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Eco-friendly management of late blight of potato using *Trichoderma* spp. and plant extracts

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Abstract
The Potato (*Solanum tuberosum* L.) ranks fourth in the world in terms of importance following wheat, maize and rice. Potato is a rich and balanced source of starch, vitamins and different minerals for many communities in almost all over the world. One of the main reasons of low productivity of potato is the late blight disease incited by *Phytopthora infestans*. For this study local variety (3797) was used and grown at the Organic Research Farm, Karguanji, Department of Plant Pathology, Institute of Agricultural Sciences, Bundelkhand University, Jhansi campus during *rabi* 2021-22. Different *Trichoderma* spp. and plant extracts was used to observe its effect on plant growth and yield characters. The maximum tubers yield (12.90 t/ha) was recorded in treatment T_1- *Trichoderma viride* and minimum tubers yield (8.69 t/ha) was recorded in control treatment. Among plant extracts, maximum tuber yield (12.61 t/ha) was found with Tulsi leaf extract treated plots. Thus, this investigation showed that bio-agents and plant extracts are highly effective in combating late blight of potato and it may be a better alternative in place of chemical for sustainable agriculture.

Keywords: *Phytopthora infestans*, potato, *Trichoderma*, management

1. Introduction
Potato (*Solanum tuberosum* L.) popularly known as "The King of Vegetables" has emerged as fourth most important food crop in India. Because of the potato's high dry matter, eatable energy and eatable protein contents, it a superior vegetable in terms of nutrition and a staple food not only in our nation but also around the world, It yields more dry matter, edible energy, and edible protein in a shorter amount of time than cereals like rice and wheat since it is a short-duration crop. So, the potato could prove to be a beneficial source for ensuring the nation's nutritional security. Area and production of potato has increased at a very fast rate in the country for last four to five decades. Total area under potato cultivation in India is about 2.2 million hectares with a production of 53.39 million tons during 2021-22 (Anonymous, 2022) [1], among which states of Uttar Pradesh, West Bengal and Bihar holds major share of the production.

Around 1 million people died in the Great Irish Famine due to starvation which was brought on by potato late blight, and another million were compelled to leave the country. The population of Ireland is still less than three-quarters of what it was at the start of the 1840s. (Yoshida *et al*., 2013) [2]. Late blight of potato affects all plant parts especially leaves, stem and tubers. On the leaf surface, the infection results in production of pale green water-soaked spots of 2- 10 mm size, which usually appears on the leaf tips and margin. Spots can form anywhere on the leaves during moist weather. Later, light brown lesions start to appear on the stem and petioles also. As these lesions grow larger and encircle the stem and petioles, they break them and kill the entire plant. The typical late blight symptoms, such as dark discolouration of the flesh, are seen on the tubers.

The disease causes the most severe losses to potato crops in Sub-Saharan Africa (44%), followed by Latin America (36%), the Caribbean (36%), South-East Asia (35%), South-West Asia (19%), and the Middle East and North Africa (9%) due to late blight (CIP, 1997) [3]. Several workers have investigated details about multiple aspects of late blight. (Singh and Bhat, 2003; Fry, 2008; Cooke *et al*., 2011) [4, 5, 6]. In the 20th century, countries in Europe and America countries began using botanicals to manage late bight in an environmentally conscious way. Plant extracts of five different plant materials *viz. Brassica nigra*, *Cinnamomum camphora*, *Eupatorium adenophorum*, *Lantana camara* and *Melia azedarach* considerably inhibited the growth of the *P. infestans* and were found effective for the

management of disease (Maharjan *et al*., 2010) [7]. Although biological control occurs naturally however, in numerous cases, the knowledge essential to explain how biological control works and how abiotic or biotic components might be changed to influence the economic control of a disease is not yet readily available. *Trichoderma* spp. are among of the most significant bio-control agents for plant pathogenic fungus. Rapid growth, tufting or postulating, repeatedly branched conidiophore with phialides, and hyaline or green conidia formed in slimy heads are all typical characteristics of *Trichoderma* (Alexopoulus *et al*., 1996) [8]. Therefore, study on efficacy of plant extracts and *Trichoderma* spp. in management of late blight of potato was conducted under natural epiphytotic conditions in field.

2. Material and Methods

The experiment was carried out at the Organic Research Farm in Karguanji, Jhansi during the rabi season (2021–2022) under natural epiphytotic conditions. Total nine treatments were laid out in a Randomized Block Design with three replications per treatment. The Jhansi is situated at an elevation of 271 m above mean sea level (24 "11" N latitude and 78 "17" E longitude). Bundelkhand region of India's central plains contain 13 districts with total of 7.08 million hectares (ha) area. Among which six (MP) districts cover 4.12 million ha and seven (UP) districts 2.94 million ha. The potato tubers of the susceptible varieties (3797) were procured from local market of Jhansi for conducting the experiment. In the state of Uttar Pradesh,

2.1 Collection and identification of the pathogen

The samples of late blight of potato infected leaves were collected form Organic Research Farm of IAS, Bundelkhand University, Jhansi (U.P.) during *rabi* season 2021-22. The infected younger leaves were collected based on the pathological symptoms described by Agrios, (2005) [9]. From infected samples, *Phytophthora infestans* was isolated and identified based on its morphology, cultural traits, and pathogenic behavior toward the host referring to description given by Erwin *et al*. (1983) [10].

2.2 Preparation of culture media

Selective tomato based culture media was prepared for the isolation of *P. Infestans* (tomato juice: 200 ml, calcium carbonate: 0.04 g, Agar powder: 20 g, distilled water: 800ml). Fresh, healthy tomatoes were purchased and properly washed under running water before being cleaned with distilled water to eliminate dust and other debris. Tomatoes were then cut into the small pieces and grinded in a mixer grinder. The resultant slurry was put through a sieve to remove big bits of tissues. 200ml filtrate was measured in a measuring cylinder and final volume made up to 1 litre by adding the distilled water. Agar powder (20 g) calcium carbonate (0.04g) was added slowly and the solution was boiled for some time. The ready media was then poured in conical flasks and autoclave at 15 lb/inch^2 pressure (15 psi) for 20 minutes at 121 °C.

2.3 Efficacy of Bio-agents

Four isolates of *Trichoderma* spp. (T*richoderma harzianum, Trichoderma viride, Trichoderma asperellum and Trichoderma citrinoviride*) selected for the present study was identified from Indian Type Culture Collection (ITCC), Division of Plant Pathology, Indian Agriculture Research Institute, New Delhi, India.

2.4 Mass multiplication of *Trichoderma* sp. isolates

Trichoderma sp. isolates were mass-multiplicated using wheat grains.

Grains were steeped in water for 12 hours and then spread on a clean sheet to drain out the excess water. After mixing 2% dextrose the air dried grains were then filled in conical flasks. Sterilization of wheat grain filled conical flasks was done in autoclave at 121 °C temperature at 15 psi pressure for 20 minutes. These sterilized conical flasks containing grains were inoculated with actively growing culture of *Trichoderma* sp. then put in BOD incubator at 26±2 °C.

2.5 Efficacy of bio-against against *Phytophthora infestans*

The sterilized soil was well mixed with 1% (w/w) pure inoculum of the different *Trichoderma* sp. viz., *T. viride, T. asperellum, T. citrinoviride* and *T. harzianum.* The *Trichoderma* inoculated soil was placed in plots (3×3 m^2) after 7 days to allow the *Trichoderma* sp. to establish well in soil. Approx. 50 percent soil moisture was maintained by adding water once a day.

2.6 Preparation of plant extracts

Water extract of the leaves of four different plants viz., Neem (*Azadirachta indica*), Tulsi (*Ocimum tenuiflorum*), Datura (*Datura stramonium*) and Lemon Grass (*Cymbopogon citratus*) were prepared to evaluate their efficacy against *Phytopthora infestans*. Fresh leaves were gathered, and they were properly cleaned with pure water. A pestle and mortar was used to crush 100 g of each cleaned plant material with an equal amount (100 ml) of sterilized water (1:1 w/v) before being heated at 80 °C for 10 minutes. The materials were purified using bilayered muslin cloth, thereafter with sterilized Whatman No. 1 filter paper and the resultant got was 100% plant extract.

2.7 Application of plant extracts on potato

The plant extracts were applied at the concentration of 10% as foliar spray (30 ml/plant). The first foliar spray of the plant extracts was done on seventh week i.e. 45 days after sowing (DAS) and the second spray was done after 15 days of the first spray. The disease severity was recorded in each plot by calculating per cent disease index (PDI) using standard 0-9 rating scale given by (Malcolimson, 1976) [11] and using formula McKinney (1923) [12] and subjected to ANOVA.

$$\text{Disease index (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of samples observed} \times \text{maximum disease grade}} \times 100$$

3. Results and Discussion

Observations on plant growth and yield parameters upon application of plant extracts and bio-agents under *in vivo* conditions are being presented as under

3.1 Efficacy of *Trichoderma* spp. and plant extracts on plant growth and yield attributes

3.1.1 Plant height (cm)

The highest plant height was measured in T_1- *T. viride*, 5.65, 17.72 and 33.30 cm followed by T_5- neem extract, 3.65, 16.52 and 29.66 cm and T_2- *T. asperellum*, 5.57, 16.54 and 32.37 cm at 30, 45 and 60 DAS, respectively whereas, minimum lowest plant height was measured in T_9- Control, 3.07, 11.22 and 25.33 cm at 30, 45 and 60 DAS, respectively (Table 1). Similar effect on plant height of potato was also reported by Maharajan *et al.* (2010) [13].

Table 1: Efficacy of *Trichoderma* spp. and plant extract on plant height of potato

Treatments	Plant height (cm)		
	30 DAS	45 DAS	60 DAS
T_1- *T. viride*	5.65	17.72	32.80
T_2- *T. asperellum*	5.57	16.54	32.37
T_3-*T. citrinoviride*	4.68	15.79	33.30
T_4- *T. harzianum*	4.12	14.67	31.29
T_5- Neem leaf extract	3.65	16.52	29.66
T_6- Tulsi leaf extract	4.45	12.44	28.70
T_7- Datura leaf extract	3.63	15.51	31.85
T_8-Lemon grass leaf extract	3.85	13.53	27.75
T_9- Control	3.07	11.22	25.33
S.Em±	0.14	0.19	0.14
C.D. at 5%	0.43	0.57	0.42

3.1.2 Number of branches and tubers per plant

Maximum branches per plant was found in T_1- *T. viride* (4.05) followed by T_5- Neem extract (3.83) and T_2- *T. asperellum* (3.47) and proved significantly superior among other treatments (Table 2) and minimum number of branches per plant was observed in treatment T_9- Control (2.62). Similar findings were also reported by Abd-El-Khair and Haggag (2007) [14]; Maharajan *et al.* (2010) [13]. The most tubers per plant were o with in T_1- *Trichoderma viride*, (15.81) followed by T_5- Neem extract, (17.40) after harvesting and minimum number tubers per plant were found in T_9- Control (11.58).

Table 2: Efficacy of *Trichoderma* spp. and plant extract on number of branches and tubers per plant of potato

Treatment	Number of branches per plant	Number of tubers per plant
T_1- *T. viride*	4.05	15.81
T_2- *T. asperellum*	3.47	14.70
T_3-*T. citrinoviride*	4.20	16.23
T_4- *T. harzianum*	4.37	14.39
T_5- Neem leaf extract	3.83	17.40
T_6- Tulsi leaf extract	3.25	12.23
T_7- Datura leaf extract	5.37	15.62
T_8- Lemon grass leaf extract	4.46	16.42
T_9- Control	2.62	11.58
S.Em±	0.11	0.11
C.D. at 5%	0.32	0.34

3.1.3 Yield attributes

The maximum tubers yield was recorded with T_1- *T. viride*, (12.90 t ha^{-1}) followed by T_6- Tulsi extract (12.61 t ha^{-1}) and T_7- Datura extract (12.50 t ha^{-1}) proved significantly superior in comparison to other treatments and the minimum yield was found in T_9- Control, (8.69 ha^{-1}) and showed in Table 3. Similar findings were reported by other workers also (Malathi and Jeyaranjan, 1995; Hamed, 1999) [15, 16]. The maximum per cent incidence in tubers yield was found in T_1- *T. viride*, (48.45%) followed by T_6- Tulsi leaf extract, (45.11%) and T_7- Datura leaf extract (43.84%). These treatments proved significantly superior to rest of the treatments and minimum per cent increase in tuber yield was observed with T_8- Lemon grass leaf extract (2.19%). Similarly, Saikia and Azad (1999) [17] and Muthukumar *et al.* (2011) [18] also observed similar trends of increase in potato tuber yield.

Table 3: Efficacy of *Trichoderma* spp. and plant extracts on tuber yield of potato

Treatment	Tuber yield (t/ha)	Increase over control (%)
T_1- *T. viride*	12.90	48.45
T_2- *T. asperellum*	10.95	26.01
T_3-*T. citrinoviride*	9.41	8.29
T_4- *T. harzianum*	9.32	7.25
T_5- Neem leaf extract	8.90	2.41
T_6- Tulsi leaf extract	12.61	45.11
T_7- Datura leaf extract	12.50	43.84
T_8- Lemon grass leaf extract	8.88	2.19
T_9- Control	8.69	-
S.Em±	0.17	-
C.D. at 5%	0.51	-

3.2 Effectiveness of *Trichoderma* spp. and plant extracts on PDI of late blight in potato

The minimum PDI of disease was recorded in T_1- *T. viride* (11.29%) followed by T_5- Neem leaf extract (12.60%) and T_2- *T. asperellum* (12.72%) and maximum PDI of late blight of potato was calculated with T_9- Control (27.70%) (Table 4). Similar results have also been reported by Rani *et al.* (2006) [19] in evaluation of the efficacy of five neem formulations (Tricure, Neem Gold, Floriguard, Vegfru-guard, Neem) and one botanical extract (Wanis) against late blight of potato (*Phytophthora infestans*) in Cv. Kufri Bahar. Other workers have also reported similar findings of reduction of diseases caused by *Phytophthora* through *Trichoderma* and plant extracts (Ephrem *et al*., 2011; Sharma *et al*., 2014; Gupta, 2016) [20, 21, 22].

The maximum per cent disease control was recorded with T_1- *T. viride* (59.24%) followed by T_5- Neem leaf extract (54.51 %) and T_2- *T. asperellum* (54.08%) that proved significantly superior to other treatments. Whereas, minimum disease control (%) was found in T_6-Tulsi leaf extract (18.74%) during the experiment (Table 4). Similar funding on potato was supported by Malathi and Jeyaranjan, 1995 [15]. Blaeser *et al.* (1998) [23] investigated 35 plant extracts for their ability to inhibit *Phytophthora infestans*. Of the 35 extracts tested, 32% demonstrated efficacies between 50 and 80%, and 5% demonstrated efficacies more than 80%.

Table 4: Efficacy of *Trichoderma* spp. and plant extracts on PDI of late blight in potato

Treatment	Disease index (%)	Disease control (%)
T_1- *T. viride*	11.29	59.24
T_2- *T. asperellum*	12.72	54.08
T_3-*T. citrinoviride*	14.54	47.51
T_4- *T. harzianum*	15.80	42.96
T_5- Neem leaf extract	12.60	54.51
T_6- Tulsi leaf extract	18.74	32.35
T_7- Datura leaf extract	16.50	40.43
T_8- Lemon grass leaf extract	14.70	46.93
T_9- Control	27.70	-
S.Em±	0.17	-
C.D. at 5%	0.52	-

4. Conclusion

Based on the results of the current research, it has been established that the *Trichoderma viride* was most effective among bio-agents and plant extract also exhibited efficacy in managing the late blight of potato as well as increasing yield of potato tubers. Therefore, *T. viride* may be recommended as soul or a component in integrated disease management for management of late blight particularly in potato crop.

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