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Research Article

Effects of botanical extracts and antibiotic on incidence and severity of bacterial blight of cotton incited by *Xanthomonas axonopodis* pv. *Malvacearum*

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Abstract

A multilocational field trial was conducted to investigate the effect of some plant extracts *(Bolanite aegyptica, Eucalyptus camaldulensis and Citrus aurantium)* and a synthetic antibiotic (Streptomycin) on the incidence and severity of angular leaf spot on five cotton genotypes namely SAMCOT-8, SAMCOT-9, SAMCOT-10, SAMCOT-11 and SAMCOT-12 in Yola and Jalingo, both located in the northern guinea savannah zone of Nigeria. The experiment was carried out on a split-plot design having three replications with cotton genotypes on the main plots and plant extracts on the sub-plots. Data collected on the incidence and severity of the disease were analysed using the Generalized Linear Model procedure and means separation at 5% level of probability by Duncan Multiple Range Test. Findings of the study revealed that Streptomycin and *Bolanite aegyptiaca* were able to reduce the disease incidence by 18.12% from 58.69% in Yola at 13 WAS. In Jalingo however, *B. aegyptiaca* was found to have reduced the disease severity from 52.12% to 19.69% at 13 WAS. SAMCOT-8 consistently recorded lower incidence and severity means in Yola and Jalingo of 47.96%; 59.12% and 40.82%; 39.70% while SAMCOT-12 recorded higher means of 53.29%; 59.82% and 48.03%; 46.70% respectively.

Introduction

Cotton (*Gossypium hirsutum* L. var. *latifolium* Hutch.) is a widely known among the oldest plant fibers being cultivated in different part of the world, its application not limited to producing natural raw materials for use in the textile industry, but also the application of its products in the food industry such as production of edible oil, pharmaceutical industry for drug formulations and agriculture for animal feed oil. Guo et al., (2003) reported that cotton remains the most sort after fibre discovered in the planet earth. According to him 8000 years after the discovery of its first use there was no other fibre that came close to reproducing all of its required characteristics combined. The present production rate of cotton is approximately 25.10 million tons, produced by virtually more than 60 countries on five continents in the planet earth. China, India, United States, Brazil

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and Pakistan are the best countries producing cotton with high quality and quantity (Thompson P.B, 2012). The plant is a shrub commonly found in the tropical and sub-tropical parts of the world. The great variety of wild cotton species is mostly found in Mexico, then by Australia followed by Africa (Wendel & Grover (2015); Soladoye & Chukwuma (2012)).

Bacterial blight is a disease caused by *Xanthomonas axonopodis* pv. *malvacearum* with great economic importance causing havoc across countries growing cotton all over the world, even though its importance varies from place to place due to the differences in climatic conditions (Partzsch et al., 2019). However, the disease is characterized by attacking plant at different stages of growth and development, affecting the stems, leaves, bracts and bolls, its causes seedling blight, black arm, angular leaf spot and boll lesions among others (Sharlaach et al., 2013). The quantity of cotton yield the world losses due to bacterial blight range between 1% and 27% depending on the cultivar and crop age (Atiq et al., 2014). Looking at the destructive and damaging effect of the bacterial blight disease in the world of cotton production, scientists advocates the use of chemicals in the past in other to manage the disease. The use of chemical as a control of plant diseases is practiced with greater intensity more especially in the economically developed countries, where agriculture is more improved with the aid of technology.

However, Juroszek & Tiedemann (2011) stated if appropriate measure is not taken the abusive uses of fungicides can increase production cost, and also can cost the society and the environment. In view of this therefore, this study was undertaken to evaluate the potential antibacterial properties of three plant extracts alongside a standard synthetic antibiotic on incidence and severity of angular leaf spot of cotton.

Materials and methods

The experiment consisted of five (5) different cotton genotypes namely SAMCOT-8, SAMCOT-9, SAMCOT-10, SAMCOT-11 and SAMCOT-12 allocated to the main plot and five (5) materials comprising of an antibiotic (Streptomycin sulphate), three (3) aqueous plant extracts and distilled water (control) allocated to the sub plots. Plots size of 4 m x 2 m with a plant spacing of 90 cm x 45 cm was maintained in both locations. The aqueous plant extracts and the streptomycin sulphate at 50% concentration were applied lightly with hand on the cotton seeds as dressing chemical. Four (4) the seeds were spread per stand and then thinned to 2 plants after observation. Other agronomic practices such as weeding, insect pest control and fertilizer application were carried out in both locations throughout the experiment.

Medium preparation and pathogen isolation

MacConkey Agar was used in the preparation, 29 g of sample dissolved in 500 ml of distilled water. The mixture was thoroughly shaken and then autoclaved for 25 minutes at 115 °C. The medium was then allowed to cool down and solidify.

Infected leaf samples collected from the experimental plots were thoroughly washed with distilled water in the laboratory. Small leaf samples (about 4 mm^2) from the infected leaves were cut and placed aseptically in sterile Petri dishes which contains the medium, this was incubated at 35 ^{0}C for 48 hours. The growth of the bacterial colonies was observed to obtain pure culture. The pure culture was obtained by streaking a loopful of the suspension on another plate of MacConkey agar in Petri dishes incubated at 35 ^{0}C for 72 hours. Repeated sub-culturing separated single colonies that were most dominant and were preserved on agar slant and kept in a refrigerator until used (Rajput et al., 2017).

Inoculation of pathogen on cotton plants

A pressurized hand sprayer was used to spray the isolated bacterial pathogen suspended in distilled water at a concentration of 10^8 cfu ml^{-1} under the leaves surface of the cotton plants so as to give equal chances of infection to the plants.

Disease assessment

The severity of the parameter was determined by assessing ten (10) tagged plants in each plot at weekly intervals beginning from 7 WAS to 1 3 week after sowing (WAS) of spot size and level of damage on the leaves using the modified scale of 1-6 : 1= no visible symptom of the disease; 2= small pin point reddish brown spots less than 0.5 mm; 3 lesions about 0.5 -1.5 mm in size, wet slightly coalescing; 4 = lesions 1.5 - 3.0 mm in size, slightly angular, sunken and some coalescing; 5 lesions 5 - 7 mm in size, angular with bulging coalescing and senescence of leaves and 6 = lesions 10 mm in size, extensive coalescing, leaves yellowish brown in most cases and some dropping.

The rating scale used for determining resistance based on means of disease severity at 13 WAS is modified scale of Awurum and Emechebe (2001) as below:

Ratings	Mean disease severity
Highly resistant	0%
Resistant	1-10%
Slightly resistant	11-20%
Moderately resistant	21-40%
Susceptible	41-65%
Highly susceptible	above 65%

The formula below was used to estimate percentage disease reduction against the control

$$\text{Disease reduction} = \frac{\text{Incidence in control} - \text{Incidence in treatment}}{\text{Incidence in control}} \text{x100}$$

Data analysis

Analysis of variance was used on the parameters collected using the Generalized Linear Model (GLM) procedure of statistical analysis system which is more suitable for the split plot design. Factorial Randomized block Design was used for means.

Results and discussion

Effects of plant extracts on the incidence of angular leaf spot

Result presented in Table 1 shows a gradual rise in the percentage disease incidence with a significant difference (p<0.05) between varieties at 10 WAS in Yola. SAMCOT-10 was found to have the lowest incidence of 30.37% while SAMCOT-11 recorded the highest incidence of 37.55%. At 13 WAS however, SAMCOT-8 recorded the incidence of 47.96% while SAMCOT-12 had the highest incidence of 53.29%. The result also showed a highly significant difference (p<0.01) between the plant extracts at 7 WAS with Streptomycin recording the lowest incidence of 10.31% followed by *B. aegyptiaca* (12.78%) and E. *camaldulensis* (13.86%). At 13 WAS, Streptomycin and *B. aegyptiaca* recorded the lowest incidence of 48.20%. The control was found to record the highest mean value of 58.69%.

Table 2 showed no significant difference among the varieties in Jalingo, though SAMCOT-8 and SAMCOT-9 recorded the lowest means of 12.14% as against 14.09% observed in SAMCOT-11 at 7 WAS. The result further revealed that SAMCOT-9 has the lowest mean about 57.77%, SAMCOT-11on the other hand recorded the highest mean of 60.13% at 13 WAS.

Furthermore, the result revealed a significant difference (p<0.05) at 8 WAS where Streptomycin gave the lowest incidence mean rating of 13.76% then *E. camaldulensis* (16.11%) and *B. aegyptiaca* (18.59%) the Control was found to record the highest mean of 24.69%. At 13 WAS, Streptomycin recorded the least mean rate of 50.82% then *B. aegyptiaca* (54.82%) and the control recorded the highest mean rate of 68.82% respectively.

Effects of plant extracts on severity of angular leaf spot

The result revealed no significant difference between the varieties in Yola as presented in Table 3. However, SAMCOT-8 recorded the lowest means of 13.88% at 7 WAS and 40.82% at 13 WAS while SAMCOT-12 had the highest with 17.25% and 48.03% respectively. A significant difference was observed between plant extracts where *B. aegyptiaca* had the mean of 15.69% at 7 WAS and 44.69% at 13 WAS the Control was found to have the highest mean rate of 18.58% and 52.37% at 7 and 13 WAS respectively. Streptomycin recorded the lowest means at 7 and 13 WAS with 13.80% and 43.87% respectively.

Table 4 presents Jalingo result with significant difference observed between varieties. Lowest mean value of 19.24% and 39.70% of

21.66% were observed on SAMCOT-8 at 7 and 13 WAS respectively. At 13 WAS however, SAMCOT-12 recorded the highest value of 46.70%. Result between plant extracts revealed a highly significant difference at (p=<0.01) at 9-13 WAS where *E. camaldulensis* recorded a lower mean of 18.70% at 7 WAS and the Control recorded the highest mean of 24.16%. At 13 WAS however, *B. aegyptiaca* recorded 41.86% which is the lowest value while the Control recorded the highest mean of 52.12

Table 1. Mean effects of plant extracts on incidence of angular leaf spot at 7-13 WAS in Yola

Varieties	7 WAS	8 WAS	9 WAS	10 WAS	11 WAS	12 WAS	13 WAS
SAMCOT- 8	14.24[b]	27.94[a]	30.20[a]	33.74[a]	36.90[ab]	45.36[a]	47.96[b]
SAMCOT- 9	17.09[ab]	23.73[bc]	27.89[ab]	32.60[b]	36.82[ab]	41.94[ab]	48.04[b]
SAMCOT -10	16.57[ab]	21.02[c]	26.40[b]	30.37[b]	33.58[b]	40.00[b]	52.49[ab]
SAMCOT -11	16.91[ab]	25.07[ab]	29.29[ab]	37.55[a]	39.06[a]	43.12[ab]	51.80[ab]
SAMCOT -12	18.51[a]	26.61[a]	26.96[ab]	30.56[b]	35.73[ab]	42.61[ab]	53.29[a]
Prob. of F.	0.0840	0.0007	0.1333	0.0002	0.0687	0.2086	0.0613
<u>Plant extracts</u>							
C. aurantium	16,64[b]	25.95[ab]	28.50[b]	34.43[b]	37.61[b]	44.39[b]	54.74[b]
B. aegyptiaca	12.78[cd]	22.30[cd]	26.40[bc]	30.51[c]	34.18[bc]	38.49[c]	48.20[c]
E. camaldulensis	13.96[bc]	24.63[bc]	27.85[bc]	32.49[bc]	36.12[bc]	41.64[bc]	51.46[bc]
S. sulphate	10.31[d]	20.60[d]	24.51[c]	29.23[c]	32.76[c]	38.10[c]	48.20[c]
Control	21.64[a]	28.60[a]	33.85[a]	38.02[a]	41.42[a]	50.40[a]	58.69[a]
Mean	15.06	24.47	28.15	32.97	36.42	42.60	52.26
CV	24.25	16.35	15.99	13.16	13.88	14.26	12.44
Prob. of F.	<u><</u>0.0001	<u><</u>0.0001	<u><</u>0.0001	<u><</u>0.0001	0.0003	<u><</u>0.0001	<u><</u>0.0001
Var. x Trt.	0.9872	0.8907	0.8015	0.9460	0.8626	0.9327	0.9984

Means with the same letter(s) in the same column are not significantly different at p=≥0.05 or p=≤0.01

Table 2. Mean effects of plant extracts on severity of angular leaf spot at 7-13 WAS in Yola

Variety	7 WAS	8 WAS	9 WAS	10 WAS	11 WAS	12 WAS	13 WAS
SAMCOT – 8	13.88[a]	18.78[a]	25.99[a]	29.09[a]	35.28[a]	38.17[a]	40.82[a]
SAMCOT – 9	16.60[a]	19.79[a]	22.27[a]	28.83[a]	32.66[a]	38.72[a]	45.39[a]
SAMCOT -10	16.83[a]	19.56[a]	22.37[a]	28.69[a]	33.79[a]	41.11[a]	47.39[a]
SAMCOT -11	15.69[a]	18.16[a]	21.69[a]	27.83[a]	34.38[a]	41.77[a]	47.89[a]
SAMCOT -12	17.25[a]	19.71[a]	22.86[a]	28.07[a]	33.71[a]	40.65[a]	43.03[a]
Prob. of F.	0.7835	0.6235	0.7938	0.8166	0.6835	0.6716	0.6618
<u>Plant extracts</u>							
C. aurantium	17.57[a]	19.16[ab]	23.16[a]	28.64[ab]	34.13[ab]	41.86[ab]	47.84[b]
B. aegyptiaca	15.69[cb]	19.36[cb]	21.48[ab]	27.26[bc]	31.70[bc]	37.89[bc]	40.69[b]
E. camaldulensis	16.59[ab]	19.21[ab]	21.64[ab]	28.66[ab]	33.14[bc]	40.11[bc]	44.76[b]
S. sulphate	13.80[b]	17.01[b]	19.61[b]	25.04[c]	30.47[c]	36.98[c]	39.87[b]
Control	18.58[a]	21.26[a]	21.28[a]	30.89[a]	37.38[a]	45.57[a]	52.37[a]
Mean	16.45	19.20	22.03	28.10	33.36	40.48	45.11
CV	23.31	17.51	19.48	15.47	13.30	14.50	11.32
Prob. of F.	0.0154	0.0271	0.0497	0.0111	0.0013	0.0033	0.0006
Var. x Trt.	1.0000	0.9404	0.9972	1.0000	0.9990	1.0000	0.9995

Means with the same letter(s) in the same column are not significantly different at p=≥0.05 or p=≤0.01

Table 3. Mean effects of plant extracts on incidence of angular leaf spot at 7-13 WAS in Jalingo

Varieties	7 WAS	8 WAS	9 WAS	10 WAS	11 WAS	12 WAS	13 WAS
SAMCOT -8	12.14[a]	20.28[a]	26.46[a]	33.64[a]	41.21[ab]	50.06[a]	5912 [a]
SAMCOT -9	12.14[a]	18.87[a]	26.30[a]	33.24[a]	41.26[ab]	48.51[a]	57.77 [a]
SAMCOT-10	13.53[a]	18.06[a]	23.86[a]	31.47[a]	38.57[a]	47.78[a]	58.16 [a]
SAMCOT -11	14.09[a]	18.63[a]	24.93[a]	32.14[a]	40.55[ab]	48.46 [a]	60.13 [a]
SAMCOT -12	13.59[a]	19.61[a]	26.86[a]	33.17[a]	42.82[a]	52.34 [a]	59.82 [a]
Prob. of F.	0.9368	0.9094	0.7029	0.7054	0.1992	0.1940	0.5759
Plant extracts							
C. aurantium	14.80[ab]	22.28[ab]	27.31[b]	34.04 [b]	42.32[b]	51.29[b]	61.50[b]
B. aegyptiaca	13.64[abc]	18.59[bc]	22.71[c]	30.36 [bc]	38.42[c]	45.93[cd]	54.41[c]
E. camaldulensis	11.44[bc]	16.11[c]	24.95[bc]	31.94 [bc]	40.76[bc]	49.89[bc]	59.41[b]
S. sulphate	7.87[c]	13.76[c]	20.75[c]	28.43 [c]	34.72[d]	49.89[d]	50.82[d]
Control	17.73[a]	24.69[a]	31.96[a]	39.43 [a]	48.18[a]	42.17[d]	68.82[a]
Mean	13.10	19.09	25.54	32.84	40.88	57.89[a]	59.00
CV	59.70	35.31	22.85	15.85	11.65	49.43	7.83
Prob. of F.	0.0167	0.0003	$\leq$0.0001	$\leq$0.0001	$\leq$0.0001	$\leq$0.0001	$\leq$0.0001
Var. x Trt.	0.9999	1.0000	0.9999	0.9973	0.9786	0.9495	0.9982

Means with the same letter(s) in the same column are not significantly different at p=$\geq$0.05 or p=≤0.01

Table 4. Mean effects of plant extracts on severity of angular leaf spot at 7-13 WAS in Jalingo

Varieties	7 WAS	8 WAS	9 WAS	10 WAS	11 WAS	12 WAS	13 WAS
SAMCOT- 8	19.24[a]	25.76[a]	28.99[a]	31.42[a]	34.94[a]	37.04[a]	39.70[a]
SAMCOT -9	21.66[a]	25.03[a]	28.02[a]	31.31[a]	35.06[a]	40.04[a]	44.89[a]
SAMCOT -10	21.13[a]	25.29[a]	28.95[a]	32.25[a]	35.73[a]	40.60[a]	45.77[a]
SAMCOT -11	20.39[a]	24.59[a]	28.04[a]	30.21[a]	34.68[a]	38.78[a]	43.72[a]
SAMCOT -12	20.50[a]	26.25[a]	29.48[a]	33.12[a]	36.71[a]	41.78[a]	46.70[a]
Prob. of F.	0.7735	0.6891	0.5935	0.2805	0.4593	0.3220	0.3337
Plant extracts							
C. aurantium	22.07[ab]	26.19[ab]	29.13[b]	32.14[b]	35.29[b]	40.31[b]	45.12[b]
B. aegyptiaca	20.38[abc]	23.82[bc]	26.50[bc]	28.87[c]	32.21[b]	37.22[bc]	40.88[b]
E. camaldulensis	18.70[bc]	24.51[bc]	27.70[bc]	30.62[bc]	33.72[b]	38.82[bc]	43.51[b]
S. sulphate	17.61[c]	22.02[c]	24.92[c]	28.30[c]	32.00[b]	36.83[c]	38.86[b]
Control	24.16[a]	28.32[a]	33.24[a]	37.39[a]	42.91[a]	47.02[a]	52.12[a]
Mean	20.58	24.97	28.30	31.46	35.22	40.04	44.10
CV	25.59	18.73	15.63	13.24	12.06	10.64	9.69
Prob. of F.	0.0104	0.0078	$\leq$0.0001	$\leq$0.0001	$\leq$0.0001	$\leq$0.0001	$\leq$0.0001
Var. x Trt.	1.0000	1.0000	0.9999	0.9987	0.9957	0.9218	0.9023

Means with the same letter(s) in the same column are not significantly different at p=$\geq$0.05 or p=≤0.01

Discussion

Bacterial blight incidence and severity and its consequent manifestations especially angular leaf spot may affect photosynthesis and in turn decrease assimilates to the plant (Awurum & Emechebe, 2001). The result of the study shows a gradual progress in the incidence and severity of angular leaf spot at the early stage of the plant growth but progresses sharply towards maturity. This may be related to varying environmental conditions on components of the infection cycle of the disease in both locations which is being enhanced by the application of plant extracts (Marri et al., 2012). It was revealed that SAMCOT-8 consistently maintained low incidence and severity of bacterial blight in both locations which may likely be attributed to the moderate resistance of the variety to the various manifestations of the bacterial blight disease and also to influence of environmental factors (Atungwu et al., 2011). The higher percentage mean on SAMCOT-11 agreed with the report by Marri et al. (2012) on susceptibility of the variety to *Xanthomonas axonopodis* p.v*. malvacearum*. Also, the variation in the percentage incidence may be attributed to the difference in levels of pre-disposing factors of rainfall, temperature and relative humidity prevailing during growth period of the plants which are principal to incidence and severity of the bacterial blight disease as reported by Hutin et al. (2015). The findings from the study also revealed that incidence and severity of the angular leaf spot was suppressed with the use of the plant extracts as dressing materials when compared with the control. This suppressive activity may be attributed to the antimicrobial constituents in these extracts that had the potential to reduce foliage infection. This is supported by Jalloul et al. (2015) who reported that aqueous extracts of *A. sativum* and *A. cepa* applied as foliar spray induces systemic resistance on leaves of cotton to a challenge infection and reduce the number of lesions by up to 73% when compared with water treated control. This gave more ground to the result obtained in the two locations, which revealed *B. aegyptiaca* and *E. camaldulensis* to have proved very effective, close to Streptomycin in reducing the angular leaf spot disease incidence and severity at 7 WAS and 13 WAS when compared with the Control which recorded higher percentage. The result corroborates with the findings of Rajput et al. (2015) who reported the bacterial potential and potency of *D. metel* extract which resulted in better inhibition of *X. axonopodis* pv. *malvacearum* compared to Bronopol. The result also agreed with Opara et al. (2010) who reported the bacterial potential and potency of *C. sinensis, P. guineese and C. citrates* in comparison with standard pesticides in reducing both disease incidence and severity of bacterial leaf blight and at the same time enhancing growth and yield of cocoyam. They concluded that the significant reduction in disease incidence and severity Shown that formulations of plant extracts could have important roles in biologically based strategies for control of diseases caused by *Xanthomonas campestis* pv *dieffenbachiae*. Jayalakshmi et al. (2011) evaluated the antibacterial activity of *Prosopis juliflora* against *X. axonopodis* pv. *malvacearum* and was observed that it inhibited the growth of the pathogen by 71-74%. This work is similar to those reported by earlier workers (Opara and Obani, 2010) which showed that some indigenous plant species could serve as potential antimicrobial agents against bacterial pathogens, indicating that some natural antimicrobial active ingredients are indeed contained in them. Similar observation was also reported by Opara and Obani (2010) on bacterial blight of egg plants. These plants extracts are likely to contain antimicrobial constituents such as alkaloids, flavonaids, saponins, tannins, phenols, terpenoids and glycosides in which their antimicrobial activities may be linked (Soladoye and Chukwunma, 2012).

Conclusion

From the findings of the study, it could be concluded that the plant extracts (*B. aegyptiaca, E. camaldulensis and Citrus aurantium*) and the synthetic antibiotic were able to reduce the incidence and severity of angular leaf spot of cotton compared to the untreated control. The reduction of the incidence and severity of angular leaf spot occurred at a different percentage rate, this means that there is varying levels of active antimicrobial substances**.** Further investigations are therefore suggested with the view of enhancing their effectiveness as in formulation, storage and application.

Author declaration

Authors declare that there is no conflict of interest. BI (Post Graduate Student) conducted field experiments and recorded field observations. HN (Professor of Pathology) conceived the idea and supervised the experiment and wrote the concept and discussion. LYB (Biotechnologist) wrote the draft authenticate the manuscript, carried out the validation and reliability of the research instrument and result, TSG worked on the data analysis and references. AM (Lecturer of Plant Pathology) advised about the laboratory technique and conducted manuscript proofreading before submission. All authors read and approved the final version of the manuscript.

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