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Bio-efficacy of *Pseudomonas fluorescens* 1.75% WP (Bio Cure-B) as a bio-control agent against early blight (*Alternaria solani*) disease of tomato under field condition

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ABSTRACT: Tomato early leaf blight, caused by *Alternaria solani*, is one of the most destructive diseases, causing significant losses in tomato production. In the absence of resistant cultivars, the treatment of tomato early blight disease has mainly relied on the use of chemical fungicides. In disease management, the use of biological control agents has been recognised as a viable alternative to chemicals fungicides. Rhizobacteria that promote plant growth are important in the control of plant diseases. Pseudomonads have long been known to promote plant growth while also delaying disease onset. The current study looked at the effects of *Pseudomonas fluorescens* on tomato early blight disease and the effect on tomato plant yield components. The field experiment was set up in a randomized block design with seven treatments and three replications at the Regional Research Sub-Station (R & L Zone), Bidhan Chandra Krishi Viswavidyalaya, Sekhampur, Birbhum, West Bengal, India, during Rabi, 2016-17 and Rabi, 2017-18. Powder formulation of *Pseudomonas fluorescens* 1.75% (Bio Cure B) as a seed treatment at 5g/kg of seeds and three foliar sprays at 3 kg/ha from 50 days after transplanting at 15 day intervals reduced disease (44.33 %) and increased yield (37.52 %). There was a disease reduction (36.32 %) and yield increase (33.68 %) with three foliar spray @ 3 kg /ha from 50 days after transplanting at 15 day intervals. The current findings demonstrated a promising approach to biological control of early blight disease using *Pseudomonas fluorescens* 1.75% (Bio Cure B). The findings of this study could be used to develop an effective and environment friendly strategy for the management of tomato early blight disease.

Keywords: Biological control, early blight, *Pseudomonas fluorescens*, tomato.

INTRODUCTION

The tomato (*Solanum lycopersicum*), a Solanaceae plant, is a short-duration remunerative vegetable with high nutritive value and antioxidant properties (Azeez *et al.*, 2019). It has several health benefits and is high in vitamin A, B, C, minerals, organic acids, and sugar (Salim *et al.*, 2017). Tomatoes are also used in a variety of food products, including ketchup, soup, paste, and powder (Manivannan and Tholkappian, 2013). *Alternaria solani* is known to cause early blight of tomato (Rotem, 1994), which is economically one of the most important tomato diseases worldwide, causing significant yield reductions of 35 to 78% (Jones, 1991). Early blight is common in the tropics and temperate zones (Waals *et al.*, 2004). The disease spreads throughout the country, resulting in a loss of yield. The yield loss in tomatoes due to early blight disease ranged from 0.75 to 0.77 t/ha for every 1% increase in disease severity (Saha and Das, 2012). In tomatoes, yield losses of up to 86 percent (50-86 %) have been reported (Mathur and Sekhawat, 1986). A 1% increase in disease intensity reduces yield by 1.36 percent, and severe disease can lead to crop failure (Ngoc *et al.*, 2013). According to a survey and assessment of losses in West Bengal, India, blight caused by *Alternaria species* was the most common fungal disease, with crop losses in the field ranging from 70 to 100 % (Kanjilal *et al.*, 2000). Because of its complete defoliation, the disease is the most damaging to tomatoes (Peralta *et al.*, 2005). The primary hosts of disease are tomato, potato, brinjal, chilli, and black nightshade (Pscheidt, 1985). Cucumber, zinnia, wild cabbage, and horsenettle are other non-solanaceous hosts (Neergaard, 1945). Thomma (2003) revealed that *Alternaria* spp. produced melanin in their spores as well as host-specific and nonspecific toxins such as alternaric acid. Plant pathologists are increasingly concerned about the use of

potentially hazardous fungicides for the treatment of *A. solani* since it could lead to the pathogen developing fungicidal resistance (Rai *et al.,* 2020). As a result, the unique strategy uses a small amount of chemicals to reduce pollution risks and management costs. *Pseudomonas spp.* are Gram-negative bacteria that live in the soil and have properties that make them useful for plant disease biocontrol (Palleroni, 2008). Several types of these bacteria have been shown to generate antibiotics that inhibit the growth of phytopathogens (Yang and Cao, 2012). Duke *et al.,* (2017) mentioned that *Pseudomonas spp.* has the property of surface colonisation. Pseudomonas is known for producing a wide range of antifungal and plant growth-promoting compounds, including VOCs (Yan *et al.,* 2017).

In the absence of resistant cultivars, the treatment of tomato early blight disease has primarily relied on the use of synthetic fungicides. The current study looked at the effects of *Pseudomonas fluorescens* 1.75% (Bio Cure B) on tomato early blight disease and the effect on tomato plant yield components. The current findings demonstrated a promising approach to biological control of early blight disease using *Pseudomonas fluorescens* 1.75% (Bio Cure B). These findings could be used to create an efficient and environment friendly strategy for managing early blight disease in tomatoes.

MATERIALS AND METHODS

A bio-efficacy trial was conducted to assess the efficacy of *Pseudomonas fluorescens* 1.75 % WP formulation (Bio Cure B) supplied by M/s T. Stanes & Company Private Limited, Coimbatore, Tamil Nadu, in managing *Alternaria solani*, which causes Early leaf blight in tomato crops. The field experiment was set at the Regional Research Sub-Station (R & L Zone), Bidhan Chandra Krishi Viswavidyalaya, Sekhampur, Birbhum, West Bengal, India, during Rabi 2016-17 and Rabi 2017-18. There were three replications laid out in a randomized block design, having a block size of 5 m × 4 m and a spacing of 60 cm × 45 cm, and the a mlik variety was taken. The crop was maintained with judicious agronomic practices.

Table 1: Treatments details.

Treatment No.	Treatment	Dose (kg/ha)	Methods of application
T_1	*P. fluorescens* 1.75 % WP (Bio Cure B)	5 g /kg	Seed treatment
T_2	*P. fluorescens* 1.75 % WP (Bio Cure B)	1 kg /ha	Three foliar sprays from 50 days after transplanting at 15 days interval
T_3	*P. fluorescens* 1.75 % WP (Bio Cure B)	2 kg /ha	Three foliar sprays from 50 days after transplanting at 15 days interval
T_4	*P. fluorescens* 1.75 % WP (Bio Cure B)	3 kg /ha	Three foliar sprays from 50 days after transplanting at 15 days interval
T_5	*P. fluorescens* 1.75 % WP (Bio Cure B)	5 g/ Kg seeds + 3 kg / ha	Seed treatment + Three foliar sprays from 50 days after transplanting at 15 days interval
T_6	Mancozeb	2 g /kg + 2 g /lit	2 g per kg of seeds + Three foliar sprays from 50 days after transplanting at 15 days interval
T_7	Control	--	-

According to Latha *et al.,* (2009), disease severity was assessed on a scale of 0 to 9 where: 0 = healthy; 1 = 1-5 %; 2 = 6-10 %; 3 = 11-%; 5 = 26-50 %, 7 = 51-75 %, and 9 = > 76 % of the leaf area infected with early blight symptoms. The formula for calculating percent disease severity (PDI) was PDI = [Sum of numerical rating/total number of observations taken x maximum disease score] × 100.

RESULTS AND DISCUSSION

A. Early blight disease severity

Among the biological treatments, *Pseudomonas fluorescens* 1.75 % WP (Bio-cure -B), when applied as seed treatment 5g /kg of seeds for 1 hour + Three foliar sprays @ 3 kg /ha from 50 days after transplanting at 15 days interval (T_5), recorded the least mean PDI of 29.56 which differed significantly from the untreated control (53.20%). In case of chemical control, Mancozeb 75 % WP as seed treatment @ 2 g per kg of seeds + 2 g /lit foliar spray recorded a mean PDI of 20.40. *Pseudomonas fluorescens* 1.75 % WP applied as seed treatment @ 5 g /kg of seeds for 1 hour + Three foliar sprays @ 3 kg /ha from 50 days after transplanting at 15 days interval was revealed to be superior to other environmentally friendly treatments. Observation on the mean PDI of early blight of tomato disease revealed that *Pseudomonas fluorescens* 1.75 % WP as three foliar sprays @ 3 kg /ha from 50 days after transplanting at 15 days interval (T_4) recorded the mean PDI of 33.89 followed by *Pseudomonas fluorescens* 1.75 % WP as three foliar sprays @ 2 kg /ha from 50 days after transplanting at 15 days interval (T_3). *Pseudomonas fluorescens* 1.75 % WP as three foliar sprays @ 3 kg /ha from 50 days after transplanting at 15 days interval (T_2) and *Pseudomonas fluorescens* 1.75 % WP as Seed treatment @ 5 gm /kg of seed for 1 hour (T_1) treated plots recorded mean PDI of 42.65 and 44.12 respectively.

B. Characteristics of plant growth and yield

Plant height, stem diameter, number of leaves/plant, number of branches/plant, number of fruits/plant, and fruit weight (g/plant) were all measured during both seasons. Under field conditions, the application of *Pseudomonas fluorescens* significantly increased the growth and yield attributing characteristics of tomato compared to the untreated control.

During the field test application of *Pseudomonas fluorescens* 1.75 % WP as seed treatment @ 5g /kg of seeds for 1 hour + Three foliar sprays @ 3 kg /ha from 50 days after transplanting at 15 days interval recorded the maximum plant height (101.93 cm), stem diameter (1.13 cm), number of branches (7.60/plant) in tomato in comparison to other treatments. Control plants were documented only 94.8 cm of plant height, 0.99 cm of stem diameter, 6.47 number of branches/plant. The fruit yield data was recorded in the both season trials. Under field conditions, the powder formulation of *Pseudomonas fluorescens* 1.75 % WP was found to significantly increase the number of fruits per plant and fruit yield of tomatoes. Chemical treatment i.e. Mancozeb 75 % WP as seed treatment @ 2 g per kg of seeds + 2g /lit foliar spray yielded the highest fruit yield of 88.46 t/ha, followed by *Pseudomonas fluorescens* 1.75 % WP @ seed treatment 5 g /kg of seeds for 1 hour + Three foliar sprays @ 3 kg /ha from 50 days after transplanting at 15 days interval yielded 76.58 t/ha. The untreated control plants had the lowest fruit yield of 55.62 t/ha.

This could be due to the antagonistic effect of bio-fungicide, which could cause systemic resistance to early blight disease in tomatoes. Many strains of *P. fluorescens* have been used as seed treatments or foliar applications to establish systemic resistance in tomatoes against early blight, leaf blight, root-knot disease, and bacterial wilt. (Babu *et al.*, 2000; Latha *et al.*, 2009; Kaur *et al.*, 2016). *Pseudomonas fluoroscens* has previously been shown to be effective as a potential biocontrol agent by Babu *et al.*, (2000), Ngoc *et al.*, (2013), and Mahapatra and Swain (2013), which is consistent with the current study. *Pseudomonas* spp. are particularly suitable as an agricultural bio-control agent because they can use many exudates compounds as a natural source (Lugtenberg *et al.*, 1999), which are abundantly present in natural soils, particularly on plant root systems, have a high growth rate, possess diverse mechanisms of action against phytopathogens, including the production of antagonistic metabolites (Rhodes and Powel, 1994), and are capable of inducing systemic (Van Loon *et al.* 1998). Globally, the use of biological control agents as an alternative to synthetic chemicals in the control of plant diseases is currently being advocated (Ganeshan and Kumar, 2005). *Pseudomonas species* affect plant growth by inhibiting fungal plant pathogens (Moore *et al.*, 2006). Biological disease control by antagonistic microorganisms has emerged as the most effective alternative to synthetic chemical pesticides (Almayehu, 2014). It has been observed that increasing levels of defense-related enzymes result in greater disease resistance (Ramamoorthy and Samiyappan 2001). It confirms that preceding treatment of bio-inoculants in various combinations and sets of applications activates the plant's natural defensive mechanism, resulting in increased production of defense-related chemicals and enzymes (Chin *et al.*, 2000).

Table 2: Effect of *Pseudomonas fluorescens* 1.75 % WP (Bio cure–B) against early blight disease of Tomato (Two years pooled data).

Tr. No.	Treatments	Wilt Incidence	
		Percent disease severity	Percent disease control
T_1	*Pseudomonas fluorescens* 1.75 % WP as Seed treatment @ 5 g /kg of seed for 1 hour	44.12 (41.6)	17.07
T_2	*Pseudomonas fluorescens* 1.75 % WP as three foliar spray @ 1 kg /ha from 50 days after transplanting at 15 days interval	42.65 (40.8)	19.83
T_3	*Pseudomonas fluorescens* 1.75 % WP as three foliar spray @ 2 kg /ha from 50 days after transplanting at 15 days interval	39.32 (38.8)	26.09
T_4	*Pseudomonas fluorescens* 1.75 % WP as three foliar spray @ 3 kg /ha from 50 days after transplanting at 15 days interval	33.89 (35.6)	36.32
T_5	*Pseudomonas fluorescens* 1.75 % WP as seed treatment 5g /kg of seeds for 1 hour + Three foliar spray @ 3 kg /ha from 50 days after transplanting at 15 days interval	29.56 (32.9)	44.33
T_6	Mancozeb 75 % WP as seed treatment @ 2 g per kg of seeds + 2 g /lit foliar spray	20.40 (26.9)	61.65
T_7	Control	53.20 (46.8)	0.00
	SEm (±)	**0.96**	
	CD (P=0.05)	**2.84**	

Values in parentheses are arcsine-transformed values.

The use of antagonistic microorganisms to control crop diseases has proven to be the most effective alternative to manmade chemical pesticides (Alemayehu, 2014). *P. fluorescens* and other antagonistic bacteria strains have been widely engaged for biological control of fungal, viral, and bacterial diseases (Raupach and Kloepper, 1998; Salaheddin *et al.*, 2010). Plant pathogens are inhibited and plant growth is stimulated by metabolites secreted by *Pseudomonas* strains. As a result, the lower disease severity observed in the current study on tomato plants treated with 'Bio cure B' may be attributed to the production of metabolites that inhibit *Alternaria solani* growth.

Table 3: Effect of *Pseudomonas fluorescens* 1.75 % WP (Bio cure–B) on plant growth promotion(Two years pooled data).

Tr. No.	Treatment details	Pl. height (cm)	Stem diameter (cm)	No. of leaves/ plant	No. of branches/ plant
T_1	*Pseudomonas fluorescens* 1.75 % WP as Seed treatment @ 5 g /kg of seed for 1 hour	96.17	1.03	86.67	6.73
T_2	*Pseudomonas fluorescens* 1.75 % WP as three foliar spray @ 1 kg /ha from 50 days after transplanting at 15 days interval	97.80	1.05	90.00	6.87
T_3	*Pseudomonas fluorescens* 1.75 % WP as three foliar spray @ 2 kg /ha from 50 days after transplanting at 15 days interval	98.67	1.06	93.33	6.93
T_4	*Pseudomonas fluorescens* 1.75 % WP as three foliar spray @ 3 kg /ha from 50 days after transplanting at 15 days interval	99.30	1.07	95.00	7.22
T_5	*Pseudomonas fluorescens* 1.75 % WP as seed treatment 5g /kg of seeds for 1 hour + Three foliar spray @ 3 kg /ha from 50 days after transplanting at 15 days interval	101.93	1.13	100.67	7.60
T_6	Mancozeb 75 % WP as seed treatment @ 2 g per kg of seeds + 2 g /lit foliar spray	100.27	1.09	98.33	7.45
T_7	Control	94.80	0.99	83.67	6.47
	SEm (±)	**0.87**	**0.23**	**1.23**	**0.34**
	CD (P=0.05)	**2.56**	**0.69**	**3.61**	**1.00**

Table 4: Effect of *Pseudomonas fluorescens 1.75 % WP (Bio cure–B)* on yield attributing characters of Tomato (Two years pooled data).

Tr. No.	Treatment details	No. of Fruits / Plant	Weight of fruits / plant (g)	Yield (t / ha)	Yield increase (%)
T_1	*Pseudomonas fluorescens* 1.75 % WP as Seed treatment @ 5 g /kg of seed for 1 hour	43.00	1005.00	61.28	10.18
T_2	*Pseudomonas fluorescens* 1.75 % WP as three foliar spray @ 1 kg /ha from 50 days after transplanting at 15 days interval	55.00	1098.33	64.97	16.81
T_3	*Pseudomonas fluorescens* 1.75 % WP as three foliar spray @ 2 kg /ha from 50 days after transplanting at 15 days interval	58.67	1110.33	70.42	26.61
T_4	*Pseudomonas fluorescens* 1.75 % WP as three foliar spray @ 3 kg /ha from 50 days after transplanting at 15 days interval	61.00	1134.67	74.15	33.32
T_5	*Pseudomonas fluorescens* 1.75 % WP as seed treatment 5g /kg of seeds for 1 hour + Three foliar spray @ 3 kg /ha from 50 days after transplanting at 15 days interval	67.00	1189.33	76.58	37.68
T_6	Mancozeb 75 % WP as seed treatment @ 2 g per kg of seeds + 2 g /lit foliar spray	64.33	1150.00	88.46	59.04
T_7	Control	32.33	885.67	55.62	0.00
	SEm (±)	**1.60**	**10.32**	**1.30**	
	CD (P=0.05)	**4.72**	**30.36**	**3.82**	

CONCLUSION

Pseudomonas fluorescens 1.75 % WP (Bio Cure B) as seed treatment @ 5g /kg of seeds and three foliar sprays @ 3 kg /ha from 50 days after transplanting at 15 day intervals was very effective and eco-friendly management strategy against tomato early blight disease in west Bengal condition.

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