# EVALUATION OF PROPHYLACTIC APPLICATION OF ACIBENZOLAR-S-METHYL FOR SYSTEMIC ACQUIRED RESISTANCE AGAINST BACTERIAL SPOT OF TOMATO

**Ganiyu, S. A.[*1], Agbolade, J. O.[2], Popoola, A. R.[3], Imonmion, J. E.[3], Uzoemeka, I. P. [3], Edun, O. O. and Akinfisoye, B. E.[3]**

[1]Department of Agronomy, Federal University of Kashere, P.M.B. 0182, Gombe State, Nigeria

[2]Department of Plant Science and Biotechnology, Federal University, Oye-Ekiti, Ekiti State, Nigeria

[3]Department of Crop Protection, Federal University of Agriculture, Abeokuta, P.M.B. 2240, Ogun State, Nigeria. *Author for correspondence: ganiyu.sikiru@yahoo.com; +2348136392050

## SUMMARY

**One of the synthetic compounds of plant origin that activates plant resistance is acibenzolar-s-methyl (ASM). A preliminary prophylactic measure against bacterial spot disease of tomato was carried out through foliar spray of ASM before transplanting at 25 and 50 ppm concentrations on Kerewa, NG/DE/MAR/01/019 and UTC-18 tomato genotypes while untreated tomato plants served as control. Results showed that the highest disease incidence (95.62%) was recorded in untreated NG/DE/MAR/01/019 which was significantly higher ($p \leq 0.05$) than 10.01%, incidence observed in UTC-18 at 50 ppm. Disease severity of 0.33 observed in Kerewa at 50 ppm was significantly lower ($p \leq 0.05$) than 4.93 severity recorded in NG/DE/MAR/01/019 in plots without ASM application. Kerewa treated with 50 ppm concentration yielded 64.16 g/plant which was significantly higher ($p \leq 0.05$) than 12.31 g/plant yields obtained from in plots without ASM application. The study revealed the promising prophylactic foliar application of ASM at 50 ppm which could be an effective measure for tomato growers against yield loss caused by bacterial spot disease of tomato.**

**Keywords:** Bacterial spot, incidence, plant activator, prophylactic, severity, tomato.

**TOMATO** is one of the most important edible and nutritious vegetable crops, widely cultivated in tropical, sub-tropical and temperate climates of the world. It is consumed fresh or as processed products such as canned tomato, sauce, juice ketchup, stews and soup (9). Consumption of tomato on daily basis has a factor of encouragement for tomato farmers in Nigeria to always grow it every season. In fact, studies have shown that consumption of raw tomato and tomato- based products is associated with a reduced risk of cancer and cardiovascular diseases (3, 4).

However, tomato is being attacked by pests and diseases (11) which consequently lead to significant yield loss annually worldwide (5) and this bottleneck may require integrated pest and disease management options (18). The disease could cause economic losses of yield up to 50% (6) while Jibrin *et al.* (7) affirmed that the disease, bacterial spot, in Nigeria is common. It first appears on the leaves as dark water-soaked circular spot (17) of which lesions are usually less than 3 mm in diameter, later become angular, turning brown to black which eventually become dry and fall from stem (19).

Though, bacterial diseases are managed with synthetic copper based chemical compounds, management is generally inadequate due to prevalence copper-resistant strains and weather conditions that often favour bacterial diseases development in the field (17). The use of chemicals is also harmful to both plants and human beings (13). Some resistant tomato cultivars are available, but they generally unacceptable by growers and commercial tomato industries due to poor horticultural quality, for example small fruits, and the varying levels of disease resistance in different locations. Acibenzolar-s-methyl (ASM) has been used to manage bacterial spot disease but all these management strategies applied ASM while tomato was on the field. Obradovic *et al.* (14) observed that field application of ASM was not as effective for controlling bacterial spot. Huang et al. (6) reported that biweekly applications of ASM did not significantly reduce disease development or the final disease severity of bacterial spot compared with the copper-mancozeb standard or the untreated control. They stressed that optimizing the application frequency and rate of ASM might improve the overall effectiveness of ASM when used alone or in combination with other strategies to manage bacterial spot of tomato. Obradovic *et al.* (15) had also indicated that ASM induced hypersensitive reaction (HR) of tomato plant against *X. campestris* pv. *vesicatoria* by preventing the infection process. Therefore, actions should be directed towards helping farmers, small scale farmers inclusive, in identifying and overcoming constraints that reduce the production of tomato (20). Due to its resistance to bactericides, bacteria leaf spot caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) is a serious threat to tomato farmers (2) and the use of induced resistance in the plants would be a promising environment-friendly strategy for controlling plant diseases (1). Thus, the experiment was out to evaluate the effect of preventive application of acibenzolar-s-methyl (ASM) on growth, incidence and severity of bacterial spot of tomato.

## MATERIALS AND METHODS

### Experimental site and design

This experiment was carried out in the year 2016, at the FUNAAB-TETFund Research Field and Tissue Culture Laboratory of the Department of Crop Protection, College of Plant Science and Crop Production of the Federal University of Agriculture, Abeokuta (FUNAAB). Experiment was laid out in Randomized Complete Block Design (RCBD) with three replicates.

### Source of seeds and treatments

Seeds of three susceptible tomato genotypes (Kerewa**,** NG/DE/MAR/01/019 and UTC-18), to bacterial spot, from our previous experiment were collected from tomato germplasm collection Centre in Tissue Culture Laboratory, Department of Crop Protection, FUNAAB. The treatments consisted of the three tomato genotypes and acibenzolar-s-methyl with two concentration levels (25 and 50 ppm) while the untreated control transplants received no acibenzolar-s-methyl (0 ppm).

### Isolation and pathogenicity test of *Xanthomonas campestris pv. vesicatoria*

Isolation was carried in the laboratory from infected tomato leaf samples with bacterial spot. Tomato leaf samples infected with bacterial spot from previous experimental field were cut into pieces using a sterile scalpel, surface sterilized with 1% NaOCl and rinsed in three changes of sterile distilled water. Segments from diseased tomato plant parts were placed on nutrient agar in Petri dish. Isolates from nutrient agar were sub-cultured on yeast dextrose carbonate (YDC) agar and incubated at 28 °C for 48 hrs. Purified culture of *Xanthomonas campestris* pv. *vesicatoria* was grown on nutrient agar for 24 h at 28 °C. Inoculum concentration ($10^8$ cfu/ml) was prepared from 24 hr-old bacterial culture through serial dilution. For pathogenicity, on 3-week-old tomato

Kerewa cultivar, bacterial suspension ($1 \times 10^8$ cfu/ml) was sprayed onto three plants of each tomato genotype till run-off. Control plants were sprayed with sterile distilled water. Plants were maintained in a growth chamber at 27-28 °C and relative humidity of 85-90%. Inoculated plants were immediately enclosed in polyethylene bags. Symptom was monitored daily.

**Pre-nursery and nursery treatments**

Sandy-loam soil was steam-sterilized at 120°C for 30 minutes. The sterilized soil was kept inside polythene bags for one week before use. Clean and undamaged tomato seeds were sorted by hand and were surface sterilised with 1% NaOCl for 5 minutes, rinsed in three changes of sterile distilled water and air-dried at room temperature. Seeds were sown in nursery trays containing sterilized soil. Tomato seedlings were watered when necessary using hand-held knapsack sprayer.

**Induction of systemic acquired resistance against Bacterial spot disease**

Each experimental plot was 3 m x 2 m in dimension and consisted of 30 plants per plot with inter and intra row spacing of 60 cm by 50 cm respectively. At fourth week in the nursery, before transplanting, acibenzolar-s-methyl (ASM) of 25 and 50 ppm concentrations were sprayed on tomato seedlings till run-off using hand-held knapsack sprayer. This was done three days before inoculation with *Xanthomonas campestris* pv. *vesicatoria* and kept asdone in pathogenicity study. Control seedlings received no acibenzolar-s-methyl. At the end of fourth week, seedlings were transplanted to the field.

**Data collection and analysis**

Data were collected on disease incidence and disease severity while agronomic data included plant height (cm), number of leaves/plant, number of branches/plant and tomato fruit yield (g/plant). Disease incidence was calculated as the percentage of infected plant while severity was evaluated on 0-5 scale according to Popoola et al. (17) after modification in order to accommodate decimal values; where 0= no symptom; 1=0.01-20% foliage affected; 2=20.01-40% foliage affected; 3=40.01-60% foliage affected; 4=60.01-80% foliage affected; 5=80.01-100% affected. Data were subjected to Analysis of Variance (ANOVA) and means separated by the Duncan's Multiple Range Test ($p \leq 0.05$) using SAS 9.1 for windows.

## RESULTS

### Isolation and pathogenicity test of *Xanthomonas campestris* pv. *vesicatoria*

Colonies appeared large, yellow, smooth-domed and mucoid-fluidal on YDC medium. The two purified isolates were pathogenic on tomato plant and formed water-soak lesions on leaves 48 - 96 hrs after inoculation while plants sprayed with sterile distilled water showed no symptom.

### Induction of systemic acquired resistance against Bacterial spot disease

The highest plant height (78.26 cm) was recorded in Kerewa treated with 25 ppm concentration, followed by52.05 cm in untreated NG/DE/MAR/01/019 which was not different from 50.73 cm and 47.17 cm observed in untreated Kerewa and UTC-18 respectively (Table 1). Variations in growth parameters might not be due to the treatment but largely to be genetic variability of the tomato used. Number of leaves/plant also varied considerably to some extent, Kerewa at 25 ppm concentration recorded highest mean value of number of leaves/plant of 188.67 which was significantly different from other tomato genotypes in control and treated plots except untreated plot of the same Kerewa which had 96.67 number of leaves/plant. Number of branches/ plant also varied with Kerewa taken the lead with 9.00 number of branches when 25 ppm was applied and this was significantly different from the least value (2.66) recorded in UTC-18 when 25 ppm acibenzolar-s- methyl concentration was applied. In all parameters measured, the least performance was observed in UTC-18 genotype.

Table 2 shows the response of tomato genotypes to disease incidence and severity as well as fruit yield. Disease incidence ranged from 10.01 to 95.62%. Highest disease incidence (95.62%) was recorded in untreated control plot that contained NG/DE/MAR/01/019 followed by 85.67% in control plot contained Kerewa. They were not significantly different ($p \geq 0.05$) from each other but were both significantly different ($p \leq 0.05$) from disease incidence observed in various concentrations. The least incidence (10.01%) was found in UTC-18 when acibenzolar-s- methyl was applied at 50 ppm concentration, though, not significantly different ($p \geq 0.05$) from 10.25% and 20.45% disease incidence at the same concentration (50 ppm) recorded in Kerewa and NG/DE/MAR/01/019 genotypes respectively. Disease severity ranged from 0.33 to 4.93. The highest severity was found in NG/DE/MAR/01/019 in untreated plot, followed by 4.90 in plots that contained Kerewa with no application of acibenzolar-s- methyl. At 50 ppm, the least bacterial spot disease severity (0.33) was observed in Kerewa plot and was not different ($p \geq 0.05$) from 1.17 at 50 ppm acibenzolar-s- methyl application and 1.33 and 1.13 in UTC-18 plot when ASM was applied at 25 and 50 ppm respectively. Kerewa, NG/DE/MAR/01/019 and UTC-18 yielded 64.16, 60.74 and 60.33 g/plant respectively when ASM was applied at 50 ppm concentration. These values were different significantly ($p \leq 0.05$) from 12.31, 21.88 and 27.07 g/plant from the plots contained Kerewa, NG/DE/MAR/01/019 and UTC-18 respectively when there was no acibenzolar-s- methyl application (Table 2). The results confirmed that the highest efficacy (88.04%) of ASM on bacterial spot incidence was obtained in Kerewa followed by UTC-18(81.80%) and NG/DE/MAR/01/019 (78.61%), all at 50 ppm concentration. Its efficacy on severity ranged from 56.43-93.27%. Figure 1indicates the trend of disease incidence of bacterial spot of tomato from 4 to 8 weeks after transplanting (WAT). There was no occurrence of bacterial spot from 4WAT to 7 WAT, 4-6 WAT and 4-6 WAT when acibenzolar-s- methyl was applied at 50 ppm in all the plots. The same trend was followed in disease severity (Figure 2). Generally, at 25 and 50 ppm concentrations, incidence and severity of bacterial spot were significantly low compared with untreated control plots.

**Table 1:** Effect of acibenzolar-s-methyl on plant height, number of leaves and number of branches of tomato plant

| Genotype | Concentration (ppm) | Plant height(cm) | Number of leaves/plant | Number of branches/plant |
|---|---|---|---|---|
| Kerewa | 0 | $50.73^{ab}$ | $96.67^{ab}$ | $6.00^{ab}$ |
| | 25 | $78.26^{a}$ | $188.67^{a}$ | $9.00^{a}$ |
| | 50 | $47.71^{ab}$ | $70.67^{b}$ | $5.00^{ab}$ |
| NG/DE/MAR/01/019 | 0 | $52.05^{ab}$ | $73.67^{b}$ | $3.67^{b}$ |
| | 25 | $34.00^{b}$ | $34.00^{b}$ | $3.00^{b}$ |
| | 50 | $44.33^{b}$ | $41.67^{b}$ | $4.17^{b}$ |
| UTC-18 | 0 | $47.17^{ab}$ | $30.83^{c}$ | $3.05^{b}$ |
| | 25 | $39.38^{b}$ | $29.58^{bc}$ | $2.66^{c}$ |
| | 50 | $44.17^{b}$ | $31.42^{c}$ | $3.19^{b}$ |

Values with the same superscript within each column are not significantly different by DMRT at ($p \leq 0.05$)

**Table 2:** Effect of acibenzolar-s-methyl on bacterial spot incidence, severity and yield of tomato plant

| Genotype | Concentration (ppm) | Leaf infection | | | | Yield (g/plant) |
|---|---|---|---|---|---|---|
| | | Incidence (%) | Efficacy | Severity | Efficacy | |
| Kerewa | 0 | $85.67^{a}$ | - | $4.90^{a}$ | - | $12.31^{c}$ |
| | 25 | $20.22^{bc}$ | 76.40 | $1.00^{bc}$ | 79.59 | $59.8^{ab}$ |
| | 50 | $10.25^{c}$ | 88.04 | $0.33^{c}$ | 93.27 | $64.16^{a}$ |
| NG/DE/MAR/01/019 | 0 | $95.62^{a}$ | - | $4.93^{a}$ | - | $21.88^{c}$ |
| | 25 | $35.67^{bc}$ | 62.70 | $2.00^{bc}$ | 59.43 | $52.55^{b}$ |
| | 50 | $20.45^{c}$ | 78.61 | $1.17^{c}$ | 76.27 | $60.74^{a}$ |
| UTC-18 | 0 | $55.01^{b}$ | - | $3.07^{b}$ | - | $27.07^{c}$ |
| | 25 | $18.98^{bc}$ | 65.50 | $1.33^{c}$ | 56.68 | $51.54^{b}$ |
| | 50 | $10.01^{c}$ | 81.80 | $1.13^{c}$ | 63.19 | $60.33^{a}$ |

Values with the same superscript within each column are not significantly different by DMRT at ($p \leq 0.05$)

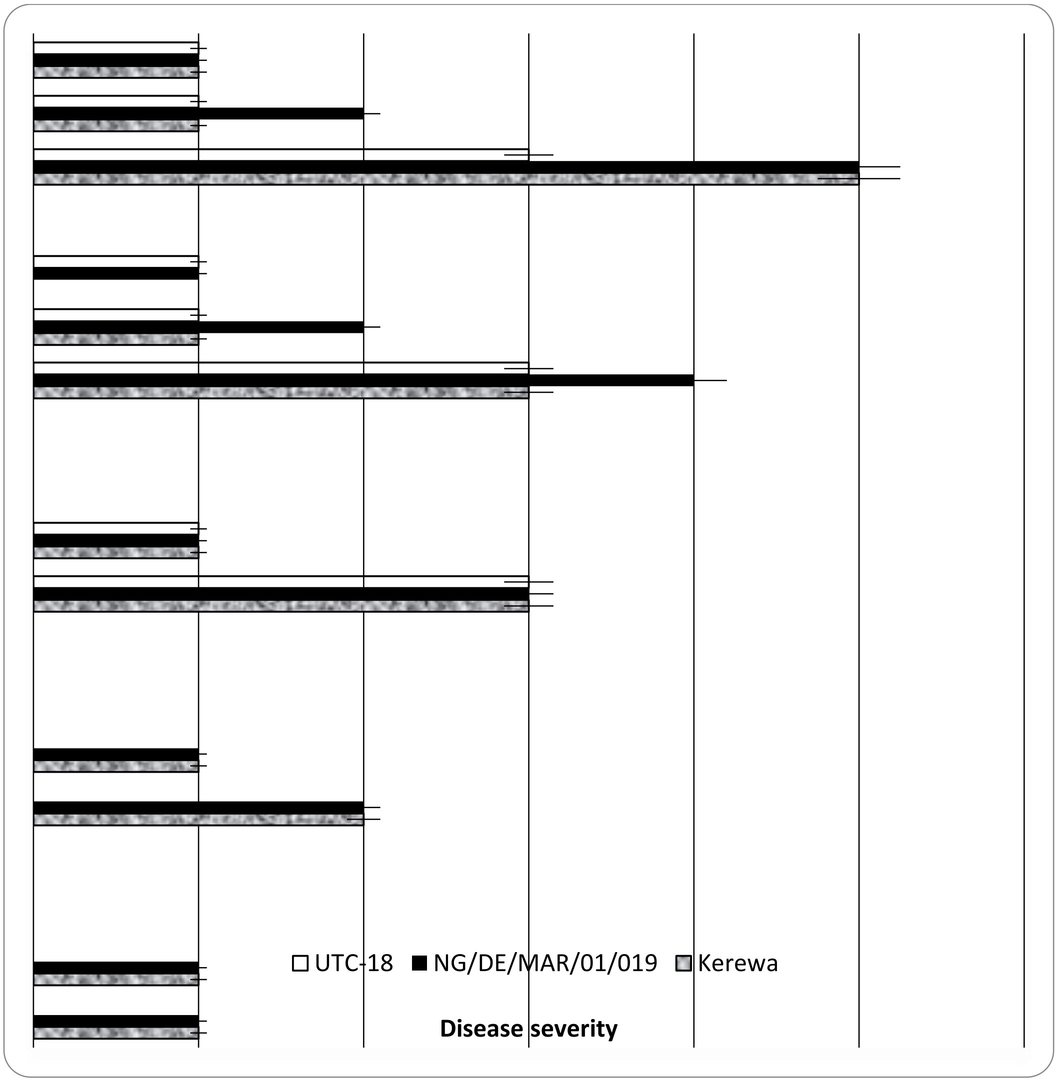


**Figure 1:** Influence of acibenzolar-s-methyl on the incidence of bacterial spot of tomato from 4 to 8 weeks after transplanting


**Figure 2:** Influence of acibenzolar-s-methyl on the severity of bacterial spot of tomato from 4 to 8 weeks after transplanting

## DISCUSSION

Isolated bacteria from diseased plant tissues were pathogenic to tomato plant. This was in consonance with the report of Mohammed (12). Variations in growth parameters might not be due to the treatment but largely to be genetic variability of the tomato used. Bacterial spot disease can cause serious economic losses to tomato and pepper growers by reducing yield and fruit quality (8**).** Based on the results obtained in this experiment, acibenzolar-s-methyl applied to tomato seedlings before transplanting had significant reduction in bacterial spot incidence and severity compared with untreated control plots. Acibenzolar-s-methyl applied at 25 and 50 ppm concentrations prevented the incidence and severity of bacterial spot to minimal levels in the three tomato genotypes used. This was in line with the results of Buonaurio *et al.* (1) that pepper plants were protected systemically and locally when treated with acibenzolar-s-methyl. The results were also consistent with previous findings of Oliveira and Nishijima (16) who reiterated that application of acibenzolar-s-methyl induced partial systemic resistance in papaya against black spot disease. Also, based on the results of Louws *et al.* (10), acibenzolar-s-methyl proved to be an effective new tool for tomato growers to manage bacterial spot of tomatoes. The weights of fruits harvested from acibenzolar-s-methyl treated plots were significantly higher than the weight of fruits harvested from plots without ASM application.

## CONCLUSION

The compound did not result in observable phytotoxicity on the plant. The study therefore revealed that prophylactic application of acibenzolar-s-methyl tomato plants against bacterial spot is a promising measure. Thus, foliar application of acibenzolar-s-methyl at 50 ppm could be an advantage for tomato growers to manage tomato bacterial spot disease where *Xanthomonas campestris* pv. *vesicatoria* is endemic.

## REFERENCES

1. **Buonaurio, R., Scarponi, L., Ferrara, M., Sidoti, P. and Bertona, A. 2002.** Induction of systemic acquired resistance in pepper plants by acibenzolar-s-methyl against bacterial spot disease. *European Journal of Plant Pathology* 108: 41–49.
2. **Buonaurio, R., Stravato, V.M. and Scortichini, M. 1994.** Characterization of *Xanthomonas campestris pv. vesicatoria* from Capsicum *annuum* L. in Italy. *Plant Disease* 78: 296–299.
3. **Clinton, S. K. 1998.** Lycopene: Chemistry, biology and implications for human health and diseases. *Nutrition Review* 56:35–51.
4. **Giovannucci, E., Rimm, E. B., Liu, Y., Stampfer, M. J. and Willett W. C. 2002.** A prospective study of tomato products, lycopene, and prostate cancer risk. *Journal of National Cancer Institute* 94(5):391-398.
5. **Helliwell, E. E., Wang, Q. and Yang, Y. 2013.** Transgenic rice with inducible ethylene production exhibits broad-spectrum disease resistance to the fungal pathogens *Magnaporthe oryzae* and *Rhizoctonia solani*. *Plant Biotechnology Journal* 11: 33- 42.
6. **Huang, C.H., Vallad, G. E., Zhang, S., Wen, A., Balogh, B., Figueiredo, J. F. L., Behlau, F., Jones, J. B., Momol, M. T., and Olson, S. M. 2012.** Effect of application frequency and reduced rates of acibenzolar-*S*-methyl on the field efficacy of induced resistance against bacterial spot on tomato. Plant Dis. 96:221- 227.
7. **Jibrin, M. O., Timilsina, S., Potnis, N., Minsavage, G. V., Shenge, K.C., Akpa, A.D. Alegbejo, M.D., Beed, F., Vallad, G.E., and Jones, J.B. 2014.** First report of *Xanthomonas euvesicatoria* causing bacterial spot diease in Pepper Northwestern Nigeria. *Plant disease* 98(10):1426. http://dx.doi.org/10.1094/PDIS-06-14-0586-PDN
8. **Jones, L. B., Woltz, S. S., Jones, J. P. and Portier, K. L. 1991.** Population dynamics of *Xanthomonas campestris* pv. *vesicatoria* on tomato leaflets treated with copper bactericides. *Phytopathology* 81:714-719.
9. **Lenucci, M. S., Cadinu, D., Taurino, M., Piro, G. and Dalessandro, G. 2006.** Antioxidant composition in

cherry and high-pigment tomato cultivars. *Journal of Agriculture and Food Chemistry* 54:2606-2613.

10. **Louws, F. J., Wilson, M., Campbell, H. L., Cuppels, D. A., Jones, J. B., Shoemaker, P. B., Sahin, F., and Miller, S. A. 2001.** Field control of bacterial spot and bacterial speck of tomato using a plant activator. Plant Dis. 85:481-488.
11. **Maerere, A. P., Sibuga, K. P. and Mwajombe, K. K. 2006.** Baseline survey report of tomato production in Mvomero District, Morogoro region, Tanzania. Sokoine University of Agriculture Faculty of Agriculture, Morogoro, pp. 1-31.
12. **Mohammed, A. A. 2011.** Pathogenic variability among five bacterial isolates of *Xanthomonas campestris* pv. *vesicatoria*, causing spot disease on tomato and their response to salicylic acid. *Journal of the Saudi Society of Agricultural Sciences* 10: 47–51
13. **Muhammad, A., Sidra, A., Nasir, A. R., Muhammad, R. B., Nazir, J., Ehteshamul, H., Waseem, A. and Babar, K. 2017.** Induction of systemic resistance in cotton against bacterial blight and its effect onyield. *International Journal of Biology and Biotechnology* 14 (4): 591-595
14. **Obradovic, A., Jones, J. B., Momol, M. T., Balogh, B., and Olson, S. M. 2004.** Management of tomato bacterial spot in the field by foliar applications of bacteriophages and SAR inducers. Plant Dis. 88:736-740.
15. **Obradovic, A., Jones, J. B., Momol, M. T., Olson, S. M., Jackson, L. E., Balogh, B., Guven, K. and Iriarte, F. B. 2005.** Integration of biological control agents and systemic acquired resistance inducers against bacterial spot on tomato. Plant Dis. 89:712-716.
16. **Oliveira, A. A. R. and Nishijima, W. 2014.** Induction of resistance to Papaya black spot elicited by acibenzolar-s- methyl. *Plant Pathology Journal* 13(2):120-124
17. **Popoola, A.R., Ganiyu, S.A., Babalola, O.A. and Ayo-John E. I. 2011.** Effect of tillage methods, varieties and compost amendments on bacterial leaf spot, leaf speck and fruit yield of two tomato

varieties. *Nigerian Journal of Plant Protection*, published by the Nigerian Society for Plant Protection, 25(1):127-138

**18. Raini, R., Hoffmann, V. and Zebitz, C.P.W. 2005.** Integrated Pest Management (IPM) and Information Flow: Case study tomato Stakeholders practices in Kenya, Tropentag, Stuttgart-Hohenheim 1-4.

**19. Satish, S., Raveesha, K. A. and Janardhana, G. R. 1999.** Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris* pathovars. *Applied Microbiology* 28:145-150

**20. Tijani, A.A., Ayanwale, A.O.S. and Baruwaa O. I. 2010.** Profitability and constraints of tomato production under tropical conditions. *International Journal of Vegetable Science* 16(2):128-133.