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Azadirachta indica Inhibited Phytopathogenic Fungi of Sorghum (*Sorghum bicolor*. L. Moench)

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Abstract: Sorghum is a dietary staple cereal which could serve as effective substitute for maize, but the incidence of soil borne phytopathogenic fungi is fast becoming an imminent threat to the production of this important crop and it thus, worth investigating. The known phytopathogenic fungi namely; *Fusarium solani*, *Fusarium verticillioides* and *Curvalaria lunata* were evaluated separately and in combination on two Sorghum cultivars namely; *Sorghum vulgare* and *Sorghum bicolor* in completely randomized factorial design experiment with three replications. Further investigation using *Azadirachta indica* as phytofungicide was also carried out. Data were collected, statistically analyzed and interpreted for meaningful conclusion. *Sorghum bicolour* showed higher susceptibility to the interactions of *F. solani* x *F. verticillioides*, while *F. solani* x *C. lunata* showed significant ($p<0.05$) pathogenic effects on percentage germination, seedlings growth and disease incidence on the two varieties of Sorghum seedlings. The extracts of *Azadirachta indica* at 5% g/ml concentration significantly ($p<0.05$) antagonized the treatment of *F. verticillioides* alone and in interaction with *F. solani* x *C. lunata* on both varieties of Sorghum seedlings. Similarly, *C. lunata* alone and in interaction with *F. verticillioides* were significantly antagonized in *Sorghum vulgare*, while *F. solani* alone and in interaction with *C. lunata* were antagonized in *Sorghum bicolor*. However, *F. verticillioides* x *F. solani* and *F. verticillioides* x *C. lunata* resisted the phytofungicidal control of extract on the two varieties. Thus, *Azadirachta indica* showed considerably control on the soil borne phytopathogenic fungi of Sorghum seedlings.

Key words: Sorghum · *Azadirachta Indica* · Phytopathogenic Fungi · Sucesptibility · Biocontrol · Phytofungicides

INTRODUCTION

Sorghum is a genus of numerous species of annual grasses raised for grain or fodder. It is quantitatively the world's fifth most important cereal crop after wheat, maize, rice and barley. The cultivated races, *Sorghum bicolor* subspecies *bicolour* [1] which are distinguished by the grain shape, glumes and panicle are classified into five primary varieties namely *Sorghum bicolor, Sorghum caudatum, Sorghum durra*, *Guinea Sorghum* and *Sorghum kafir* and ten intermediate varieties which are all combinations of the basic varieties [2]. Sorghum is the dietary staple of more than 500 million people in more than 30 countries [3] and together with millet, they represents Africa's most important contribution to world food supply [4]. This cereal crop which is the second most important cereal grain in Africa after maize, has spread in southern Asia and the Americas but Africa is still the largest producer of Sorghum with the productions of 21.9 million tonnes annually which is equivalent to 39.044% of world production. Nigeria with 9.394% world production of Sorghum is ranked the fourth largest producer [5] in the world after USA (17.341%), India (12.923%) and Mexico (10.888%). The grain has higher protein and fat content compare to maize and it is used as food, fodder and the production of alcoholic beverages, breads and pancakes [6].

Sorghum is drought, heat-tolerant and has special adaptations to weather extremes [7], this makes it an important crop in the dry areas especially in arid regions where the grain is one of the staples for poor and rural people [4]. Although, Sorghum is an economically important cereal that adapts to adverse weather conditions, yet, its continual production is threatened by

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the pathogenic organisms which causes various diseases such as; seed and seedling diseases, root rots, stalk rots, foliar diseases caused by fungi, virus diseases, bacteria diseases, downy mildews [8] but fungal contamination constitutes a major biotic constraint to Sorghum improvement and production worldwide [2]. The fungal diseases of Sorghum has not only led to decrease in production, but has also discouraged the cultivation of this important crop.

As agriculture production intensified over the past three decades, farmers became more dependent on agrochemicals as a relatively reliable method of crop protection helping in the economic stability of their operations [9]. The quest for pesticide free food across the globe coupled with the increasing cost of pesticides which even constitutes menace to the environment, causing several negative effects, including development of pathogen resistance to the applied agents and their non-target environmental impacts [10, 11] has led to the shift in the crop protection methods. Biological control is thus being considered as an alternative or a supplemental way of reducing the use of chemicals in the agriculture [11, 12] since plants produces a variety of secondary metabolites that are bioactive and thus may have inhibitory effects on soil borne pathogenic bacteria and fungi [13]. However, this study is an important step in the development of plant based pesticides which are eco-friendly approach for the management of phytopathogenic fungi that constitutes menace in Sorghum cultivation. Therefore this study evaluates the effects of *Fusarium solani*, *Fusarium verticillioides* and *Curvalaria lunata* pathogens singly and in combination; coupled with the effect of *Azadirachta indica* extract on these soil-borne phytopathogenic fungi causing diseases on Sorghum seedlings.

MATERIALS AND METHODS

Source of Planting Materials: This research was conducted in the year 2011/2012, the two varieties of Sorghum seeds used were *Sorghum vulgare* (NG/SA/JAN/09/136) and *Sorghum bicolor* (NG/SA/JAN/09/088) obtained from National Centre for Genetics and Biotechnology (NACGRAB) Ibadan, Oyo state, Nigeria.

Source of Phytopathogenic Fungi: The isolates of phytopathogenic fungi; *Fusarium solani*, *Fusarium verticillioides* and *Curvalaria lunata* were obtained from the Plant pathology/ Mycology laboratory, Department of Botany, University of Ibadan, Nigeria.

Source of Plant Extract: The fresh leaves of *Azadirachta indica* used for the biocontrol experiment were obtained from the Botanical Garden of Department of Botany, University of Ibadan, Nigeria.

Preparation and Quantification of Inoculum: The isolates of phytopathogenic fungi; *Fusarium solani*, *Fusarium verticillioides* and *Curvalaria lunata* were subcultured on Potato Dextrose Agar (PDA) to obtain pure culture prior inoculum size determination; already identified fungi species were harvested separately in already sterilized labelled beakers, the solution was sieved with double folded cheese cloth to allow the passage of fungal spores, counted separately for each fungus and re-adjusted to 1 x 10^6 spores / ml using haematocytometer. Inoculums of the fungal pathogens in equal volume (50 ml) with respect to the factorial experiment design were prepared for screen house experiment.

Preparation of Plant Extract: One hundred grams (100g) of sorted clean leaves of *Azadirachta indica* was weighed and soaked in 5% Sodium hypochlorite (NaOCl) solution for 5 minutes. The leaves were then rinsed in three exchanges of sterile w ater, air-dried and blended in a sterilized electric blender, 200ml sterilized distilled water was used to prepare 100g of the leaves, giving a concentration of 0.5g/ml. The blended slurry was aseptically sieved with sterilized muslin cloth.

Seed and Soil Sterilization: The viable Sorghum seeds were treated separately with 5% NaOCl solution for 3 minutes, then rinsed in two exchanges of sterile distilled water and air dried in laminar flow for 2 hours. The top soil of 0-15cm deep was collected from the farm of Botany Department, University of Ibadan, Nigeria. The soil was sterilized in an electric soil sterilizer.

Screen House Experiment: Seeded pots were inoculated with 10ml of already quantified (1 x 10^6 spores/ml) *Fusarium solani*, *Fusarium verticillioides* and *Curvalaria lunata* alone and in factorial combinations. This was followed after the third day (3rd day) by inoculation with 10ml of plant extracts of *Azadirachta indica* at concentration levels of 5% g/ml. The control experiment involves the treatment with sterile distilled water only. Management practices such as thinning, wetting and weeding were carried out and data on the number of germinated seeds, numbers of infested seedlings, seedlings height (cm) and number of leaves were obtained at day 7 and 14.

Disease Assessment: The percentage germination of the Sorghum seeds was determined according to [14] as;

$$\text{Percentage germination} = \frac{\text{Number of seed germinated}}{\text{Total number of seed planted}} \times 100$$

While percentage disease infection was calculated using the method of [15];

$$\text{Percentage infection} = \frac{\text{Number of seedling infected}}{\text{Total Number of seedlings}} \times 100$$

Statistical Analysis: The data were statistically analyzed using SAS [16]. The least significant different (LSD) test at 0.05 level of significant was used to compare treatment means for each parameter.

RESULTS

The phytopathogenic fungi showed significant ($p<0.05$) reduction in the growth of the two sorghum varieties, *F. solani* treated plants expressed the most inhibitory effects on the germination of *Sorghum vulgare* at day 7 (33.3%), day 14 (53.3%) and *Sorghum bicolor* at day 7 (36.7%), day 14 (70%). The pathogenic interaction involving; *F. solani x C. lunata* was the most virulent on the Sorghum varieties and significantly ($p<0.05$) inhibited the germination rate at day 14 on the Sorghum varieties to 53.3%. *F. verticillioides x F. solani* caused reduced germination percentage at day 7 (40%, 46.7%) and day 14 (60%, 66.7%) on *S. vulgare* and *S. bicolor* respectively, whereas, the untreated plants (control) at day 14 recorded 93.3% and 100% on *S. vulgare* and *S. bicolor* respectively. However, effects of the phytopathogens on the germination rates were significantly ($p<0.05$) suppressed by the extracts of *Azadarichta indica*. The extracts significantly ($p<0.05$) raised the germination rates of plants treated with *F. solani* which was the most virulent on *S. bicolor* to 83.2% at day 7 and 83.3% at day 14. The extracts produced significant ($p<0.05$) increase in the germination rates of other treatments (Table 1).

F. verticillioides showed higher and significant ($p<0.05$) disease severity rate of 53.2% and 20% on *S. bicolor* (53.2%) and *S. vulgare* respectively. This was followed by *F. solani* on *S. bicolor* (42.9%) and *S. vulgare* (38.9%). Whereas, the pathogenic interaction of *F. solani* x *C. lunata* and *F. verticillioides* x *C. lunata* also constituted significant ($p<0.05$) pathogenic effects on the two Sorghum varieties compared to the control (untreated plants) (Figure 1).

The effect of the pathogens and extract treatment showed no significant ($p<0.05$) difference from the control on the number of leaves produced on the two varieties of Sorghum. The plants treated with *C. lunata, F. verticillioides x F. solani* and *F. verticillioides x F. solani x C. lunata* showed no significant ($p<0.05$) pathogenic effect on *S. vulgare*. Whereas, *S. bicolor* variety treated with the phytopathogens expressed significant ($p<0.05$) reduction in the plant heights with the exception of *F. verticillioides x F. solani x C. lunata* (8.6cm) that was not significantly different from the control (8.5cm). The extracts recorded significant increase in the number of leaves and plant height on both Sorghum varieties. *Sorghum bicolor* showed higher resistance to the phytopathogens and its growth was more enhanced by the plant extracts (Table 2).

The pathogenic effects of *F. verticillioides* and *F. verticillioides x F. solani x C. lunata* were the most significantly ($p<0.05$) antagonized on both varieties of Sorghum by the extracts of *A. indica*. While the effects of

Table 1: Effect of phytopathogenic fungi and Azadirachta indica on germination of Sorghum seeds

	Treatment							
	Effect of phytopathogenic fungi on Sorghum seed germination				Effect of Azadirachta indica extracts and Phytopathogenic fungi on Sorghum seed germination			
	Sorghum vulgare		Sorghum bicolour		Sorghum vulgare		Sorghum bicolour	
Day	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
Control	90.0a	93.3a	90a	100a	96.7a	100a	99.9a	100a
F. verticillioides	33.3b	90b	60c	66.7d	96.7a	96.7b	93.3c	93.3c
F. solani	86.7b	53.3g	36.7f	70dc	90c	90cd	83.2c	83.3e
C. lunata	86.7b	73.3e	60c	80b	96.7a	100a	90b	96.7b
F. verticillioides and F. solani	76.7d	83.3d	26.7b	70c	93.3b	90d	93.3a	100a
F. verticillioides and C. lunata	40f	60f	46.7e	66.7d	83.3e	80d	89.9d	90d
F. solani and C. lunata	70e	53.3g	33.3g	53.3e	86.7d	96.7b	100a	100a
F. verticillioides, F. solani and C. lunata	83.3c	86.7c	50.1d	70c	90c	90c	96.7a	100a

The significant differences at $p < 0.05$ are indicated by different letters. Values in the same row followed by the same letter are not significantly ($p<0.05$) different from each other.

Table 2: Phytopathogenic fungi and Azadirachta indica effect on the number of leaves and plant height of Sorghum seedlings

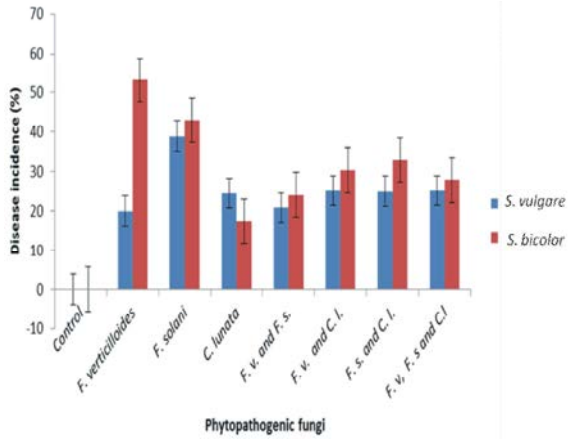
	Treatment							
	Effect of Azadirachta indica extracts and Phytopathogenic fungi on Sorghum number of leaves and plant height after 21 days				Effect of phytopathogenic fungi on number of leaaves and plant height of Sorghum after 14 days			
	Sorghum bicolor		Sorghum vulgare		Sorghum bicolour		Sorghum vulgare	
Growth characteristics	Seedling height (cm)	Number of leaves	Seedling height (cm)	Number of leaves	Seedling height (cm)	Number of leaves	Seedling height (cm)	Number of leaves
Control	12.8c	5.0a	10.5a	5.0a	8.5b	4.0a	9.9c	4.0a
F. verticillioides	12.9b	5.0a	11.7b	4.8a	6.6a	3.9a	9.7c	4.0a
F. solani	11.5f	5.0a	12.4c	5.0a	7.4ab	3.6a	8.9e	3.7a
C. lunata	13.6a	5.0a	11.8b	5.0a	7.5ab	3.7a	10.4b	3.8a
F. verticillioides and F. solani	11.6f	5.0a	11.8b	5.0a`	6.8a	3.3a	10.6a	3.8a
F. verticillioides and C. lunata	12.2e	5.0a	11.6b	5.0a	7.1a	3.9a	9.4d	3.7a
F. solani and C. lunata	12.4d	5.0a	12.0bc	5.0a	6.1a	3.7a	9.8c	3.9a
F. verticillioides, F. solani and C. lunata	13.7a	5.0a	10.8ab	4.9a	8.6bc	3.9a	10.3b	3.9a

The significant differences at $p < 0.05$ are indicated by different letters. Values in the same row followed by the same letter are not significantly ($p<0.05$) different from each other.

Table 3: Disease severity based on interaction of Azadirachta indica and phytopathogenic fungi of Sorghum seedlings.

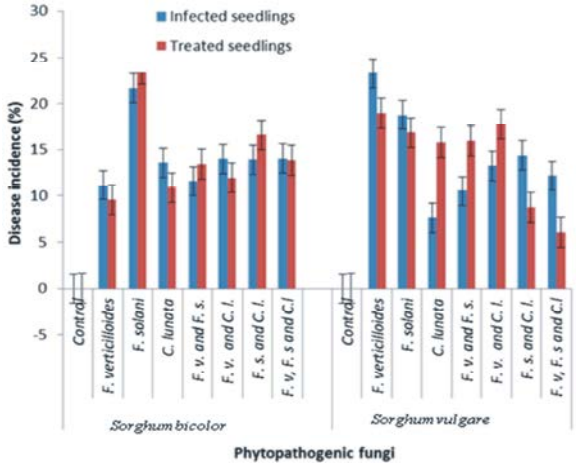
		Day 14		Day 21	
Treatment		Sorghum vulgare	Sorghum bicolour	Sorghum vulgare	Sorghum bicolour
Control	+ AZI	0.0e	0.0e	0.0h	0.0h
F. verticillioides	+ AZI	6.7b	3.3d	10.1g	21.8a
F. solani	+ AZI	3.7d	8.5b	24.8a	19.4c
C. lunata	+ AZI	6.7b	3.7c	11.5f	18.1e
F. v. and F. s.	+ AZI	0.0e	3.3d	14.1d	18.3d
F. v. and C. l.	+ AZI	4.8c	10.1a	12.5e	20.4b
F. s. and C. l.	+ AZI	6.7b	3.3d	17.5b	10.1f
F. v, F. s and C.l	+ AZI	7.9a	3.3d	14.5c	7.1g

The significant differences at $p < 0.05$ are indicated by different letters. Values in the same column followed by the same letter are not significantly ($p<0.05$) different from each other F. v = Fusarium verticillioides, F. s = Fusarium solani and C. l = Curvularia lunata, AZI = Azadirachta indica



F. v = Fusarium verticillioides, F. s = Fusarium solani and C. l = Curvularia lunata.

Fig. 1: Percentage disease incidence of phytopathogenic fungi on Sorghum seedlings at day 14.



F. v = Fusarium verticillioides, F. s = Fusarium solani and C. l = Curvularia lunata.

Fig. 2: Percentage disease incidence of Azadirachta indica and phytopathogenic fungi on Sorghum seedlings at day 14.

C. lunata and *F. verticillioides x C. lunata* were significantly ($p<0.05$) suppressed on *Sorghum vulgare, F. solani* and *F. solani* x *C. lunata* were more controlled on *S. bicolor*. However, *F. verticillioides* x *F. solani* treated plants did not showed significant ($p<0.05$) control of the extracts. *S. bicolor* responded more significantly ($p<0.05$) to the treatment of *A. indica* extract (Figure 2).

The disease severity assessed at day 14 on *F. verticillioides x F. solani* treated plants in *S. vulgare* variety showed no significant ($p<0.05$) difference when compared to the control but other treatments expressed various levels of significance effects. However, highest disease severity was recorded in *F. solani* treated plants at day 21 in *S. vulgare* (24.8%) and *Sorghum bicolor* (19.4%) varieties. Followed by *F. verticillioides* on *S. bicolor* (21.8%) and *F. verticillioides* x *C. lunata* on *S. bicolor* (20.4%), while the least disease severity was expressed by the treatments of; *F. verticillioides* x *F. solani x C. lunata* on *S. bicolor* at day 21 (7.1%) (Table 3).

DISCUSSION

Fungi belonging to more than 40 genera were reported to be associated with the diseases of Sorghum especially molded grains [17] which included but not limited to Aspergillus sp. Fusarium sp. *Alternaria alternata, Chaetomium globosum, Curvularia lunata*, *Drechslera halodes*, *Rhizopus stolonifer* and *Trichothecium roseum* [18]. These are the common occurrences that had also been reported by Martin *et al.* [19]. However, the fungi that infect Sorghum during early stages of development are; *Fusarium moniliforme* Sheld. *Curvularia lunata* (Wakker) Boedijn, *Fusarium pallidoroseum* Berk. and Rav. and *Phoma sorghina* (Sacc.) Boerema Dorenbosch and van Kesteren [17, 20]. The pathogenicity test of the fungi conducted in the screenhouse in respect to this study showed that the treatments of *F. verticillioides* alone, interactions of *F. verticillioides* x *C. lunata* and also *F. solani* x *C. lunata,* caused significant reduction in Sorghum seed germination. Producing symptoms such as seedling decay, wilting, root and stalk rot which was similar to the symptoms earlier reported [21, 22]. This resulted in more than 40% germination decline with emphasis on the reduced plant height at the seedling stage, which corroborated with the findings of Yates *et al*. [23] who reported that *F. verticillioides* affects the thickness, height, weight and leaf length of seedlings developed from infected seeds, thus, affirms the identity of *F. verticillioides* as a pathogen of Sorghum [24]. This claim was however, validated by Bandyopadhyay *et al.* [17] who described *Fusarium moniliforme* and *Curvularia lunata* as phytopathogenic fungi of economic importance.

F. verticillioides and *F. solani* expressed the highest disease incidence on the Sorghum varieties in this study. The pathogenic effects of these species have been verified by Vesna *et al.* [25] who reported the pathogenic effect of *F. verticillioides* isolated from wheat and maize grain on maize seedlings. Berna *et al*. [26] also affirmed this claim in their study, although presented *F. subglutinans, F. oxysporum*, *F. equiseti, F. acuminatum, F. solani, F. verticillioides* as weak pathogens against the winter wheat, but affirms the pathogenic potentials of *F. verticillioides* and *F. solani*. The interactions of the pathogens which exerts disease incidence (25.2%) on *Sorghum vulgare* were found to express higher pathogenic effect on *Sorghum bicolor*. The interactions of *F. solani* and *C. lunata* expressed the highest (32.9%) disease incidence compared to *F. verticillioides* (53.2%), *F. solani* (42.9%) and *C. lunata* (17.4%) respectively. This result was similar to the findings of Rajput *et al.* [27] who reported the interactions of *Rhizoctonia solani* and *C. lunata* as pathogenic on Shisham seedlings (Dalbergia sissoo Roxb.). In this research, *C. lunata* interacted with *F. solani* or *F. verticillioides* expressed a less observable disease incidence and severity. Thus, suggest that continuous occurrence of *C. lunata* in combination with *F. solani* or *F. verticillioides* may likely not lead to severe disease incidence, thus, the effect may be insignificant to Sorghum production. This further agreed with the report of Rajput *et al.* [27] that *R. solani* and *C. lunata* either completely failed or caused very rare infection on their test plants. *F. solani* was also reported to produce moderate infection on Shisham seedlings when inoculated with either *R. solani* or *C. lunata*.

The antagonistic activities of aqueous extracts of *Azadirachta indica* on the phytopathogenic fungi at 0.5g/ml concentration, was justifiable in this investigation. This could be attributed to the antimicrobial properties of *Azadirachta indica* extracts as reported by Locke [28]. The germination percentage was enhanced with the least germination rate observed in the interactions of *F. verticillioides x C. lunata* (80%) for *Sorghum vulgare* and *F. solani* alone (83.3%) on *Sorghum bicolour.* The agronomic characteristics (the seedlings height and number of leaves) were also appreciably increased. This is in line with the findings of Ibtihaj Mukhtar Ahmed [29] who claims that apart from the aqueous extracts of *Azadirachta indica* been environmentally safe, it

eliminates or reduces the incidence of the economic important pathogens and also increases seed germination, growth and yield of Sorghum plant. However, plant extracts had been reported effective in the control of pathogenic fungi [30] of Sorghum [31], thus, extracts of *Azadirachta indica* at 0.5g/ml concentration significantly reduced the disease incidence of pathogenic fungi of Sorghum. This was in consonance with the findings of Akanmu *et al.* [32] that reported the effectiveness of *A. indica* at 0.5% concentration in the control of *Pseudomonas corrugata*, the causative pathogen of tomato pith necrosis. The importance of plant extracts was also intensified by Satish *et al.* [33] that tested fifty two plants from different families for their antifungal potentials against eight important species of *Aspergillus* spp.

The treatment of *F. verticillioides* x *F. solani* resist the control effects of *Azadirachta indica* extracts in this study. This could be due to the higher virulence conferred by the interaction of the two most pathogenic fungi recorded in the pathogenicity test on Sorghum varieties. Similar occurrence had also been reported by Somda *et al.* [31] on the resistance of *Collectotrichum graminicola*, *Phoma sorghina* and *Fusarium moniliforme* to the control effect of *Azadirachta indica* and *Eucalyptus camaldulensis* extracts. The efficacy of the extracts of *A. indica* as phytofungicide in this study showed that, proper utilisation of *A. indica* would not only control the phytopathogens, but would also help to sustain *Sorghum* production in Nigeria.

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