NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 53 Number 2, August 2022 Pg. 297-302
Available online at: http://www.ajol.info/index.php/naj
https://www.naj.asn.org.ng

Sclerotium Rot Disease Management in Sunflower (*Helianthus annuus,* L.) with Sawdust or Ash Extract

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Abstract
The potency of sawdust or ash extracts of *Gmelina arborea* and *Ficus exasperata* to manage Sclerotium rot disease of two sunflower varieties (SAMSUN 1 and SAMSUN 2) was investigated. Sunflower plants were sprayed with 50% extracts once every three weeks after planting. Results showed that all extracts significantly (p<0.05) reduced the incidence and severity of the disease. Specifically, ash extracts of *G. arborea* and *F. exasperata* reduced the incidence of Sclerotium rot disease on sunflower plants and minimized disease development, thereby, preventing achene and flower infection. Seeds from the plots sprayed with ash extracts of *F. exaspera*ta recorded significant lower fungal infection ranging from 23.78 to 40.04% compared to unsprayed plots (70.29 to71.27%). Similarly, germination percentage of seeds from sprayed plots was significantly (p<0.05) higher (range: 67.21 to 89.71%) than that of control (15.09 to 20.14%). Seed stored with extracts had a significantly (p<0.05) lower fungal incidence (13.61 to 16.11%) than that without extract (21.81 to 24.11%). Likewise, extract-stored seeds recorded significantly (p<0.05) higher germination (69.11and 98.14%) than control (17.26 and 20.17%). In conclusion, sawdust or ash extracts of *G. arborea* and *F. exasperata* were comparable to Benlate (Benzimidazoles 50% WP) in their effect to suppress Sclerotium rot disease in sunflower varieties; therefore they hold promising prospects for use in the management of sunflower Sclerotium rot disease.

Keywords: Ash, Fungicides, Sawdust, Sunflower and Sclerotium rot

Introduction

Sunflower (*Helianthus annuus* L.) is one of the important oilseed crops of the world (Ahmad *et al*., 2011). It is easily distinguished from other cultivated crops by its single term and very prominent inflorescence called capitulum and it's quite rustic and very adaptable to diverse environmental conditions and management practices (Martin *et al*., 2012). This crop has shown distinct superiority to other oilseed crops because of its wider adaptability to different agro-climatic conditions, high oil production per unit area, short duration, high yielding and ability to withstand drought relative to other rain-fed crops, particularly under delayed sowing situation (Tonev, 2006). The seed is rich in high percentage of oil (48-53%), protein (14-19%), crude fibre (16-27%), ash (2-3%), soluble sugar (7-9%) and hull (21-27%) (Rosa *et al*., 2009). Sunflower seeds and oil are on high demand all over the world because of its nutritional benefits and quality of edible oil (Groove and Summer, 2005). Sclerotium rot disease caused by *Sclerotium rolfsii* Sacc., however, is one of the major problems in the cultivation of this crop (Bhutta *et al*., 1995). This disease is common in most sunflower growing areas of the world (Cother, 2000) and particularly severe in the tropical and subtropical areas (Harikrishnan and Del Río, 2006). The disease which affects sunflower plant as early as 3 weeks after planting (WAP) starts as elliptical lesion at the base of the stem. The lesion eventually girdles the stem and extends for 10cm or more and as a result of large quantities of oxalic acid and polygalacturonase produced by the pathogen; there is extensive rotting of the plant. Severe infection at plant maturity causes damage of sunflower achene and yield loses which ranged from 45.8 to 69.5% (Shukla, 2005).

A vast array of pesticides are being used to control plant diseases (Karuna *et al*., 2012). These pesticides prevent or reduce infections by utilizing various principles of disease control. However, the intensive and indiscriminate use of pesticides in agriculture has caused many problems to the environment such as

water, soil, animals and food contamination, elimination of non-target organisms and development of resistance by phytopathogens (Di Piero *et al*., 2010). In attempt to minimize the negative impact of pesticides usage, this study was carried out to determine the efficacy of sawdust and ash extracts of two tropical plant species for controlling of *Alternaria* leaf blight disease of sunflower.

Materials and Methods

Source and preparation of extract

Wood products comprising sawdust of *Gmelina arborea* and *Ficus exasperata* were obtained from saw mill industries located at Abeokuta Metropolis, Ogun State. Part of each saw dust was sun-dried and burnt using a pre-fabricated incinerator (12 – 100kg/hour) modelled according to PATH (2010). The tropical trees were selected because some have been associated with antimicrobial, traditional healing and crop protection activities (Shaikh and Sahera, 2016). Fifty grams of *G, arborea* (sawdust or ash) or *F. exasperata* (sawdust or ash) were weighed with sensitive weighing balance (Model: PL203; Surinda and Company, Ambala). Weighed samples were each mixed with 1000ml of sterile distilled water, soaked for 24 hours and sieved through 1 mm cheese cloth for extracts.

Field experiment

A field experiment in Randomized Complete Block Design with three replicates was conducted at the Teaching and Research Farm, Federal University of Agriculture, Abeokuta (7°15′N; 3°25′E) Ogun State, Nigeria. Experimental site was ploughed, weeded and well tilled. The treatments consists of four sawdust or ash extract, a synthetic fungicide Benlate (Benzimidazoles 50% WP) and an unsprayed control. Two improved sunflower varieties developed in Nigeria: SAMSUN 1 and SAMSUN 2 were obtained from the National Cereals Research Institute, Samaru, Kaduna State, Nigeria. The seeds were sown in drills during 2014 early cropping season (March) in plots of size 5 x 4m, manual weeding done three weeks after sowing and other standard cultural practices for growing sunflower applied. The plant extracts in 100%(w/v) concentration were sprayed using a pneumatic hand sprayer from three WAP, that is, before disease development and was repeated at two weeks' interval until 11 WAP. The synthetic fungicide, Benlate, was sprayed at the recommended rate of 2.3 g/L at 5 WAP and 8 WAP, while the control was left unsprayed. The plants were examined for disease symptoms weekly and quantitative assessments (number of plants infected) were made until 12 WAP. Assessment of the number of infected plants was done in two permanent, randomly placed quadrats (100 x 100cm) per plot. The total number of plants and number infected in a quadrat were counted and the percentage disease incidence worked out. Number of leaves infected was obtained from five randomly tagged plants per plot and expressed as percentage of the total number of leaves. Disease severity was assessed by counting the number of plants with lesions on their leaves at 13 WAP and rating the symptom expression on a 0 to 6 scale (Table 1). For this, five plants per plot were selected and on each plant, the number of lesions on plant stand was counted, while another set of five random plants per plot was used for disease rating. Achenes from sprayed and unsprayed plots were harvested, seeds were removed and weighed to determine seed yield in kg/ha.

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected leaves per plant}}{\text{Total number of leaves per plant in the plot}} \times 100$$

$$\text{Disease severity} = \frac{\Sigma n \times 100}{N \times S}$$

Where; Σ = Summation, n = number of infected leaves, N = Number of leaves assessed, S = Maximum numerical value/grade

Seed viability and incidence of mycoflora

Four hundred seeds from each treatment were plated on blotter paper at the rate of 20 seeds per Petri-dish (9cm diameter). The plates were placed in an incubator and temperature was set at 28±2°C for 7 days with 12hrs alternating cycles of light and darkness, and observations on seed germination fungal infection were noted.

Seed treatment with sawdust or ash

Two hundred grams of seed samples of the two sunflower cultivars (not from the batch harvested in the experiment described above) were stored with 5g of the test plants in plastic containers for 30 and 60 days. The same grams of seeds were stored with Benlate (5mg) in a plastic container for 30 and 60 days respectively and used as controls. Four hundred seeds from each treatment were plated at the rate of 20 seeds per Petri dish (9cm-diameter). The factorial set of treatments consisting of sawdust or ash or fungicides and a control (where no extract is added) were arranged in a completely randomized design replicated three times. Seeds were incubated as above and assayed for fungal incidence and germination.

Statistical analysis

All data collected were subjected to Analysis of Variance (ANOVA) using "CoStat software" (CoStat, 2005) and mean separation was done using Duncan's Multiple Range Test (DMRT) or Least Significant Difference (LSD).

Results and Discussion

Results

Generally, disease incidence was reduced by the application of sawdust or ash extracts (Table 2). Percentage of plants infected in both sunflower varieties was significantly ($p \leq 0.05$) reduced by the two extract forms. SAMSUN 1 sunflower plant treated with *F. exasperata* ash extract recorded significantly ($p \leq 0.05$) lower Sclerotium rot incidence than with other extracts, while, synthetic fungicide- Benlate also effectively minimized the spread of disease, it was statistically at par with that of *G. arborea* (sawdust), *G. arborea* (ash)

and *F. exasperata* (sawdust). Incidence of Sclerotium rot disease on SAMSUN 2 sunflower plant was lowest with *F. exasperata* (ash) treatment. However, it was comparable to that treated with *F. exasperata* (sawdust) extract and synthetic fungicide spray. Untreated sunflower plants had significantly ($p \leq 0.05$) higher disease incidence than those treated (Table 2). Disease index score showed that plots of SAMSUN 1 treated sawdust or ash extracts of *F. exasperata* had only "Minor infection" characterized by widened brown spots with traces of yellow background on the leaves and by implication stem was not affected which could lead to necrotic tissues causing rot. Similarly, plots of SAMSUN 2 treated with sawdust and ash extracts of *F. exasperata* showed similar disease index score. Furthermore, SAMSUM 2 plots treated with sawdust or ash of *G. arborea* or synthetic fungicides showed "Moderate infection" and consequently protected the achenes against infection. Conversely, untreated plots showed "Very severe infections" (Table 2). Coincidentally, incidence of fungi on sunflower seeds from sprayed plots was between 23.78 and 40.04% (Table 3), which was significantly lower than that of control (70.29 and 71.27%) and by implication, the achenes are probably not infected in the sprayed plots. The outcome of seed test revealed that germination of seeds from extract-sprayed plots was significantly ($p \leq 0.05$) greater (67.21 and 89.71%) than that of unsprayed plots (15.09 and 20.14). Comparative analysis of data in Table 3 further showed that total grain yields from plots sprayed with extracts of *F. exasperata* (1,690 and 1,810kg/ha) or *G. arborea* (1,593 and 1,790kg/ha) were significantly higher than that of unsprayed plots (1,290 and 1,300kg/ha). The higher yield levels (1,750 to 1,810kg/ha) recorded in the extract-treated SAMSUN 1 plots were also comparable to that of Benlate-sprayed plots (1,700kg/ha). However, yield level of *F. exasperata* extract-treated SAMSUN 2 plots were significantly ($p \leq 0.05$) higher than that of Benlate-sprayed plots. There were variabilities in the fungal incidence and germination of seeds stored with sawdust or ash of *G. arborea* and *F. exasperata* for 30 and 60 days respectively (Table 4). Generally, incidence of fungi decreased with increase in storage period but seed germination increased with increase in storage period of the treated seeds. However, reverse was the case for untreated sunflower seeds. SAMSUN 1 seeds stored with *F. exasperata* (ash) had significantly ($p \leq 0.05$) lower fungal incidence than other stored seeds at the end of 60 days. Benlate-stored SAMSUN 1 seeds recorded a comparable incidence of fungi with that stored with *G. arborea* (sawdust). Similarly, germination was significantly higher in SAMSUN 1 seeds stored with *F. exasperata* at the end of storage period. The same trend followed for SAMSUN 2 but with averagely high fungal incidence and low seed germination (Table 4).

Discussion

Outcome of the study confirms that extracts of *G. arborea* (saw dust) and *F. exasperata* (ash) reduced the incidence and severity of Sclerotium disease of sunflower. This might be due to efficacy of extracts to inhibit spore germination of pathogens as plant extracts interfered with disease development through the effect of their antifungal constituents. Reduction in Sclerotium disease incidence of sunflower by the sawdust or ash extracts was consistent with earlier reports that many plant products contain antimicrobial properties that have the qualities to control plant diseases (Das *et al*., 2010; Bajpai and Kang, 2010). Five constituents of *Gmelina arborea* isolated and identified as (+)-7-O-ethyl arboreol, (+)-paulownin, (+)-gmelinol, (+)-epieudesmin and (−)-β-sitosterol has been reported to be antifungal in nature (Kawamura *et al*., 2004). Similarly, antifungal constituents which include; ficusamide, furanocoumarins, (*S*)-(_) oxypeucedanin hydrate, (*R*)-(_) oxypeucedanin hydrate and bergapten (5-methoxypsoralen) were isolated from *F. Exasperata* (Adesope *et al*., 2006). Lawal *et al*. (2012) had reported the antifungal effect of the foliar spray of root bark of *G. arborea* extract against *Aspergillus niger* and *Aspergillus flavus*. Eno *et al*. (2016) also reported that extract of *G. arborea* showed great potentials of fungicidal properties and abilities to control anthracnose disease of cowpea on the field. The enhanced seed germination in harvested seeds from extract treated plots could be attributed to low seed infection by the pathogens. Mahrotra and Aggarwal (2003) reported that fungi are able to retard seed germination through softening and necrosis of tissues. Moss and Smith (2006) confirmed the association of seed borne fungi with seed viability, wilting of plant and stem flaccidity. Enikuomehin *et al*. (2010) indicated that viability of sesame seed depends on infection level. Reduction in microbial infection and improved germination of seeds stored with sawdust or ash of *G. arborea* and *F. exasperata* might be due to the inability of seedborne fungi to grow under treatment condition. This is in line with earlier findings of Egbontan *et al*. (2020) that extracts of *G. arborea* and *F. exasperata* inhibited mycelial growth of fungi associated with improved varieties of sunflower. The result also corroborated the report of Enikuomehin and Peters (2002) that extracts of *Ocimum gratissimum, Azadirachta indica,* and *Mangifera indica* reduced mycoflora load of sesame seeds through a possible reduction in capsule infection. In addition, Enikuomehin, (2005) reported that seed treatment by soaking in plant extracts for 30 min can be advocated as preventive measure against cercospora leaf spot of sesame. The comparable higher yield levels between extract-treated and Benlate sprayed plots implied prominent advantages for certain sawdust or ash extracts in restraining disease development and promoting sunflower yields at lower costs.

Conclusion

The study presents the potential of sawdust or ash extract of *G. arborea* and *F. exasperata* in controlling Sclerotium rot disease of sunflower. Further studies are, however, necessary to determine the minimum concentration required for maximum disease control as well as the frequency and mode of application of the different plant extracts.

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Table 1: Severity scale of Sclerotium rot disease of sunflower

Grade/Numerical value	Plant tissue damage (%)	Rating	Symptom expression
0	0	No disease	Spotless leaves and stem
1	1 – 20.9	Trace infection	Scattered pin point brown spot on the stem
2	21 – 40.9	Minor infection	Widened brown spots on stem
3	41 – 60.9	Moderate infection	Coalescing of brown spots to form extended necrotic areas on stem tissues
4	61 – 80.9	Severe infection	Extensive browning and necrosis of foliar tissue which tends to wilting of the entire leaf and stem
5	81 – 100	Very severe infection	All features above with brown spot on the achene/head

Modification of Sangeetha and Siddaramaiah (2007) disease severity assessment scale for Sclerotium rot of sunflower

Table 2: Effect of field spray with sawdust or ash extracts on the incidence and severity of Sclerotium rot disease in two cultivars of sunflower (SAMSUN 1 and SAMSUN 2) at Abeokuta, Nigeria

Treatment	Disease incidence (%) Plant infected		Severity assessment Disease score (S.E)[1]	
	SAMSUN1	SAMSUN2	SAMSUN1	SAMSUN2
Gmelina arborea (sawdust)	45.68^{b}	42.21^{b}	1.2±0.34	3.0±0.41
Gmelina arborea (ash)	48.18^{b}	40.12^{b}	1.4±0.12	3.4±0.71
Ficus exasperata (sawdust)	35.23^{b}	32.41^{bc}	1.1±0.13	3.3±0.11
Ficus exasperata (ash)	29.41^{c}	25.52^{c}	1.3±0.51	2.2±0.32
Benlate	43.13^{b}	40.67^{bc}	3.1±0.43	3.4±0.51
Control	95.84^{a}	97.43^{a}	4.0±0.32	4.0±0.41

Values with different superscripts in the same column are significantly different ($p<0.05$) in Duncan's Multiple Range Test. Data are means ± standard error of visual severity scores obtained 13 weeks after planting (WAP) from 15 plants per cultivar and within each treatment category. Plant were scored on a 0–6 scale (see text for details)

Table 3: Seed mycoflora, germination of harvested seeds and grain yield of two varieties of sunflower (SAMSUN 1 and SAMSUN 2) as affected by different sawdust or ash extract sprays at Abeokuta in Nigeria

Treatment	Fungal incidence		Seed germination		Grain yield (kg/ha)	
	SAMSUN1	SAMSUN2	SAMSUN1	SAMSUN2	SAMSUN1	SAMSUN2
Gmelina arborea (sawdust)	35.45^{b}	40.04^{b}	75.90^{a}	67.21^{a}	1,750^{a}	1,593^{b}
Gmelina arborea (ash)	30.90^{b}	34.89^{b}	82.12^{a}	72.17^{a}	1,790^{a}	1,670^{b}
Ficus exasperata (sawdust)	29.17^{b}	31.63^{b}	85.32^{a}	75.11^{a}	1,765^{a}	1,690^{ab}
Ficus exasperata (ash)	23.78^{b}	24.42^{b}	89.71^{a}	77.63^{a}	1,810^{a}	1,740^{a}
Benlate	30.10^{b}	33.38^{b}	83.21^{a}	69.21^{a}	1,700^{a}	1,620^{b}
Control	70.29^{a}	71.27^{a}	20.14^{b}	15.09^{b}	1,290^{b}	1,300^{c}

Table 4: Fungal incidence and germination of seeds soaked with 100% sawdust or ash extracts of tropical trees for different durations on two sunflower varieties

Duration of treatment/plant species	Sunflower varieties			
	SAMSUN 1		SAMSUN 2	
	Fungal incidence (%)	Germination (%)	Fungal incidence (%)	Germination (%)
30 days				
Gmelina arborea (sawdust)	13.61	79.21	16.11	69.23
Gmelina arborea (ash)	11.02	81.39	15.89	73.10
Ficus exasperata (sawdust)	9.90	82.21	12.90	78.18
Ficus exasperata (ash)	7.22	89.12	9.72	80.64
Benlate	12.50	79.16	16.10	70.43
Control	25.19	22.51	31.19	19.25
LSD (P≤0.05)	2.19	2.74	3.02	3.12
60 days				
Gmelina arborea (sawdust)	9.39	80.92	15.19	70.92
Gmelina arborea (ash)	9.12	85.12	12.15	85.12
Ficus exasperata (sawdust)	7.74	92.89	10.14	85.94
Ficus exasperata (ash)	6.30	98.14	8.79	86.14
Benlate	9.23	90.02	14.23	78.24
Control	24.11	20.17	21.81	17.26
LSD (P≤0.05)	1.73	1.03	3.27	5.13