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Performance of Some Plant Extracts and Pesticides in the Control of Bacterial Spot Diseases of *Solanum*

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Abstract: Pot trials were conducted at Michael Okpara University of Agriculture Umudike, Abia State, Nigeria to investigate the performance of four plant extracts (*Azadirachta indica*, *Garcinia kola*, *Zingiber officinale* and *Allium sativum*) and three synthetic pesticides (furadan, benomyl and streptomycin sulphate) for the control of bacterial leaf spot of two varieties of *Solanum* (*S. gilo* and *S. torvum*). The experiment was laid out in a Completely Randomized Design (CRD) and replicated three times. The results showed that *A. indica* and *Z. officinale* at 5 g L^{-1} were as good as the synthetic pesticides such as benomyl (0.1 g L^{-1}) in reducing bacterial leaf spot disease severity of the two varieties; *S. gilo* (2.52) and *S. torvum* (3.48) in contrast with the control experiment, water (4.94-5.52). In this study, some plant extracts can serve as alternatives to the synthetic pesticides in the control of bacterial leaf spot disease of *Solanum* without any adverse effect on crop yield and yield parameters. The use of plant extracts therefore can be recommended to farmers considering their environmentally friendliness and availability.

Key words: Plant extracts, synthetic pesticides, *Solanum*, bacterial leaf spot, inoculum

INTRODUCTION

Bacterial leaf spot of *Solanum* incited by the bacterium *Xanthomonas campestris* pv. *vesicatoria* has resulted to 40-70% crop loss in Nigeria (Valencia, 1997). The bacterium gets entrance into the leaves through the water pores or wounds and progress to the vascular systems. Veins are blackened with leaf tissue browning in a v-shape (Singh, 1998; Romain and Raemakers, 2001). It has a wide range of hosts especially of solanaceaeous plants including tomato and pepper. This disease is a direct effect of toxins or tabtoxin produced by the bacterium. The bacteria persist for a few months in crop refuse and on seed and enter through stomatal cavities (Nardozzi and Kopiski, 2003).

Control of bacterial leaf spot is usually by application of artificial fungicides and bactericides available in the markets however, nature supplies a reasonable number of plant products that have useful properties for crop protection, which are often neglected in favour of commercial products (Reddy *et al*., 2009). These natural substances have useful properties: they are quite specific and cause little disturbance to the natural balance between living organisms. They are cheap and can be produced by farmers from local sources. They are often harmless to humans and animals and are rarely toxic to plants when compared with artificial fungicides (Amadioha, 2004; Opara and Wokocha, 2008).

Azadirachta indica (neem tree) contains substances like terpenoids, *nimbin, azadirone, azadirachtin* etc. and these substances are useful in anti microbial activities. The *azadirachtin* can be extracted from the fruits and the leaves. It is also an insect repellant and can retard insect reproduction (Rembold, 1989; Jotwani and Sircar, 1993; Bankole, 1997; Stoll, 2000; Romain and Raemakers, 2001). *Allium sativum* (garlic) contains a significant antibiotic compound called *allicin*. It is effective against a broad range of bacteria species at dilutions of 1:10 and most bacteria are shown to be susceptible to *allicin*. Fresh garlic extracts have also been found to be effective against many fungal species and have been used to protect plants and stored foods (Stoll, 1998).

Zingiber officinale (ginger) contains gingerols and polyphenol compounds (antioxidants) which have many medicinal properties, e.g., the rhizome is effective against many diseases that affect cultivated pest Crops (Stoilova *et al*., 2007). Also, plant species like *Piper*, *Xylopia*, *Gongronema*, *Latifolium*, *Citrus*, *Bryophyllum*, *Pinnatum*, *Vernonia amygdalina*, *Chrysanthemum* and *Ocimum* have been reported to be promising species as

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crop protection (Jacobson, 1989; Stoll, 1998, 2000; Okonkwo, 2001; Opara and Wokocha, 2008). In this investigation therefore, the performance of various plant extracts and synthetic pesticides on bacterial leaf spot of *Solanum* were carried out.

MATERIALS AND METHODS

Soil preparation and sterilization: Top moist soil was collected from the farm nursery site of Michael Okpara University of Agriculture. The soil was well-drained sandy loam and was mixed with poultry dropping to improve its organic matter content for good growth and fine river sand to improve the porosity at a ratio of 3:2:1, respectively. The soil mixture was moistened and then put into a cut drum, covered and heated until it reached a temperature of 80°C and maintained at this temperature for to 20 min and then allowed to cool down before transferring into plastic pots of about 20 cm diameter.

Nursery preparation and transplanting: The pots were watered for 7 days before sowing the seeds of *Solanum* the fruit (*S. gilo*) and the leafy (*S. torvum*) garden egg obtained from the National Institute for Horticultural Research (NIHORT) Okigwe. The seeds were first sown in the nursery pots which were kept in the nursery and watered twice daily before transplanting into the experimental pots. The pots were covered with mulching materials to reduce too much heat and to conserve soil moisture. Four days after germination the mulch materials were removed to prevent damping off and death of seedling (Greensil, 1968).

The experiment was arranged in a Completely Randomized Design (CRD) with three replicates and eight treatments. The *Solanum* seedlings were transplanted into all the experimental pots two per pot, which was later thinned down to one plant per pot after establishing. Before transplanting the pots were well watered and also after transplanting and the experimental pots were weeded by handpicking the weeds regularly.

Preparation of plant extracts: Four plants which were tested are: neem seed (*Azadirachta indica*), bitter kola (*Garcinia kola*), ginger (*Zingiber officinale*) and garlic (*Allium sativum*). About 1.0 kg of each fresh plant material was sourced from the local environment and National Root Crop Research Institute (NRCRI), Umudike. The plant materials were washed and sun dried before being grinded into powder. About 50 g powder of each plant material was dissolved in 1000 mL of sterile water, allowed for 24 h and filtered with filter paper (White man) and the filtrate used as the plant extract (Stoll, 1998, 2000; Anonymous, 2000). Also, the synthetic pesticides which include furadan, benomyl and streptomycin sulphate, were used at recommended rate by dissolving 5 g of the products in 1000 mL of sterile water.

Preparation of culture media and bacterial inoculum: The media used for isolating of bacteria was nutrient agar, prepared by weighing out 7 g of nutrient agar powder into a conical flask and was dissolved with 250 mL of sterile water. The mixture was thoroughly shaken and melted over hot plate and then autoclaved at 120°C for 15I bs/pressure for 30 min. This was followed by the dispensation of the medium about 15 mL into twelve petri dishes which was allowed to cooled down and solidify, before the culture plates were kept in an incubator at 28-30°C for 24 h.

Infected leaves collected from the field were washed with sterile water and sterilized in 70% absolute alcohol and then crushed in a drop of water and allowed to stay for 30 min to enable the bacteria to multiply.

The bacterial suspension formed was streaked on Nutrient Agar (NA) in petri dishes using a flamed wire loop and incubated for 48 h at 30°C, after which the pure culture was dissolved in sterile water using the serial dilution method to obtain a concentration of 10^8 cfu mL^{-1}.

The inoculation of the bacterial suspension onto the nutrient agar was then carried out and kept in an inoculation chamber in the laboratory at 28°C for 48 h. Prior to culturing, the inoculation chamber was mobbed with 70% absolute alcohol to prevent contamination. The inoculated media were kept in the chamber upside down for 8 h to enhance drying of the surface agar in the petri dishes.

Pathogenicity test and inoculation of seedlings: This was done by injecting 1 mL of dissolved bacterial suspension (10^8 cfu mL^{-1}) into the vein of the leaf of eggplant seedling using the hypodermic syringe and needle at the underside of the leaf. Also, 1 mL of sterile water was injected into the other side of the same leaf and allowed for 12 h under shade to test the virulence of the bacterial organism.

Also inoculation test was conducted using *Solanum* seedlings 2 weeks old in plastic pots arranged in Completely Randomized Design (CRD) replicated three times and this gave a total of seven treatments and a control experiment as follows: i.e., neem *Azadirachta indica* seed, ginger (*Zingiber officinale*), bitter kola (*Garcinia kola*), garlic (*Allium sativum*), furadan, benomyl, streptomycin sulphate and sterile water as the control.

The *Solanum* seedlings were inoculated with the prepared bacterial inoculum of a concentration 10^8 cful mL^{-1} before the application of plant extracts. The seedlings were inoculated by spraying the bacterial inoculum in the evening using a hand atomizer/hand sprayer. The leaves and emerging shoots were also sprayed until there was a run-off. The seedling were covered with transparent polythene bag to create a humid condition and allowed 48 h for the inoculum to incubate as stated by Jones *et al*. (1981).

Disease assessment and data collection: Severity score was based on the scale of 0-6 as follows:

0 = Leaves without spot
1 = 1-3 spots on leaves
2 = $^1/_5$ of the leaves covered with spots
3 = $^1/_3$ of the leaves covered with spots
4 = $^1/_2$ of the leaves covered with spots
5 = $^2/_3$ of the leaves affected
6 = The entire leaf area affected

Data on growth and yield parameters were collected from one week after transplanting based on: plant height (cm), stem diameter (cm), number of 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. All data collected were statistically analyzed at probability of 5% significance (p = 0.05).

RESULTS AND DISCUSSION

The results of the data analysis are presented in Table 1, which showed that for the control of the leaf spot in the variety *S. gilo* (fruit garden egg), *A. indica* (2.82) gave the best reduction of the disease severity among the four plant extracts tested and was as good as the synthetic pesticides like streptomycin (1.48) and benomyl (1.93) at p = 0.05. While for the variety *S. torvum* (the leafy garden egg), *Z. officinale* was the most effective in controlling the disease severity when compared with other three extracts and proved more superior than the control (sterile water). The results from this study showed that the plant extracts have bactericidal effect just like the synthetic pesticides. The bactericidal effects of the plant extracts on the leaf spot disease of *Solanum* are in agreement with the documentations of Stoll (1998) and Amelio (1999) which showed that they contain significant antibiotics and some medicinal properties. The effectiveness of these plant extracts could also be attributed to the bioactivity of the constituents of the plant materials (Bankole, 1997).

The data also showed that in terms of mean number of leaves *A. indica* (20.67 and 54.56 for *S. gilo* and *S. torvum*, respectively) just like streptomycin (16.78 for *S. gilo* and 51.11 for *S. torvum*) gave the highest mean number of leaves along with other synthetic pesticides. Other growth parameters analyzed included stem diameter, plant height and number of branches. In the case of stem diameter all the extracts tested performed as good as the synthetic pesticides without significant differences (p = 0.05). While in terms of plant height it was observed that *S. indica* (54.24 and 64.53 for *S. gilo* and *S. torvum* respectively) was the only extract that was at par with synthetic pesticides however, although other plant extracts still performed better than sterile water, the control experiment (p = 0.05). For number of branches *S. indica* and *Z. officinale* were in the same rank with the synthetic pesticides while others still proved superior when compared with the control (water).

The results also showed that in terms of average fruit yield *Z. officinale* and *A. indica* gave high yield (4.32-4.45 g) for the two *Solanum* varieties which was statistically the same (p = 0.05) as plants treated with synthetic pesticides (4.23-4.52 g) as shown in Fig. 1. In this investigation only *G. kola* did not improve fruit yield (p = 0.05) when compared with the control experiment (water).

Table 1: Effect of plant extracts and synthetic pesticides on growth and yield parameters of the two varieties of *Solanum*

	Disease severity		No. of leaf		Stem Diameter (cm)		Plant Ht (cm)		No. of Branches	
Treatments*	*S. gilo*	*S. torvum*	*S. gilo*	*S. torvum*	*S. gilo*	*S. torvum*	*S. gilo*	*S. torvum*	*S. gilo*	*S. torvum*
A. indica	2.52^{b}	3.48^{c}	11.67^{c}	54.88^{a}	0.81^{ab}	0.93^{ab}	54.24^{ab}	64.53^{ab}	4.44^{a}	13.00^{a}
A. sativum	3.82^{ab}	3.89^{bc}	20.67^{a}	57.56^{a}	0.87^{ab}	0.94^{ab}	40.89^{cd}	58.00^{c}	1.78^{cd}	8.78^{cd}
Z. officinale	2.56^{b}	3.18^{c}	11.56^{c}	44.33^{bc}	0.80^{ab}	0.88^{bc}	47.20^{bc}	54.00^{d}	2.67^{bc}	11.89^{ab}
G. kola	4.41^{ab}	4.22^{ab}	7.89^{d}	46.78^{b}	0. 76^{ab}	1.01^{a}	46.64^{c}	57.90^{cd}	1.22^{d}	11.56^{ab}
Furadan	1.96^{b}	3.44^{C}	11.89^{c}	47.11^{c}	0.91^{ab}	1.01^{a}	54.56^{a}	60.41^{bc}	1.78^{cd}	11.00^{ab}
Benomyl	1.93^{b}	3.19^{c}	10.11^{cd}	22.78^{d}	0.94^{a}	0.98^{ab}	54.60^{a}	67.12^{a}	1.33^{c}	9.89^{bc}
Streptomycin	1.48^{b}	2.11^{c}	16.78^{b}	51.11^{ab}	0.86^{ab}	1.00^{ab}	33.16^{e}	58.47^{bcd}	3.33^{b}	11.44^{ab}
Water	5.52^{a}	4.94^{a}	9.89^{cd}	37.89^{c}	0.51^{c}	0.80^{c}	37.61^{de}	62.27^{abc}	1.00^{d}	7.33^{d}

*Column means with same letters are not significantly different (p = 0.05)

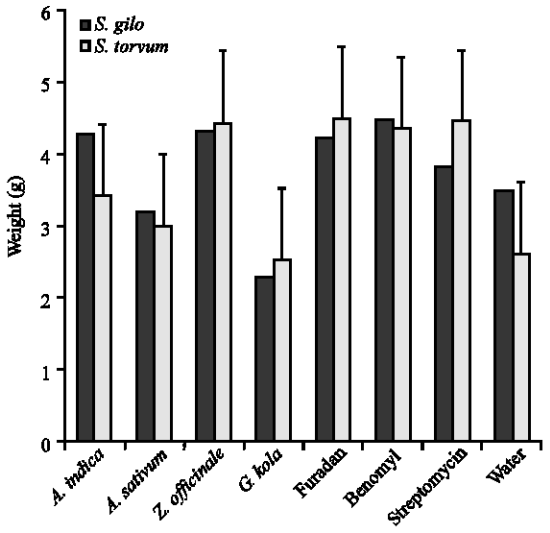

Fig. 1: Effect of plant extracts and synthetic pesticides on fruit yield of the two varieties of *Solanum*

The poor performance recorded by the control experiment (water) in the yield and growth parameters when compared with the treated experiments (plant extracts and synthetic pesticides) was as attributed to reduced photosynthetic surface of the leaves of the affected crops caused by the infection of bacterial spot disease pathogen growing on the leaves as reported by Reddy *et al*. (2009).

CONCLUSION

This study reveals that bacterial leaf spot of *Solanum* can be completely inhibited with the plant extracts (e.g., *A. indica* or *Z. officinale*) in same manner as the synthetic pesticides (benomyl), however, plant extracts are preferable because of their non toxic activities and their environmental friendliness (Reddy *et al*., 2009). The inhibitory activity of plant extracts is likely due to antimicrobial components in plant extracts.

Therefore, there arises the need to adopt cheaper and environmental friendly control measures which are available to the resource poor farmers which helps to enhance crop production and at the same time reduce bacterial leaf spot disease.

Therefore, there is a need to explore the potential usage of these antimicrobial compounds to control plant bacterial diseases as reported by some earlier workers in different crops (Singh, 1998; Amadioha, 2004).

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