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Rapid Laboratory Evaluation of Fungicides against *Phytophthora infestans* Causing Late Blight of Potato

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Authors' contributions

This work was carried out in collaboration among all authors. Author DCK designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author BM managed the literature searches, analyses of the study performed the spectroscopy analysis and both author BM and NKP managed the experimental process. Authors DCK and BM identified the species of the plant pathogen. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aim: To develop method for rapid and simple laboratory evaluation of fungicides against *Phytophthora infestans*, the causal pathogen of late blight of potato.
Study Design: Descriptive statistics and analysis of variance. Each treatment was replicated thrice.
Place and Duration of Study: Department of Plant Protection, Palli-Siksha Bhavana (Institute of Agriculture), Visva-Bharati, India in February, 2014.
Methodology: Small portion of potato leaf tissue from advancing margin of late bight lesion is placed on water since *Phytophthora infestans* (Mont.) de Bary grow well in water producing hypha and sporangium. Using this property, performance of fungicides on mycelial growth and sporangia production/formation was assayed by placing a small bit of infected leaf tissue in different

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concentration of fungicides.
Results: Among the seventeen fungicides tested, chlorothalonil, fenamidone + mancozeb and tricyclazole inhibited mycelial growth and sporangia production but dimethomorph, tebuconazole + trifloxystrobin inhibited sporangia production only. Metalaxyl + mancozeb totally inhibited mycelial growth at higher concentration.
Conclusion: This method can be useful for evaluating and comparing performance of different fungicides and even same formulations of a fungicide, produced by different companies within a very short time period. The experimental work can be completed within 48 –72h if late blight infected leaves are available.

Keywords: Phytophthora infestans; *late blight; potato; rapid screening; fungicides.*

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important crops in India. In West Bengal, potato occupies nearly 386.61 thousand hectare with a production of 11591.30 thousand tones during 2012-13 [1]. Late blight disease appears as mild to severe form every year. Growers claim that some of the fungicides marketed in this area for management of the late blight are not performing well [2]. Methods already standardized for laboratory evaluation of fungicides against *Phytophthora infestans* (Mont.) de Bary require good laboratory facilities [3] and also time. This creates an interest to develop method for rapid and simple laboratory evaluation of fungicides against *P. infestans*, the causal pathogen of late blight of potato.

2. MATERIALS AND METHODS

White growth of *P. infestans* is visible on the under surface of late blight infected potato leaves at advancing margin of the lesion. If a portion of the leaf tissue is taken from that area (containing both healthy and diseased tissue) and placed in water in sterile Petri dish, the pathogen grows beyond the area of the leaf tissue and produce good mycelial growth in water leading to sporangia formation. Microscopic observation is possible by placing such Petri dish under microscope (Compound light microscope, 10X objective lens).

Seventeen fungicides including protective, systemic fungicides and combination products were selected for the study (Table 1). Two non-target fungicides (carbendazim, tricyclazole) were included for comparison. These two fungicides are not marketed for controlling late blight disease of potato. Aqueous suspension of commercial preparation was used for evaluation. Diseased potato leaves containing white mycelial growth underside were collected in February 2014 from infected field in morning hours. Leaf bits were prepared measuring nearly 1.0cm x 0.5cm containing both diseased and healthy tissue. Three such bits were placed in a Petri dish containing fungicide suspension or non-sterile filtered tap water (Aquaguard^{TM} Classic - a complete 3-stage water purification system). Initially fungicide suspension/water was shacked for better contact with the leaf bits and the process was repeated after three hours. There were three replications for each treatment. The plates were incubated at room temperature for 48h and then observed under microscope to record the extent of mycelial growth and sporangia formation in fungicide suspension or water. A rating scale was also prepared to record the extent of mycelial growth and sporangia formation as follows: no mycelial growth or sporangium formation = zero (0); very scanty/very few = < 5; scanty/few = between 5 and 10; medium = between 10 and 30; high/huge between 30 and 50, and profuse => 50. Descriptive statistics was used to calculate mean, standard deviation, coefficient of variation from the experimental data (Table 1). Apart from these, analysis of variance was also carried out in completely randomized design (CRD) to compare the treatment means.

3. RESULTS AND DISCUSSION

Good variation was recorded (Table 1) in respect to effect of different fungicides on mycelial growth and sporangia formation. Among the fungicides carbendazim did not have any effect on *P. infestans*. Metalaxyl showed more or less similar effect. However, metalaxyl when associated with mancozeb inhibited mycelial growth and sporangia formation. Copper oxychloride, mancozeb, zineb, metiram and combination product of iprovalicarb + propineb inhibited mycelial growth only at higher

concentration. Chlorothalonil, fenamidone + mancozeb and tricyclazole inhibited both mycelial growth and sporangia production while dimethomorph and tebuconazole + trifloxystrobin inhibited sporangia production only. Other fungicides *viz.* Thiram, Azoxystrobin, Carboxin + Thiram, Cymoxanil + Mancozeb had not any remarkable effect on mycelial growth and sporangia production of the fungus (Table 2).

Table 1. Effect of fungicides against *P. infestans* in aqueous environment

Fungicides	Active ingredient and formulation	Extent of mycelial growth % concentration of fungicide formulation			Extent of sporangia formation % concentration of fungicide formulation		
		0.1	0.05	0.025	0.1	0.05	0.025
Blitox (Rallis India Limited)	Copper oxychloride 50% WP	Scanty	Medium	Profuse	Very few	Few	Medium
Shaktiman Mancozeb (Indo Gulf Fertilizers)	Mancozeb 75% WP	Very scanty	Medium	Medium	Nil	Medium	Medium
Indofil Z-78 (Indofil Industries Ltd.)	Zineb 75% WP	Scanty	Medium	Medium	Nil	Very few	Very few
Devithiram (Devidayal (sales) Ltd.)	Thiram 75% DS	Medium	Medium	Profuse	Nil	Very few	Very few
Ovate (Cheminova India Ltd.)	Chlorothalonil 75% WP	Very scanty	Scanty	Scanty	Nil	Nil	Nil
Pack-Up (Krishi Rasayan Exports Pvt. Ltd.)	Metiram 70% WG	Scanty	Medium	High	Few	Few	Medium
Shine – 35 (Fil Industries Ltd.)	Metalaxyl 35% WS	Profuse	Profuse	Profuse	Nil	Medium	Medium
Amister (Syngenta India Ltd.)	Azoxystrobin 23% SC	Medium	Medium	Medium	Very few	Few	Few
Acrobat (BASF India Ltd.)	Dimethomorph 50% WP	Medium	Medium	Profuse	Nil	Nil	Nil
Bavistin (BASF India Ltd.)	Carbendazim 50% WP	Profuse	Profuse	Profuse	Medium	Profuse	Profuse
Trikaal (Devidayal Agro Chemicals)	Tricyclazole 75% WP	Very Scanty	Scanty	Scanty	Nil	Nil	Very few
Vitavax Power (Dhanuka Agritech Ltd.)	Carboxin 37.5% + Thiram 37.5% WS	Scanty	Medium	Profuse	Very few	Few	Huge
Krilaxyl Gold (Krishi Rasayan Exports Pvt. Ltd.)	Metalaxyl 8% + Mancozeb 64% WP	Nil	Scanty	Medium	Nil	Very few	Few
Curzet (Dupont)	Cymoxanil 8% + Mancozeb 64% WP	Medium	Profuse	Profuse	Very few	Medium	Medium
Sectin (Bayer Crop Science)	Fenamidone 10% + Mancozeb 50% WG	Scanty	Scanty	Scanty	Nil	Nil	Nil
Melody Duo (Bayer Crop Science)	Iprovalicarb 5.5% + Propineb 61.25% WP	Scanty	Profuse	Profuse	Nil	Medium	Medium
Nativo (Bayer Crop Science)	Tebuconazole 50% + Trifloxystrobin 25% WG	Medium	Profuse	Profuse	Nil	Nil	Nil
Water (untreated control)	Non-sterile filtered tap water	Profuse	Profuse	Profuse	Profuse	Profuse	Profuse

Table 2. Effect of fungicides on mycelial growth and sporangia formation of *P. infestans*

Treatments	Extent of mycelial growth (no.) at different concentrations of fungicides			Extent of sporangia formation (no.) at different concentrations of fungicides		
	0.1%	0.05%	0.025%	0.1%	0.05%	0.025%
Copper oxychloride	6.67 (2.67)	14.67 (3.89)	56.67 (7.55)	3.67 (2.03)	8.33 (2.97)	19.67 (4.48)
Mancozeb	3.33 (1.95)	12.67 (3.62)	14.33 (3.85)	0.00 (0.70)	15.00 (3.92)	22.33 (4.77)
Zineb	6.67 (2.67)	21.67 (4.69)	19.33 (4.45)	0.00 (0.70)	3.00 (1.87)	3.67 (2.03)
Thiram	13.33 (3.71)	22.00 (4.74)	67.33 (8.22)	0.00 (0.70)	3.67 (2.03)	4.00 (2.12)
Chlorothalonil	2.33 (1.67)	6.33 (2.61)	7.67 (2.85)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Metiram	6.33 (2.61)	17.67 (4.25)	37.33 (6.14)	6.33 (2.61)	8.00 (2.91)	19.00 (4.41)
Metalaxyl	71.67 (8.49)	92.00 (9.61)	99.33 (9.99)	0.00 (0.70)	26.00 (5.14)	28.67 (5.40)
Azoxystrobin	17.67 (4.25)	22.00 (4.74)	26.67 (5.21)	3.33 (1.95)	6.33 (2.61)	7.67 (2.85)
Dimethomorph	25.00 (5.04)	27.00 (5.24)	68.67 (8.31)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Tricyclazole	3.33 (1.95)	7.67 (2.85)	8.67 (3.02)	0.00 (0.70)	0.00 (0.70)	2.33 (1.67)
Carboxin + Thiram	7.00 (2.73)	23.33 (4.88)	77.67 (8.83)	3.67 (2.03)	7.33 (2.79)	38.33 (6.22)
Metalaxyl + Mancozeb	0.00 (0.70)	8.00 (2.91)	21.67 (4.69)	0.00 (0.70)	3.67 (2.03)	8.00 (2.91)
Cymoxanil + Mancozeb	24.33 (4.97)	76.33 (8.76)	84.67 (9.22)	4.00 (2.12)	27.00 (5.24)	28.67 (5.40)
Fenamidone + Mancozeb	6.00 (2.54)	7.67 (2.85)	8.67 (3.02)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)
Iprovalicarb + Propineb	8.00 (2.91)	62.67 (7.94)	77.33 (8.82)	0.00 (0.70)	15.67 (4.01)	25.00 (5.04)
Tebuconazole + Trifloxystrobin	26.00 (5.14)	70.67 (8.43)	82.00 (9.08)	0.00(0.70)	0.00 (0.70)	0.00 (0.70)
Carbendazim (Check)	97.67(9.90)	103.00 (10.17)	115.67 (10.77)	27.67 (5.30)	69.00 (8.33)	84.33 (9.20)
SEm (±)	0.11	0.12	0.13	0.39	0.89	0.88
CD (*p*=0.05)	0.33	0.36	0.37	0.11	0.25	0.25

Table 3 revealed that median value (7.0) was very low compared to overall mean (19.14) which indicated that the overall mean was affected by a few extreme values (profuse mycelial growth) recorded at higher concentration (0.1%) in some treatments. The above result also showed that the mycelial growth in presence of half of the fungicides tested was scanty. Again, the standard deviation (26.38), and the coefficient of variation (137.87) were very high indicating a big variation in mycelial growth among the treatments. Besides, with the decreased of fungicidal concentrations coefficient of variation and difference between overall mean and median were decreased. It specified that at lower concentrations the mycelial growth in all the treatments was significantly increased resulting in reduction of variation among the treatments. Similar trend was recorded in sporangia production as well. However, median value of sporangia production in all the concentrations was low as computed in mycelial growth and indicated no sporangia production in half of the fungicides tested. Profuse and uncountable growth of mycelia and sporangia was observed in untreated control and was not included in statistical calculation. Earlier, similar method was utilized successfully in selecting fungicides for management of fruit and vine rot of pointed gourd caused by *Phytophthora melonis* [4]. In this case infected fruit tissue was used. Khatua et al. [5] tested performance of the fungicides against *Phytopthora nicitianae* in aqueous environment using mycelial disc from agar medium as inoculum. After the incidence of late blight of potato in a particular area, present method can be useful for comparing performance of different fungicides and even same formulations of a fungicide, produced by different companies within 48 –72h.

4. CONCLUSION

This method can be useful for evaluating and comparing performance of different fungicides and even same formulations of a fungicide, produced by different concerns within a very short time period. The experimental work can be completed within 48 –72h if late blight infected leaves are available.

Table 3. Effect of different doses of fungicides on mycelial growth and sporangia formation of *P. infestans*

Treatments	Extent of mycelial growth (no.) at different concentrations of fungicides			Extent of sporangia formation (no.) at different concentrations of fungicides		
	0.1%	0.05%	0.025%	0.1%	0.05%	0.025%
	Treatment mean (average of three replications)					
Copper oxychloride	6.67	14.67	56.67	3.67	8.33	19.67
Mancozeb	3.33	12.67	14.33	0.00	15.00	22.33
Zineb	6.67	21.67	19.33	0.00	3.00	3.67
Thiram	13.33	22.00	67.33	0.00	3.67	4.00
Chlorothalonil	2.33	6.33	7.67	0.00	0.00	0.00
Metiram	6.33	17.67	37.33	6.33	8.00	19.00
Metalaxyl	71.67	92.00	99.33	0.00	26.00	28.67
Azoxystrobin	17.67	22.00	26.67	3.33	6.33	7.67
Dimethomorph	25.00	27.00	68.67	0.00	0.00	0.00
Carbendazim	97.67	103.00	115.67	27.67	69.00	84.33
Tricyclazole	3.33	7.67	8.67	0.00	0.00	2.33
Carboxin + Thiram	7.00	23.33	77.67	3.67	7.33	38.33
Metalaxyl + Mancozeb	0.00	8.00	21.67	0.00	3.67	8.00
Cymoxanil + Mancozeb	24.33	76.33	84.67	4.00	27.00	28.67
Fenamidone + Mancozeb	6.00	7.67	8.67	0.00	0.00	0.00
Iprovalicarb + Propineb	8.00	62.67	77.33	0.00	15.67	25.00
Tebuconazole + Trifloxystrobin	26.00	70.67	82.00	0.00	0.00	0.00
Overall mean	19.14	35.02	51.39	2.86	11.35	17.16
Median	7.00	22.00	56.67	0.00	6.33	8.00
SD	26.38	32.20	35.42	6.71	17.15	21.29
CV (%)	137.87	91.96	68.92	234.37	151.10	124.07

CONSENT

All authors declare that written informed consent was obtained for publication of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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