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Efficacy of different *Trichoderma* spp. and Fungicides against late blight of potato caused by *Phytophthora infestans* (Mont.) de-Bary

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Abstract

The potato (*Solanum tuberosum*) is one of the most important vegetable crops in the world, belonging to the family solanaceae and is an important starchy food crop in both sub-tropical and temperate regions. Potato plants are subjected to attack by numerous diseases wherever the crop is grown. Among them, late blight of potato caused by *Phytophthora infestans* (Mont.) de-Bary is of major cause of concern in potato production at present. An experiment was conducted in the Department of Plant Pathology, College of Agriculture, C S A U A & T, Kanpur. During Rabi season 2016-17 to control. The antifungal activity of different fungicides was evaluated *in-vitro* through Food poison technique. The experimental finding showed that radial growth of mycelium of *Phytophthora infestans* was inhibited by fungicides over control. At 100 ppm, the minimum radial growth of mycelium was found in Equation Pro treatment as 5.3, 8.2, 11.4, 14.2, 16.2, 18.4 and 22.6 mm over control against 12.3, 19.1, 26.1, 35.9, 42.5, 51.4 and 64.8 mm at 1, 2, 3, 4, 5, 6 and 7 days after inoculation respectively. Similarly, 500 and 1000 ppm the minimum radial growth of mycelium was found in Equation Pro treatment. Efficacy of bio-control agents on the radial growth of *Phytophthora infestans* was evaluated using Dual Culture Methods. Among the different concentrations 1000 ppm was found most effective than 100 and 500 ppm. Among the different bio-agents, *T. harzianum* able to reduced maximum radial mycelial growth of fungus showing 4.6, 8.8, 10.1, 13.2, 15.6, 19.3 and 23.5 mm against 12.3, 19.1, 26.1, 35.9, 42.5, 51.4 and 64.8 mm at 1, 2, 3, 4, 5, 6 and 7 days after inoculation, respectively.

Keywords: *Trichoderma* spp, fungicides, potato, *Phytophthora infestans* and growth of mycelium

Introduction

Potato (*Solanum tuberosum* L.) belongs to the family *Solanaceae*, considered as "King of vegetables". It is an important tuberous crop cultivated for vegetable, food and several other processed products. It can be grown in wider range of altitude, latitude and climatic conditions. The English word "Potato" comes from Spanish word "patata" (the name used in Spain). Food and Agriculture Organization of UN has declared potato as 'Food for future'. (Hawkes, 1989)^[16]. The origins of the potato can be traced back to South American natives in 5000 B.C. in the highlands of the Peruvian Andes Mountains. Around 1570, the potato reached Europe with the returning Spanish explorers. There are more than 160 wild potato species and most of them contain high levels of alkaloids. The first edible potato is considered to have been cultivated 4000 years ago in Peru (Hijmans and Spooner, 2001)^[17]. Potato represents valuable sources of nutrient in a balance diet. The average nutritional value of 200g potato is about Calories: 200, Protein: 4.6g, Carbohydrate: 51g, Fat: 0.2g, Cholesterol free and good source of dietary fiber with 4-8g, Niacin: 16mg, Vitamin B6: 7mg, Vitamin C: 26mg Iron: 2.75mg, Magnesium: 55mg, Thiamine: 22mg and Pantothenic acid: 1.12 (USDA 2014)^[32].

Now a days potato is also gained a considerable importance as an export crop to European markets and other parts of the world which is considered an important source for national income. (El-Sirafy *et al.*, 2008; El-Mougy, 2009)^[9, 8]. India also exports potato to Nepal (32.1%), Sri Lanka (30.4%), Russia (18.6%), Malaysia (5.8%), Mauritius (4.9%) and many other countries of the world. It is the world's fourth important food crop after wheat, rice and maize because of its higher yield potential and high nutritive value. Recently, potato is grown in about 150 countries of the world and the total area under potato production during 2011 was about 19.33 million hectares with a total production of about 19.33 million tons which is

consumed by over a billion of peoples across the globe, of which half are in the developing countries (Sumit *et al.*, 2018) [30]. The five major potato producing country in the world are China (96.01 MT), India (45.9 MT), Russia (30.9 MT), Ukraine (23.0 MT) and USA (19.9 MT) during 2013-14 (FAOSTAT 2016). The production and productivity of potato is quite impressive. During 2004, global potato production was recorded as 336 MT which significantly increased to 374 MT, with more than 80% production recorded in Europe and Asia (Axel *et al.*, 2012; FAOSTAT 2016). India produced 41.565 MT of potato from an area of 18.87 lakh hectares of land during 2011-12 (Gracy *et al.*, 2013) [12]. In India, the leading potato producing states are Uttar Pradesh 33% followed by West Bengal 22%, Bihar 16%, Madhya Pradesh 6%, Gujarat 5%, Punjab 5%, Assam 2%, Haryana 2%, Karnataka 1%, Jharkhand 1%, others 7% which constitute about 100% of the total domestic potato production (Indian Horticulture Database - 2014, NHB) [18]. From the last few years, potato production in Uttar Pradesh has caught the attention of many prospective food processing entrepreneurs and even the Netherlands ambassador Alphonsus Stoelinga, during his recent visit to Lucknow, had said that his country was looking for setting up potato processing centre in Uttar Pradesh (Gracy *et al.*, 2013) [12].

Potato suffers from a number of diseases caused like, early blight, late blight, leaf spot, dry rot, charcoal rot, black scurf, common scab, soft rot, leaf roll *etc.* Among them, late blight caused by *Phytophthora infestans* (Mont.) de Bary is the most important and most destructive disease of potato causes "Irish Famine in 1845". The disease is distributed all over the world like North & South America, Europe, Asian continents (Fry *et al.*, 1993; Chowdappa *et al.*, 2013) [10, 5]. In last few years, late blight has become a significant epidemic problem in North Africa and Morocco (Sedgui *et al.*, 1999; Andrivon *et al.*, 2007) [28, 3], Tunisia (Jmour and Hamada, 2006; Harbaoui *et al.*, 2013) [19, 14] and Algeria (Corbière *et al.*, 2010) [6]. The annual economic losses caused by the disease world wide have been about 170 billion US dollars (Haverkort *et al.*, 2009; Wu and Gui *et al.*, 2012) [15, 33]. In India, the losses caused by the disease are 10-20% in Uttar Pradesh, 10-15% in West Bengal and Punjab, 10-15% in Karnataka and Uttarakhand also have been reported during 2013-14 (Lal *et al.*, 2016) [21] but the amount of losses depends on the variety and plant protection measures adopted.

Materials and Methods

Collection of infected plant samples

Late blight infected leaves were collected from the potato field at Vegetable Research Farm, C. S. Azad University of Agriculture and Technology, Kanpur. Infected leaves with sporulation lesions were taken from the field and washed in sterilized water and stored in BOD for further study.

Preparation of culture media

The *Phytophthora infestans* causes late blight of potato belongs to Class Oomycetes and generally not grown on Potato Dextrose Agar Media. Therefore, following selective culture media are being used to isolate the fungus.

Tomato based specific media

Tomato juice medium

Tomato juice	-	250ml
Calcium carbonate	-	0.04gm
Agar powder	-	20gm
Dextrose	-	20gm

Distilled water - 1000ml

The tomato dextrose agar medium prepared was sterilized at 121.6 °C, 15 psi. For 15 minutes in an autoclave.

Procedure

Fresh and healthy tomato were collected from market and washed thoroughly in running tap water and then distilled water to remove dust and foreign matter from the surface. 250 gm tomato was taken and cut into the small pieces and grinding with electric mixer or Oster food blender. The obtained slurry was passed through a sieve with a pore size of 1.5×1.5 mm to remove large pieces of tissues. The filtrate was measured in a measuring cylinder and final volume made up to 1 lit by adding more distilled water. It was again poured in sauce pan and heated. 20 gm agar powder was added slowly in heating juice. The solution was boiled for some time till it tends to solidify on cooling. The prepared media was then poured in four conical flask of about 200 ml. 10 ml of media was poured in 10 culture tubes. Both conical flask and culture tubes were plugged with non-absorbent cotton and mouth was wrapped with butter paper and rubber band. The culture tubes were placed vertically in wire baskets. The media in flask and culture tubes were then auto claved at 15 lb/inch² pressure (15 psi) for 20 minutes at 121.6 °C.

Isolations of pathogen

A small piece of infected leaf from border of sporulating lesion along with some healthy green tissue was cut and dipped in mercuric chloride solution (0.1%) for 30 seconds followed by rinse in sterilized distilled water thrice and dried off with sterilized filter paper. The small pieces were then placed on tomato extract based media which was previously pour in sterilized in Petri plates. The plates were then incubated at 18± 1 °C. The Petri plates were observed daily to find out the presence of mycelium around the leave bits. As soon as the mycelia growth is notices around the bits, the pathogen was purified by hyphal tip culture method.

Purification of *Phytophthora infestans*

The white mycelial bits of *Phytophthora infestans* was removed from the margin of fungal colony and then transferred to another Petri-plate which was previously poured with sterilized tomato extract based medium. After purification, the pure culture of *Phytophthora infestans* was transferred on slant medium and incubated at 15-18 °C in darkness till full growth. The culture was then transferred into the incubator at 10-12 °C for further use.

Identifications of *Phytophthora infestans*

The isolated pathogen was identified on the basis of its morphological, cultural characters and pathogenic behaviour towards the host. *P. infestans* belong to the class Oomycetes. The vegetation is mycelium characterized by the absence of cross walls, along with both asexual and sexual reproduction occurs. The sporangiophores and sporangia emerge at asexual reproduction phase. The sporangia are lemon shape, measurement of 21- 38µm× 12-23µm. Sporangia develop at the end of these sporangiophores. The pathogen was found to produce the characteristics leaf blight symptoms on the affected plants. The isolation pathogen was identified on the basis of its morphological and cultural characters and pathogenic behaviour towards the host.

Maintenance of the culture

After confirmation of isolated pathogen as *P. infestans*, the pure culture was transferred on media slant and maintain in the BOD at 10 - 12°C for further study.

Pathogenicity test

The pathogenicity test of isolated fungus was conducted on healthy potato plants in order to establish the pathogenic nature of the fungus. The pathogenicity was tested according to Koch's postulates (1882).

The earthen pots of 30cm diameter were taken to conduct the present experiment. Initially the pots were filled with sterilized soil and water was added to bring the soil under good tilt condition. The healthy tubers of potato variety *Kufri Bahar* (3797) were placed in these pots and were allowed to grow for one month. The homogenized spore suspension was prepared in sterilized water from 7 days old culture of *P. infestans*. The suspension was sprayed on one month old potato plants @ 2ml/plant. The inoculated plants were placed on the bench of glass house. After 2-3 days, the plants began to show the symptoms of blight. The inoculated plants showed pale to dark green spots occur at the leaf tips and margins that change into brown or black lesions later. These lesions are not delimited in size and enlarged rapidly in a favourable weather. On the lower side of leaves, a white mildew appears on the surface of lesions where the pale and purplish tissues join. These symptoms confirmed that the blighting was caused by *P. infestans*.

Comparative evaluation of fungicides against *Phytophthora infestans* (in-vitro)

Seven fungicides belonging to different groups viz were screened against the pathogen under laboratory conditions to find out their relative efficacy in inhibiting the growth of the pathogen in culture by the "Poison Food Technique" (Schmitz, 1930) [26]. Required quantity of each fungicide was incorporated in already prepared two per cent tomato extract medium prior to solidification and thoroughly mixed them by shaking prior to pouring in sterilized Petri plates. The medium was allowed to solidify and then 5 mm bits of fungal culture from seven days old culture were placed at the centre of Petri plates.

The fungal disc was reversed so that the pathogen could come in direct contact with the medium. Three replications were kept for each treatment. The Petri plates were incubated at 18±1°C. One set of control was maintained in which the medium was not mixed with any fungicide but simply inoculated with the pathogen. The data on radial growth of fungal colony was measured in mm after every 24 hours till the control Petri plates were not filled up. The per cent inhibition over control was calculated by the following formula as given by Bliss (1934) [4].

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Percent inhibition in mycelia growth

C = Growth of pathogen in control plates.

T = Growth of pathogen in treated plates.

Results and Discussion

The preliminary works have been undertaken for comparative evaluation of different, bio-agents and chemicals against *P. infestans*, *in-vitro* through Poison Food Techniques. The data presented in Table-1 showed that the minimum radial growth of mycelium was found in Equation Pro treatment, representing 5.3, 8.2, 11.4, 14.2, 16.2, 18.4 and 22.6 mm at 1, 2, 3, 4, 5, 6 and 7 days after inoculation against 12.3, 19.1, 26.1, 35.9, 42.5, 51.4 and 64.8 mm in case of control at 100 ppm. Similarly at 500 and 1000 ppm, the minimum radial growth were noted from Equation Pro treatments. From the (Table 2,3), it is also cleared that among the different concentrations, 1000 ppm is found most effective to reduce the mycelial growth of *P. infestans*. Similar findings were also reported by several workers against several pathogenic fungi. (Kone *et al.*, 2009, Alex, 2019, Ziv and Zitter 1992) [20, 2, 35]. The bicarbonates salts has been shown to have a profound inhibitory effect on several fungi and causes the collapse of hyphal walls and shrinkage of conidia (Punja and Grogan, 1982; Ziv and Zitter, 1992; Abd-El-Kareem, 2012; and Montaser Fawzy *et al.*, 2012) [24, 35, 1, 23]. Fungicides of different formulations of inorganic and organic materials which have the potentials of growth inhibition, killing of zoospores and zoospores and mycelium of the causative organism (Song *et al.*, 2003) [29].

Efficacy of bio-control agents viz *Trichoderma harzianum*, *T. viride*, *T. koningii* and *T. virens* on the growth of *P. infestans* was evaluated using Dual Culture Technique. The result presented on the table-4 showed that all the treatment significantly reduced the radial growth of mycelial of the pathogens. Among the different bio-agents, *T. harzianum* able to reduced maximum radial mycelial growth of fungus showing 4.6, 8.8, 10.1, 13.2, 15.6, 19.3 and 23.5 mm over control as 12.3, 19.1, 26.1, 35.9, 42.5, 51.4 and 64.8 at 1, 2, 3, 4, 5, 6 and 7 days after inoculation, representing 63.73% inhibition over control at 7 days of inoculation. Shailbala and Pundhir, (2007) [27] and Glare *et al.*, (2012) [11] found that among the seven potato phylloplane fungi, only three fungi viz., *Fusarium* spp, *Trichoderma* spp, *Aspergillus* spp showed antagonistic potential against *P. infestans*, causal agent of late blight of potato. Many bio-agents i.e. *Trichoderma viride*, *Penicillium viridicatum*, *P. aurantiogriseum*, *Chetomium brasiliense* (Gupta *et al.*, 2004) [13], *Acremonium strictum* (CPRI, 1999) [7], *Myrothecium varrucaria* and *P. aurantiogriseum* (Roy *et al.*, 1991) [25] showed antagonistic effect against *P. infestans* in lab studies. The antagonistic activities of *Pseudomonas fluorescens*, *Pseudomonas* sp. *Aspergillus flavus*, *A. niger*, *Penicillium* sp., *T. virens* and *T. harzianum* showed positive inhibition of mycelial growth of *P. infestans*, *Fusarium* spp and *Rhizoctonia solani* under *in-vitro* conditions (Lal *et al.*, 2013) [22]. *Bacillus* sp. were used for managing late blight disease of potato *in-vitro* (Sunaina *et al.*, 2005) [31]. Yuan Hang *et al.*, 2014 [34] also reported that *T. koningiopsis* and *T. asperellum* were effective against *P. infestans* under both laboratory and field conditions.

Table 1: Evaluation of different fungicides against *Phytophthora infestans* at the concentration of 100 ppm (*in-vitro*)

Treatments	Radial mycelial growth at different days after inoculation (mm)							Per cent inhibition over control at 7 DAI
	1 DAI	2 DAI	3 DAI	4 DAI	5 DAI	6 DAI	7 DAI	
Krilaxyl	6.8	10.6	15.2	17.4	20.5	22.1	28.1	56.64
Equation Pro	5.3	8.2	11.4	14.2	16.2	18.4	22.6	65.12
Zampro	6.4	9.6	13.1	16.1	19.4	21.3	26.2	59.57
Matco	7.3	10.9	15.9	17.9	21.6	22.8	29.7	54.17
Curzate	5.8	8.6	12.3	15.5	18.3	19.5	24.4	62.34
Combi Plus	8.1	12.5	16.4	19.7	23.8	24.6	31.2	51.85

Indofil M-45	8.5	13.4	18.2	22.7	25.1	27.7	34.5	46.75
Control	12.3	19.1	26.1	35.9	42.5	51.4	64.8	-
SEm±	0.063	0.191	0.268	0.326	0.403	0.278	0.495	-
CD at 5%	0.190	0.577	0.812	0.987	1.218	0.842	1.497	-

Table 2: Evaluation of different fungicides against *Phytophthora infestans* at the concentration of 500 ppm (*in vitro*)

Treatments	Radial mycelial growth at different days after inoculation (mm)							Per cent inhibition over control at 7 DAI
	1 DAI	2 DAI	3 DAI	4 DAI	5 DAI	6 DAI	7 DAI	
Krilaxyl	4.8	8.6	12.5	15.6	18.4	21.2	26.4	59.25
Equation pro	3.1	5.8	8.2	11.3	15.4	17.3	20.5	68.36
Zampro	4.2	9.6	11.1	13.4	17.4	20.7	24.6	62.03
Matco	5.3	9.4	13.9	17.7	19.1	22.4	28.5	56.02
Curzate	3.5	6.4	8.7	12.4	16.3	18.2	22.8	64.81
Combi Plus	6.1	10.5	13.4	18.9	21.2	23.6	31.8	50.92
Indofil M-45	6.5	11.4	15.2	20.7	24.0	26.4	33.6	48.14
Control	12.3	19.1	26.1	35.9	42.5	51.4	64.8	-
SEm±	0.076	0.143	0.221	0.342	0.264	0.374	0.471	-
CD at 5%	0.229	0.433	0.667	1.035	0.797	1.130	1.423	-

Table 3: Evaluation of different fungicides against *Phytophthora infestans* at the concentration of 1000 ppm (*in vitro*)

Treatments	Radial mycelial growth at different days after inoculation (mm)							Per cent inhibition over control at 7 DAI
	1 DAI	2 DAI	3 DAI	4 DAI	5 DAI	6 DAI	7 DAI	
Krilasxyl	2.8	4.6	7.0	11.4	14.2	17.1	21.5	66.82
Equation pro	1.8	2.5	3.7	6.0	10.1	12.4	15.5	76.08
Zampro	2.3	4.3	5.8	9.6	12.2	15.3	19.3	70.21
Matco	3.3	5.5	9.1	13.2	16.1	19.4	23.6	63.58
Curzate	1.9	2.7	5.7	8.4	11.5	14.0	17.2	73.46
Combi Plus	4.1	7.3	10.2	14.6	18.8	22.6	26.8	58.64
Indofil M-45	5.5	9.0	11.5	16.0	19.0	25.0	32.6	49.69
Control	12.3	19.1	26.1	35.9	42.5	51.4	64.8	-
SEm±	0.086	0.171	0.165	0.350	0.293	0.446	0.402	-
CD at 5%	0.260	0.518	0.498	1.057	0.887	1.349	1.215	-

Table 4: Evaluation of different bio-agents against *Phytophthora infestans* (*in-vitro*)

Treatments	Radial mycelial growth at different days after inoculation (mm)							Per cent inhibition over control at 7 DAI
	1 DAI	2 DAI	3 DAI	4 DAI	5 DAI	6 DAI	7 DAI	
<i>T. harzianum</i>	4.6	8.8	10.1	13.2	15.6	19.3	23.5	63.73
<i>T. viride</i>	5.3	9.5	11.3	13.5	16.4	20.4	24.3	62.50
<i>T. koningii</i>	5.7	10.3	13.6	14.7	17.2	22.6	26.9	58.49
<i>T. virens</i>	6.2	10.9	14.8	16.4	18.5	24.5	28.6	55.86
Control	12.3	19.1	26.1	35.9	42.5	51.4	64.8	-
SEm±	0.178	0.251	0.288	0.194	0.537	0.455	0.645	-
CD at 5%	0.567	0.754	0.920	0.620	1.713	1.454	2.060	-

Conclusion

The experimental finding showed that radial growth of mycelium of *P. infestans* was inhibited by fungicides over control. At 100 ppm, the minimum radial growth of mycelium was found in Equation Pro treatment as 5.3, 8.2, 11.4, 14.2, 16.2, 18.4 and 22.6 mm over control against 12.3, 19.1, 26.1, 35.9, 42.5, 51.4 and 64.8 mm. Similarly, 500 and 1000 ppm the minimum radial growth of mycelium was found in Equation Pro treatment. Among the different bio-agents, *T. harzianum* able to reduced maximum radial mycelial growth of fungus showing 4.6, 8.8, 10.1, 13.2, 15.6, 19.3 and 23.5 mm against 12.3, 19.1, 26.1, 35.9, 42.5, 51.4 and 64.8 mm at 1, 2, 3, 4, 5, 6 and 7 days after inoculation, respectively.

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