

## Effects of biocontrol agents on Early Blight Disease of potato in field

YUMKHAIBAM SONIA SHAHNI<sup>1</sup>, SUSANTA BANIK<sup>1</sup>, NAROLA PONGENER<sup>1</sup>, PANKAJ NEOG<sup>2</sup> AND A. P. SINGH<sup>3</sup>

<sup>1</sup>Department of Plant Pathology, <sup>2</sup>Department of Entomology, <sup>3</sup>Department of Agronomy School of Agricultural Science and Rural development, Nagaland University, Medziphema campus- 797106, Nagaland.

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Two biocontrol agents *Trichoderma harzianum*, *Pseudomonas fluorescens* and the chemical fungicide copper oxychloride were selected to evaluate the efficacy against Early Blight of potato under field condition. The most effective treatment against Early Blight of potato was observed to be tuber treatment with *T. harzianum* (PDI-17.50%) followed by tuber treatment with *P. fluorescens* (PDI-25.00%). The experiment on effect of biocontrol agents on germination of potato tubers was found statistically non-significant. The effect of the biocontrol agents on plant height was found statistically significant only at 40 DAS, while plant height recorded at 20 DAS and 80 DAS were non-significant. All the treatments showed significantly higher plant height compared to control at 40 DAS except T<sub>5</sub> (Tuber treatment *P. fluorescens* @ 8g/kg+ copper oxychloride @ 4g/l of water). The maximum potato yield (kg/ha) was obtained from T<sub>1</sub> (tuber treatment with *T. harzianum*@ 8g/kg) i.e., 6,875 kg/ha followed by T<sub>5</sub> (Tuber treatment with *P. fluorescens* @ 8g/kg + copper oxychloride @ 4g/l of water) (6458 kg/ha) and T<sub>4</sub> (Tuber treatment with *T. harzianum* @ 8g/kg+ copper oxychloride@ 4g/l of water) (6250 kg/ha) which are statistically significant when compared to the control treatment (3958 kg/ha).

**Keywords:** *Alternaria solani*, biological control, disease management, Early Blight, potato, *Pseudomonas fluorescens*, *Trichoderma*

### INTRODUCTION

Early blight or *Alternaria* blight is widely distributed and is the most important foliage diseases. This pathogen was reported in India for the first time from Farukhabad district of Uttar Pradesh. This pathogen is responsible for causing leaf and stem disease both on potato and tomato.

The symptoms include the appearance of dark, elongated or circular lesions with concentric rings and a yellow halo appearance around them. Early blight caused by two species of the genus *Alternaria* (*A. solani* and *A. alternata*) occurs worldwide on potato crops, particularly in the regions with higher temperatures and alternating periods of dry weather, low in organic matter (Pasche *et al.* 2004). At the growing stage, high infection may

reduce the yield by about 20-50% . It causes great damage of plant tissues due to the toxins released by pathogen (Devi *et al.* 2010). In Manipur climatic condition, mainly late and early blight, wilt and *Curvularia* diseases occurred on potato crop (Regar *et al.* 2020).

Management of Early Blight disease is done predominantly by application of fungicides. Biological methods using biocontrol agents provide a powerful alternative to the use of chemicals. Due to this, there is an increasing interest in obtaining alternative anti-microbial agents (biocontrol agents) for use in plant disease control systems. (Aldiba and Escov, 2019). Biocontrol agents are found to be the most effective and eco-friendly. Moreover, they are easily biodegradable, non-phytotoxic, systemic and safe to environment (Devi *et al.* 2017). The combination of biocontrol agents with fungicides would provide similar disease suppression as achieved with the higher fungicides

\*Correspondence: ysonia4u@gmail.com

used (Monte, 2001). In the present study, an attempt was made to evaluate the performance of biocontrol control agents for management of early blight disease of potato in field condition.

## MATERIALS AND METHODS

The experiment was carried out in the plain areas of Kiyamgei Awang Leikai, Imphal East district. Kufri Naveen potato variety was selected and the seed tubers were collected from the Regional Potato Farm at Mao, Senapati district through the State Horticulture Department Imphal, Manipur. For *in-vivo* study biocontrol agents *Trichoderma harzianum* (GUARD- T 1% WP powder, manufactured by IIHR, TH-2 Satrain Accession No. ITCC No.-6888) and *Pseudomonas fluorescens* 1% WP powder manufactured by TNAU were used. Fungicide, copper oxychloride (Blitox 50 WP) was also used as a chemical check.

The experiment was carried out in Randomized Block Design consisting of six different treatments with four replications each. The total field area was 140 m<sup>2</sup> with plot size of 3m x 2m. 24 plots were maintained with the spacing of 60 x 20 cm. The various treatment combinations used in the study were as follows: T0 : Control, T1 : Tuber treated with *Trichoderma harzianum* (TH) @ 8g/kg, T2, : Tuber treatment with *Pseudomonas fluorescens* (PF) @8g/kg, T3 : Two foliar applications of copper oxychloride (CoC) (Blitox 50 WP) @ 0.4% i.e. 4g/l of water, T4 @8g/kg+ two foliar spray of CoC @4g/l of water, T5: Tuber treatment with *Pseudomonas fluorescens* @ 8g/kg+ two foliar applications of CoC @ 4g/l of water.

Potato tubers were treated with *Trichoderma harzianum* and *Pseudomonas fluorescens* @ 8g / kg each (80g each for 10kg of tuber seeds) for 30 minutes, after which the tubers were air-dried at room temperature under shade before sowing.

Fungicide was prepared by mixing thoroughly with the required quantity of water, i.e., 4g/l of water. Fungicide was applied just after the first incidence of early blight disease on leaves exhibiting the characteristic symptoms. Two foliar sprays were also given at 65 DAS and 80 DAS. For the control

treatment in each block, spraying was done with plain water only. Irrigation was done at regular interval after 15 DAS and weeding was done five times after 20, 40, 60, 80 and 100 DAS.

### **Measurement of the Disease(% leaf area disease)**

Three readings at different stages at 50, 65 and 80 DAS were taken from each treatment. Five selected plants were tagged and the readings were taken on those days, respectively. The disease incidence from each selected plant was examined and scored for disease incidence on a 0-5 scale (Horsefall and Barette, 1945).

The Mckinney (1923) formula is used to calculate the Per cent Disease Index,

$$PDI = \frac{\sum \text{all numerical ratings}}{\text{Total number of leaves observed} \times \text{maximum disease rating (S)}} \times 100$$

### **Germination percentage**

The observations on germination of potato tubers were recorded at 16 DAS and the germination percent value was calculated by using the formula,

$$\text{Germination percentage} = \frac{\text{Number of germinated Seed}}{\text{Number of seeds planted}} \times 100$$

### **Plant height**

The height of potato plants was measured in centimeters from the ground level up to the base of the terminal fully opened leaves. The observations were taken after germination at 20 DAS and also at 40 DAS and 80 DAS with the help of a linear scale.

### **Yield**

The yield of potatoes per plot from each treatment was recorded and the data was expressed in kilogram per ha.

$$\text{Yield (Kg / ha)} = \frac{\text{Yield per plot}}{\text{Area of plot}} \times 10,000$$

## RESULTS AND DISCUSSION

### **Efficacy of biocontrol agents and copper oxychloride against early blight disease of potato**

The data presented in (Table1) depict that seed treatment with *Trichoderma harzianum* and *Pseudomonas fluorescens* 8g/kg each, recorded the least disease severity with 17.50% and 25.00% respectively compared to 72.08% in control at 80 DAS.

Tuber treatment with *T. harzianum* was significantly superior to all the other treatments except *P. fluorescens*. The effect of two other treatments viz., foliar application of COC @4g/l (33.75%) and tuber treatment with *T. harzianum* @ 8g/kg + COC @ 4g/l (32.50%) were statistically at par with T<sub>2</sub> i.e., tuber treatment with *P. fluorescens* @ 8g/kg (25.00%).

Similar findings were reported by Verma *et al.* (2018) and Lal *et al.* (2013) who found that *Trichoderma harzianum* and *Pseudomonas fluorescens* were most effective against the pathogen *A. solani*. It is also reported that highest percentage of mycelial inhibition of *A. solani* was given by *T. viride* (91.88%) and *T. harzianum*

(80.11%) compared to the control treatment *in vitro*. They also revealed that the collapsed hyphae and sunken conidia of *A. solani* were due to antagonistic effect of *T. viride* and *T. harzianum* when observed under scanning electron microscope.

### **Efficacy of biocontrol agents on growth parameters of potato plants**

#### **Potato germination**

Potato seed germination was found to be enhanced in all the treatments compared to control (Table 2). Tuber treatment with *Pseudomonas fluorescens* recorded better germination (100%) compared to control treatment (90%).

#### **Height of potato plant**

The effects of various treatments on the height of potato plant were statistically non-significant at 20 and 80 DAS (Table 3). The effects of the treatments were found to be significantly different only after 40 DAS. The maximum height of potato plant (31.50 cm) was observed in T<sub>2</sub> (tuber treatment with *P. fluorescens* @ 8 g/kg seed) which is significantly superior to control where the plant

**Table 1:** Effects of biocontrol agents on severity of early blight disease of potato

Treatment	Per cent disease index (PDI)		
	50 DAS	65 DAS	80 DAS
T0 -Control (Water)	20.00 (4.45)	34.75 (36.06)	72.08 (58.50)
T1 -Tuber treatment with <i>Trichoderma harzianum</i> @ 8g/kg	4.58 (1.88)	5.83 (10.68)	17.50 (24.44)
T2 -Tuber treatment with <i>Pseudomonas fluorescens</i> @ 8g/kg	0.00 (0.70)	6.25 (8.58)	25.00 (29.36)
T3 -Foliar application with Copper Oxychloride @ 4g/l of water	3.75 (1.51)	22.50 (28.05)	33.75 (35.20)
T4 -Tuber treatment with <i>Trichoderma harzianum</i> @ 8g/kg + Copper Oxychloride @ 4g/l of water	5.00 (1.66)	30.00 (32.89)	32.50 (34.60)
T5 -Tuber treatment with <i>Pseudomonas fluorescens</i> @8g/kg + Copper Oxychloride@ 4g/l of water	0.00 (0.70)	22.50 (27.24)	38.75 (38.45)
SEm ±	0.57	4.80	3.63
C.D.(p=0.05)	1.84	14.01	10.96

Note: Figures in the parenthesis denote square root (50 DAS) and arc sin transformed (65 DAS and 80 DAS) values

**Table 2:** Effects of biocontrol agents on potato seed germination

Treatments	Germination percentage (%) at 16 DAS
T0 -Control (water)	90.00 (9.52)
T1 -Tuber treatment with <i>Trichoderma harzianum</i> @ 8g/kg	98.75 (9.98)
T2 -Tuber treatment with <i>Pseudomonas fluorescens</i> @ 8g/kg	100.00 (10.05)
T3 -Foliar application with Copper Oxychloride @ 4g/l of water	97.50 (9.92)
T4 -Tuber treatment <i>Trichoderma harzianum</i> @ 8g/kg+ Copper Oxychloride @ 4g/l of water	98.75 (9.98)
T5 -Tuber treatment <i>Pseudomonas fluorescens</i> @ 8g/kg+ Copper Oxychloride @ 4g/l of water	97.50 (9.92)
SEm ±	0.139
C.D.(p=0.05)	N/A

Figures in the parenthesis denote square root transformed values

**Table 3:** Effects of biocontrol agents on height of potato plant

Treatments	Plant height (cm)		
	20 DAS	40 DAS	80 DAS
T0 -Control (Water)	3.25	18.75	24.75
T1 -Tuber treatment with <i>Trichoderma harzianum</i> @ 8g/kg	4.25	27.50	28.75
T2 -Tuber treatment with <i>Pseudomonas fluorescens</i> @ 8g/kg	4.25	31.50	33.50
T3 -Foliar application with Copper Oxychloride @ 4g/l of water	3.25	30.75	32.50
T4 -Tuber treatment <i>Trichoderma harzianum</i> @ 8g/kg+ Copper Oxychloride @ 4g/l of water	4.00	29.00	29.75
T5 -Tuber treatment <i>Pseudomonas fluorescens</i> @ 8g/kg+ Copper Oxychloride @ 4g/l of water	5.50	22.50	25.00
SEm ±	0.58	2.47	2.35
C.D.(p=0.05)	N/A	7.53	N/A

DAS – Days after Sowing

height of only 18.75 cm were recorded. Lal (2018) also noted that growth parameters of potato crop including fresh shoot weight, number and weight of potato tubers were enhanced with the treatment of *P. fluorescens* on potato crop.

### **Effects of biocontrol agents on yield of potato**

It is clear from the data given in Table 4 that the maximum yield of 6875 kg/ha was recorded in T1 treatment (tuber treatment with *T. harzianum* @

**Table 4:** Effects of biocontrol agents on yield of potato (Kg/ha)

Treatments	Yield (Kg/ha)
T0 -Control (Water)	3,958.50
T1 -Tuber treatment with <i>Trichoderma harzianum</i> @ 8g/kg	6,875.00
T2 -Tuber treatment with <i>Pseudomonas fluorescens</i> @ 8g/kg	5,625.00
T3 -Foliar application with Copper Oxychloride @4g/l of water	4,208.25
T4 -Tuber treatment with <i>Trichoderma harzianum</i> @ 8g/kg+ Copper Oxychloride @ 4g/l of water	6,250.00
T5 -Tuber treatment with <i>Pseudomonas fluorescens</i> @ 8g/kg + Copper Oxychloride @ 4g/l of water	6,458.50
SEm ±	687.98
C.D.(p=0.05)	2,092.71

8g/kg) followed by T<sub>5</sub> (tuber treatment with *P. fluorescens* @ 8g/kg + copper oxychloride @ 4g/l of water) (6458 kg/ha) and T<sub>4</sub> (tuber treatment with *T. harzianum* @ 8g/kg+ copper oxychloride @ 4g / l of water) (6250 kg/ha) which are significantly superior to yield in control treatment (3958 kg/ha). The treatment T<sub>2</sub> (tuber treatment with *P. fluorescens* @ 8g/kg) and T<sub>3</sub> (foliar application with copper oxychloride @4g/l of water) recorded the potato yield of 5625 and 4208kg/ha respectively which are statistically *at par* with the control treatment. Similar findings were reported by Murmuet *al.* (2015) who found that *T. viride* exhibited best performance in terms of reducing early blight of potato and production of economic yield (25.51t/ha) which is followed by *P. fluorescens* (23.65t/ha). Mane *et al.* (2014) and Lal *et al.* (2016) also found that the bioagents *T. harzianum* and *P. fluorescens* when applied as seed treatment followed by foliar spray were effective in reducing the disease intensity of early blight of potato and also increasing tuber yield. The yield increased obtained from our study might have resulted either from suppression of pathogen infection from direct interactions between *Trichoderma* strains and potato plants in terms of hormonal regulation or

nutrient acquisition or possibly a combination of these effect (Bhattacharjee and Dey, 2014).

## CONCLUSION

It may be concluded from the present study that the biocontrol agents under study *T. harzianum* and *P. fluorescens* were found effective in improving growth parameters and yield of potato plant as well as reducing the incidence of early blight disease in field. To buttress the existing experimental results in future, similar experiment can be carried out with the priority on finding out more productive management strategies with the combination of various bioagents in various potato growing regions. The use of biocontrol agents in certain combination practices which includes soil treatment, seed treatment and foliar spray of it, can be more effective with lesser risk to the ecosystem and cost efficient.

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