



Comparitive Evaluation of Different Fungicides against *Phytophthora infestans* (Mont.) de Bary. Causing Late Blight of Potato

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SK and SKB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors VTC, KL and VK managed the analyses of the study. Author AB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Evaluation of various fungicides and their effectiveness at different doses against *Phytophthora infestans*, the causal pathogen of late blight disease of potato.

Study Design: Descriptive statistics was used and each treatment was replicated thrice using complete randomize design.

Place and Duration of Study: Department of Plant Pathology, CSA University of Agriculture & Technology, Kanpur, Uttar Pradesh, India in October 2016.

Methodology: We followed "Food Poison Technique" [1] to find out the relative efficacy of various fungicides in Inhibiting the growth of pathogen.

A small piece of infected leaf from border of sporulating lesion along with some healthy green tissue was cut and Sterilized. These small pieces were then placed on tomato extract based media which was previously poured in sterilized Petri plates. As soon as the mycelia growth is noticed around the bits, the pathogen was purified by hyphal tip culture method. Mycelial bits of 5 mm from

the pure culture were placed on the tomato extract media which was incorporated with various fungicides at different doses.

Results: Among the five different fungicides Metalaxyl inhibited the mycelial growth upto 99.2% over the control at 300 ppm concentration followed by 98.44% inhibition at 300 ppm concentration mancozeb at 400 ppm concentration inhibited the radial growth upto 96.62% followed by 95.27% inhibition at 300 ppm concentration.

Conclusion: This method can be used for Analysing the efficacy of various fungicides against different pathogens within a very short period of time.

Keywords: Phytophthora infestans; potato; disease severity; fungicides.

1. INTRODUCTION

Late blight of potato caused by *Phytophthora infestans* (Mont.) de Bary is one of the most serious diseases of potato that caused Irish Famine in 1845. The pathogen is perpetuated through soil and seed tubers through production of resting spore i.e. Oospore. Several management practices like host resistance, cultural adjustments, biological management and use of fungicides have been adopted so far to minimize the disease. Cultural practice like field sanitation, summer ploughing, soil solarisation, soil amendments and crop rotation etc. can minimize the possibility of disease but cannot completely control the disease in standing crops. Another alternative method of disease management strategy is biological control. In this context, *Trichoderma harzianum*, *Trichoderma viride*, *Chaetomium globosum*, *Gliocladium virens* etc. have been exploited for management of diseases but biological control alone cannot manage the disease completely because a little fluctuation in temperature, pH, moisture etc. largely affects the efficacy of bio agent. The use of resistant variety is another important method which is reliable and cheap for management of plant disease but due to development of new races of pathogen, the resistant variety becomes susceptible one. Hence, the use of fungicides is the last and only method for management of plant disease. [2] reported that the disease can efficiently be managed by spraying fungicide in early growth period (seedling stage to budding stage) and in late growth period (flowering stage to maturing stage). [3] also reported that four sprays of Mancozeb at 8-10 days interval or two sprays of Mancozeb and one spray of Metalaxyl + Mancozeb were more accurate for controlling late blight. [4] screened fifteen fungicides against late blight and reported that new fungicides JE-874 at 10 ml/10 L, Curzate at 3 g/10 L and Melody at 5 g/10 showed 15.66%, 14.23% and 7.56% reduction in disease severity while Mancozeb at 0.2% and Ridomil MZ-72 at

0.2% sprayed plot showed 1.25% and 1.00% reduction disease severity.

2. MATERIALS AND METHODS

The present investigations was done at the Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology Kanpur during October 2016. The procedure and techniques applied during the course of investigations were elucidated as below.

2.1 Isolation, Purification, Identification and Maintenance of *Phytophthora infestans*

2.1.1 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 sporulating lesions were taken from the field and washed in sterilized water. The leaves were then placed in a humidity chamber with the leaf axial side up. They were incubated at 18±1°C in BOD (Biological oxygen demand), until sporulation appeared. Small pieces of infected tissue along with healthy portion from the sporulating border were cut and placed under potato slices in empty sterilized Petri-plates. The Petri-plates were incubated at 18± 1°C for 10 days until there was a growth of abundant mycelium on the upper side of the slice. Mycelium was taken from the tuber slices by using sterilized needle and transferred on the selective medium.

2.1.2 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 rinsed 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 Petri plates. The plates were then incubated at $18 \pm 1^\circ\text{C}$. The Petri plates were observed daily to find out the presence of mycelium around the leave bits. As soon as the mycelia growth is observed around the bits, the pathogen was purified by hyphal tip culture method.

2.1.3 Purification of *Phytophthora infestans*

The white mycelial bits of *P. 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 *P. infestans* was transferred on slant medium and incubated at $15-18^\circ\text{C}$ in darkness till full growth. The culture was then transferred into the incubator at $10-12^\circ\text{C}$ for further use.

2.1.4 Identifications of *Phytophthora infestans*

The isolated pathogen was identified on the basis of its morphological and cultural characters and pathogenic behaviour towards the host. *P. infestans* belong to the class Oomycetes. The vegetative mycelium is characterized by the absence of cross walls, along with both asexual and sexual spores. The sporangiophores and sporangia emerge at asexual reproduction phase. The sporangia are pear shape, measurement of $21-38 \mu\text{m} \times 12-23 \mu\text{m}$. Sporangia develop at the end of these sporangiophores. Oospores are found at sexual reproduction. When mycelia of different mating types of the fungus grow together, one of them may form antheridia and the other oogonia. The oogonium grows through the antheridium, allowing fertilization. The fertilized oogonium develops into a thick – walled oospore, while the oospore is orange red, nearly round – shaped, with measurement of $28-32 \mu\text{m}$. The pathogen was found to produce the characteristics leaf spot symptoms on the affected plants.

2.1.5 In vitro activity of fungicides against *Phytophthora infestans*

Five fungicides belonging to different groups 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 "Food Poison Technique" [1]. Recommended 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 six treatments. The Petri plates were incubated at $18 \pm 1^\circ\text{C}$. 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. Radial growth of the mycelium was recorded. The per cent inhibition over control was calculated by the following formula as given by Bliss (1934).

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

where,

I = Per cent inhibition in mycelia growth
C = Growth of pathogen in control plates

3. RESULTS AND DISCUSSION

3.1 Effect of bavistin 50% wettable powder on radial growth of *Phytophthora infestans* at 72 hrs. (in vitro)

Bavistin is a systemic fungicide with protective action. Bavistin was evaluated *in vitro* against *P. infestans* by Poison Food Technique at 200, 400, 600, 800 and 1000 part per million concentrations after 72 hrs. of incubation.

Data presented in Table 1 showed that minimum radial growth of 26 mm in diameter with 48% inhibition over control was recorded at 1000 ppm concentration followed by 800 ppm (31 mm, 38% inhibition), 600 ppm (36 mm 28% inhibition), 400 ppm (41 mm 18% inhibition), 200 ppm with (47 mm 6% inhibition). From the table, it is clear that concentration of fungicide is inversely proportion with radial growth of mycelium. On the other hand, per cent inhibition of mycelium growth varied from 6–48%. Bavistin is moderately effective against *P. infestans* at recommended dose. Statistical analysis of the data revealed that each treatment varied invariably and significantly at 0.05 level of significance.

3.2 Effect of Metalaxyl 50% Wettable Powder on Radial Growth of *Phytophthora infestans* at 72 hrs. (in vitro)

Metalaxyl is a non-systemic fungicide with protective and curative action. Metalaxyl was evaluated *in vitro* against *P. infestans* by Poison Food Technique at 50, 100, 200, 300 and 400 ppm concentrations after 72 hrs. of incubation .

Data presented in Table 2 indicated that minimum radial growth of 0.50 mm in diameter was recorded at 300 ppm concentration which was followed by 200 ppm, 100 ppm and 50 ppm. However, no mycelial growth was recorded at 400 ppm. As per concern of per cent inhibition, the highest of 100% inhibition was recorded from 400 ppm concentration of metalaxyl. [5] reported that metalaxyl at 25 mg/ml Supressed *in vitro* sporangium germination of *Phytophthora infestans*. From the table, it is also clear from Table 2 that there was significance differences at 0.05 level of significance among all the treatments.

3.3 Effect of Tebuconazole 50% Wettable Powder on Radial Growth of *Phytophthora infestans* at 72 hrs. (in vitro)

Tubaconazole is a systemic fungicide with protective and curative action. Tubaconazole was evaluated *in vitro* against *P. infestans* by Poison Food Technique at 200, 400, 600, 800 and 1000 ppm concentrations after 72 hrs. of incubation.

Data presented in Table 3 indicated that minimum radial growth of 30.16 mm in diameter of mycelium growth was recorded at 1000 ppm concentration, which is inhibited by 28.19% over control. The rest of the treatments like 800 ppm, 600 ppm, 400 ppm and 200 ppm also inhibited the mycelium growth of fungi, showing 23.57%, 18.76%, 11.30% and 5.35% inhibition respectively. Tubaconazole is moderately effective against *P. infestans* at recommended dose. Mondal et al. (2014) reported that Tebuconazole +Trifloxystrobin inhibited the sporangia production at 0.1%. It is also clear from the Table 3 that each treatment varied in

variably and significantly at 0.05 level of significance.

3.4 Effect of Mancozeb 75% Wettable Powder on Radial growth of at 72 hrs *Phytophthora infestans* (in vitro)

Mancozeb was evaluated in vitro against *P. infestans* by Poison Food Technique at 50, 100, 200, 300 and 400 ppm concentrations after 72 hrs. of incubation.

Data presented in Table 4 indicated that minimum radial growth (2.20 mm) of 96.62 % inhibition was recorded at 400 ppm concentration followed by 300 ppm (3.08 mm, 95.27%), 200 ppm (4.12 mm, 93.67%), 100 ppm (6.20 mm, 90.47), 50 ppm (8.10 mm, 87.56%) respectively as compare to control . Mancozeb is moderately effective against *P. infestans* at recommended dose. Mondal et al. (2014) reported that metalaxyl in association with mancozeb inhibited the mycelial growth It is also clear from the Table 4 that each treatment varied in variably and significantly at 0.05 level of significance.

3.5 Effect of Tricyclozole 75 WP on Radial Growth of *Phytophthora infestans* at 72 hrs (in vitro)

Tricyclozole is a systemic fungicide with protective and curative action. Tricyclozole was evaluated *in vitro* against *P. infestans* by Poison Food Technique at 200, 400, 600, 800 and 1000 ppm concentrations after 72 hrs. of incubation.

Data presented in Table 5 indicated that minimum radial growth of 33.22 mm in diameter of mycelium growth with was recorded at 1000 ppm concentration which is inhibited upto 41.86% over control. The rest of the treatments like 800 ppm, 600 ppm, 400 ppm and 200 ppm also influenced the mycelium growth of fungi showing 36.00 mm, 40.42 mm, 43.00 mm and 46.12 mm respectively. [6] reported that tricyclazole inhibited both mycelial growth and sporangia production at 0.1%.Tricyclozole is moderately effective against *P. infestans* at recommended dose. It is also cleared from the table that each treatment varied in variably and significantly at 0.05 level of significance.

Table 1. Effect of Bavistin 50% WP on radial growth of *Phytophthora infestans* at 72 hrs

Concentration (ppm)	Radial growth(mm)	Inhibition %
200	47.0	6
400	41.0	18
600	36.0	28
800	31.0	38
1000	26.0	48
Control	50.0	
CD (0.05)	2.556	
SE(d)	1.160	
C.V.%	3.692	

Table 2. Effect of Metalaxyl 50% WP on radial growth of *Phytophthora infestans* at 72 hrs

Concentration (ppm)	Radial growth(mm)	Inhibition %
50	4.75	92.59
100	3.00	95.32
200	1.00	98.44
300	0.50	99.22
400	0.00	100
Control	64.12	
CD (0.05)	1.704	
SE(d)	0.774	
C.V.%	7.749	

Table 3. Effect of Tubaconazole 50% WP on radial growth of *Phytophthora infestans* at 72 hrs

Concentration (ppm)	Radial growth(mm)	Inhibition %
200	39.75	5.35
400	37.25	11.30
600	34.12	18.76
800	32.10	23.57
1000	30.16	28.19
Control	42.00	
CD (0.05)	2.344	
SE(d)	1.064	
C.V.%	3.630	

Table 4. Effect of Mancozeb 75% WP on radial growth of *Phytophthora infestans* at 72 hrs

Concentration (ppm)	Radial growth(mm)	Inhibition %
50	8.10	87.56
100	6.20	90.47
200	4.12	93.67
300	3.08	95.27
400	2.20	96.62
Control	65.12	
CD (0.05)	1.751	
SE(d)	0.795	
C.V.%	6.571	

Table 5. Effect of Tricyclozole75% WP on radial growth of *Phytophthora infestans* at 72 hrs

Concentration (ppm)	Radial growth(mm)	Inhibition %
200	46.12	19.28
400	43.00	24.74
600	40.42	29.26
800	36.00	36.99
1000	33.22	41.86
Control	57.14	
CD (0.05)	2.811	
SE(d)	1.276	
C.V.%	3.665	

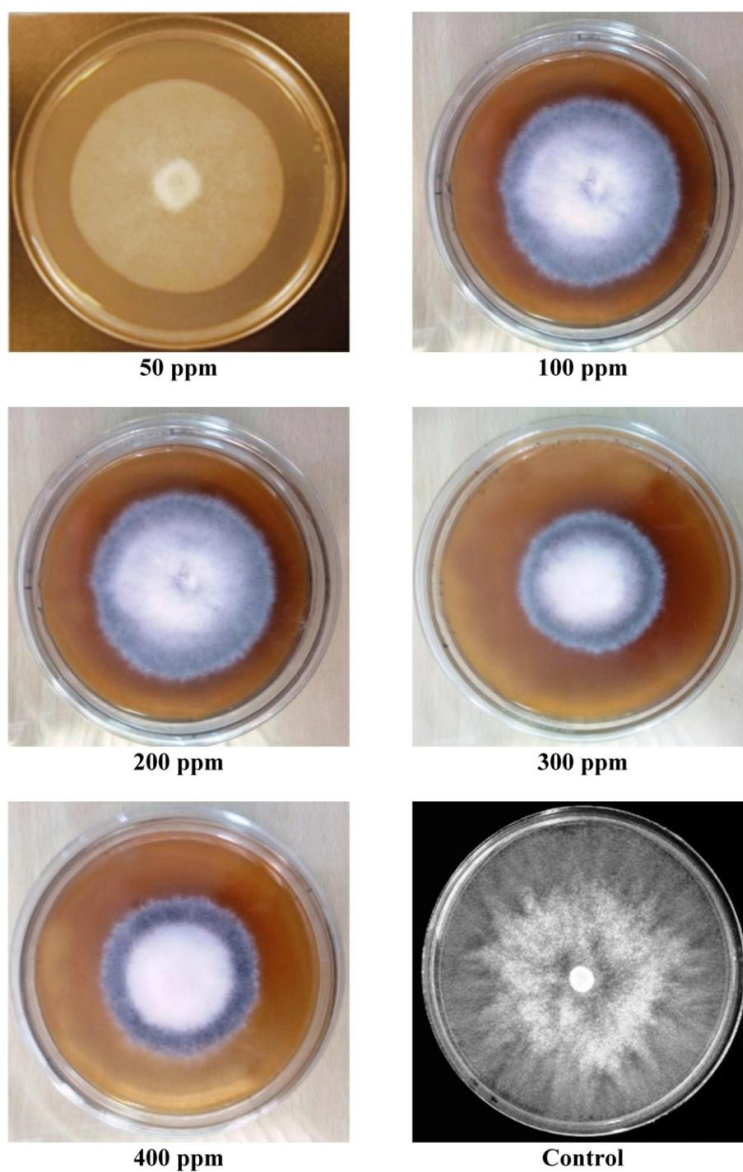


Fig. 1. Effect of different concentration of Metalaxyl on mycelial growth of *Phytophthora infestans*

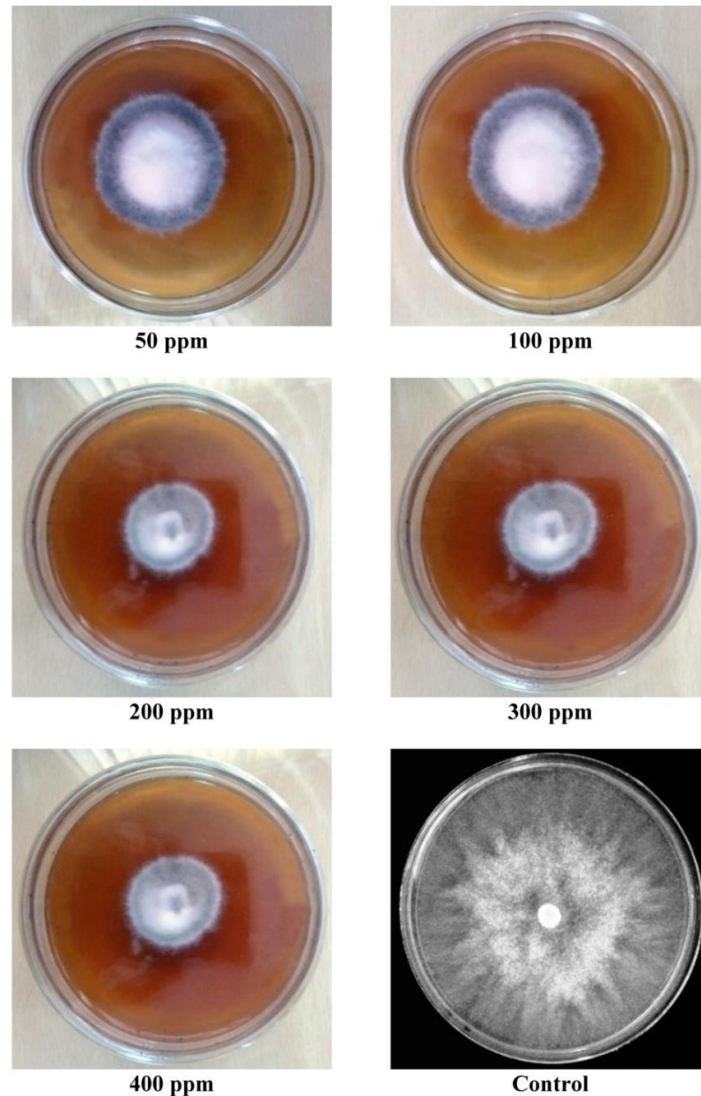


Fig. 2. Effect of different concentration of Mancozeb on mycelial growth of *Phytophthora infestans*

4. CONCLUSION

The pathogen *Phytophthora infestans* was isolated from potato leaves showing typical late blight symptoms. The fungus growing on tomato extract media produced white colored mycelium, distinctive sympodial sporangiophores with pear shaped sporangia having distinct papilla. The description of the fungus agreed with the description given by Common wealth Mycological Institute, Kew, Surrey, England, [7]. Thus the pathogen causing late blight of potato has been identified as *P. infestans*. Successful pathogenicity of the fungus on potato was proved

following Koch's postulates by inoculating the spore suspension.

Among the five different fungicides tested on mycelial growth of the *P. infestans*, the minimum radial growth of 0.50 mm in diameter was recorded at 300 ppm concentration of metalaxyl which was followed by 200 ppm, 100 ppm and 50 ppm. However, no mycelial growth was recorded at 400 ppm. As per concern of per cent inhibition the highest of 100% inhibition was recorded from 400 ppm concentration of Metalaxyl followed by Mancozeb of 2.20 mm diameter with 96.62% inhibition at 400 ppm concentration.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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