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Effectiveness of *Pseudomonas* species in the management of tomato early blight pathogen *Alternaria solani*

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Management of tomato early blight pathogen (*Alternaria solani*) has relied principally on application of synthetic fungicides. The use of biological control agents has been recognized as a viable option to synthetic chemicals in plant disease control. The present study evaluated the effects of *Pseudomonas fluorescens, P. aeruginosa, P. putida,* and *P. cepacia* on tomato early blight pathogen and investigated their efficacy on the yield components and yield of tomato plants infected with *A. solani.* Tomato seeds raised in the nursery beds were transplanted to the main field after three weeks. The experiment was laid in a randomized complete block. Treatment application was done by immersion of roots of three-week-old seedlings of tomato in *Pseudomonas* solution prior to transplanting. The treatments consisted of plots treated with *P. fluorescens, P. aeruginosa, P. putida, P. cepacia* and the control. Data were collected on disease severity, plant height, stem girth, number of fruits per plant, fruit length and fruit weight. Tomato plants in the control plot had significantly ($p \leq 0.05$) higher disease severity (52.0) There was no significant difference in the severities of early blight on tomato plants treated with *P. fluorescens*, *P. putida* and *P. cepacia*. Tomato plants treated with *P. aeruginosa* had significantly ($p \leq 0.05$) higher height (39.0 cm) than all other treatments. There was no significance difference in the fruit weights of tomato plants treated with *P. fluorescens*, *P. aeruginosa*, *P. putida,* and *P. cepacia*. Tomato plants in the control plots had a significantly lower fruit weight (69.5 g). Findings of the present study demonstrated a promising approach of biological control of early blight pathogen with *Pseudomonas* species. Results of this work could be used as bedrock for formulation of an effective and eco-friendly strategy for the management of early blight disease.

Key words: Tomato, *Alternaria solani*, *Pseudomonas*, biological control, early blight.

INTRODUCTION

Tomato *(Solanum lycopersicum)* is the most important vegetable crop grown in Nigeria (Usman and Bakari, 2013; Nwosu et al., 2014). The vegetable plays an important role in human nutrition, providing essential amino acids, vitamins and minerals (Sainju et al., 2003). Tomato contains lycopene, a very potent antioxidant that prevents cancers (Agarwal and Rao, 2000). Nigeria is the thirteenth largest producer of tomatoes in the world and the leading country in Sub-Saharan Africa, with a production estimate of 1.7 million tons per annum (Donkoh et al., 2013). In spite of this high ranking, the country is the largest importer of tomato paste in the world (Nwosu et al., 2014).

Optimum productivity and economic value of tomato is hindered by an array of abiotic and biotic factors. The main constraints include low level of technology, declining soil fertility, socio-economic factors, as well as pests and diseases (Tanzubil and Boatbil, 2014).

Pests and diseases have been identified as the major biotic factor limiting tomato production in Nigeria. Early blight, incited by the fungus, *Alternaria solani* is the most destructive disease of tomato, causing significant reduction in the quantity and quality of fruit yields (Tewari and Vishunavat, 2012). The disease is characterized by the appearance of brown to dark brown necrotic spots with concentric rings on foliage, stem and fruits (Chohan et al., 2015). Yield losses of about 80% have been attributed to early blight disease.

The variability in pathogenic isolates of *A. solani*, prolonged disease cycle phase and broad host range have made the control of early blight very difficult (Chohan et al., 2015). Management of the disease has relied principally on the application of synthetic fungicides. However, continuous use of agrochemicals for controlling the disease may pose several problems like toxicity to non-target organisms, development of resistance in the populations of the pathogen and environmental pollution (Varma et al., 2008). The use of environmentally safer microbial bioagents (antagonists) is considered as an effective alternative tool which can be exploited for the management of crop diseases (Varma et al., 2008; Singh et al., 2015). The use of plant growth promoting rhizobacteria, *Pseudomonas fluorescens* (Migula) is one of the promising biological control modules, and its commercial formulations have been tested against several crop diseases caused by pathogens (Kaur et al., 2016).

The use of biological control agents has been recognized as a viable option to synthetic chemicals in plant disease control and is currently being advocated worldwide (Ganeshan and Kumar, 2005; Salaheddin et al., 2010). Bioagents possess antagonistic effects against pathogen of plants (Muriungi et al., 2013). Inducing crops with *Pseudomonas* species to improve disease resistance has showcased the potential of developing plant protection products from biological control agents. Biocontrol treatments of plant diseases with *P. fluorescens* have proved to be eco-friendly and effective against many plant pathogens and have been considered as a long term solution to management of plant diseases (Varma et al., 2008; Kaur et al., 2016). Kaur et al. (2016) reported 39 to 46% reduction of tomato early blight disease with talc based formulation of *P. fluorescens*.

The present study was therefore carried out to evaluate the effects of *P. fluorescens, P. aeruginosa, P. putida,* and *P. cepacia* on tomato early blight pathogen and investigated their efficacy on the yield components and yield of tomato plants infected with early blight disease.

MATERIALS AND METHODS

Site description

The study was carried out at the Teaching and Research Farm of Landmark University, Omu-Aran, Kwara State, Nigeria. Omu-Aran is located in the North central part of Nigeria in the South-Eastern direction of Ilorin and has a latitude 8.9`N and longitude 50`61 E. The annual rainfall pattern of the area is 600 to 1,500 mm between the months of April and October with peaks in June. The humidity ranges from 50% in the dry season to about 85% during wet season.

Source of materials

The seeds of tomato cultivar used in this study were obtained from the Teaching and Research Farm of Landmark University, Omu-Aran, Nigeria while the four *Pseudomonas* species, that is, *P. fluorescens, P. aeruginosa, P. putida* and *P. cepacia*, were obtained from the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria.

Experimental design and treatment application

A total land area of 270 m^2, partitioned into forty- five beds was used for the experiment. Each bed measured 2 m × 3 m. Each plot was separated from the adjacent bed by 0.5 m alley. The experiment was laid in a randomized complete block design with five treatments replicated three times. The seeds were raised in nursery beds for three weeks and the seedlings transplanted to the main field at 0.3 m and 0.5 m intra- and inter-row spacings, respectively.

Inoculation of the biocontrol agent was done by immersion of roots of three-week-old seedlings of tomato in *Pseudomonas* solution (8 g *Psf* powder L^{-1} of water) for 5 min prior to transplanting while roots of seedlings of plants in the control plots were dipped in water. The treatments consisted of plots treated with *P. fluorescens, P. aeruginosa, P. putida, P. cepacia* and the control. The tomato plants were monitored for symptoms of *Alternaria solani* infection, that is, brown to dark brown necrotic spots with concentric rings on foliage, stem and fruits.

Data collection and analysis

Data were collected on disease severity, plant height, stem girth, number of fruits per plant, fruit length and fruit weight. Disease severity assessment was carried out on a scale of 0 to 9 according to Latha et al. (2009) where: 0 = healthy; 1 = 1-5%; 2 = 6-10%; 3 =

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11-25%; 5 = 26-50%, 7 = 51-75%, and 9 = > 76% of the leaf area infected with early blight symptoms.

Fruit length was measured with a vernier caliper. For yield determination, tomato fruits were harvested and weighed as and when ripe until the experiment was terminated at 24 weeks after transplanting.

The data collected were subjected to analysis of variance (ANOVA) and the means were separated using Duncan multiple range test (DMRT) at 5% probability level.

RESULTS

Disease severity

Tomato plants in the control plot had significantly ($p \leq 0.05$) higher disease severity (52.0) There was no significant difference in the severities of early blight on tomato plants treated with *P*. *fluorescens*, *P*. *putida* and *P. cepacia*. Tomato plants in plots treated with *P. aeruginosa* had significantly lower early blight severity (11.0).

Stem girth

There was no significant difference in the stem girths of tomato plants treated with *P. aeruginosa*, *P. putida*, *P*. *cepacia* and the control.

Plant height

Tomato plants treated with *P*. *aeruginosa* had significantly ($p \leq 0.05$) higher height (39.0 cm) than all other treatments. This was followed by tomato plants in plots treated with *P. putida* which has a height of 35.7cm. There was no significant difference in the heights of tomato plants treated with *P*. *fluorescens* and the control, which have heights of 30.0 and 31.7 cm, respectively. Tomato plants treated with *P. cepacia* had significantly lower plant height (24.0 cm).

Fruit length

There was no significant ($p \leq 0.05$) difference in the fruit lengths of the tomato plants treated with *Pseudomonas* species and the control. However, tomato plants in plots treated with *P*. *cepacia* had a numerically higher fruit length (3.1 cm).

Number of fruits

There was no significant ($p \leq 0.05$) difference in the number of fruits on tomato plants treated with *P. aeruginosa, P. putida* and *P. cepacia*. Similarly, no significance difference was observed in the number of fruits on tomato plants treated with *P. fluorescens* and the control, although tomato plants in the control plots had the least number of fruits (6.33).

Fruit weight

There was no significance ($p \leq 0.05$) difference in the fruit weights of tomato plants treated with *P. fluorescens*, *P. aeruginosa*, *P. putida* and *P. cepacia*. However, tomato plants treated with *P*. *aeruginosa* had a numerically higher fruit weight (202.82 g). Tomato plants in the control plots had a significantly lower fruit weight (69.53 g).

DISCUSSION

The present study investigated the effect of *P. fluorescens, P. aeruginosa, P. putida* and *P. cepacia* on early blight pathogen and assessed their effects on plant height, stem girth, fruit length, number of fruits and fruit weight of tomato plants infected with early blight disease. The results showed that the tomato plants treated with the *Pseudomonas* species had lower disease severities at the different weeks of assessment compared with the control.

Pseudomonas species influence plant growth through inhibition of fungal plant pathogens and by their effects on the roots of plants (Moore et al., 2006). Certain strains of *Pseudomonas* secrete metabolites that inhibit plant pathogens and stimulate plant growth. Thus, the lower disease severities recorded on tomato plants treated with *Pseudomonas* species in the current study might be attributed to the production of metabolites that inhibited the growth of *A. solani*.

The rhizosphere (zone around the roots of plants) is noted for intense microbial activity, arising from secretion of organic and amino acids by plants. *Pseudomonas* species are among the most effective rhizosphere colonizers of soil (Lugtenberg et al., 2001). They possess traits that enable them to exploit the resources in the rhizosphere. Species of this biocontrol agent also effectively inhibit the colonization of plants by other microorganisms (Brianciotto et al., 1996). According to Moore et al. (2006), *P. putida*, *P. fluorescens*, as well as other *Pseudomonas* species colonize and proliferate quickly in the rhizosphere of a number of agriculturally important plants. Furthermore, *P. fluorescens*, *P. putida* and *P. cepacia* produce antibiotics like pyrol, nitrin, oomycin-A and hormone such as indole acetic acid, gibberellic acid and siderophores that inhibit the growth of pathogens (Bhattacharjee and Dey, 2014). These attributes might have assisted the *Pseudomonas* species used in the current study to inhibit *A*. *solani* establishment in the rhizosphere of the tomato plants.

P. putida is a versatile, aggressive colonizer that establishes itself and persists within the rhizosphere and in bulk soils at high cell densities (Molina et al., 2000).

Table 1. Effect of four *Pseudomonas* species on disease severity, stem girth, plant height, fruit length, number of fruits and fruit weight of tomato plants infected with early blight pathogen (*A. solani*).

Treatment	Parameters					
	Disease severity	Stem girth (cm)	Plant height (cm)	Fruit length (cm)	Number of fruits	Fruit weight (g)
P. fluorescens	16.0[b]	2.3[b]	30.0[c]	3.0[a]	8.4[b]	113.1[ab]
P. aeruginosa	11.0[c]	2.5[ab]	39.0[a]	2.5[a]	25.4[a]	202.8[a]
P. putida	15.3[b]	2.4[ab]	35.7[b]	2.1[a]	14.6[ab]	130.6[ab]
P. cepacia	15.7[b]	2.4[ab]	24.0[d]	3.1[a]	13.6[ab]	103.4[ab]
Control	52.0[a]	2.5[a]	31.7[c]	2.9[a]	6.3[b]	69.5[c]

Values are means of three replicates. Means in the same column followed by the same alphabet are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

This trait was demonstrated by this strain in the current study as the lowest early blight severity was recorded at maturity stage of the tomato plants at 9 weeks after transplanting (Table 1). Features that contribute to rhizosphere and soil fitness of *P. putida* include adhesion and colonization abilities, antibiotic production, resistance to multiple antibiotics, capability to utilize seed and root exudates, production and utilization of siderophores, and ability to cope with oxidative stresses (Jataraf et al., 2005; Jorjani et al., 2011).

Biological control of crop diseases by antagonistic microorganisms has become the most effective alternative to synthetic chemical pesticides (Alemayehu, 2014). Among the antagonistic bacteria, several strains of *P. fluorescens*, *P. putida, P. cepacia* and *P. aeruginosa* have been widely used for biological control of fungal, viral and bacterial pathogens (Raupach and Kloepper, 1998; Vidhyasekaran and Muthamilan 1999; Salaheddin et al., 2010). The mechanisms employed by bacterial antagonists for plant disease control include antibiosis, siderophore production, enzyme secretion, hormone production, competition for nutrients and space, activation of plant defenses and inducing systemic resistance in host plants (Duffy et al., 2003; Jataraf et al., 2005; Jorjani et al., 2011).

Conclusion

Findings of the present study demonstrated a promising approach of biological control of early blight pathogen with *Pseudomonas* species. Results of this work could be used as bedrock for formulation of an effective and eco-friendly strategy for the management of early blight disease.

CONFLICT OF INTERESTS

The authors of this manuscript declare that there is no conflict of interest.

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