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Survey on the Severity of *Xanthomonas Axonopodis* PV Malvacearum and its Management in Adamawa State, Nigeria

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ABSTRACT: *Prevalence survey on bacterial blight disease of cotton were carried out in some cotton growing locations in Adamawa State in 2018 to determine the severity of the disease in the study areas and the best combinations of cotton genotypes and plant extracts in the management of the disease. Results of the survey revealed the presence of the disease with variable percentage severities with Yola South recording the highest severity of 67.03%, followed by Guyuk (58.24%) while the least severity of 35.97% was recorded in Lamurde Local Government Area respectively. Results further showed that the combination of SAMCOT 8 cotton variety with Allium sativum as plant extract (50%) gave the best control of the disease during the study. This study has revealed the presence of the diseases in these locations with varying levels of severities. Further studies of the disease and other diseases in other locations needs to be carried out so as to determine the most effective management strategy.*

KEYWORDS: survey, severity, management, cotton and bacterial blight

INTRODUCTION

Bacterial blight of cotton caused by *Xanthomonas axonopodis* pv. *malvacearum* is the most serious disease which occurs in all cotton growing regions of the world (Nahunnaro, 2007). The disease develop symptoms on cotton plants as seedling blight, angular leafspot, vein blight, black arm and boll rot (Nahunnaro *et al.*, 2007). Free water is required for foliar infection and secondary spread is favoured by high humidity following periods of wind and rain which distribute the bacteria within the crop canopy. Provided the relative humidity is 85 %, the optimum temperature for disease development is around 36^{o}C (Steve, 2004: Tuti *et al.*, 2015. The first symptoms appear on the margins of the veintral side of cotyledons as small water soaked and circular spots. The lesions extend inwards and later the cotyledons are distorted. The pathogen then reaches the stem through petioles, resulting to death of the seedling. This phase of the disease is the most obvious symptoms on all cultivars. The lesion are initially minute, water soaked, and scattered on the under surface of young leaves which increases in size (up to 5mm in diameter), first turn brown then black and become visible on the upper surface of the leaves (Jeevan, 1986). Black arm phase of this disease first appears as lesion infection on petioles and stem in form of elongated, grayish to sooty black

lesions. The stems show deep cracks and are liable to break off during strong wind, causing considerable damage to fruiting branches or complete death of the plant (Aygan and Arkan, 2007). On the bolls, the symptoms first appeared as small round water soaked and raised spots. The lesions gradually become irregular in shape and turn brown-black. Infected bolls open prematurely revealing stained lint, with reduced market value. The pathogen then became seed borne as a result of intra embryonic infections (Verma, 1986; Nahunnaro *et al.,* 2007; Gwary *et al.*, 2009). Yield losses due to bacterial leaf blight have been estimated up to 50 % under field conditions (Watkins, 1981, Nahunnaro, 2007, Kalpana *et al.,* 2004, Mishra and Krishna, 2001 and Sandipan *et al.,* 2016) and also affect the quality of lint (Sharma and Chauhan, 1985).

In Nigeria, the disease is reported in the northern part of the country where environmental conditions are favorable for cotton cultivation (Poswal and Erinle, 1984; Nahunnaro *et al*., 2007; Ogunjobi *et al.,* 2010 and Ajene *et al.*, 2014). At present, quick and effective management of plant diseases and microbial contamination in several agricultural commodities is generally achieved by the use of synthetic pesticides (Shragia, 1975; Agrios, 1997). However, the incessant and indiscriminate application of these chemical pesticides has caused health hazards in animals and humans due to their residual toxicity. Considering the deleterious effects of these pesticides on life supporting system therefore, there is an urgent need for alternative agents which will equally be effective and eco-friendly for the management of plant pathogenic micro-organisms including *Xanthomonas axonopodis* pv *malvacearum*.

It is for this reasons therefore this study was conducted with the objectives to determine the prevalence of the disease in the study area, to evaluate the efficacy of some selected plant materials in the management of *X. axonopodis* pv. *malvacearum* of cotton and to identify the best combination of cotton variety/varieties and plant extract(s) in the management of the pathogen in the study area

MATERIALS AND METHODS

Study Area.

This study was conducted in two phases namely; survey and field trail from June – November 2018. The survey was conducted in three Local Government Areas of Adamawa State (Yola South, Guyuk and Lamurde) that are into cotton production (Figure 1, Table 1) to determine the severity of the disease while the laboratory experiment was conducted at the Microbiology Department and field trial was conducted at the Teaching and Research Farm of Agricultural Technology Department, Federal Polytechnic Mubi. Mubi is located between latitude 10^{o} 11′ N and 9^{o} 26′ N and longitude 13^{o} 1′ and 13^{o} 44′ E (Adebayo and Tukur, 1999).

Field Survey

The field survey was purposive and conducted through the administration of a total of ninety (90)) questionnaires which were distributed (30 per Local Government Area) to cotton farmers/growers. An assessment of severity of the disease was done by four random quadrants (1m x 1m) per farm

according to Eman (2011). Plants were examined at two weeks intervals as from 4 - 12 weeks after sowing (WAS). The percentage severities of the disease obtained from each of the surveyed farms were used to calculate the average severity of the disease for each of the Local Government Area .Disease severity were calculated using the formula:

$$\text{Disease Severity} = \frac{Scores\ of\ individual\ ratings}{Total\ Number\ of\ plants\ assessed\ x\ highest\ score\ in\ the\ ratings} \text{ x } 100 \text{ (Poswal, 1988)}$$

Thereafter, diseased and infected plant parts (leaves and stems) from each of the surveyed farms were collected in clean polyethene bags and taken to the laboratory for further examinations

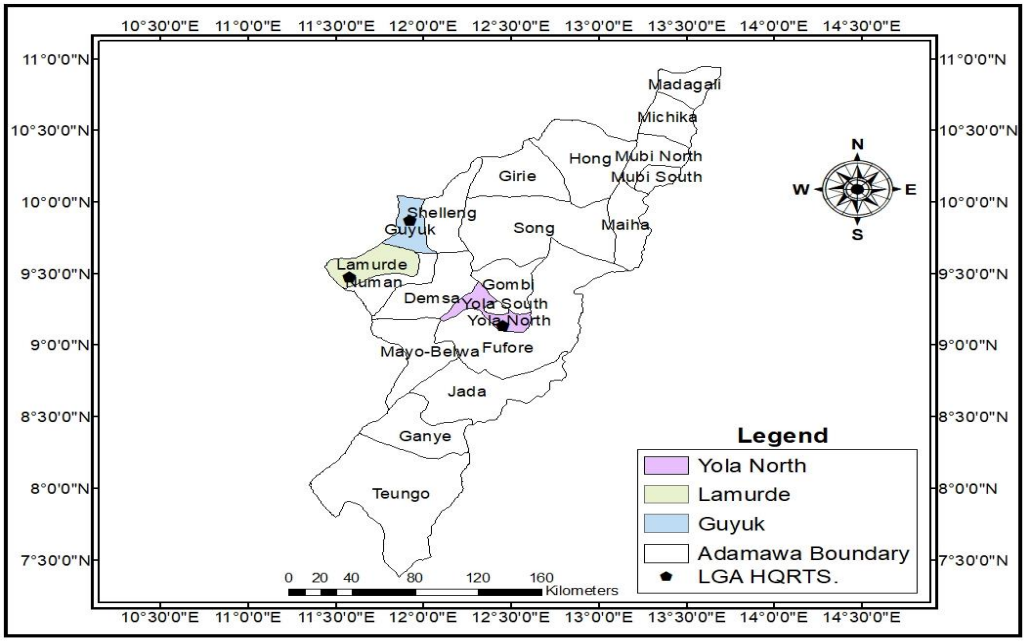

Figure 1: Map of Adamawa State showing the surveyed Local Governments Areas (FMIC 2018).

Table 1: **Location and Number of Farms Surveyed.**

State	Local Govt Area/Location	Number of farms surveyed
Adamawa	**Guyuk**	
	Banjiram	10
	Bobini	10
	Chikila	10
	Lamurde	
	Gyawana	10
	Lafia	10
	Lamurde	10
	Yola South	

Ngurore	10
Hosere	10
Ganigaku	10

Laboratory Experiment

Preparation of Medium and Isolation of *Xanthomonas anoxopodis* pv. *malvacearum*.

Preparation of medium for isolation was done based on procedures described by (Schaad, 2001), gram staining tests were carried out base on methods of Hayward and Waterson, (1964) and confirmed with a KOH test according to the method of Ryu, (1940). Pathogenecity test was performed according to the method described by (Jagtap *et al., 2012*)

Collection and Preparation of Plant Materials

The plant materials; onion bulb (*Allium cepa)* and garlic clove (*A. sativum),* were obtained from Mubi Main Market, while bitter leaf (*Vermonia amagdalina*) and tobacco leaves (*Nicotania tabaccum)* were sourced from surrounding gardens in Mubi. The plant materials were rinsed with 10 % sodium hypochlorite (NaOCl) and air dried. 200g of the respective plant material was pounded into paste using mortar and pestle. Thereafter, 500 ml of distilled water was added to the paste each making suspensions which were allowed to stand for 24 hours after which the contents were filtered using a muslin cloth and kept aseptically in glass bottles for later use in the experiments. The 50% concentration of the various plant extracts were prepared by taking 50 ml of the stock solution and dissolving it in 100 ml of distilled water, while 100 ml of distilled water will be used for the control in the experiment.

Field Trail:

The field trail consisted of five (5) different varieties of cotton (SAMCOT-8, SAMCOT-9, SAMCOT-10, SAMCOT-11 and SAMCOT-12) collected from the Cotton Breeding Section of the Institute of Agricultural Research, Ahmadu Bello University Zaria and four (4) plants extracts (*Allium cepa, A. sativum, Vernonia magdalina, Nicotinia tabaccum* and distilled water which serve as control). The design used for conducting the experiment was split-plot design replicated three (3) times with varieties allocated to the main plots while the plant extracts (treatments) were allocated to the sub-plots. The experimental field measured 29 m x 59 m (1711 m^2) with plots measuring 5 m x 3 m, with alleys of 1m pathway between plots and replicates. All agronomic practices appropriate for cotton production such as land preparation, sowing at recommended rate and distance, weeding, pest control, fertilizer application and harvesting were adapted (Idem, 1999; CDC, 2007).

The isolated bacterial pathogens (10^8cfu/ml) were suspended in distilled water and later sprayed under the leaves surface of the plants at 5 WAS using a pressurized hand sprayer to increase the chance of infection by the pathogen. The inoculations were done in the evening while plants were examined regularly for infection at weekly intervals beginning from 6 WAS and two weeks after

inoculation up to 13 WAS. The plant extracts prepared were applied unto the inoculated plants at 5 WAS and at period of bolls formation in the morning using a knapsack sprayer.

Data collection

Data were collected on Percentage (%) severity of the disease from the surveyed areas and also during field trail. The data collected were analyzed using the Generalized Linear Model (GLM) procedure of Statistical Analysis System (SAS) (1996) appropriate for Randomized Complete Block Design (RCBD) for the survey and Split-Plot Design for the field trail and means separation was carried out using the Least Significant Difference (LSD) for the survey and Duncan Multiple Range Test (DMRT) for the field trail at 5 % level of probability.

RESULTS AND DISCUSSIONS

The results on the morphological characteristics of the various isolates collected showed that the bacterial cells were rod shaped with rounded ends, cells appeared singly and also in pairs, gram negative, capsulated, non-spore forming with, single polar flagellum measuring 0.4 – 0.25 µm 1.25 – 3.00 µm in size. The cells readily stained with common stains such as crystal violet and gentian violet. On YDCA medium, the isolates differed greatly in respect of colony colour where they exhibited pale yellow and light yellow. The colonies appearances revealed either a convex or slightly raised surface and had a highly mucoid texture. Pathogenicity test yielded characteristic symptoms as small, water soaked, brown to black colored lesions, which later on developed into angular to irregular shaped spots along the veins and veinlets of the leaf lamina leading to marginal necrosis. The isolates obtained from the artificially inoculated plants, yielded the bacterial colonies similar to the original ones.

During the survey, results (Figure 2) showed higher significant variations ($P \leq 0.01$) in the presence of the disease in the three (3) Local Governments Areas surveyed with also a progressive increase in the severity of the disease over time. At 4 WAS, results indicated that Lamurde obtained the least severity of 10.15% while the highest of 21.54% was recorded in Guyuk. At 8 WAS, significantly higher differences ($P \leq 0.01$) were observed amongst the Local Government Areas with Lamurde obtaining the lowest severity of 19.84%, while the highest severity of 40.53% at the same period was obtained in Guyuk. Similarly at 12 WAS, significantly higher differences ($P \leq 0.01$) were observed between the locations with Lamurde recording the least severity of 35.97% while the highest disease severity of 58.24% was recorded in Guyuk. Results obtained from these survey generally revealed that nearly all fields or farms studied had significant levels of severity of the disease ranging between 10 -50 % in Yola, 3.33-53.33% in lamurde and 23.33-50% in Guyuk local government.

Weather data in the locations surveyed revealed variations and fluctuations in temperature between the months of July-and September falling between 19.55 ^{o}C and 27.29 ^{o}C, relative humidity fluctuates between 82.39, 86.77 and 81.84% while rainfall was between 7.58 and 15.86 mm which

all falls within the appropriate ranges required by the pathogen for infection. These conducive weather elements might have been responsible for inciting the disease resulting into higher incidence and severity during the study period. This survey is in tandem with Wheeler *et al.* (2007), (Jagtapa et al.(2012) , Nahunnaro *et al.* (2012) and Tuti *et al.* (2015) who reported that the principal factors influencing *Xam* incidence and severity in cotton were rainfall, relative humidity, temperature, solar radiation, quantity of the inoculum and the resistant gene in the genotypes. Also cropping pattern of the farmers in these locations plays a role in the presence and spread of these disease as most of the farmers do not use seed dressing chemicals before sowing and do not adopt close season practices after harvest which might be responsible for the continued presence of the pathogen/inoculums on their fields. This survey agreed with Tiwari *et al.* (2004) who observed that using seed dressing fungicides before sowing reduces incidence of grey leafspot of sorghum by 5-13% and severity by 7%, respectively. They further reported that logistic infection rate was significantly lower in plants grown from seeds treated with appropriate seed dressing fungicides and that seedling establishment and grain yield were increased. Chaube and Singh (2001) also reported that continuous and wide spread annual cultivation of any crop over the season and years without observing close season practices will build up inoculum level to such an extent that the epidemic will become common phenomena.

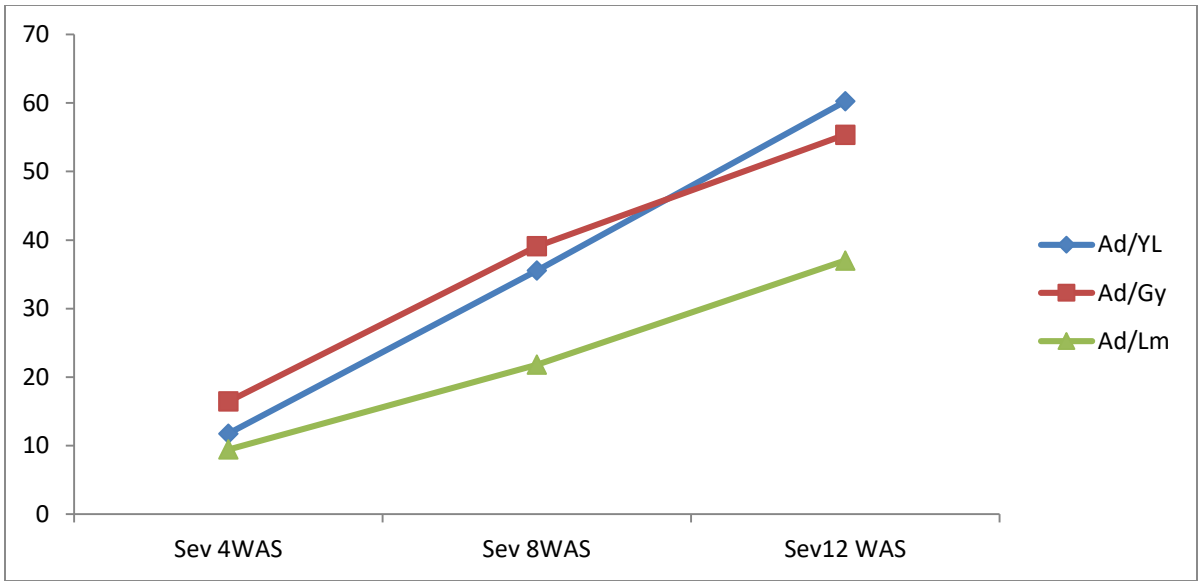

Fig 2: Mean Severity of Angular leafspot disease of cotton from the three (3) Local Government Surveyed

Effects of Varieties and Plant Extracts on Severity of Angular Leafspot at 7 – 13 WAS

The result for the effects of varieties and plant extracts on severity of angular leafspot (Figure 3) revealed a gradual increase in the severity of the disease with highly significant ($P \leq 0.01$) variation from 8 – 13 WAS. The lowest severity of 12.72% was observed on SAMCOT-10 while SAMCOT-12 had the highest severity of 14.20% at 7 WAS. At 13 WAS, SAMCOT-8 recorded the lowest

severity of 40% while 53.62% was observed on SAMCOT-9. The results further revealed highly significant ($P \leq 0.01$) difference among the plant extracts on the severity of the disease. *A. sativum* recorded a lower mean value of 10.13% and 38% at 7 and 13 WAS, followed by *A. cepa* with 11.66% and 40% at the same period with the control having the highest mean values of 20.27% and 70.42% at 7 and 13 WAS respectively.

The inhibitory effects of *A. sativum* and *A. cepa* on angular leaf spot im this study may be attributed to the presence of some fungicidal constituents that had the potential to reduce foliage infection. This finding agreed with that of Satya *et al.* (2007), Bawa and Nahunnaro, 2016 who reported that aqueous extracts from *A. sativum* and *A. cepa* when 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% compared with water treated control plants. They further reported that the treated leaves exhibited significantly high activity of enzymes phenylananine, amino-lyase, peroxidase and polyphenol oxidase along with rapid accumulation of phenolics. This finding indicates that *A. Sativum* extracts can be used as an excellent alternative to chemicals in the control of bacterial blight of cotton as it proves effective as foliar spray with cupravit + streptomycine sulphate which recorded 4.21% reduction in incidence and severity of angular leaf spot as reported by Islam *et al.* (2003).

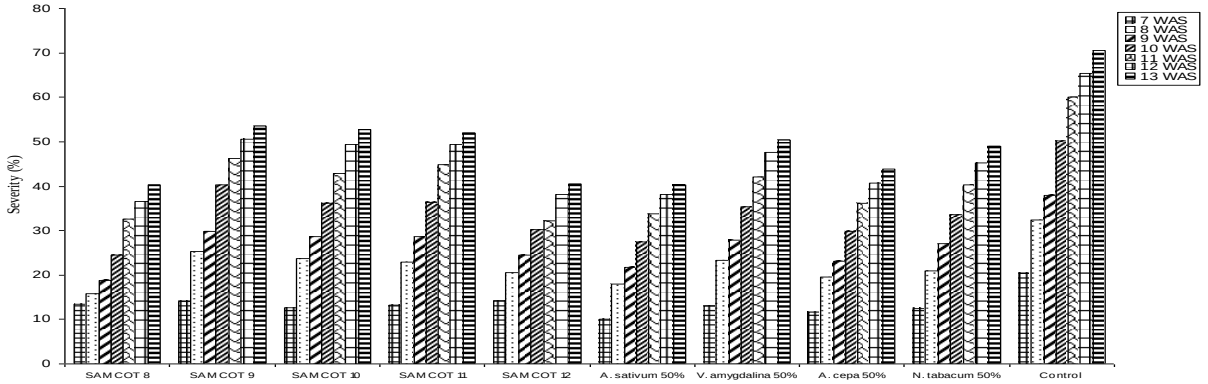

Fig 5: Effects of varieties and plant extract on Severity of angular leaf spot at 7-13 WAS at Mubi

CONCLUSION

This study has revealed the presence of bacterial blight of cotton caused by *Xanthomonas anoxopodis* pv. *malvacearum* in the study area and equally demonstrated its management using the combination of SAMCOT 8 cotton variety and *Alllium sativum* extract at 50% concentration .

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