Evaluation of *Rhizobium etli* Strain ciat 899 as a Biocontrol in Managing Fusarium Wilt (*Fusarium oxysporum*) of Tomato (*Solanum lycopersicum l.*)

ABSTRACT

Fusarium wilt, caused by *Fusarium oxysporum*, is a devastating disease affecting tomato plants globally with fruit yield loss of about 25-55% when not properly managed. This research was conducted at the Teaching and Research Farm, Ekiti State University during the 2024 cropping season to evaluate the effectiveness of *Rhizobium etli* strain CIAT 899 as a biocontrol agent in managing Fusarium wilt. The experiment was laid out in a Complete Randomized Design (CRD) with three replicates. The results showed that the analysis of variance was significantly differences (P<0.05) between the two tomato varieties studied for all the traits studied. Significant significances were also recorded between the treatments (control, *Rhizobium etli* and Carbendazim 12% + Mancozeb 63% WP). However, There was no significant difference (P<0.05) between the Carbendazim 12% + Mancozeb 63% WP and *Rhizobium etli* for plant height, number of fruits per plant, fresh fruit weight per plant and disease rating. The findings suggest that *Rhizobium etli* strain CIAT 899 can be a valuable tool in integrated pest management strategies for controlling Fusarium wilt on tomatoes, contributing to improved crop yields and reduced environmental pollution.

Keywords: Bio-control, Fusarium wilt, Tomato, *Rhizobium etli*, Yield loss.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a fruit vegetable crop widely cultivated globally. It could either be cultivated in the open field, protected environment, or a container (Houssei *et al*., 2010). Mature tomato fruits are a major component of the human diet. The fruits can be processed into various products or consumed as fresh (Brookie *et al.*, 2018). Ripe tomato fruit is rich in vitamins A and C which are highly essential in a human's daily diet (Kapsiya *et al*. 2015). It is processed into different products locally and industrially and consumed in diverse ways. According to FAOSTAT (2018), tomato global production was estimated to be 182.256 million tonnes. It serves as a huge source of income for those involved in its production cycle ranging from agro-dealers, farmers, transporters, marketers, processors, etc. In Nigeria, the consumption rate is far beyond the production rate thereby resulting in the scarcity of the product leading to undue competition among the consumers and a price increase.

Tomato production is greatly affected by various biotic and abiotic factors. Of the various biotic factors affecting tomato production, disease is one. However, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *Lycopersici*(Sacc.) Snyder and Hansen are recognized as the most dangerous and destructive disease affecting tomato production globally (Abdelaziz *et al*., 2022). The disease is more severe in tropical regions, especially in acidic sandy soil with high rainfall (Srinivas *et al*., 2019). The severity of any disease is a factor in the susceptibility of the variety grown, the prevailing environmental conditions of the area, the extent of infection, the inoculum load, and the timing and management approach are all important information needed in combating the disease (Agbowuro *et al.,* 2020). The long-term survival of the chlamydospore of the pathogen causing fusarium wilt in plant debris, soil, farm equipment, and other surfaces makes the disease one of the most destructive diseases of tomatoes (Chang *et al*., 2018). The pathogen can cause substantial loss in tomato farms ranging from 25 to 55% (Nirmaladevi *et al*., 2016). The fungus can completely wipe off the whole farm under optimal infection conditions such as high moisture and humidity, poor soil drainage, compacted soil, and warm temperature (Muhammad *et al***.,** 2023).

Fusarium oxysporum is a filamentous plant-pathogenic fungus that causes root rot, wilting, and necrosis on host plants and is ranked fifth out of the top 10 plant pathogens of scientific/economic importance. *F. oxysporum* can produce mycotoxins, including beauvericin, fusaric acid, and fumonisins in hosts (Li *et al*., 2013)**.** Among the devastating diseases caused by plant-pathogenic fungi, *Fusarium oxysporum* is a serious threat to tomato production and remains challenging to manage.

In the infection process, *F. oxysporum* employs a number of secretion systems and elicits a variety of virulence factors, such as mycotoxins, effector proteins, and plant cell wall-degrading enzymes (CWDEs), to subvert target host cells. It is a soil-borne fungal pathogen that causes a disease known as *Fusarium* wilt in tomato plants. This disease can significantly affect the growth and yield of tomato plants. Biological control is an alternative strategy to control disease-causing pathogens by using antagonistic nonpathogenic microorganisms, that have the potency to minimize or recover the harmful effects in numerous crops (Agbowuro *et al*., 2021). Recent studies favoured the adoption of biological control as a safety approach for humans and the environment in the control *F. oxysporum* (Abdelaziz *et al.,* 2022). Stimulated resistance is the physiological state produced by specific eco-friendly stimuli that have antimicrobial potency against a broad range of plant pathogens, including fungi (Mohammed *et al.*, 2019). Plant growth can be enhanced by non-pathogenic fungi through different mechanisms, such as systemic resistance stimulation and plant nutrition improvement, in addition to their toxicity to plant pathogens (Agbowuro *et al*., 2021). Therefore, this experiment aims to evaluate the effectiveness of *Rhizobium etli*strain CIAT 899 as a biocontrol agent in managing Fusarium wilt of tomatoes.

2.0 MATERIALS AND METHOD

2.1 Description of the Study Sites

The experiment was conducted at the Teaching and Research Farm, Ekiti State University, Ado-Ekiti, Nigeria. Ado-Ekiti is located in the vegetation that is intermediate between the forest and savannah zone of Nigeria and the coordinates of the experimental site are 7°37'N and 5°13'E. The vegetation existing at the experimental site was Mexican sunflower (*Tithonia diversifolia*), Bill goat weed (*Ageratum conyzoides*), milkweed (*Euphorbia heterophylla*), and guinea grass (*Megathyrsus maximus*). The site has been previously used for growing arable crops like maize, cassava, and vegetables. The site had been allowed to fallow for two years before the experiment. The soil in the experimental site is well-drained and sandy loam in nature.

2.2 Soil Sampling and Analysis of the Evaluation Site

At the experimental sites, ten soil core samples were collected randomly at a depth of 0-30 cm with sterilized soil auger before land preparation. The cores were thoroughly mixed to form a composite sample. The composite soil samples from each experimental site were packed in separate well-labelled polythene bags. The composite soil samples were thoroughly air-dried, crushed and sieved with a 2 mm sieve and kept inside laboratory cupboard before analysis. The physiochemical analysis of the soil samples was carried out to determine the physical and chemical properties of soil at the Department of Environmental Toxicology Laboratory, Elizade University, Nigeria. Beckam pH meter was used to determine the pH of the soil.

2.3 Experimental Materials

Two (2) tomato varieties (Padma F1 and platinum F1) produced by Premier Seed Nigeria Limited, Kaduna, and East-West Seed Company, Philippines respectively were used for the experiment. Saaf fungicide (Carbendazim 12% + Mancozeb 63% WP) used was gotten from Ondo State Government Ministry of Agriculture, Akure. *Rhizobium etli* and *Fusarium oxysporum* were obtained from the Microbiology labouratory, International Institute of Tropical Agriculture, Ibadan.

2.4 Treatment, Field Experimental Design and Cultivation Condition

The experiment was laid out in a Complete Randomized Design (CRD) with three replicates. The soil was obtained at the Teaching and Research Farm, Ekiti State University, Ado-Ekiti. The soil was sterilized to get rid of pathogens. 5kg of sterilized soil was packed into 10 litres of perforated pots and labelled accordingly. The seeds were surface sterilized with hydrogen peroxide solution, nursed on sterilized cocopeat for 21 days and transplanted. The pots were kept weed-free throughout the experiment. Split bamboo poles were used as stakes for the tomato plant. At three-week intervals, insect pests were controlled with 30 and 70 ml of Lamda cyhalothrine and Cypermethrin plus Dimethoate in 16 litres of Knapsack sprayer.

2.5 Inoculation Procedure

The pathogen (*Fusarium oxysporum*) was added to the soil in a pot experiment for 3 weeks before transplanting the tomato seedlings to ensure pathogen colonization in the soil. The biocontrol agent (*Rhizobium etli*) was added one (1) week after transplanting at the rate of 1 x 106 spores per ml using a haemocytometer. Carbendazim 12% + Mancozeb 63% WP at the rate of 50g per 15 litres of water was applied using a knapsack sprayer at one-week intervals according to the design of the Fungicide company (UPL brand).

2.6 Data collection and analysis

Data were collected on the following parameters plant height, number of leaves per plant, number of flowers per plant and fruit weight per plant. Data were analyzed using the International Rice Research Institute Statistical Tools for Agricultural Research (IRRI STAR 2014) software. Means were separated using Tukey’s Significant Test for differences at $P<0.05$.

3.0 RESULTS AND DISCUSSION

3.1 Soil Physiochemical Properties

Table 1 presents the physical and chemical properties of the soil at the experimental sites. The pH values for the soil were 5.62. The textural class was sandy loam. Total carbon (%) was 0.89. The total nitrogen values and Nitrogen were 1.66 and 0.11. Phosphorus was measured in mg/kg while Ca2+ and Mg 2+ were measured in mol/kg. The soil pH value in the soil is slightly acidic which is still within an acceptable range (Aune and Lal, 1997). The pH will still make soil nutrients readily available for plant root uptake (Golla, 2019). Texture is a vital soil property that determines the water and nutrient-holding capacity of the soil which invariably enhances plant growth and development. The textural class of the soil at the experimental site potential to hold water with high humus content.

3.2 Mean Squares for the Studied Traits in Field Trial

Mean squares for all the studied parameters are shown in Table 2. The mean squares due to varieties and treatments for all the studied traits were significantly different (P>0.05) for the traits studied. Varieties and treatment interaction were significantly different for all the traits though at different levels. For treatment, there were significant differences for all traits studied though at different levels except for the number of leaves per plant. The level of significance is a pointer to the degree of genetic variability in the genetic materials which provides a good opportunity for varietal improvement of the crop (Akinyosoye *et al*., 2017).

3.3 Mean performance for agronomic traits for two tomato varieties grown under *Fusarium oxysporum* pressure

The mean performance for agronomic traits for two tomato varieties grown under *Fusarium oxysporum* pressure is present in Table 3. The result shows that plant height is significantly different (P>0.05) among the treatments. However, there is no significant difference between *Rhizobium etli* and (SAAF) Carbendazim 12% + Mancozeb 63% WP for plant height. The number of fruits per plant, fresh fruit weight per plant and score rating was not significantly different (P>0.05) for the SAAF fungicide (Carbendazim 12% + Mancozeb 63% WP). SAAF fungicide (Carbendazim 12% + Mancozeb 63% WP (55.33) had the highest number of leaves followed by *Rhizobium etli* (52.00).

Table1: Physiochemical properties of the experimental sites

Properties	Environments
	Ado-Ekiti
Sand (%)	59.7
Clay (%)	20.3
Silt (%)	20.0
Texture	Sandy loam
pH (H_2O)	5.62
Total Carbon (%)	0.87
Organic matter (%)	1.66
Nitrogen (%)	0.11
Phosphorus(mg/kg)	9.60
Ca^{2+} (mol/kg)	1.41
Mg^{2+} (mol/kg)	0.70

Table 2**:** Mean square for agronomic traits of the tomato varieties grown under *Fusarium oxysporum* pressure

SV	DF	PH (cm)	NLP^{-1}	NFP^{-1}	$FFWP^{-1}$ (cm)	Disease Rating
Rep	2	526.88	25.16	41.16	0.58	1.55
Var	1	128.00**	392.00**	392.00**	0.15**	2.00**
Trt	2	117.05**	15.16	15.16*	3.15**	5.55**
Var x Trt	2	6.50**	6.16**	6.16**	0.27**	2.00**
Error	17					

*, ** *significant at 5% and 1% level respectively*. Rep.: Replication; Var.: variety; Trt.: Treatment; PH: Plant height; NLP^{-1}: Number of leaves per plant; NFP: Number of fruit per plant; $FFWP^{-1}$: Fresh fruit weight per plant (g).

The number of fruits per plant and fresh fruit weight per plant mean value was high in *Rhizobium etli*. For score rating, there is no significant difference between *Rhizobium etli* and Carbendazim 12% + Mancozeb 63% WP. Haas and Défago, *et al*., (2005), *Rhizobium etli* produces compounds that inhibit the growth of microbes. These compounds include phenazine antibiotics, a broad-spectrum antifungal that inhibit the growth and development of various plant pathogens.

Table 3: The mean performance for agronomic traits for tomato varieties grown under *Fusarium oxysporum* pressure.

Treatment	PH (cm)	NLP^{-1}	NFP^{-1}	$FFWP^{-1}$	SCORE RATING
Control	76.17b	51.68b	13.33b	0.90b	6.33a
SAAF	82.50a	55.33a	26.39a	2.42a	4.67b
Rhizobium etli	84.67a	52.00b	27.51a	2.61a	4.90b

Note: Means with the same letter (s) in each Column are not significantly different (P<0.05) according to Duncan's Multiple Range Test (DMRT). PH: Plant height; NLP-1: Number of leaves per plant; NFP: Number of fruit per plant; $FFWP^{-1}$: Fresh fruit weight per plant; SAAF: Carbendazim 12% + Mancozeb 63% WP.

The results show significant reductions in disease severity and incidence, as well as improvements in plant growth and soil microbial balance. Rhizobium etli offers a sustainable and environmentally friendly alternative to chemical control methods, making it an attractive solution for farmers and agricultural practitioners. By adopting Rhizobium etli and integrating it with other integrated pest management (IPM) strategies, we can promote sustainable agriculture practices and reduce the impact of Fusarium wilt on tomato production.

4.0 CONCLUSION

Farmers are encouraged to adopt *Rhizobium etli* strain CIAT 899 as a biocontrol agent for managing Fusarium wilt. It should be noted combination of *Rhizobium etli* with other IPM strategies for optimal disease management can be effective in pest suppression to boost the production and yield potential of tomatoes (*Solanum lycopersicum L.*). Further research can be conducted to investigate the effectiveness of *Rhizobium etli* against other tomato diseases and in different environmental conditions to develop cost-effective methods for large-scale production and distribution of *Rhizobium etli*.

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