective with 40.11% mycelial inhibition of the fungus. However, extracts of *M. indica* and *Allium sativum* were also very effective against the pathogen with inhibitory values of 88.21 and 80.56%, respectively. There was no mycelial reduction in the negative control, while the positive check showed a 100% mycelial inhibition.



a) **b)**

Figure 1. Cultural characteristics(a) and macro conidium (b) of *Curvularia* sp.

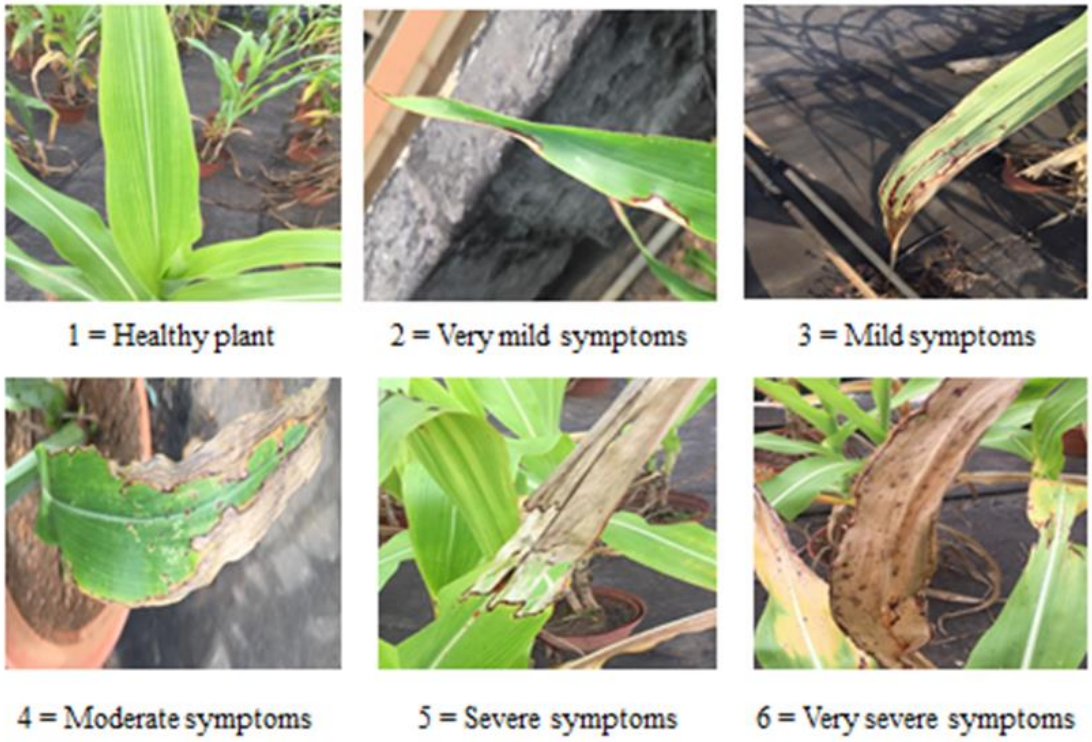


Figure 2. Disease severity rating among sorghum plants inoculated with *Curvularia* sp

Table 2. Inhibitory effect of plant extracts on radial mycelial growth of *Curvularia* sp.

	Extract concentration (%)					
Extract	5	10	20	30	40	50
Azadirachtha indica	7.44b	12.73c	20.01d	27.13d	37.14cd	58.63cd
Carica papaya	8.82b	15.73bc	19.76d	25.44d	33.62d	53.31d
Moringa oleifera	15.23ab	21.66b	33.54b	50.11bc	77.11ab	88.21ab
Zingiber officinale	10.55b	16.32bc	21.44cd	30.84cd	42.34c	64.91c
Allium sativum	17.49ab	22.53b	29.69bc	46.36c	66.41bc	80.56b
Chromolaena odorata	5.11b	12.12c	18.33fg	22.55g	29.55de	51.41d
Syzigium aromaticum	15.77ab	28.72ab	43.97ab	60.77ab	81.78ab	90.61ab
Ocimum gratissimum	4.89b	11.11c	24.77c	29.66cd	37.31cd	40.11e
Vernonia amygdalina	7.33b	10.61c	16.42d	22.31de	41.66c	45.71de
Jatropha curcas	17.21ab	23.74b	34.82b	55.64b	70.38b	76.37bc
Redforce	100a	100a	100a	100a	100a	100a
Control	0.0bc	0.0cd	0.0de	0.0e	0.0e	0.0ef
CV (%)	7.08	5.77	11.30	8.60	3.08	4.82

Means followed by same letter along a column are not significantly different using Duncan Multiple Range Test (DMRT) at $p<0.05$

Disease incidence among treatments was significantly lower at the vegetative growth stage (Table 3) Plants that were inoculated with the pathogen and treated with *Allium sativum* and *Syzygium aromaticum* extracts were asymptomatic during the first 35 days of growth (vegetative growth). Similarly, plants that were inoculated and treated with Redforce fungicide did not exhibit leaf blight symptoms. However, control plants that were inoculated and untreated had a higher incidence of 14.41% which was significantly higher than other treatments. At panicle initiation stage, disease incidence varied between 3.58 and 38.77%. Plants that were inoculated and treated with S. *aromaticum* extract had the lowest incidence of 3.58% relative to the control with 36.77% incidence. Treatments with *Ocimum gratissimum* extract were least effective with 22.11% incidence. Disease incidence in control plants was significantly higher than other treatments. The trend was similar at grain filling stage with plants that were inoculated and treated with *S. aromaticum* having the lowest incidence of 10.31% relative to control (66.72%). Disease severity was also significantly higher among plants that were inoculated with *Curvularia* sp. but untreated with plant extracts.

Table 3. Effect of plant extracts on incidence and severity of leaf blight disease of sorghum at different growth stages

	Disease incidence		**Disease severity**			
Treatment	Vegetative	Panicle initiation	Grain filling	Vegetative	Panicle initiation	Grain filling
Azadirachtha indica	6.06d	13.30de	34.71e	1.72abc	3.09ab	4.32ab
Carica papaya	8.03c	15.08d	43.80d	1.03c	3.94a	4.52ab
Moringa oleifera	1.33e	8.44g	21.06g	1.22bc	1.71b	1.98
Zingiber officinale	11.04b	17.61c	31.44f	2.13a	2.33ab	4.92ab
Allium sativum	0.0e	11.17ef	14.22i	1.0c	1.82b	2.04c
Chromolaena odorata	0.77e	12.53ef	35.88e	1.08c	2.11ab	4.44ab
Syzygium aromaticum	0.0e	3.58h	10.31j	1.0c	1.43b	2.33c
Ocimum gratissimum	10.31b	22.11b	49.77c	2.32a	3.46ab	5.11ab
Vernonia amygdalina	5.03d	20.31b	54.71b	2.01ab	3.15ab	5.52a
Jatropha curcas	0.82e	10.22fg	18.22h	1.09c	2.14ab	3.51bc
Redforce	0.0e	1.34i	5.03k	1.0c	1.08b	2.01c
Control	14.41a	38.77a	66.72a	2.45a	4.01a	5.92a
CV (%)	6.72	5.03	8.31	3.47	4.11	3.75

Means followed by same letter along a column are not significantly different using Duncan Multiple Range Test (DMRT) at $p<0.05$.

At the vegetative stage, plant height varied between 22.77 and 36.41 cm within the first 35 days of growth (Table4) Plants that were inoculated and treated did not differ significantly ($p>0.05$) from uninoculated control plants The number of leaves per plant varied from 11.09-13.77 at the grain filling growth stage. Similarly, there was no significant difference in the effect of treatments on other growth parameters such as stem number of leaves and stem diameter.

The number of panicles per spikelet varied between 4.28 and 8.05. Sorghum plants that were inoculated and treated with *V. amygdalina* extract had the highest number of 8 panicles (Table 5). This was followed by *S. aromaticum* and *O. gratissimum* with 7.28 and 7.98 panicles respectively. There was no significant difference ($p>0.05$) in the effect of treatments on panicle length and grain weight. Grain yield per hectare varied between 2277.05- 3576 .7kg/ha^{-1}. Sorghum plants that

were inoculated with the pathogen and treated with *S. aromaticum* extract produced the highest yield of 3576 .7kg/ha^{-1} relative to the control which had 2277.04 kg/ha-1. Grain yield per hectare and harvest index was significantly (p>0.05) higher in plants that were inoculated and treated than the untreated control.

Table 4. Effect of treatments on growth of sorghum inoculated with *Curvularia* sp.

	Plant height (cm)			Number of leaves			Stem girth (cm)		
Treatment	Vege-tative	Panicle initiation	Grain filling	Vege-tative	Panicle initiation	Grain filling	Vege-tative	Panicle initiation	Grain filling
Azadirachtha indica	31.41abcd	80.81cd	172.31de	6.31a	10.67a	12.82abc	0.33a	0.44a	0.65a
Carica papaya	26.91cd	77.24de	168.99e	5.84a	9.22a	11.46bc	0.41a	0.55a	0.71a
Moringa oleifera	35.80ab	70.65f	181.11ab	8.51a	9.22a	11.09c	0.21a	0.38a	0.59a
Zingiber officinale	31.85abcd	83.21c	161.52f	6.34a	11.41a	13.42abc	0.27a	0.43a	0.63a
Allium sativum	36.41a	92.35a	178.44bc	7.52a	11.33a	14.01a	0.31a	0.38a	0.52a
Chromolaena odorata	27.79abcd	82.51c	164.28f	8.11a	9.70a	13.77ab	0.25a	0.42a	0.74a
Syzygium aromaticum	30.11abcd	88.11b	184.55a	6.41a	8.81a	12.65abc	0.37a	0.42a	0.60a
Ocimum gratissimum	25.62d	79.71cd	161.33f	7.21a	10.08a	12.33abc	0.30a	0.39a	0.53a
Vernonia amygdalina	35.50ab	82.17c	170.90e	8.02a	10.71a	13.22abc	0.28a	0.51a	0.55a
Jatropha curcas	29.94abcd	75.52e	175.91cd	7.24a	11.44a	13.71ab	0.25a	0.55a	0.61a
Redforce	34.09ab	90.23a	177.20bc	8.22a	11.78a	13.05abc	0.49a	0.43a	0.74a
Control	28.41abcd	80.36cd	171.24e	7.28a	11.33a	12.81abc	0.29a	0.44a	0.65a
CV(%)	5.80	4.45	6.01	4.82	3.77	6.02	4.01	4.20	3.32

Multiple Range Test (DMRT) at p<0.05

Table 5. Effect of treatments on yield sorghum inoculated with *Curvularia* sp.

Treatment	Panicle Number	Panicle length(cm)	Grain weight (g)	Grain yield t/ha^{-1}	Harvest index (%)
Azadirachtha indica	5.28bcd	24.51g	21.88e	2345.51ef	22.40f
Carica papaya	7.40a	31.77c	23.67de	2876.77cd	26.53de
Moringa oleifera	4.77cd	27.40ef	30.43a	3001.80bc	30.31bc
Zingiber officinale	8.05a	36.13b	26.44bc	2564.31de	24.82def
Allium sativum	5.33bcd	30.80cd	30.05a	3272.40b	33.22b
Chromolaena odorata	5.01cd	40.66a	27.33b	1873.22gh	21.72f
Syzygium aromaticum	4.28d	25.41fg	24.21cd	3576.79a	39.70a
Ocimum gratissimum	7.04ab	38.90a	30.91a	2539.01de	26.11de
Vernonia amygdalina	8.40a	28.41de	25.64cd	2052.11fg	27.88cd
Jatropha curcas	6.61abc	32.44b	24.32cd	3172.44bc	23.66f
Redforce	6.90ab	35.68b	33.02a	3360.72ab	34.21b
Control	7.01ab	32.60b	16.69f	1671.04h	30.44bc
CV (%)	4.71	6.88	5.94	11.32	7.08

Means followed by same letter along a column are not significantly different using Duncan Multiple Range Test (DMRT) at p<0.05

DISCUSSION

Leaf blight disease of sorghum constitutes significant threat to production wherever the crop is grown worldwide. However, it is most destructive in the tropics and sub-tropics where the prevailing warm and humid environmental conditions encourage epidemiology of the disease. The use of synthetic fungicides remains the most effective method of mitigating the effect of the disease on crop yield since most of the cultural practices have not provided adequate control. The mycelial growth of *Curvularia* sp. on PDA occurred over the temperature range of 28-30°C with an optimal temperature of 28°C, forming colonies that were grey on PDA and dark underneath. They were blunt, branched, hyaline and septate. These results agree with the findings of Dai et al. (2019) that reported matured conidia of *Curvularia* to be slightly curved, blunt round on the two sides of the cell, pale brown to brown and non-transparent with 3–4 septations. *Curvularia* sp. was pathogenic among *Aspergillus* spp., *Rhizopus* spp., and *Penicillum* spp. that were associated with sorghum leaf blight disease. Pathogenicity of isolates showed identical blight symptoms on sorghum plants that were inoculated with *Curvularia* sp. as those observed initially on naturally infected leaves at 14-28 days after inoculation, while plants that were sprayed with sterile distilled water were asymptomatic. The re-isolation of fungi from all the lesions and subsequent culturing on PDA showed similar features as the initial isolates thus confirming Kock's postulates. These results are consistent with previous findings of Dai et al. (2019) that reported *Curvularia coicis* as the causative agent of leaf blight disease of pearl barley based on morphological characteristics and sequencing of the ribosomal DNA internal transcribed spacer (rDNA-ITS). Similarly, the severity of *Curvularia* stem blight disease had been reported in cassava fields (Msikita et al., (2007). However, pathogenicity of *Curvularia* isolates in this study disagrees with earlier reports of Beshir et al. (2015) and Singh et al. (2019) that reported *Exserohilum turcicum* as the causal organism of sorghum leaf blight disease. Also *Curvularia lunata* had been reported as seed-borne pathogen of sorghum (Grish et al., 2011; Deanna et al., 2013). The disparity may be attributed to anthropogenic activities such as indiscriminate use of synthetic fungicides and the effect of climate change that have facilitated the emergence of resistant and more virulent races of pathogens.

All the plant extracts that were evaluated in the *in vitro* bioassay significantly ($p<0.05$) reduced the radial mycelial growth of the pathogen and efficacy increased with concentration. Plant extracts have been used in the management of leaf spot disease and the prevention of its transmission from naturally infected seeds to sorghum plant organs and grains in the field (Bonzi et al., 2012), root rot disease of cowpea and okra (Darwa et al., 2010), *Phytophthora nicotiana* causing leaf blight disease of tobacco (Bowers and Locke, 2004) and tuber rot disease of sweet potato (Dania and Thomas, 2019a). The use of botanicals in disease control has been reported to confer resistance on plants (Guleira and Kumar, 2006; Latha et al., 2009) and the reduction in fungal sporulation in the laboratory as well as in field conditions (Deepak et al., 2005).

The incidence and severity of leaf blight disease were significantly lower in sorghum plants that were inoculated with the pathogen and treated than the untreated controls. At maturity stage, disease incidence was lowest in plants that were inoculated and treated with *S. aromatic* and *A. sativum* extracts with incidences of 10.31 and 14.22% respectively. Hamini-Kadar et al. (2014) had reported the antifungal activity of the essential oil of clove (*S. aromaticum*) in the management of phytopathogenic fungi of tomato. In a related development, Suleiman et al. (2019) reported the efficacy of ethanol extract of *S. aromaticum* as biocontrol agent in the *in vitro* control of postharvest pathogens of tomato and potato. Disegha et al. (2015) reported the effectiveness of aqueous and ethanolic extracts of *A. sativum* in the control of *Aspergillus* and *Penicillium* species.

Sorghum plants that were inoculated with the test pathogen and untreated had the lowest

yield of 2277.04 kg/ha^{-1}, which was significantly lower than those that were inoculated and treated. Leaf blight is a foliar disease that is characterized by extensive necrotic lesions on affected leaves and this limits the leaf surface area for photosynthesis and overall yield of diseased plants. Therefore, the low yield in the control plants may be attributed to the effect of the blight disease on the leaves which reduced its chlorophyll content. Sorghum plants that were inoculated with the pathogen and treated with *S. aromaticum* extract produced the highest yield of 3576.7kg/ha^{-1} . These results are consistent with the previous findings of Latha et al. (2009) that reported the application of *S. aromaticum* as foliar spray increased systemic resistance to the early blight pathogen, *Alternaria solani* and ultimate yield in tomato.

CONCLUSION

Curvularia sp., is often a seed-borne pathogen of sorghum and infection is initiated on the grains in the field and there is the likelihood of seed-to-plant transmission. Therefore, planting of healthy seeds from reputable agricultural research institutes will be very useful in reducing the incidence of leaf blight disease. This study has shown that the application of plant extracts as foliar spray significantly reduced the incidence and severity of sorghum leaf blight disease and their effectiveness compared favourably with the synthethic Redforce fungicide. Therefore, these botanicals are promising candidates in the formulation of an integrated approach for the management of the disease.

REFERENCES

[1] Aliyi, T., Chala, A. and Bacha, N. Survey of *Turcicum* leaf blight (*Exserohilum turcicum*) on maize (*Zea mays*) in major highland and mid altitudes of maize growing agro-ecologies of Western part of Oromia, Ethiopia. African Journal of Plant Science, 2018. **12**(10):264-276.

[2] Al-Sadi, A.M., and Deadman, M.L. Influence of seed-borne *Cochliobolus sativus* (anamorph *Bipolaris sorokiniana*) on crown rot and root rot of barley and wheat. Journal of Phytopathology, 2010. **158**(10): 683–690.

[3] Barnett HL, Hunter BB. Illustrated genera of imperfect fungi. 4th ed. St. Paul, MN: APS Press. 1998. 218pp.

[4] Beshir1, M.M., Ahmed, N.E., Ali, AM., Babiker, I.H., Rubaihayo, P. and Okori, P. Prevalence and severity of sorghum leaf blight in the sorghum growing areas of Central Sudan. Wudpecker Journal of Agricultural Research, 2015. **4**(4):054 - 060

[5] Biya, M. Determination of Nitrogen and Phosphorous Fertilizer Requirement for Sorghum (*Sorghum bicolor* (L.) Production in Kersa- Jimma Zone of Ethiopia, East Africa. Journal of Biology, Agriculture and Healthcare, 2018. **8**(7): 14-22.

[6] Bowers, J. H., and Locke, J. C. Effect of formulated plant extracts and oils on population density of *Phytophthora nicotianae* in soil and control of *Phytophthora* Blight in the Greenhouse. Plant Disease, 2004, **88**(1), 11–16.

[7] Dania, V. O. and Thomas, A. S. Bioactivity and Phytochemical Composition of *Moringa oleifera* in the management of fugal rot disease of orange-flesh sweet potato. Nigerian Journal of Mycology, 2019a, **11**:26-45

[8] Dania, V. O. and Omidiora, J. A. Combination of biological control agents and garlic (*Allium sativum*) extract in reducing damping-off disease of tomato. Bangladesh Journal of Agricultural Research. 2019b. **44**.3:553-567.

[9] Dania, V. O. and Gbadamosi, L. O. Efficacy of combined application of *Trichoderma asperellum* NG T158, *Aloe vera* leaf extract and poultry manure for the management of *Colletotrichum lindemuthianum* causing anthracnose disease on cowpea, Archives of Phytopathology and Plant Protection 2019c. **52**:1-2:90-107

[10] Dai, Yu-Li, Lin Gan, Fu-Ru Chen & Xiu-Juan Yang. Leaf blight causedby *Curvulariacoicis* on Chinese pearl barley (*Coixchinensis*) in Fujian Province, China, Canadian Journal of Plant Pathology, 2019. **41**(1): 1-7.

[11] Dawar, S., Khaliq, S. and Tariq, M. Comparative effect of plant extract of *Datua alba* Nees and *Cynodon dactylon* (L). Pers., alone or in combination with microbial antagonists for control of root rot disease of cow pea and okra. Pakistan Journal of Botany, 2010. **42**(2):1273–1279.

[12] Deanna L., Funnell-Harris, L. K. and Pedersen, J.F. Isolation and characterization of the grain mold fungi *Cochliobolus* and *Alternaria* spp. from sorghum using semiselective media and DNA sequence analyses. Canadian Journal of Microbiology, 2013. **59**:87-96

[13] Deepak, S.A., Oros, G., Sathyanarayana, S.G., Shetty, N.P., Shetty, H.S. Shashikanth, S. 2005. Antisporulant activity of leaf extracts of Indian plants against *Sclerospora graminicola* causing downy mildew disease of pearl millet. Archives of Phytopathology and Plant Protection, 2005. **38**(1):31–39.

[14] Disegha, G,C. and Akani, N.P. Antimitotic Effect of *Allium Sativum* on Selected Fungi. Current Studies in Comparative Education, Science and Technology, 2015. **2**(2) 228-244.
[15] FAOSTA. Database Collections, FAO. Available online: http://www.fao.org/faostat/en/#data (accessed on 15 December 2015).
[16] Funnell, D.L., and Pedersen, J.F. Association of plant colour and pericarp colour with colonization of grain by members of *Fusarium* and *Alternaria* in near-isogenic sorghum lines. Plant Disease, 2006. **90**(4): 411–418.
[17] Girish, G.A, Baig, M.M.V. Anitha, K. and Chakrabarty, S.K. 2011. *Curvularia* species detected in sorghum seeds collected from Marathwada region of Maharashtra Indian Journal of Plant Protection, 2011. **39** (4):299-303.
[18] Gomez, K.A. and Gomez, A. Statistical procedure for agricultural research. 2ndedn. John Wiley and Sons Inc. New York. 1984. 740pp.
[19] Guleria, S. and Kumar, A. *Azadirachta indica* leaf extract induces resistance in sesame against *Alternaria* leaf spot disease. Journal of Cell Molecular Biolology, 2006. **5**:81–86.
[20] Hamini-Kadar, N , Hamdane, F., Boutoutaou, R., Kihal, M. and Henni, J. E. Antifungal activity of clove (*Syzygium aromaticum* L.) essential oil against phytopathogenic fungi of tomato (*Solanum lycopersicum* L) in Algeria. Journal of Experimental Biology and Agricultural Sciences, 2014. **2**(5): 447-454.
[21] Jadon, KS and Shah, R. Antifungal activity of different plant extracts against *Drechslera bicolor* causing leaf blight of bell pepper. Archives of Phytopathology and Plant Protection, 2012. **44**:1–12.
[22] Javaid, A. and Saddique, A. Control of charcoal rot fungus *Macrophomina phaseolina* by extracts of *Datura metel*. Natural Products Research, 2011. **1**:1–6.
[23] Latha, P., Anand, T., Ragupathi, N., Prakasham, V. and Samiyappan, R. Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and zimmu leaf extract against *Alternaria solani*. Biological Control, 2009. **50**(2):85–93.
[24] Mohan, S.M., Chakravarthi, D.V.N., Satish, K., Madhusudhana, R., Rathore, S., Srinivas, G., Mani, N.S., Mathur, K., Reddy, N.R. and Seetharama, N. Identification of quantitative trait loci associated with resistance to foliar diseases in sorghum (*Sorghum bicolor* (L.) Moench). Euphytica, 2010, **176**: 199-211.
[25] Msikita, W., Baimey, H., and James, B. D. Severity of *Curvularia* stem blight disease of cassava in West Africa. Plant Disease, 2007. **91**:1430-1435
[26] Murillo-Williams, A. and Munkvold, G. Systemic infection by *Fusarium verticillioides* in maize plants grown under three temperature regimes. Plant Disease, 2008. **92**(12): 1695–1700.
[27] Navi, S.S., Bandyopadhyay, R., Reddy, R.K., Thakur, R.P., and Yang, X.B. Effects of wetness duration and grain development stages on sorghum grain mold infection. Plant Disease, 2005. **89**(8):872–878.
[28] Nwanosike, M.R.O., Mabagala, R.B. and Kusolwa, P.M. Disease Intensity and Distribution of *Exserohilum turcicum* Incitant of northern leaf blight of maize in Tanzania. International Journal of Pure and Applied Biosciences, 2015. **3**(5):1-13.
[29] Ogolla, F., Muraya, M.M. and Onyango, B.O. Incidence and severity of *Turcicum* leaf blight caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs on sorghum populations in different regions of Tharaka Nithi County, Kenya. Journal of Scientific and Engineering Research, 2019. **6**(1):104-111
[30] Rajeshwar, R.P., Narayan, P.R. and Ranga, R.R. *Turcicum* leaf blight of maize incited by *Exserohilum turcicum*: A review. International Journal of Applird Biology and Pharmaceutical Technology. 2014. **5**(1): 54-59.
[31] SAS Institute. SAS/STAT. Guide for personal computers, version 9.1 ed. Cary, NC: SAS Institute. 2002.
[32] Sharma, R., Rao, V.P., Upadhyaya, H.D., Gopal R.V., and Thakur, R.P. Resistance to grain mold and downy mildew in a mini-core collection of sorghum germplasm. Plant Disease, 2010. **94**(4): 439–444
[33] Shirsath, L. P., Patil, S. P. and Patil, U. K. Incidence of leaf spot disease on cotton caused by *Curvularia verruculosa* and role of its hydrolytic enzymes in pathogenesis. Physiology and Molecular Biology of Plants, 2018. **24**(4): 711–714.
[34] Singh, H. and Garampalli, R.H. Screening of plant extracts for organic management of downy mildew of sorghum. Archives of Phytopathology and Plant Protection, 2012. **45**(14):1732–1740
[35] Singh,V., Shekhar, M,. Rakesh, M, Singh. N. and Sunaina, B.T. *Exserohilum turcicum* Incidence on *Zea mays* in India: Epidemiology and Management Strategies to minimize Yield Losses. Chapter 13 Adaptive Crop Protection Management Strategies Editors: D. Prasad (Eds). 2019 pp. 158-172
[36] Suleiman, W.B., El Bous, M.M. M. El Sai., H., El Baz, G. *In vitro* evaluation of *Syzygium aromaticum* L. ethanol extract as biocontrol agent against postharvest tomato and potato diseases. Egyptian Journal of Botany, 2019. **51** (1): 81 – 94
[37] USDA. World Agricultural Production; Circular Series WAP 3–19; USDA:Washington, DC, USA, 2019. 17pp.
[38] Watanabe, T. Pictorial Atlas of Soil and Seed Fungi. Morphologies of cultured fungi and key to species. 2nd edition. CRC Press LLC. NW Corporate Blvd., Boca Raton, Florida. 2000. pp.42-235.