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 crops glo with fruit yield loss of about 25-55% when not properly managed. A 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 in tomatoes. The experiment was laid out in a Complete Randomized Design (CRD) with three replicates. The results showed that *Rhizobium etli* significantly reduced disease incidence and severity and promoted plant growth, and improved soil microbial balance. The study demonstrated the potential of *Rhizobium etli* as a sustainable and environmentally friendly alternative to chemical control methods. 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: Fusarium wilt; Tomato; Rhizobium

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a fruit vegetable crop that is widely cultivated globally. It could either be cultivated in the open field, protected environment, or in a container (Houssei *et al*., 2010). Mature tomato fruits are a major component of the human diet. The fruits can be processed into various types of 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 who are 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 to 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, diseases of various kinds are 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 and Mostafa *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.,* 2020a). The long-term survival of the chlamydospore of the pathogen causing fusarium wilt in the plant debris, soil, farm equipment, and other surfaces makes the disease to become 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 in a large number of host plants and is ranked fifth out of the top 10 plant pathogens of scientific/economic importance (Dean *et al*., 2012). *F. oxysporum* can produce mycotoxins, including beauvericin (Li *et al*., 2013), fusaric acid (López-Díaz *et al.,* 2018), and fumonisins which contribute to pathogenicity in hosts**.** Among the devastating diseases caused by plant pathogenic fungi, *Fusarium oxysporum* holds an important place and remains challenging to be managed (Wanjohi *et al*., 2018). *Fusarium oxysporum* of tomato is soil borne disease and the pathogen is soil inhabitant therefore using chemicals for the management of the fungus is neither economical nor practical (Dignam *et al*., 2016).

This soilborne asexual fungus is known to include both pathogenic and nonpathogenic strains. During 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 (Ma *et al*., 2013). 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 of the Fusarium wilts by using Antagonistic nonpathogenic microorganisms, that have potency to minimize or recover the harmful effects in numerous crops (Glick *et al.,* 2012). Recent studies favored the application of biological control as safety approach for human and environment in the control *F. oxysporum* in (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 (Farrag *et al.,* 2017). Therefore, this experiment aims at evaluating the effectiveness of *Rhizobium etli* strain CIAT 899 as a biocontrol agent in managing Fusarium wilt of tomatoes.

MATERIALS AND METHOD

Description of the Study Sites

The experiment was carried out 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 the cultivation of 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.

Soil Sampling and Analysis of the Evaluation Site

At the experimental sites, ten soil core samples were collected randomly at the depth of 0-30 cm with sterilized soil auger before land preparation. The cores were thoroughly mixed together to form composite sample. The composite soil samples from each experimental site were packed in a separate well labeled polythene bags. The composite soil samples were thoroughly air dried, crushed and sieved with 2mm sieve and kept inside laboratory cupboard prior analysis. The physiochemical analysis of the soil samples were 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.

Experimental Materials

Two (2) tomato varieties (Padma F_1 and platinum F_1) produced by Premier Seed Nigeria Limited, Kaduna, and East-West Seed Company, Philippines respectively. Saaf fungicide (Carbendazim 12% + Mancozeb 63% WP) were used which were gotten from Everything Proper Goods, Ibadan. The *Rhizobium etli* and the *Fusarium oxysporum* were gotten from Microbiology labouratory, IITA, Ibadan.

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 labeled accordingly. The seeds were surface sterilized with the use of hydrogen peroxide solution and 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 the use of 30 and 70 ml of Lamda cyhalothrine and Cypermethrin plus Dimethoate respectively in 16 litres of Knapsack sprayer.

Inoculation Procedure

The pathogen (*Fusarium oxysporum*) was added to the soil in a pot experiment for 3 weeks before transplanting of the tomato seedlings to ensure pathogen colonization in the soil. The biocontrol agent (*Rhizobium etli*) were added after one (1) week after transplanting at the rate of

1 x 10^6 spores per ml using a haemocytometer. Carbendazim 12% + Mancozeb 63% WP at the rate of 50g per 15 litres of water was applied using knapsack sprayer at one week interval according to the design of the Fungicide company (UPL brand).

Data collection and analysis

Data were collected on the following parameters plant height, number of leaf per plant, number of flower 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 $P<0.05$.

RESULTS AND DISCUSSION

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 Ca^{2+} 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. The pH will still make soil nutrients readily available for plant root uptake. 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.

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

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 all 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 level except for number of leaves per plant. The level of significance is a pointer to the degree of genetic variability in the genetic materials which provide a good opportunity for varietal improvement of the crop (Akinyosoye *et. al*., 2017). The level of significance in treatment shows different among the treatment in inhibiting the disease.

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)	SCORE 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).

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 significant different ($P>0.05$) among the treatments. However, there is no significant different between *Rhizobium etli* and (SAAF) Carbendazim 12% + Mancozeb 63% WP for plant height. Number of fruits per plant, fresh fruit weight per plant and score rating were not significant difference ($P>0.05$) for 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). Number fruits per plant and fresh fruit weight per plant mean value was high in *Rhizobium etli.* For score rating, there is no significant different between *Rhizobium etli* and Carbendazim 12% + Mancozeb 63% WP. Haas and Défago, *et al*., (2005), *Rhizobium etli* produces compounds that inhibit the growth of microbial. 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 two 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 IPM strategies, we can promote sustainable agriculture practices and reduce the impact of Fusarium wilt on tomato production.

According to the result and research findings, Farmers in the research or experimental region are thereby 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 (Integrated Pest Management) strategies for optimal disease management can be effectiveness in pest suppression in order to boost production and yield potential of tomato (*Solanum lycopersicum L.*). Further research can be conducted to investigate the effectiveness of Rhizobium etli against other tomato diseases and in different environmental conditions in order to develop cost-effective methods for large-scale production and distribution of Rhizobium etli.

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