Scholars Journal of Agriculture and Veterinary Sciences (SJAVS) **e-ISSN 2348–1854**
Abbreviated Key Title: Sch. J. Agric. Vet. Sci. **p-ISSN 2348–8883**

A Unit of Scholars Academic and Scientific Society, India
(An International Publisher for Academic and Scientific Resources)

Inhibitory Effect of Some Plant Extracts in The Control of Bacterial Leaf Spot Pathogen of Okra (*Abelmoschus esculentus* (L.) Moench)

Opara Emma Umunna*[1], Onuoha, Chioma Progress[2]
[1]Department of Plant Health Management,[2]Department of Agronomy, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

Original Research Article

***Corresponding author**
Opara Emma Umunna

Article History
Received: 20.11.2017
Accepted: 26.11.2017
Published: 30.01.2018

DOI:
10.36347/sjavs.2018.v05i01.004

Abstract: This study examined the antibacterial effect of some seed plant extracts including the seeds of pawpaw, water melon, orange and *Moringa* against leaf spot disease of okra (*Abelmoschus esculentus* (L.) Moench) in Umudike, South Eastern Nigeria. It was found that all plant seed extracts tested at the same concentration (20%) were effective to varying degrees in reducing the disease incidence and severity. The best results however, were observed in paw-paw seed extract at concentration of 20% followed by water melon seed extract also the seed extracts contributed positively in promoting plant height and yield and suppressed the disease in the field. There was no significant difference in number of leaves and number of branches after the application of seed extracts. The study therefore recommends the application of seed extracts in order to reduce the action of leaf spot bacteria on okra in the field.
Keywords: Pathogenicity test, inoculum concentration, disease assessment, bacteria suspension.

INTRODUCTION

Okra is one of the most highly consumed vegetables and product food source in Nigeria. However, bacterial diseases such as bacterial wilt and especially bacterial spot have led to major loss in production of this important vegetable crop. The bacterial leaf spot of okra has been reported to be most important foliar diseases causing significant losses in yield and in qualityof okra (*Abelmoschus esculuntus* (L.) Moench) in many parts of the country. Bacterial spot is most commonly caused by *Xanthomonas arboricola* pv. *pruni*, *X. campestris* pv. *pruni* and also by *X. axonopodis*. This disease is also referred to as bacterial shoot hole disease [1]. Bacterial spot occurs on leaves, twigs, and branches and nectarines and fruits. Bacterial leaf spot disease of okra can results to 40-70% crop loss in Nigeria[2].

The bacterium gets entrance into the leaves through the water pores or wounds and progresses to the vascular systems [1]. Young leaves are most susceptible before becoming fully expanded, the leaf symptoms are usually visible 5 to 14 days after infection. Rapid symptom expression is dependent on warm temperatures. The most obvious leaf symptoms of leaf spot of okra include yellowing, chlorotic leaves with angular lesions at the leaf tip, mid-rib, and/or along the leaf margins. Infected leaves frequently experience premature drop [3]. Developing foliar lesions are water-soaked, sometimes grayish colored, and angular in shape, being delimited by the vein-lets of the leaf. Initially, individual lesions are only 1 to 2 mm (pencil point) in size, usually expanding from 2 to 3 mm, but seldom exceeding 5 mm [4]. As lesions age, centers may become dark or purple in color and necrotic but when lesion covers the center a hole may appear and abscission occurs as a result. The very earliest leaves to emerge in spring, on some varieties before bloom, can become infected and serve as secondary sources of inoculum for later emerging leaves and fruits [5].

A combination of copper compounds and fungicides e.g. maneb and mancozeb were used against *X. axonopodis*. However, this measure of control remains ineffective under warm and humid climatic conditions. Consequently, biological control agents such as *Pseudomonas* species and glycinecin a derivative from the bacterial strain *X.* pv. *glycines* were also found to be effective in controlling bacterial spot of tomato [6]. However, plants extracts are the most important sources of control agents ever known for instance ground tobacco (*Nicotinia tabacum*) has been utilized for control of pests of agricultural crops [7]. Pyrethrins (*Chrysanthemium cinerariarium*) and their related synthetics have been commercially developed

and used for pest control. Ever since then several plant-based products such as Ryania (*Ryania speciosa*) sabadilla (*Schoenocaulon officinale*) with its synthetic derivatives as well as several neem-based products. Neem seed contains 18 active principles amongst them being *Azadirachtin* [8]. At present many other botanical pesticides extracted from grape fruit seeds and garlic is also being marketed commercially as pest management chemicals [9].This work aimed at investigating the anti microbial activities of some plants extracts against bacterial leaf spot disease of Okra in Umudike, South Eastern Nigeria.

MATERIALS AND METHODS

Experimental Site

The study was conducted with field soils collected from the Michael Okpara University of Agriculture, Research farms, Umudike which is located on latitude 5^{o} 28′ N and longitude 7^{o} 32′ E, with an elevation of 122m above sea level. The area is characterized by uniform mean temperature ranging from 27^{o}C to 30^{o}C all through the year, with annual rainfall of 2,200 mm per annum. The rainfall pattern is bimodal; a long wet season from April to July is interrupted by a short "August brake". This is followed by another short rainy season from September to October or early November, dry season stretches from early November to March [2].

Sources of the experimental materials

The okra seed was collected from the National Agricultural Seed Council, Federal Ministry of Agriculture and Water Resources Umudike, Abia State. About 1.0kg of each fresh plant material that was used as plant extract was sourced from the local environment and from National Root Crop Research Institute (NRCRI), Umudike.

Soil collection and analysis

Soil samples were collected from a depth (0-15cm), sieved through a 2mm mesh sieve and weighed out into perforated containers and Soil chemical properties analyzed at the Soil laboratory of National Root Crop Research Institute (NRCRI), Umudike. The experiment was conducted using a Randomized Complete Block Design (RCBD) with three (3) replicates.

Preparation of plant extracts

The seed extracts used in the treatments were five; water melon (*Citrullus lanatus)*, pawpaw seed *(Carica papaya)*, orange (*Citrus sinensis*) seed, *Moringa oleifera* seed, macozeb (a positive check) and sterile (a control). Fresh seeds of these plants were washed air dried and grinded into powder. About 200g powder of each plant material was dissolved in 1000ml of sterile water, allowed for 24hours after which it was filtered and the filtrate are used as plant seed extract [10,11] while 5g of mancozeb was dissolved in 1000ml of sterile water. Data on growth and yield parameters were collected every two weeks interval after planting.

STATISTICAL ANALYSIS

All the data collected were subjected to the analysis of variance (ANOVA) using SAS (2009) version. The significant differences between the means were separated by Fisher's Least Significant Difference (LSD) at 5% probability level.

Pathogenicity test and inoculation of seedlings

This was done by injecting 1ml of dissolved bacterial suspension (10^{8}cfu ml^{-1}) into the vein of the leaf of okra plant seedling using the hypodermic syringe and needle at the underside of the leaf. Also, 1mlof sterile water will be injected into the other side of the same leaf as a check and allowed for 12 hours under shade to test the virulence of the bacterial organism.

The okra seedlings were initially inoculated with the prepared bacterial inoculum concentration of 10^{8}cfu ml^{-1} (pre-inoculation) before application of plant extracts. The seedlings were inoculated by spraying the bacterial inoculum in the evening using hand atomizer/hand sprayer. The leaves and emerging shoots were also sprayed until a run-off. The seedlings were covered with moist transparent polythene bag to create a partial humid condition (70-80%) and allowed for 48 hours for the pathogens to incubate as stated by Opara and Obani [1].

Disease assessment and data collection

Severity score was based on scale of 0-6 as follows:

0=leaves without spot
1=1-3 spots on leaves
2=1/5 of the leaves cover with spots
3=1/3 of the leaves cover with spots
4=1/2 of the leaves cover with spots
5=2/3 of the leaves affected
6=the entire leaf area affected/dead

Data on growth and yield parameters were collected at weekly interval after treatment based on: Plant height (cm), stem diameter (cm), number leaves and dry matter weight (g). The disease severity assessment in the field was based on the first four leaves starting from the youngest open foliage of each plant.

Statistical Analysis

All the data were collected subjected to analysis of variance (ANOVA) using SAS 2005 model. The significant differences between the means were separated by Least Significant Difference (LSD) at 5% probability level.

RESULTS AND DISCUSSION

Table 1 shows the plant height, number of leaves, and number of branches and disease incidence

of okra treated with different plant seed extracts. The result of the analysis showed that no significant difference in plant height existed between okra plants treated with water melon seed, pawpaw seed, orange seed and moringa seed and macozeb on the other hand. It was observed from the result that plants treated with pawpaw seed extracts had the highest plant height (10.10cm) while the least plant height was obtained from those treated with orange seed extract (7.30cm) for the growth period. The result of the analysis implies that plant extract used in the control of bacterial leaf spot of okra did not promote plant height beyond what is obtained in the control. The reports of Amadioha and Obi [12] showed high inhibitory effect of plant seed extracts on bacterial control which was attributed to the presence of secondary metabolites of plants used in his study. Pawpaw seed extract showed more remarkable effect on plant height than other plant seed extracts. This may be possibly due to the active compounds found within the seeds of pawpaw that is, glycosides and caricin. Other essential biologically active compounds include alkaloids, carpaine, pseudocarpaine, flavanols, butanoic acid, tannis, linalool, benzlglucosinolate, cis and trans-linalool, terpenoids, alpha-palmitic. Some of these compounds are effective super oxide antioxidants with ability to inhibit microbial growth by reacting with the cell components. Aqueous, n-hexane and ethanol extract of *Carica papaya* leaves was investigated by Enyiukwu and Awurum [3] for antibacterial activity of some bacteria; *S. aureus, B. subtilis, E. coli,* and *Pseudomonas auruginasa* and it was observed that the seed extracts were able to inhibit all the bacteria tested.

The seed extract treatment did not show any significant difference on the number of branches and number of leaves among the treated plants. To measure the disease severity of plants which have been infected by plant bacterial pathogens, a scoring pattern was evolved to measure the Severity of disease and whether to classify the plant to be either susceptible or resistant to the disease is based on the score (%). The scale was designed to determine the percentage of plant disease infection and also to compensate for human error. From Table1, disease incidence was higher in plants treated with mancozeb (20.33%) while the least was obtained from plants treated orange and pawpaw seed extracts at 9.90% and 9.90% respectively.

Table 1: Effect of Plant Extract on Disease Incidence and Growth Parameters of Okra

Treatment	PlantHeight (cm)	No. Of Leaves	No. Of Branches	%Disease Incidence
water melon seed extract	9.52	3.43	4.77	15.43
Carica papaya seed extract	10.10	2.87	4.10	9.90
Citrus sinesis seed extract	7.30	3.23	4.43	9.90
Moringa Oliefera seed extract	7.62	3.33	4.47	14.20
Mancozeb	8.27	3.67	4.90	20.33
Control	8.00	4.00	5.10	17.27
LSD (P=0.05)	**2.69***	**NS**	**NS**	**10.09***

* Significant; **NS=** Not Significant (P=0.05)

Copper based pesticides and foliar sprays cause bacteria to develop resistance, with their slow degradation causing damage to soil and the beneficial microbes present in soil [13]. Therefore, other environmental friendly methods are being encouraged such as plant extracts/oils, since they contain an array of secondary metabolites which are cost effective and an eco-friendly alternative to chemical compounds Plant extracts/oils degrade easily without affecting soil pH and due to the presence of various compounds; it becomes difficult for the pathogen to acquire resistance [6].

The seed extracts influenced bacterial growth either directly by inhibition of bacterial growth or indirectly through activation of plant immune system, which activates production of plant secondary metabolites having antibacterial activity [14]. Therefore, in the present study an attempt has been made to evaluate the effect of various plant seed extracts on the control of bacterial leaf spot of okra. Several medicinal plant's compounds/extracts reported to possess antibacterial potential were discovered through *in vitro* assays but were not evaluated *in vivo* and thus have not been utilized to their fullest potential[5,15].

Many plant extracts have been reported as biopesticides that inhibit spore germination and mycelial growth of pathogenic fungi [12]. These crude extracts were evaluated *in vitro* for activities against germination of the spores and mycelial growth of the

pathogens, and later *in vivo* inhibition of the development and spread of the organisms in actual field conditions. They have been found effective against seed inhabiting (seedborne) pathogens, soil dwelling (soilborne) biotic agents, and nutrient and water uptake impairing organisms such as the wilt-inducing *Fusarium oxysporium* of egg plants and against phyto-fungal pathogens of *Amaranthus*, legumes, tomato, yam and Pawpaw. They have also proven effective for arresting the development and spread of bacteria-induced diseases of vegetables and tuber crops[12,14]. Several plant extracts have been evaluated and found efficacious against pre- and post-emergence damping-off, post-harvest rots and transit-decay inciting pathogens [12,14]. For example, recently phytochemicals from some tropical plants (*Carica papaya* and *Piper guineense*) strongly retarded the germination of spores of *Colletotrichum destructivum* [16]. Enyiukwu and Awurum [3] reported the isolation of *Botrydiplodia theobromae* as the most virulent pathogen amongst other rot fungi from cocoyam corms in Southeast Nigeria and that extracts from *Allium sativum* and *Azadirachta indica* were fungitoxic against them. Similarly, Amadioha, [4] indicated also that 30% strength of extracts of *Alchornea cordifolia* leaves reduced development of rot in mechanically injured and artificially inoculated sweet potato by the same organism to the tune of 46%. In an evaluation in sorghum, *Cymbopogon citratus* (30% strength) completely inhibited the growth of *Colletotrichum graminicola* and *Phoma sorghomi* causing seed and seedling rots in cereal plants.

The use of these natural products for pathogenic disease management is particularly important and necessary in the developing countries like Nigeria where synthetic fungicides are not only unavailable but also as farmers who produce about 98% of food in the country are poorly equipped to handle them making their use uneconomic for resource-poor farmers. Plants extracts are hence suitable for exploitation as potent sources of biopesticides to reduce losses arising from pathogenic attacks on crops and stored products [2]. Gupta *et al.* [17] reported that extracts from *Eucalyptus terticornis* and *A. indica* improved seed germination and seedling vigour by decreasing the pre- and post- emergence mortality and number of seedlings showing symptoms of black mould in infected onion. Greenhouse studies conducted in Southeast Nigeria revealed that *P. guineense* and *C. papaya* extracts inhibited the development and spread of anthracnose caused by *C. destructivum* and the results compared well with a synthetic pesticides. In field trials, Enyiukwu *et al*., [18] reported extracts from *Dennettia tripetala* and *Spondias mombin* comparable in fungi toxic effects of benomyl in combating *Choanephora cucurbitarium* induced wet rot of *Amaranthus* vegetable.

CONCLUSION

The study examined the effect of plant seed extract on the control of bacterial leaf spot of okra in the study area. The study demonstrated that seed extracts of water melon, orange, and moringa contained antibacterial compounds which can be utilized in preparation of a potential phyto-bactericide to control the pathogenic bacteria that causes leaf spot of okra. It was apparent that the use of raw seed extracts of the three plants has a potential to substitute the chemical approach of controlling the disease based on the result of the study mancozeb performed at par with seed extracts.

This kind of biological approach would be economically viable and environmentally friendly. The plants are also available to all farmers including those that do not have access to other chemicals. The study did not ascertain chemical compounds that were responsible for the antibacterial activity. The research further recommends split application method to increase the efficacy of action and further research to indentify the active compounds against the leaf spot bacteria. The present study therefore investigated the influence of different plant seed extracts as compared to a chemical pesticide in the control of bacterial leaf spot diseases of okra and established the usefulness of these seed extracts over chemical applications

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