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Effect of Acibenzolar-S-Methyl on severity of Fusarium wilt and fruit yield of two tomato cultivars

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Abstract
Tomato wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*) is one of the limiting factors to tomato production in the whole world because of its negative effect on the fruit yield of tomato. The study investigated the application of Acibenzolar-S-Methyl (ASM), a plant activator that stimulates plants' defence mechanisms, at different concentrations (0, 25, 35 and 45 mg/L) on two tomato genotypes (Delila and Kerewa) using drenching method. Factorial experiments were laid out in a randomized complete block design and completely randomized design in both field and the screenhouse, respectively. Results showed that the application of ASM significantly reduced (p≤0.05) disease severity. At 9 weeks after transplanting Delila recorded severity scores of 3.00 and 3.33(screenhouse) and 0.33 (field) while Kerewa had severity scores of 1.33 and 1.50 (screenhouse) and 0.33 (field) when ASM was apllied at 35 and 45 mg/L concentrations, respectively. These values were significantly lower (p≤0.05) than those recorded in untreated plots. Delila yielded 151.23 and 151.73 kg/ha (screenhouse), and 754.70 and 795.8 kg/ha (field) while Kerewa yielded 109.60 and 124.03 kg/ha (screenhouse) and 601.10 and 279.8 kg/ha (field) when ASM was apllied at 35 and 45 mg/l concentrations, respectively. Yields in both screenhouse and the field were significantly higher (p≤0.05) than yields in the untreated control plots. The study concluded that application of ASM at 35 and 45 mg/L to tomato could enhance its resistance which would translate to fruit yield increase.

Keywords: Acibenzolar-S-methyl, Delila, drenching, *Fusarium oxysporum* f.sp. *lycopersici,* Kerewa, severity, tomato.

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Introduction

Tomato plants, scientifically known as *Solanum lycopersicum,* are among the most popular and widely cultivated vegetable plants in the world. Belonging to the nightshade family, they are native to western South America and were later introduced to other parts of the world. With their rich history and diverse varieties, tomato plants have become a staple in gardens, farms, and culinary traditions around the globe (Remison, 2005). Tomato plants are characterized by their herbaceous nature, typically growing as sprawling vines that can reach varying heights, depending on the cultivar, and growing conditions. These plants have a fascinating life cycle, starting from tiny seeds and maturing into vigorous plants with lush foliage and vibrant fruits.

One of the most remarkable features of tomato plants is their fruit, the tomato itself. These fruits vary in size, shape, and colour,

ranging from small cherry tomatoes to large beefsteak varieties. Tomatoes come in a spectrum of colours, including classic red, yellow, orange, green, and even purple. Their juicy, flavourful flesh and versatility in cooking make them a cherished ingredient in numerous cuisines worldwide (Ganiyu, 2014).

Cultivating tomato plants requires proper care, including adequate sunlight, well-drained soil, and regular watering. They thrive in warm climates, but with careful attention, they can be grown in a wide range of environments. Additionally, tomato plants are known for their susceptibility to various pests and diseases, which necessitates preventive measures and diligent monitoring to ensure healthy growth (Popoola, et al., 2012).

Fusarium oxysporum f. sp. *lycopersici*, the causal agent of fusarium wilt disease, is a soil-borne pathogen and attacks tomato cultivars. Infected plants show yellowing and wilting of leaves that progress upwards from the base of the stem and later spread to other parts of the plant. Wilted leaves usually drop prematurely. The pathogen invades the vascular tissues, produces more cells, and stops the flow of water and other nutrients to the upper parts of the plant which causes wilting and ultimately leading to death of the plant (Davies, 1982). Wilt disease in tomato results to an average yield loss of 50%, which further reduces farmer's income and family intake of vitamin A (Popoola et al., 2012). It poses a major threat to food production and food security in Sub-Saharan Africa, especially in the coastal regions (Popoola et al., 2012).

Planting of resistant cultivars remains the key strategy to control wilt caused by *F. oxysporum* (Hartman and Elphinstone 1994). Other practices have proven effective in reducing the occurrence of *Fusarium oxysporum*. For example, in some developing countries where the use of healthy seed and long crop rotations are not practical solutions to the problem of *Fusarium* wilt of tomato (FWT*),* intercropping has been used as a means of reducing soil populations of the pathogen (Hayward 1991). Removal of infected plants and burning has also shown to be very effective in disease control but not applicable to FWT. However, the use of fungicides and some plant growth regulators like Acibenzolar-S-Methyl have proven to be effective in the control of the pathogen.

Acibenzolar-S-methyl (ASM), is a synthetic analogue of salicylic acid (SA), that has recently been used to protect crops from several diseases by activating the plant defense systems (Kunz et al., 1997; Oostendorp et al., 2001). ASM has been proposed to induce systemic resistance (Hacisalihoglu et al. 2007), which is the best-known commercial resistance inducer (Dietrich et al. 2005). The compound triggers the production of pathogenesis-related proteins (PR-proteins) in the plants, thereby activating systemic acquired resistance similar to the role of salicylic acid (Kessmann et al., 1994; Lawton et al., 1996). It induces resistance in various plant species against a broad spectrum of pathogenic viruses, bacteria, fungi, and nematodes. This study was out to examine the effects of ASM on severity of *Fusarium* wilt and fruit yield of two tomato cultivars under screenhouse and field conditions grown in Abeokuta, Ogun State, Nigeria.

Materials and Methods

Experimental Site

The experiments were carried out in Tomato Screenhouse and the Teaching and Research Farm, as well as Plant Tissue Culture Laboratory of the Department of Crop Protection Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria.

Source of tomato accessions

Two tomato varieties, Delila (moderately resistant) and Kerewa (susceptible) were sourced from tomato germplasm Unit, Plant Tissue Culture Laboratory, FUNAAB.

Treatments and Experimental Designs

The treatments were two tomato varieties (Delila and Kerewa), Acibenzolar-S-methyl (ASM) at 0, 25, 35 and 45 mg/L were applied as soil drench in both field and screenhouse. Two trials with three replications were laid out in a Randomized Complete Block Design and Completely Randomized Design in both field and the screenhouse, respectively.

Soil sterilization and nursery establishment

Sandy-loam soil was steam-sterilized at 100˚C for 2 hours. The sterilized soil was kept inside polythene bags for one week before use. The plant nursery was raised, and the seedlings

were grown for 1 month before transplanting (Popoola et al, 2012).

Transplanting of tomato seedlings, preparation, and application of acibenzolar-S-methyl

Three-week-old tomato seedlings were transplanted to the screenhouse and in the field on April 20, 2016, and June 25, 2017, respectively. A wettable granular formulation of Acibenzolar-S-Methyl was weighed into a beaker using a sensitive scale (Amput electronics industries, China) and dissolved in sterile distilled water. Three concentrations were prepared 25 mg/L, 35 mg/L and 45 mg/L accordingly. Twenty-four hours after transplanting, each concentration was applied into the soil 2 cm deep at the root base of each plant using pipette.

Isolation and identification of Fusarium oxysporum f. sp. *lycopersici*

Infected tomato plants showing wilt symptoms were collected, taken to the laboratory for fungal isolation and identification. Infected samples were surface sterilized using 1% NaOCl and were further rinsed in petri dishes in three changes of sterile distilled water. Samples were plated in Petri dish containing autoclaved Potato Dextrose Agar (PDA), incubated at room temperature for 7 days. Preliminary identification of *F. oxysporum* f. sp. *lycopercisi* was conducted using morphological appearance and further characterized using taxonomic and morphological features as contained in the work of Leslie and Summerell, (2006).

Inoculation of F. oxysporum f. sp. *lycopersici*

Spore suspension was prepared and adjusted to (1.0 x 10^6) spores/ml from 7-day-old cultured fungus with the aid of haemocytometer. *F. oxysporum* f. sp. *lycopersici* spore suspensions (1.0 x 10^6 spores/ml) in distilled water were inoculated on four-week-old, already transplanted tomato plants in both the screenhouse and the field at basal root point (1 ml/hole); this was done one week after ASM application. Control pots or plots were inocculated with sterile distilled water. These steps were adopted from Animashaun et al., 2017.

Fruit yield

The following parameters were collected for yield of both tomato cultivars grown in the greenhouse and field:

Number of fruits per plant: The total number of mature fruits harvested from each plant.
Fruit weight: The weight of individual fruits measured using a digital scale.
Yield per unit area: The total weight of fruits harvested per unit area (e.g., kilograms per hectare). Mature and ripe fruits were collected in sterile bags and labelled according to plots and pots (in the screenhouse) they were collected from. They were taken to the laboratory and weighed. Fruit yield weight(s) were statistically analysed.

Data collection and analysis

Data collected on disease severity and fruit yield were subjected to Analysis of Variance (ANOVA) and means separated by Duncan's Multiple Range Test at $p \leq 0.05$ using Statistical Analysis System (SAS) 9.1 package. Disease severity was assessed using the 0–4 rating scale provided by Amini and Sidovich (2010), where 0= 0–24% of leaves yellowed; 1=25–49% of leaves yellowed; 2= 50–74% of leaves yellowed; 3=75–99% of leaves yellowed and 4=100% of leaves yellowed (dead).

Results

Table 1 shows the effect of Acibenzolar-S-Methyl (ASM) concentrations on severity of fusarium wilt in two tomato genotypes at 5, 7 and 9 Weeks After Transplanting (WAT) in both screenhouse and field trials. In screenhouse, the highest disease severity was recorded at 5 WAT (3.00) for Delila cultivar with 0 mg/L concentration which is the control pot with no ASM, while the least significant ($p \leq 0.05$) severity score (0.00) was recorded when ASM was applied at 25 mg/L, 35 mg/L and 45 mg/L concentrations in both Delila and Kerewa respectively. Similarly, the highest severity scores for 25 mg/L, 35 mg/L, and 45 mg/L were 0.66 and 1.66 at 7 and 9 WAT, respectively. In the field trial, severity scores were from 0.33-0.67 at 9 WAT when ASM was applied at 25 mg/L, 35 mg/L and 45 mg/L concentrations in both Delila and Kerewa respectively, which were though not significant ($p \geq 0.05$) from the untreated control plots (1.67).

Application of ASM influenced fruit yield significantly ($p \leq 0.05$) in both screenhouse and the field trials (Table 2). Generally, yield from field was higher than screenhouse. The

highest yield of 795.80 kg/ha was recorded in the field trial with the application of ASM at 45 mg/L in Delila followed by 754.7 recorded in Delila and 601.10 kg/ha in Kerewa treated with 35 mg/L ASM. These were significantly higher ($p \leq 0.05$) than 399.70, 428.60 and 171.60, 214.70 kg/ha recorded in Delila and Kerewa at 0 and 25 mg/L respectively. Similarly, in screenhouse, application of ASM at 35 and 45 mg/L recorded significant yield increase in both Delila and Kerewa.

Table 1: Effect of acibenzolar-s-methyl concentrations on severity of fusarium wilt in two tomato genotypes at 5, 7 and 9 WAT in both screenhouse and field trials

Cultivar	ASM (mg/L)	Disease severity					
		Screenhouse trial			Field trial		
		5WAT	7WAT	9WAT	5WAT	7WAT	9WAT
Delila	0	3.00^{a}	4.00^{a}	4.00^{a}	0.00^{a}	1.67^{a}	1.67^{a}
	25	0.00^{c}	3.33^{a}	4.00^{a}	0.00^{a}	0.67^{b}	0.33^{ab}
	35	0.00^{c}	2.66^{a}	3.00^{ab}	0.00^{a}	0.33^{ab}	0.33^{ab}
	45	0.00^{c}	1.00^{b}	3.33^{ab}	0.00^{a}	0.00^{c}	0.33^{ab}
Kerewa	0	0.66^{b}	2.33^{bc}	3.42^{ab}	0.00^{a}	1.67^{a}	1.67^{a}
	25	0.00^{c}	0.66^{b}	1.66^{c}	0.00^{a}	0.67^{b}	0.67^{ab}
	35	0.00^{c}	0.66^{b}	1.33^{c}	0.00^{a}	0.00^{c}	0.33^{ab}
	45	0.00^{c}	1.00^{b}	1.50^{c}	0.00^{a}	0.00^{c}	0.33^{ab}

Means with the same superscript in a column are not significantly different by Duncan Multiple Range Test (DMRT) at ($p \leq 0.05$); ASM: Acibenzolar-S-methyl; WAT: weeks after transplanting.

Table 2: Effect of Acibenzolar-S-methyl concentrations on fruit yield in two tomato genotypes in both screenhouse and field trials

Cultivar	ASM (mg/l)	Yield (kg/ha)	
		Screenhouse trial	Field trial
Delila	0	45.73^{d}	399.7^{bc}
	25	98.05^{b}	428.6^{b}
	35	151.23^{a}	754.7^{a}
	45	151.73^{a}	795.8^{a}
Kerewa	0	62.10^{c}	171.6^{c}
	25	65.27^{c}	214.7^{c}
	35	109.60^{b}	601.1^{a}
	45	124.03^{ab}	279.8^{bc}

Means with the same superscript in a column are not significantly different by Duncan Multiple Range Test (DMRT) at ($p \leq 0.05$); ASM: Acibenzolar-S-methyl.

Discussion

Application of acibenzolar-S-methyl (ASM) significantly reduced the severity of *Fusarium* wilt of tomato. The best-known commercial resistance inducer is ASM, which is a structural and functional analogue of salicylic acid (Dietrich et al. 2005). ASM is a plant activator that stimulates the natural, inherent defence mechanism of plants and through this, activities of *Fusarium* sp in plants are controlled (Animashaun, et al. 2017). Studies using this elicitor demonstrate rapid expression of resistance-related genes responsible to produce enzymes such as glucanases and chitinases (Dietrich et al. 2005). In support of all these assertions, severity of *Fusarium* wilt infection on tomato in this study was significantly reduced by the application of ASM at 35 and 45 mg/L concentrations, especially under field condition. Effect of reduced severity could be attributed to certain metabolic changes

through upregulating or downregulating of certain plant defence mechanism (Hayat et al., 2010). Interestingly, Animashaun et al. (2017) reiterated that ASM had been proven to induce a stronger resistance against infections in plants. Previous reports by Oostendrop et al. (2001), Hong et al. (2011) and Walters et al (2012) reported that ASM induced resistance against a wide range of pathosystems in both monocots and dicots, including fungal, bacterial, and viral pathosystems. Ganiyu (2014) also reported that foliar application of ASM reduced bacterial wilt incidence and that the activities of ASM and thymol were indicative of an integrated approach for management of various bacterial wilt of tomato.

Results from this study have pointed out the efficiency of ASM in the control of *Fusarium* wilt disease of tomato. The outcome of this experiment substantiated an earlier report by Animashaun et al. (2017) that exogenous plant growth regulator applied as soil drench at root point could reduce the effect of soil borne pathogens in plant. The significant reduction in tomato wilt severity displayed by the two tomato cultivars on application of ASM reflects its indirect suppressive capacity on pathogens.

Reduction in *Fusarium* wilt severity through the application of ASM at 35 and 45 mg/L concentrations, translated to significant fruit yield increase of the two tomato cultivars used in this experiment. Application at these rates was more effective in field trial. The low fruit yield experienced in screenhouse could have resulted from poor pollination of flowers, which agrees with experiments conducted by Pradhanang et al. (2005).

Conclusion

The study evaluated the responses of two tomato genotypes, grown in the field and screen house, to ASM application at different concentrations. The overall results of this research work revealed that application of ASM at 35 and 45 mg/L was promising in reducing the severity of tomato wilt caused by *F. oxysporum* f. sp. *Lycopersici,* and this had translated to significant fruit yield increase. Tomato farmers could therefore apply ASM at 35 and 45 mg/L to manage Fusarium wilt disease of tomato to enhance fruit yield.

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