

In Vivo Control Of Sorghum Anthracnose With Some Plant Products Using Different Application Methods

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Abstract: *In screen house experiments, the antifungal activities of nine plant extracts were evaluated as seed treatment, soil drenches and foliar sprays on anthracnose of sorghum caused by Colletotrichum graminicola. All experiments were laid out in a completely randomized design (CRD). All treatments were replicated three times. Data was subjected to analysis of variance appropriate to CRD. Mean comparisons were carried out using Duncan's Multiple Range Test (DMRT). Percentage disease incidence and severity were recorded at 45 and 65 days after sowing. Disease development was inhibited at various degrees in sorghum crops treated with the plant extracts in all three methods of evaluation. Results showed that extracts of 10% Allium sativum, Azadirachta indica leaf and seed powder, Moringa oleifera leaf powder had significant potential in controlling the disease over the others. Soil drench was observed to be the most effective in reducing the incidence at both 45 DAS and 65 DAS. When the severity of the disease was used as the parameter for measuring effectiveness of application methodology, foliar spray was the most effective in reducing disease severity at both 45 DAS and 65 DAS.*

Keywords: *Colletotrichum graminicola, plant extracts, disease and severity*

I. INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) belonging to the family Poaceae, is the 5th most important cereal crop in the world after wheat, maize, rice and barley. Nigeria, with 9.394% world production of sorghum is ranked the fourth largest producer in the world after U.S.A (17.341%), India (12.923%) and Mexico (10.888%)(Abiala *et al.*, 2013).

Fungal contamination constitutes a major biotic constraint to sorghum improvement and production worldwide. Anthracnose, caused by *Colletotrichum graminicola*, also known as red leaf blight and when it occurs on seedlings as seedling blight is the most important foliar disease on both local and improved sorghum varieties in WCA (Thomas and

Frederiksen, 1995). Anthracnose is reported to cause considerable yield losses of up to 47% in Nigeria (Gwary *et al.*, 2004; Marley, 2004) and up to 67% in other parts of West Africa (Thomas and Frederiksen, 1995).

The quest for pesticide free food which leave undesirable residues in treated food material and the environment coupled with other negative influences such as pathogen resistance, pathogen resurgence, effects on non-target species, ecological and human health concern has led to the shift in other crop protection methods. Antifungal compounds from plant origin have been reported to be a more suitable alternative (Mekonnen *et al.*, 2014; Talibi *et al.*, 2013; Zarafi and Moumoudou, 2010). A number of plants have been screened

for their antifungal activities and valuable results have been achieved.

Applications by foliar spray, soil treatment and seed treatment have been used. Findings of Harish *et al.*, (2008) demonstrated that post inoculation spray of neem cake extract on 60 day old rice plants resulted in significant disease reduction. Adandonon *et al.*, (2006) observed significant disease control in cowpea when *Moringa* extracts were applied as seed treatment for *Sclerotium* disease control in the green house. Similarly extracts of neem seeds, ginger rhizomes and *Cassia alata* leaves applied as seed treatment have been found effective in controlling seed borne pathogenic fungi of tomatoes (Zakaria, 2010). This paper reports the efficacy of some plant extracts in reducing sorghum anthracnose using different application methods.

II. MATERIALS AND METHODS

ISOLATION OF TARGET PATHOGEN

Seeds and leaves of Sorghum ICSV 400 infected by the pathogen were obtained from the Institute of Agricultural Research, Zaria. For isolation from the seeds, the seeds were first rinsed in tap water and then surface sterilized using 10% sodium hypochlorite solution for 5 minutes and thereafter rinsed three times in sterile distilled water to remove all traces of the sodium hypochlorite. The seeds were blot dry using sterilized filter papers and thereafter transferred onto plates containing potato dextrose. The plates were then incubated at room temperature (28°C) and observed periodically for fungal growth. Characteristics of the colonies were noted and those similar to that of the test fungi were sub-cultured onto fresh PDA to obtain a pure culture of the fungus. Pure cultures of the fungus were maintained on PDA slants.

For isolation from the leaves, the leaves bearing lesion were cut into tiny portions which were washed for 5 minutes in 10% sodium hypochlorite solution and thereafter rinsed three times in sterile distilled water, blotted dry using sterile filter paper and then transferred into fresh PDA. Subsequent treatments were as described for the seeds above.

ASSESSING THE POTENTIAL OF THE PLANT EXTRACTS AS SEED TREATMENT

The method used in this study was as described by Somda *et al.* (2007). Susceptible seeds of sorghum were surface sterilized in sodium hypochlorite solution and rinsed in 3 changes of distilled water. The seeds were soaked overnight in different suspensions of the plant extracts and benomyl, after which they were air dried for 2 minutes before sowing in plastic pots containing soil previously sterilized by autoclaving. In the control experiment, the seeds were dipped in sterile distilled water. Soil inoculation was done 2 days before planting by adding 3ml of the pathogen per planting hole.

All pots were arranged in completely randomized design. Management practices such as thinning, wetting and weeding were carried out and data on the incidence and severity of anthracnose were obtained at 45 and 65 DAS.

The incidence of the disease was calculated using the formula by Chaube and Pundir (2005).

$$DI = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Disease severity was scored on 10 tagged plants using the severity rating scale of:

- 1 = No symptom
- 2 = 1-5% Leaf area covered with lesions;
- 3 = 6-10% Leaf area covered with lesions;
- 4 = 11-20% Leaf area covered with lesions;
- 5 = 21-30% Leaf area covered with lesions;
- 6 = 31-40% Leaf area covered with lesions;
- 7 = 41-50% Leaf area covered with lesions;
- 8 = 51-75% Leaf area covered with lesions;
- 9 = > 75% Leaf area covered with lesions

The mean percentage severity was computed using the formula:

$$DS = \frac{\sum n}{N \times 9} \times 100$$

Where: $\sum n$ = sum of individual rating

N = total number of plants assessed

9 = the highest score on the severity scale. (Bdliya *et al.*, 2007)

ASSESSING THE POTENTIAL OF THE PLANT EXTRACTS AS SOIL DRENCH

Seeded pots were inoculated with 10ml of *C. graminicola* suspension. This was followed after the 3rd day by inoculation with 10ml of the plant extracts at different concentrations. Untreated seeds were dipped in sterile distilled water as control following the methodology of Abiala *et al.*, (2013).

ASSESSING THE POTENTIAL OF BIOFUNGICIDES AND ANTAGONISTS AS FOLIAR APPLICATION

Susceptible seeds of sorghum were surface sterilized in sodium hypochlorite solution and rinsed in 3 changes of distilled water. The seeds were sown at the rate of 3 seeds per pot. Three weeks after emergence, all the pots were sprayed with spore suspension of *C. graminicola* and immediately covered with polyethylene bags for 18 hours to provide the high humidity necessary for infection and also to prevent the spores from being washed off. 72hrs after inoculation, the plants were sprayed with plant extracts at different concentrations (Bankole and Adebajo, 1996). The disease incidence and disease severity were calculated as described above.

III. STATISTICAL ANALYSIS

Data was subjected to analysis of variance appropriate to CRD. Mean comparisons were carried out using DMRT. Statistical Package used = SAS (2002) software version 9.1 and means were separated using Duncan's Multiple Range Test at 5% level of significance.

IV. RESULTS

EFFECT OF PLANT EXTRACTS APPLIED AS SEED TREATMENT ON ANTHRACNOSE DISEASE INCIDENCE AND SEVERITY

Table 1 represents the means across all level of concentrations of plant materials with regard to the incidence and severity of anthracnose. At 45 DAS, all the plant extracts significantly reduced the incidence of the disease below that recorded in the control except *Cymbopogum citratus*, *Allium cepa*, *Moringa oleifera* seed extract and *Vernonia amygdalina* which were not significantly different from the control. The lowest incidence at 45 DAS was recorded under benomyl treatment (9.13%), followed by *Allium sativum* (18.11%). At 65 DAS, all the extracts significant reduced the incidence of the disease compared to the control. Treatment with benomyl recorded the lowest incidence (18.0%) and then *Allium sativum* (25.42%). Among the plant materials, the least effective is *Eucalyptus camuldulensis* (42.97%).

The effect of plant extracts on disease severity is also presented in the same table. At 45 DAS, all the treatments significantly reduced the disease severity in comparison to the control. The most effective material is benomyl (2.56%), the least effective of the plant materials is *Moringa oleifera* leaf powder (5.53%). At 65 DAS, all the treatments significantly reduced the severity of the disease compared to the control.

EFFECT OF PLANT EXTRACTS APPLIED AS SOIL DRENCH ON ANTHRACNOSE DISEASE INCIDENCE AND SEVERITY

It is evident from Table 2 that the test materials differed significantly on their effects on anthracnose of sorghum when applied as soil drench. At 45DAS, *Moringa oleifera* seed extract had the least disease incidence (15.97%) followed by *Allium sativum* (19.56%). The least effective plant material is *Vernonia amygdalina* with a disease incidence of 42.19%. At 65 DAS, *Moringa oleifera* seed extract treatment had the lowest incidence (27.14%) while the least effective of the plant materials at this time period was *Vernonia amygdalina* with 52.38% disease incidence.

The effect of the extract types on severity of disease is also presented in Table 2. At 45 DAS, the severities of diseases on plants treated with the different materials were significantly lower than those of the control (22.75%). The lowest severity was recorded under *Moringa oleifera* leaf powder, followed by benomyl (2.59%), the least effective of the materials is *Cymbopogum citratus* with a disease severity of 5.05%. At 65 DAS, all the treatments significantly reduce disease severity compared to the control treatment (29.50%).

EFFECT OF PLANT EXTRACTS APPLIED AS FOLIAR SPRAY ON ANTHRACNOSE DISEASE INCIDENCE AND SEVERITY

The mean values of incidence and severity across concentration levels are presented in Table 3. At 45 DAS, the lowest incidence was from benomyl treatment (18.0%) and the least effective is *Cymbopogum citratus* (40.97%). At 65 DAS,

Allium sativum was the most effective test material (11.16%) while the least effective is *Eucalyptus camuldulensis* (55.95%). All the test materials except *Cymbopogum citratus* and *Eucalyptus camuldulensis* significantly reduced the incidence of anthracnose.

With regards to disease severity, at 45 DAS, the most effective test material is *Allium sativum* (1.88%), followed by benomyl (2.60%). At 65 DAS, all the test materials significantly reduced the severity of the disease compared to the control without treatments. Among the test materials, the most effective is benomyl (2.25%) and *Allium sativum* (2.39%); the least effective is *Eucalyptus camuldulensis* (5.07%).

In general, *Allium sativum* seems to be superior to all other plant extracts in terms of disease severity at both 45 DAS and 65 DAS.

COMPARATIVE EFFICACY OF THE DIFFERENT APPLICATION METHODS TESTED

Table 4 shows the comparative efficacy of the three methods of application of test materials -seed treatments, soil drenches and foliar sprays. The analysis shows that with regards to effect on disease incidence, soil drench was the most effective in reducing the incidence at both 45 DAS and 65 DAS, followed by seed treatment while the least effective methodology was foliar spray. When the severity of the disease was used as the parameter for measuring effectiveness of application methodology, foliar spray was the most effective in reducing disease severity at both 45 DAS and 65 DAS, followed by soil drench at 45 DAS and seed treatment at 65 DAS. The interaction effects were observed to be significant for all the methods and the test material used. This indicates that whichever method that is used i.e. seed treatment, foliar spray or soil drench and any of the plant extracts used is good enough to control *C. gramimicola*.

Test material	45 DAS		65 DAS	
	Disease Incidence (%)	Disease Severity (%)	Disease Incidence (%)	Disease Severity (%)
<i>Allium sativum</i>	18.11 ^e	2.91 ^{ef}	25.42 ^{ef}	2.93 ^g
<i>Azadirachta indica</i> seed extract	20.04 ^e	3.50 ^{c-f}	29.44 ^{de}	5.86 ^f
<i>Azadirachta indica</i> leaf powder	23.61 ^{de}	4.07 ^{b-f}	25.75 ^{ef}	7.32 ^{def}
<i>Moringa oleifera</i> seed extract	25.72 ^{cde}	5.10 ^b	35.42 ^{b-e}	8.26 ^{cde}
<i>Moringa oleifera</i> leaf powder	32.25 ^{bcd}	5.53 ^b	33.81 ^{b-e}	8.35 ^{cde}
<i>Allium cepa</i>	33.81 ^{abc}	4.96 ^{bc}	35.66 ^{b-e}	10.56 ^b
<i>Cymbopogum citratus</i>	39.67 ^{ab}	4.91 ^{bc}	38.35 ^{bc}	9.39 ^{bcd}
<i>Vernonia amygdalina</i>	35.54 ^{ab}	4.91 ^{bc}	36.67 ^{bcd}	11.21 ^b
<i>Eucalyptus camuldulensis</i>	31.60 ^{bcd}	5.28 ^b	42.97 ^b	9.64 ^{bc}
Benomyl	9.13 ^f	2.56 ^f	18.00 ^f	2.43 ^g
Control	42.50 ^a	22.75 ^a	55.91 ^a	29.50 ^a
S. E. ±	2.326	0.392	2.555	0.573

Means in each column with same superscript are not significantly different at $P \geq 0.05$ using Duncan's Multiple Range Test.

Table 1: Effect of plant extracts applied as seed treatment on anthracnose disease incidence and severity

Test material	45 DAS		65 DAS	
	Disease Incidence (%)	Disease Severity (%)	Disease Incidence (%)	Disease Severity (%)
<i>Allium sativum</i>	19.56 ^{def}	3.75 ^{bcd}	28.77 ^{bcd}	7.52 ^d
<i>Azadirachta indica</i> seed extract	24.72 ^{b-e}	3.64 ^{bcd}	29.81 ^{bcd}	7.02 ^d
<i>Azadirachta indica</i> leaf powder	29.44 ^{bcd}	3.10 ^{cd}	27.30 ^{bcd}	8.42 ^{cd}
<i>Moringa oleifera</i> seed extract	15.97 ^{ef}	3.03 ^{cd}	27.14 ^{bcd}	11.15 ^{bc}
<i>Moringa oleifera</i> leaf powder	22.50 ^{b-f}	2.55 ^d	29.58 ^{bcd}	13.45 ^b
<i>Allium cepa</i>	31.81 ^b	4.52 ^{bc}	37.38 ^b	13.01 ^b
<i>Cymbopogum citratus</i>	31.25 ^{bc}	5.05 ^b	35.83 ^{bc}	12.49 ^b
<i>Vernonia amygdalina</i>	42.19 ^a	3.64 ^{bcd}	52.38 ^a	12.73 ^b
<i>Eucalyptus camuldulensis</i>	23.61 ^{b-e}	3.77 ^{bcd}	38.03 ^b	13.19 ^b
Benomyl	12.67 ^f	2.59 ^d	36.67 ^{bc}	7.59 ^d
Control	42.50 ^a	22.75 ^a	55.91 ^a	29.50 ^a
S. E. ±	2.716	0.445	3.071	0.942

Means in each column with same superscript are not significantly different at $P \geq 0.05$ using Duncan's Multiple Range Test.

Table 2: Effects of plant extracts applied as soil drench on anthracnose disease incidence and severity

Test material	45 DAS		65 DAS	
	Disease Incidence (%)	Disease Severity (%)	Disease Incidence (%)	Disease severity (%)
<i>Allium sativum</i>	21.17 ^{ef}	1.88 ^e	11.16 ^e	2.39 ^{fg}
<i>Azadirachta indica</i> seed extract	27.36 ^{cde}	3.27 ^{bcd}	28.79 ^{c-e}	3.31 ^{efg}
<i>Azadirachta indica</i> leaf powder	36.39 ^{ab}	3.58 ^{bc}	33.04 ^{cd}	4.17 ^{b-e}
<i>Moringa oleifera</i> seed extract	38.36 ^{ab}	3.24 ^{bcd}	42.50 ^b	3.09 ^{efg}
<i>Moringa oleifera</i> leaf powder	35.28 ^{abc}	3.69 ^b	36.48 ^{bc}	3.99 ^{b-e}
<i>Allium cepa</i>	25.56 ^{def}	2.65 ^{b-e}	33.45 ^{cd}	3.46 ^{d-g}
<i>Cymbopogum citratus</i>	40.97 ^{ab}	2.95 ^{b-e}	54.01 ^a	4.25 ^{b-e}
<i>Vernonia amygdalina</i>	32.64 ^{bcd}	3.40 ^{bcd}	41.94 ^b	4.77 ^{bc}
<i>Eucalyptus camuldulensis</i>	35.83 ^{ab}	2.39 ^{de}	55.95 ^a	5.07 ^b
Benomyl	18.00 ^f	2.60 ^{b-e}	20.86 ^f	2.25 ^g
Control	42.50 ^a	22.75 ^a	55.91 ^a	29.50 ^a
S. E. ±	2.243	0.287	2.076	0.311

Means in each column with same superscript are not significantly different at $P \geq 0.05$ using Duncan's Multiple Range Test.

Table 3: Effect of plant extracts applied as foliar spray on anthracnose disease incidence and severity

Treatment	45 DAS		65 DAS	
	Disease Incidence (%)	Disease Severity (%)	Disease Incidence (%)	Disease Severity (%)
Method (M)				
Seed treatment	28.45 ^b	4.85 ^a	32.57 ^b	7.96 ^b
Foliar spray	30.84 ^a	3.47 ^c	35.18 ^a	4.36 ^c
Soil drench	25.80 ^c	4.09 ^b	31.73 ^b	10.32 ^a
S. E. ±	0.694	0.108	0.683	0.188
Test material (P)				
<i>Allium sativum</i>	19.61 ^f	2.84 ^{ef}	21.78 ^{hi}	4.28 ^{gh}
<i>Azadirachta indica</i> seed extract	24.04 ^{def}	3.47 ^{b-e}	29.35 ^{fg}	5.40 ^{efg}

<i>Azadirachta indica</i> leaf powder	29.82 ^c	3.59 ^{b-e}	28.70 ^{fg}	6.64 ^{de}
<i>Moringa oleifera</i> seed extract	26.69 ^{cd}	3.79 ^{bcd}	35.02 ^{de}	7.50 ^{cd}
<i>Moringa oleifera</i> leaf powder	30.01 ^c	3.92 ^{bcd}	33.29 ^{ef}	8.59 ^{bc}
<i>Allium cepa</i>	30.39 ^c	4.04 ^{bc}	35.50 ^{de}	9.01 ^b
<i>Cymbopogum citratus</i>	37.30 ^b	4.30 ^b	42.73 ^{bc}	8.71 ^{bc}
<i>Vernonia amygdalina</i>	36.79 ^b	3.98 ^{bcd}	39.41 ^{cd}	9.57 ^b
<i>Eucalyptus camuldulensis</i>	30.35 ^c	3.81 ^{bcd}	45.65 ^b	9.30 ^{1b}
Benomyl	13.27 ^g	2.58 ^f	25.18 ^{ghi}	4.09 ^{gh}
Control	42.50 ^a	22.75 ^a	55.91 ^a	29.50 ^a
S. E. ±	1.407	0.22	1.386	0.382
Interaction				
M*P	*	*	*	*

Means in each column with same superscript are not significantly different at $P \geq 0.05$ using Duncan's Multiple Range Test.

Table 4: Comparative efficacy of application methods and test materials for control of *C. graminicola* on sorghum

V. DISCUSSION

Majority of the studies on extract evaluation against fungal pathogens have been conducted *in vitro* and this is one of the first attempts on sorghum under *in vivo* conditions, using different application methods. Results in the current study indicated that the various plant extracts tested at 2.5, 5 and 10% concentration not only suppressed growth of *C.graminicola* in the laboratory, but also controlled the disease in the screen house irrespective of the application method used. The degree of success recorded when the plant extracts were applied as foliar spray in this study was impressive. Foliar spraying requires less volume of extracts and much easier to apply thus could easily be adopted by farmers. These findings are in consonance with the earlier findings of Obi and Barriuso-Vargas (2013) who demonstrated the success of plant extracts under *in vivo* conditions in inhibiting disease development at various degrees in cowpeas. The findings are again consistent with those of Mekonnen *et al.*, (2014) who observed reduction in disease severity and control when *Vernonia amygdalina* among others, was applied as foliar spray on lemon grass rust in the green house. Onyeani and Osunlaja (2012) had earlier on demonstrated the effect of *V.amygdalina* applied as foliar spray in impeding the development and severity of anthracnose lesions incited by *C.gloeosporoides* on mango. Corroborative study from Paulert *et al.*, (2009) and Bajpai *et al.*, (2012) have also reported similar findings with plant extracts applied as foliar sprays *in vivo* on anthracnose of *Phaseolus vulgaris* and pepper anthracnose respectively.

Seed treatment is one of the most important methods of application of biological control and has been demonstrated to give good results, especially on diseases with initial infection from seed inoculation (Shovan *et al.*, 2008). In the past, seed treatments were carried out mainly by applying fungicides. However, giving the growing cost of these fungicides,

particularly in less affluent regions of the world and consumer demand for pesticide free food (Mancini and Romanazzi, 2013) several non-chemical methods of seed treatment which include seed coating with plant extracts are increasingly being developed.

The current study also demonstrated the efficacy of the various plant extracts as seed treatment for control of anthracnose disease. Seed treatments with extracts from *A.sativum* (Obagwu and Korsten, 2003), *A.indica* (Harish *et al.*, 2008), *Moringa* (Adandonon *et al.*, 2006) were effective as seed dressing phytochemicals against various pathogenic fungi. This view was supported by Ademe *et al.*, (2013) and Tegegne *et al.*, (2007) who found extracts of *Lantana camara*, *L.vivurnoides*, *Echinops* spp, *Ruta chalapensis* and *Agapanthus africanus* strongly effective as seed dressing phytochemicals. Talibi *et al.*, (2013) also working in the glass house, noted that methanol extracts of various plants potently checked the incidence and severity of citrus sour fruit compared with the control.

Present findings also confirmed the effectiveness of plant extracts applied as soil treatment. In the study by Sulaiman and Emuah (2009), ginger crude extract drenches was reported as being the best control for root rot of Cowpea.

Interestingly, important observations that must be made mention of are the significant differences between benomyl and the plant materials except garlic. Benomyl was consistent and more effective than other materials in the overall evaluation. These results confirm the observation of Onyeani and Osunlaja (2012) who found benomyl as being superior to *Anonnas quamosa*, *Azadirachta indica* and *Vernonia amygdalina* in controlling anthracnose of mango. Amadioha and Obi (1999) and Nwanosike and Adeoti (2002) however reported low percentage protection recorded by benomyl in the control of *C.lindemuthianum* on cowpea anthracnose and *Alternaria macrospora* on cotton leaf spot respectively. This may be due to development of resistance by the fungi.

In spite of the successes recorded with benomyl however, easy availability of plant species coupled with less phytotoxicity and environmental hazards make them a potential alternative. Also, fungitoxicity of botanical products are considered to be the safe means of plant disease control. Plants used in this study are readily available and their crude extracts can be easily obtained.

In the overall evaluation, neither benomyl nor any other plant material completely inhibited the manifestation of the disease *in vivo*. Factors such as degradation, hydrolysis, polymerization can affect the biological activities of certain components when in contact with plant tissue (Talibi *et al.*, 2013).

It is note worthy that except in a few cases, fungitoxicity of the plant extracts did not persist beyond 45DAS as incidence and severity were observed to increase slightly at 65DAS.

ACKNOWLEDGEMENT

We wish to express our gratitude to Mal. A. Aliyu of the Department of Crop Protection, Faculty of Agriculture, Ahmadu Bello University, Zaria for technical assistance during the course of study.

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