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EFFECTS OF PLANT EXTRACTS, A SYNTHETIC FUNGICIDE AND *Cercospora* sp. ON SESAME YIELD IN SOUTHERN AND NORTHERN SAVANNA ECOLOGIES OF NIGERIA

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ABSTRACT
Cercospora leaf spot (CLS) caused by *Cercospora* sp. on sesame constitutes one of the most important foliar diseases. The experiment was aimed at determining the effect of CLS on sesame and the effectiveness of some plant extracts and a synthetic fungicide on the disease. A field experiment was carried out at Ardo-kola (Northern Guinea Savanna) and Gassol (Southern Guinea Savanna) in 2011 and 2012, involving five plant extracts (*Azadirachta indica*, *Jatropha curcas* Linn., *Alium sativum*, *Ocimum gratissimum*, *Chromolaena odorata*) and Benomyl. Crop loss studies revealed that plant extracts and Benomyl gave 15- 21 % reduction in severity of CLS and 24 – 57 % higher yields. Plants treated with Ocimum and Chromolaena, which was at par with Benomyl, gave significant reduction of disease severity by 19 and 21 %, and highest yield increase of 57 and 45 % than unsprayed plots in Ardo-kola and Gassol respectively. Correlation studies revealed that all the yield characters considered correlated positively and highly significantly with seed yield and with themselves, except for weeks to 50 % flowering, weeks to harvest and CLS severity, which correlated negatively with the yield. The highest yield losses of unsprayed plots and the negative correlation between disease severity and seed yield indicated the need to control the leaf spot *Cercospora* sp. for enhanced seed yield and seed quality in sesame especially when grown under moist environments in ecologies where the pathogen is endemic.

KEYWORDS: *Cercospora* sp., sesame, plant extracts, synthetic fungicide, yield losses, correlations.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is an annual plant that belongs to the Pedaliaceae family (Karifiti and Deckers, 2001). Annual exports of sesame from Nigeria are valued at about US$20 million and Nigeria is one of the major suppliers of sesame seed to the world's largest importer, Japan. In response to the growing export market demand, Nigeria's production of the crop has consistently increased from about 15,000 MT in 1980, to about 100,000 MT in 2006. Of an estimated 3.5 million hectares of land available for the crop, only about 335,000 ha are currently used for sesame, suggesting a vast potential for increased production of the crop (USAID MARKETS, 2009). Nigeria exports 80,000 metric tonnes of the global export of 900,000 metric tonnes. Major destinations for export include China, Japan, Syria, European Union, Korea, Turkey and the Middle East (Tiamiyu*et al*., 2013). The wide range of sesame seeds markets are a great opportunity to earn additional foreign exchange for the country if its production potentials are exploited. Sesame oil has a composition that provides good health benefits including high levels of unsaturated fatty acids (80%) and antioxidants. The presence of some antioxidants (Sesamum, Sesamolin and Sesamol) makes the oil one of the most stable vegetable oils in the world. Moreover, the absence of linolenic acid makes the oil useful as a raw material for the production of medicinal drugs and perfumes. Possibly for this reason, sesame oil is widely considered to prevent diseases of different kinds. Cercospora leaf spot of sesame has been reported to be prevalent in all the sesame growing areas with considerable loss in yield of sesame of about 20 % (Naresh and Sangwan, 2010). It was earlier revealed in Nigeria that uncontrolled effect of the disease could lead to reduction of plant height (19.08 %), number of capsules (21.50 %), number of branches (33.40 %) and yield (54.11 %) respectively (Uwala, 1998). This research paper presents reports of the efficacy of plant extracts and a synthetic fungicide on Cercospora leaf spot disease reduction and yield increase of sesame under Northern and Southern Guinea Savanna Ecologies.

MATERIALS & METHODS

Experimental Sites

The experiment was a field experiment conducted in 2011 and 2012 rainy seasons at two locations namely: Teaching and Research Farm of Taraba State University in Ardo-Kola Local Government in NGS (Latitude 08º51′N - 08º 53′N and Longitude 011º17′E - 11º 19′E) with topography of 250 m above sea level, mean annual rainfall of 600-1000 mm and the mean annual temperature of 25 to 30 ºC (Taraba State Government Diary, 2007). The second location was at the Ministry of Agriculture Silos complex site located at Gassol, Taraba State (Latitude 08º 31′N and Longitude 10º 33′E). It has an annual average rainfall of 800-1200 mm and annual average temperature of 27 to 29

^{o}C with vegetation similar to that of Southern Guinea Savanna (S.G.S.). Rains Commence as from April and terminates in October, while dry season begins from November to March in both locations.

Collection of Sesame Plant Samples and Isolation of *Cercospora* sp.

Sesame diseased samples were collected from farms. The samples were brought into the laboratory sealed in air-tight cellophane pack, and leaves with typical leaf spot symptoms collected from surveyed fields were carefully transferred unto humid chambers made up of petri dishes lined with wet filter papers at both covers (Eman, 2011). The lesions on leaf materials were incubated in these humid chambers at room temperature for 72 hours, and examined for sporulation under Trinocular Microscope. Afterwards observed conidia from separate conidiophores were picked using glass needle (that was previously flamed and cooled in methylated spirit) and transferred aseptically in to different plates containing V8 agar using single spore technique to obtain pure cultures (Hashem and Farrag, 2005). The plates were placed on bench in a previously fumigated clean room where they could access sunlight at room temperature for 21 days. During this period cultural and morphological characteristics of the fungus were observed daily during the growth period until the cultures had fully sporulated.

Collection of Plant Materials

Leaves of *Azadirachta indica* A.Juss, *Jatropha curcas* Linn., *Ocimum gratissimum* (Linn.), *Chromolaena odorata* King and Robinson and bulbs of *Alium sativum* Linn. were collected from within and around Modibbo Adama University of Technology, Yola. The phytochemical composition of plant extracts (Table 1) were also reported (Miron *et al*., 2000 and Wei *et* al., 2011; Terezinha *et al*., 2006; Prabhu *et al*., 2009; Pierangeli *et al*., 2009; Moses *et al*., 2010; Sanjay and Kumar, 2006; Saetae and Suntornsuk, 2010).

Preparation of Cold Water Extracts

The crude cold water extracts were obtained by sterilizing the leaves in 10 % Sodium hypochlorite (NaOCl) for 1 minute, washed 5 times in distilled water and air dried for 3 weeks according to Akinbode and Ikotun (2008). Thereafter, the plant leaf materials were pounded using mortar and pestle and sieved in a 40 mm sieve. To obtain extracts, 100 g each of the powder (packaged according to plant species) was weighed in to labeled conical flasks and 100 ml distilled water added at a ratio of 1:1 w/v. This was then corked, shaken for 20 minutes to mix, allowed to stand for 24 hours at room temperature and filtered using a muslin cloth. To obtain 10 % concentration for spray, 100 ml of the filtrate was added to 900 ml of sterile water to make up to 1 litre.

Field Layout and Experimental Design

The field experiments in Ardo-Kola and Gassol were laid out using a Randomized Complete Block Design with 4 replications. Water extracts from Neem leaf (*Azadirachta indica* Juss), Jatropha leaf (*Jatropha curcas* Linn.), Garlic bulb (*Alium sativum* L.), African Basil leaf (*Ocimum gratissimum* L.) and Siam weed leaf (*Chromolaena odorata* (Linn.) King and Robinson), a synthetic fungicide, Benomyl 50 WP (500 g a.i kg^{-1}) and an unsprayed control (7 treatments) were tested. A total of 28 plots were laid out on the field. Plot size was 5 m x 4 m with inter-and intra-row spacing of 50 cm and 10 cm respectively.

Preparation of Conidial Suspension

Conidia suspension used for inoculation was obtained by carefully scraping 10 g of culture from six petri dishes into 100 ml sterile distilled water in a conical flask, using a sterile scalpel. This was thoroughly mixed with sterile glass rod, shaken vigorously for ten minutes and filtered through two layers of muslin cloth to remove mycelia fragments and made up to 100 ml suspension. The conidial concentration of $1x10^4$ conidiospores/ml was determined using a Haemocytometer.

Field Inoculation of *Cercospora* sp. Pathogen on Sesame Plants

At 3 weeks after sowing, 800 seedlings per the 20 m^2 plot were sprayed (on the abaxial and adaxial) with 50 ml suspension containing spore concentration of $1x10^4$ conidiospores/ml in distilled water of the pathogen using pressurized hand sprayer. Weekly observations for symptoms were carried out as from 4 weeks until 12 weeks after sowing (WAS).

Application of plants extracts and fungicide

Having inoculated the seedlings at 3 WAS, plant extracts (10 %) were sprayed using pressurized hand sprayer from 4 weeks after sowing (WAS) and repeated at 6, 8 and 10 WAS. The synthetic fungicide, Benomyl 50 WP (500 g a.i. /kg) was sprayed at the recommended rate of 2 kg ha^{-1} at 4 WAS and 8 WAS, while the control was left unsprayed.

Harvesting

At harvest the two border rows of each plot were not considered including the 50 cm at both border ends, there by leaving a net plot of 2.5 m x 4 m = 10 m^2. At grain maturity each plot was harvested separately using sickles. After harvesting, the plants were tied with rope into bundles and position in an erect form on tarpaulin for the capsules to be fully dried. This helped to prevent wastage of seeds and contamination by impurities (Uzoh, 1998).

DATA COLLECTED

Disease severity

The percentage coverage as related to disease score was done using a severity scale in Table 2. Severity was estimated by assessing 10 randomly tagged plants per sub plot and determining overall score according to percentage area covered using a scale of 1-7 as outlined by Enikuomehin *et al*. (2002) with some modifications at 12 WAS. This was intended to show the amount of the disease in each treatment. The following formula was used in determining the severity of infection.

$$\text{Disease severity} = \frac{\text{Sum of all disease scores}}{\text{Number of plants scored x Highest score}}\ x\ 100$$

TABLE I. Phytochemical/Chemical Composition of the Selected Plant Extracts and a Synthetic Fungicide (Benomyl)

S/No	Plant/ Chemical	Chemical name of active ingredients	Chemical family	Molecular formulae	IUPAC or Chemical name	Use against
1.	*Allium sativum*	Allicin	Organo sulfur compound (diallyl disulphide)	C6H10OS2	2-Propene-1-sulfinothioic acid S-2-propenyl ester	*Rhizoctonia* sp. and *Alternaria* sp.on Tomato
2.	*Ocimum gratissimum*	Eugenol	Phenylpropanoids	C10H12O2	4-Allyl-2-Methoxyphenol;5-Allylguaiacol	*Rhizoctonia* sp. and *Alternaria* sp.on Tomato
3.	*Chromolaena odorata*	Geijerene and Pregeijerene	Phenol	C12H18	(3S,4S)-3-Isopropenyl-4-methyl-4-vinyl-1-cyclohexene	*Colletotrichum lindemuthianum*, *Penicillium chrysogenum*, *Rhizoctonia* sp. and *Alternaria* sp.on Tomato
4.	*Azadirachta indica*	Azadirahtin	Limonoid which is a highly oxidized tetranortripenoid	C35H44O16	dimethyl(2a*R*,3*S*,4*S*,*R*,*S*,7a*S*,8*S*,10*R*,10a*S*,10b*R*)-10-(acetyloxy)-3,5-dihydroxy-4-[(1*S*,2*S*,6*S*,8*S*,9*R*,11*S*)-2-hydroxy-11-methyl-5,7,10-rioxatetracyclo[6.3.1.$0^{2,6}$.$0^{9,11}$]dodec-3-en-9-yl]-4-methyl-8-{[(2*E*)-2-methylbut-2-enoyl]oxy}octahydro-1*H*-furo[3',4':4,4a]naphtho[1,8-*bc*]furan- 5,10a(8*H*)-dicarboxylate	Induce resistance in pea (*Pisum sativum* Linn) against *Erysiphe pisi*, barley against *Drechslera graminea* and *Alternaria sesami*
5.	*Jatropha carcass*	Phorbol esters	Aromatic hydrocarbon (Terpenes)	C36H56O8	(1a*R*,1b*S*,4a*R*,7a*S*,7b*S*,8*R*,9*R*,9a*S*)-9a-(acetyloxy)-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-1a,1b,4,4a,5,7a,7b,8,9,9a-decahydro-*H*-cyclopropa[3,4]benzo[1,2-*e*]azulen-9-yl myristate	1% ethanolic solution of crude extracts caused complete inhibition of mycelial growth of *Fusarium* spp., *Pythium aphanidermatum*, *Culvularia lunata*, *Colletotrichum capsici* and *Colletotrichum gloeosporioides*
6.	Benlate	Benomyl	Carbamate fungicide	C14H18N4O3	Methyl l-(butycarbamoyl) benzimidazol-2-ylcarbamate	Benzimidazole group of highly effective broad spectrum systemic fungicide; widely used on Ascomycetes, some Basidiomycetes, and Deuteromycetes

Source: Miron *et al*., 2000 and Wei *et* al., 2011; Terezinha *et al*., 2006; Prabhu *et al*., 2009; Pierangeli *et al*., 2009; Moses *et al*., 2010; Sanjay and Kumar, 2006; Saetae and Suntornsuk, 2010

Disease reduction and yield increase

The following formulae were used to estimate percentage reduction and yield increase over the control according to Sudheendra (2005):

$$\text{Disease reduction (\%)} = \frac{\text{Disease severity in control plot} - \text{Disease severity in treated plot}}{\text{Disease severity in control plot}} x\ 100$$

$$\text{Yield increase (\%)} = \frac{\text{Yield in treated plot} - \text{Yield in untreated control plot}}{\text{Yield in untreated control plot}} x\ 100$$

Growth and yield parameters

Determination of agronomic traits was done using the 10 tagged plants that were used for disease severity score. The parameters measured included:

* Plant height (cm) was measured at 4, 6, 8, 10, 12 weeks after sowing (WAS) and at harvest by measuring the plant from ground level to the tip of each of the 10 tagged plants and the average per height determined.

*Number of branches per plant was determined by counting number of branches at 4, 6, 8, 10, 12 weeks after sowing (WAS) and at harvest on the 10 tagged plants and later averaged per plant.

*Weeks to 50% flowering was achieved by counting total number of plants and number of plants that had flowered per plot at weekly interval to determine when 50% of plants per each plot had flowered.

*Weeks to harvest was estimated when 90 % of the plants in each plot had two thirds of their capsules way up fully matured by turning yellow.

*Number of capsules per plant was achieved by counting number of capsules on each of the 10 tagged plants at harvest and finding average per plant.

TABLE 2. Disease Score for Cercospora Leaf Spot of Sesame

Scale	Disease severity (%)	Resistant Category	Rating	Leaf spot characteristics
1.	0 – 14	Immune (I)	No disease	No trace of infection
2.	14.1 – 29	Highly resistant (HR)	Hypersensitivity	Hypersensitive spot on lower leaves only
3.	29.1 – 43	Resistant (R)	Trace infection	Small lesions on lower leaves only
4.	43.1 – 57	Moderately resistant (MR)	Slight infection	Small lesions on lower and upper leaves and stem
5.	57.1 – 71	Moderately susceptible (MS)	Moderate infection	Advanced lesions[1] on upper and lower leaves, with or without new infections on stem and petiole
6.	71.1 – 86	Susceptible (S)	Severe infection	Advanced lesions on upper and lower leaves, flower, buds, stems and petiole and slight infection of pod[1]
7.	86.1 – 100	Highly susceptible (HS)	Very Severe infection	All features of 6 above with severe infection of pod

Advanced lesion is characterized by a dark to dark-brown spots with a whitish to straw-coloured or perforated entre.Source: Enikuomehin *et al*., 2002

*Number of seeds per capsule was achieved at harvest by randomly selecting twenty capsules from the ten tagged plants per plot and put in small envelops. Seeds were counted from the individually selected and threshed capsules and their means worked out to determine number of seeds per capsule.

*1000-seed weight (g) was estimated by randomly counting five sets of 1000seeds separately and the average weight recorded for each plot.

*Final seed yield (t/ha) was determined as follows:

$$\text{Seed Yield (kgha}^{1}) = \frac{\text{Seed Weight (kg) of net plot}}{\text{Harvested net plot area } (m^2)} x\ 10000m^2$$

Statistical analysis

The data obtained from disease severities and seed yield were subjected to analysis of variance (ANOVA) for RCBD and correlation studies using the generalized linear model (GLM) procedure of SAS Version 9 (SAS, 2005).

RESULTS

Estimation of loss

The relationship between CLS severity and seed yield is presented in Table 3. Results of combined means in the two locations in 2011 and 2012 revealed a reduction in disease severity over control between 11 – 19 % and 12 – 22 %, which led to yield increase ranges of 26 – 57 % and 17 – 45 % in Ardo-kola and Gassol respectively depending on the plant extract applied. Plants treated with Ocimum and Chromolaena, which was at par with Benomyl, gave better reduction of disease severity by 19 and 21 %, and gave significantly highest yield increase of 57 and 45 % than unsprayed plots in Ardo-kola and Gassol locations respectively.

Correlation studies

Tables 4 and 5 revealed highly significant negative correlation between CLS severity and plant height; branches plant^{-1}; capsules plant^{-1}; seeds capsule^{-1}; 1000-seed weight; seed yield plant^{-1}; final seed yield. Furthermore, there was significant positive correlation between final seed yield and plant height; branches plant^{-1}; capsules plant^{-1}; seeds capsule^{-1}; 1000-seed weight.

TABLE 3. Effect of CLS Severity on Seed Yield at 12 weeks after plant extracts Application in 2011 and 2012 at Ardo-kola and Gassol.

	Ardo-kola				Gassol			
Plant extracts	Severity	Reduction (%)	Seed yield (Kg ha^{-1})	Increase (%)	Severity	Reduction (%)	Seed yield (Kg ha^{-1})	Increase (%)
Neem	47.49^{b}	11.35	673.20^{c}	26.22	53.04^{b}	12.32	925.88^{b}	17.49
Jatropha	47.36^{b}	11.59	702.29^{c}	31.67	52.49^{b}	13.22	969.53^{b}	23.03
Garlic	45.06^{c}	15.89	813.07^{ab}	52.44	48.74^{c}	19.42	1085.49^{a}	37.74
Ocimum	43.35^{e}	19.08	839.06^{a}	57.31	48.51^{c}	19.80	1105.24^{a}	40.25
Chromoleana	43.91^{de}	18.03	805.93^{ab}	51.10	47.50^{c}	21.47	1142.78^{a}	44.92
Benlate	44.73^{cd}	16.50	799.48^{b}	49.89	47.27^{c}	21.85	978.92^{b}	24.22
Control	53.57^{a}	-	533.37^{d}		60.49^{a}	-	788.07^{c}	-
Mean	46.50		738.06		51.15		999.42	
CV(%)	2.72		6.20		3.83		8.75	
S.E.	1.27		45.78		1.96		87.40	

Means in the same column followed by the same superscript(s) are not significantly different (0.05) using LSD.

TABLE 4: Mean Correlation Matrix between CLS Severity and Yield Characters of Sesame and Seed Yield in Field Trial at Ardo-kola in 2011 and 2012

	Severity	Plant height	Branches per plant	Weeks to 50 % flowering	Weeks to harvest	Capsules per plant	Seeds per capsule	1000 – seed weight	Seed yield per plant	Final seed yield
Severity	1									
Plant height	-.752**	1								
Branches per plant	-.502**	.623**	1							
Weeks to 50 % flowering	.560**	-.584**	-.290**	1						
Weeks to harvest	.460**	-.561**	-.453**	.905**	1					
Capsules per plant	-.649**	.735**	.726**	-.286**	-.342**	1				
Seeds per capsule	-.718**	.767**	.778**	-.608**	-.703**	.661**	1			
1000 – seed weight	-.675**	.760**	.752**	-.325**	-.386**	.997**	.712**	1		
Seed yield per plant	-.717**	.760**	.618**	-.829**	-.876**	.523**	.877**	.572**	1	
Final seed yield	-.661**	.827**	.761**	-.576**	-.665**	.705**	.885**	.743**	.822**	1

**. Correlation is significant at the 0.01 level (2-tailed).

TABLE 5: Mean Correlation Matrix between CLS Severity and Yield Characters of Sesame and Seed Yield in Field Trial at Gassol in 2011 and 2012

	Severity	Plant height	Branches per plant	Weeks to 50 % flowering	Weeks to harvest	Capsules per plant	Seeds per capsule	1000 – seed weight	Seed yield per plant	Final seed yield
Severity	1									
Plant height	-.734**	1								
Branches per plant	-.637**	.867**	1							
Weeks to 50 % flowering	.439**	-.685**	-.685**	1						
Weeks to harvest	.379**	-.702**	-.757**	.941**	1					
Capsules per plant	-.488**	.546**	.512**	-.257**	-.305**	1				
Seeds per capsule	-.770**	.829**	.848**	-.604**	-.647**	.643**	1			
1000 – seed weight	-.784**	.690**	.561**	-.330**	-.291**	.575**	.699**	1		
Seed yield per plant	-.671**	.829**	.843**	-.767**	-.796**	.513**	.844**	.714**	1	
Final seed yield	-.387**	.656**	.687**	-.592**	-.633**	.393**	.585**	.209*	.474**	1

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

Studies on the estimation of loss indicated that various components of growth parameters most especially plant height, number of branches and final seed yield are the determinants of loss due to CLS disease infection at different severity levels. All these determinants of loss are negatively related to disease incidence and severity. The present study revealed that reduction of disease severity and yield increases over control varied depending on the efficacy of the various plant extracts on severity of the disease. The application of Ocimum and Chromolaena extracts, which is at par with Benomyl, drastically slowed down the rate of epiphytotic process, reduced the severity of the disease by 19 %, and subsequently increased yield by 57 % over unsprayed control with yield of 533 kg ha^{-1}at Ardo-kola. Similarly, the same plant extracts gave 21% reduction in severity and induced significantly better agronomic traits that increased yield by 45 % over the unsprayed control (788 kg ha^{-1}) at Gassol location in 2011 and 2012. Schneider *et al.* (1976) estimated yield loss upto 42 % due to Cercospora leafspot in cowpea and a single application of benomyl gave adequate control of this disease in Nigeria. Hartman *et al.* (1999) also estimated overall yield loss of 15 – 30 % due to Cercospora leaf blight of soybean. There was a highly significant negative correlation between Cercospora leaf spot severity and growth and yield parameters. This was a clear indication that the disease can cause significant reduction in grain yield directly proportional to the severity of infection. This is in conformity with the works of Uwala (1998) who also revealed negative correlation between disease severity and seed yield which indicated the need to control the leaf spot *Cercospora* sp. for enhanced seed yield and seed quality in sesame especially when grown under moist environments in ecologies where the pathogen is endemic.

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