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In-vitro studies on effect of fungicides against mycelial growth and sporangial germination of *Phytophthora infestans* (Mont) de Bary) causing late blight of potato

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DOI: <https://doi.org/10.22271/chemi.2020.v8.i1ae.8569>**Abstract**

Late blight of potato is most destructive disease. In the present study, a total of eleven fungicides, out of which eight systemic/translaminar fungicides cymoxanil 50 WP, cymoxanil 8 + mancozeb 72 WP, cymoxanil 8 + mancozeb 64 WP, metalaxyl 8 + mancozeb 64 WP, metalaxyl 32 WP, fenamidone 10 + mancozeb 50 WP, difenoconazole 25 EC, dimethomorph 50 WP at concentrations of 10, 50, 100, 200 and 500 $\mu\text{g ml}^{-1}$ and three protectants viz. propineb 70 WP, mancozeb 70 WP and chlorothalonil 70 WP at concentrations of 100, 200, 500, 1000 and 2000 $\mu\text{g ml}^{-1}$ were tested against the mycelia growth and sporangial germination ability on V8 agar medium by using poison food technique *in vitro*. It was found that all the tested fungicides significantly inhibited the growth of mycelium compared to the control (untreated). Among the systemic fungicides dimethomorph exhibited the minimum overall mycelial growth of 38.86 mm followed by cymoxanil 8 + mancozeb 72 WP sustaining a radial growth of 43.00 mm compared to a radial mycelial growth 85.73 mm obtained in unamended check plates. An increasing trend in inhibition of mycelial growth was observed as the concentration of each fungicide was increased, such that each fungicide at 200-500 $\mu\text{g/ml}$ exhibited more than 90 per cent inhibition of mycelia growth, compared to 10 $\mu\text{g/ml}$ which yielded less than 10 per cent inhibition. Among protectant fungicides mancozeb exhibited highest inhibition percent with minimum mycelial growth of 46.77 mm followed by propineb yielding the mycelial growth of 50.13 mm as compared to 85.73 mm growth obtained in unamended check. The results also revealed that all the fungicides significantly reduced the sporulation and germination ability of the sporangia at all the test concentrations. Among systemic fungicides, dimethomorph proved most effective allowing sporangia germination to the extent of only 31.80 percent followed by metalaxyl + mancozeb 64 WP and cymoxanil + mancozeb 72 WP with sporangia germination of 36.53-37.00 per cent as compared to 80.66 per cent germination observed in un-amended check (control) plates. Among the protectants.

Keywords: *Phytophthora infestans*, systemic fungicides, protectants, mycelial growth, sporangial germination

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important food crops worldwide which represents a valuable source of nutrients in a balanced diet. In terms of human consumption, the potato is the third most important food crop in the world, following only rice and wheat. Potato is also one of the important food crops in India. India stands second in world's potato production where it is cultivated over an area of 2.13 million hectares with a production of 43.77 million metric tonnes (Viswanath *et al.*, 2018) ^[1]. The area and production of the Potato crop in Jammu & Kashmir state are 6.9 thousand hectares and 1.27 lakh metric tonnes, respectively (Anonymous, 2015) ^[1]. Reports of complete field destruction due to late blight epidemics are relatively common. The fungus is responsible for global annual crop loss of US \$ 12 billion. Yield loss due to late blight in India varies from year to year and range from 20-75% The pathogen produces water soaked lesions with chlorotic borders that are small at first expand rapidly under humid conditions, blighting the entire plant in only a few days with subsequent rotting of the developing tubers resulting in heavy yield losses under favorable. (Sundaresha *et al.*, 2015) ^[10].

As phytophthora belongs to oomycetes fungus most of the fungicides which are used for higher fungi cannot be used against this disease. As it was known fact that among all the strategies of disease management fungicide based management is highly effective means compared to the other methods of control. And there are many fungicides which include systemic/translaminar as well as protectant in nature. As there were many previous reports of evolution of new races by this late blight pathogen due to resistance development against the fungicides, it is therefore highly recommended for the need based spray application of these fungicides and at proper doses at field level. So, before the application of fungicides at the field level, there is great necessity to evaluate them first under laboratory conditions against the pathogen under different concentrations. So the present study was taken up to evaluate different systemic/translaminar and contact fungicides at different concentrations against the mycelial growth, sporulation and sporangial germination of the pathogen.

Material and methods

Eleven fungicides (eight systemic/translaminar and three protectants) were evaluated in the laboratory for their efficacy in inhibiting the mycelial growth of the test pathogen *P. infestans* using poisoned food technique (Nene and Thapilyal, 2002) [7]. The systemic/translaminar fungicides viz., cymoxanil 50 WP, cymoxanil 8 + mancozeb 72 WP, cymoxanil 8 + mancozeb 64 WP, metalaxyl 8 + mancozeb 64 WP, metalaxyl 32 WP, fenamidone 10 + mancozeb 50 WP, difenoconazole 25 EC, dimethomorp 50 WP, were evaluated at concentrations of 10, 50, 100, 200 and 500 µg ml⁻¹ whereas the protectant fungicides viz., propineb 70 WP, mancozeb 70 WP and chlorothalonil 70 WP were evaluated at 100, 200, 500, 1000 and 2000 µg ml⁻¹. The required quantities of fungicides were separately mixed with V-8 agar medium before pouring in Petri dishes, which were stored for 24 hours at 4°C in order to stabilize the medium prior to inoculation with a 7 day old culture of *P. infestans*. Mycelial plug (0.4 cm dia) was placed at the centre of each Petri plate and incubated at 20±2°C until the culture in un-amended check plate attained full growth. At the end of incubation period, radial

colony growth (mm) was measured in each treatment and the inhibition percentage over control was calculated as follows:

$$\text{Growth inhibition (\%)} = \frac{\text{Growth in control plates} - \text{Growth in treatment plates}}{\text{Growth in control plates}} \times 100$$

Sporulation and sporangial Germination%: The sporulation percent obtained in V-8 media amended with different fungicides at different concentrations was analyzed. The sporangia were harvested from these treated media on to the Water agar plug placed on microscopic glass slides and germinated sporangia was observed under the microscope by incubating them at 18°C in the dark for 3 days. Treatments and controls were replicated three times. The number of germinating sporangia was calculated as percent of germinated sporangia relative to the total number of sporangia (depending on sporangia density). This percentage was then compared to the germination percentage of the control.

Results and discussion

In-vitro bioassay

The test pathogen *P. infestans* was allowed to grow *in vitro* on V-8 medium poisoned separately with various concentrations of different fungicides, and the inhibition of the mycelial growth was assessed after 10 days of incubation. The results revealed that all the fungicides significantly reduced the mycelial growth of the test pathogen compared to control, and the growth decreased with increase in the concentration of the test fungicides. Of the systemic fungicides (Table-1), dimethomorph exhibited the minimum overall mycelial growth of 38.86 mm followed by cymoxanil 8 + mancozeb 72 WP sustaining a radial growth of 43.00 mm compared to a radial mycelial growth 85.73 mm obtained in unamended check plates. The results further revealed an overall minimum mycelial growth (17.85 mm) at highest test concentration of 500 µg/ml. A gradual decrease in the fungicide concentration resulted in the increase in the mycelial growth such that a minimum mycelial growth (80.40mm) occurred at the test concentration of 10 µg/ml.

Table 1: Comparative mycelial growth of *Phytophthora infestans* on V-8 medium amended separately with different systemic/translaminar fungicidal concentrations

Fungicide	Radial Mycelial growth (mm) at different concentrations					
	10 µg ml ⁻¹	50 µg ml ⁻¹	100 µg ml ⁻¹	200 µg ml ⁻¹	500 µg ml ⁻¹	Mean
Cymoxanil 50 WP	79.33	77.66	49.00	12.33	10.33	45.67
Cymoxanil 8+Mancozeb 72 WP	80.33	76.00	41.00	9.67	8.00	43.00
Cymoxanil 8+ mancozeb 64 WP	80.33	77.00	45.33	10.67	8.67	44.33
Matalaxyl8+ Mancozeb 64 WP	80.30	77.00	45.30	10.00	8.00	44.13
Metalaxyl 32 WP	81.00	78.33	40.67	15.33	14.33	45.93
Fenamidone10 + Mancozeb 50 WP	79.00	78.66	50.00	12.00	10.00	45.93
Difenaconazole 25 EC	80.33	78.33	44.00	13.67	11.67	45.60
Dimethomorph 50 WP	77.67	73.33	34.67	05.00	04.00	38.86
Control	85.67	85.67	85.67	85.67	85.67	85.73
Mean	80.40	70.03	48.33	19.57	17.85	-

CD (0.05%)

Fungicide = 0.65

Concentration = 0.47

Fungicide x Concentration = 1.45

Data are mean of three replications

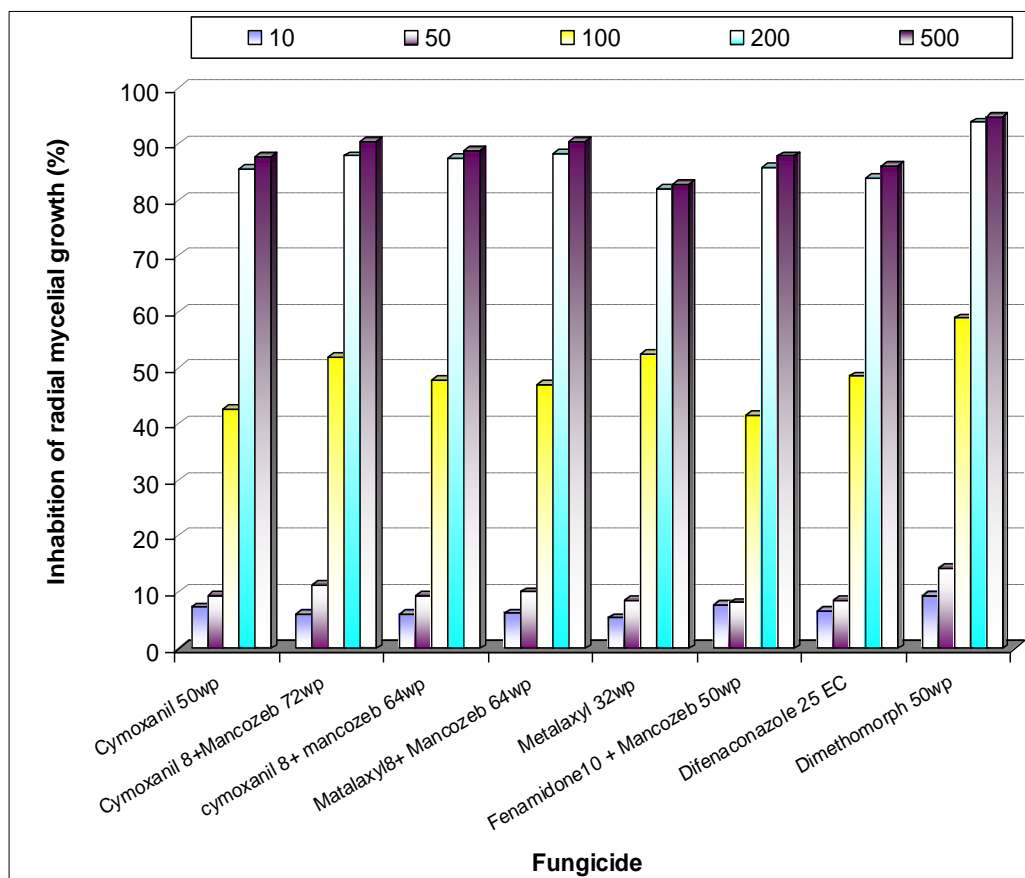


Fig 1: Inhibition% of mycelial growth of *P. infestans* at different concentrations of systemic/translaminar fungicides

There existed a significant interaction between the different fungicides and their concentrations in checking the radial mycelial growth of the test fungus. Compared to a radial mycelial growth of 85.66 mm obtained in un-amended check plates, dimethomorph 50WP resulted in the minimum radial mycelial growth of 4-5 mm only at concentration of 200-500 µg/ml followed by cymoxanil 8+mancozeb 72 WP, cymoxanil + mancozeb 64 WP and metalaxyl 8 + mancozeb 64 WP at the concentration of 500 µg/ml (8-8.67 mm). Cymoxanil 8 + mancozeb 72WP, cymoxanil 8 + mancozeb 64WP and metalaxyl 8 + mancozeb 72WP at concentrations of 200 µg/ml and Fenamidone 10 + mancozeb 50WP at concentration of 500 µg/ml were the next best fungicides harbouring *P. infestans* mycelial growth of only 9.67-10.67 mm. Except dimethomorph, all the test fungicides at the lowest test conc. of 10 µg/ml exhibited the radial mycelial growth 79.00-81.00 mm proved least effective against *P. infestans*. Perusal of the data (Fig. 1) revealed an increasing trend in inhibition of mycelia growth as the concentration of each fungicide increased, such that each fungicide at 200-500

mg/ml exhibited more than 90 per cent inhibition of mycelia growth, compared to 10 mg/ml which yielded less than 10 per cent inhibition. Dimethomorph at all the concentration was more compared to other fungicides.

The protectant fungicidal (Table-2) amendments into the substrate medium also resulted in significant reduction in radial mycelial growth of the *P. infestans*. On an average, mancozeb amendments harboured minimum mycelial growth of 46.77 mm followed by propineb amendments yielding the mycelial growth of 50.13 mm. Chlorothalonil was comparatively less effective yielding radial mycelial growth of 50.26 mm as compared to 85.73 mm growth obtained in un amended check. As with protectant fungicides, a gradual increase in fungicide concentration from 100 to 2000 µg/ml, there was a gradual decline in mycelial growth such that an average mycelial growth of 85.66 mm obtained at 100 mg/ml concentration dropped to 28.24 mm at 2000 µg/ml. A significant interaction also existed between fungicide and their concentrations.

Table 2: Radial mycelial growth of (*Phytophthora infestans*) on V-8 medium amended separately with different protectant fungicides

Fungicide	Radial mycelial growth (mm) at different concentrations					
	100 µg ml ⁻¹	200 µg ml ⁻¹	500 µg ml ⁻¹	1000 µg ml ⁻¹	2000 µg ml ⁻¹	Mean
Propineb 70 WP	80.33	75.33	55.33	30.00	10.00	50.13
Mancozeb 75 WP	77.33	72.66	50.33	27.66	5.66	46.77
Chlorothalonil 75 WP	81.33	76.66	50.66	31.00	11.66	50.26
Control	85.66	85.66	85.66	85.66	85.66	85.73

CD (0.05%)

Fungicide = 0.77

Concentration = 0.85

Fungicide x Concentration = 1.74

Data are mean of three replications

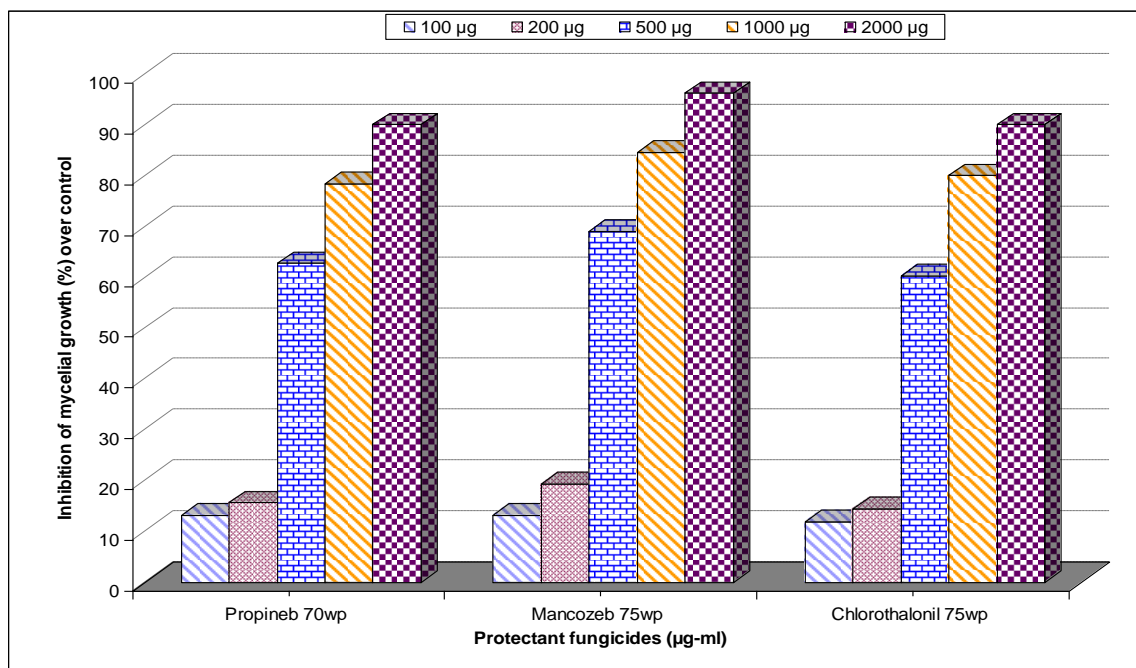


Fig 2: Inhibition % of mycelial growth of *P. infestans* by different concentrations of protectant fungicide

Mancozeb 75 WP at 2000 µ/ml was the most effective fungicide showing a radial growth of only 5.66 mm followed by propineb 70WP and chlorothalonil 75WP at the same concentration yielding mycelial growth of 10 and 11.66 mm each compared to 85.66 mm growth obtained in un amended check plates. Propineb 70WP and chlorothalonil 75 WP at 100-200 µg/ml and mancozeb at 100 µg/ml allowed the maximum growth of 75.33- 81.33 mm compared to other fungicidal concentrations and control. Insight into the data, Fig. 2 indicates mancozeb as the most effective fungitoxic compound providing more than 80 per cent mycelia growth inhibition at 1000-2000 mg/ml followed by propineb and chlorothalonil which did so at 2000 mg/ml.

The spore/conidia produced on the media amended separately with different fungicides at various concentrations were subjected to germination test. The results on the efficacy of systemic fungicides (Table-3) revealed that all the fungicides significantly reduced the germinability of the *sporangia* at all the test concentrations. On an overall basis, dimethomorph proved most effective allowing sporangia germination to the extent of only 31.80 percent followed by metalaxyl + mancozeb 64 WP and cymoxanil+ mancozeb 72 WP with sporangia germination of 36.53-37.00 per cent as compared to 80.66 per cent germination observed in un-amended check plates. A significant interaction between fungicides and their concentrations also existed. Dimethomorph proved most antisporeulant and completely restricted the sporangial germination at 200-500 µg/ml concentration followed by cymoxanil + mancozeb 72, metalaxyl +mancozeb +metalaxyl 64 and fenamidone 10 + mancozeb 50WP at 200-500 µg/ml and cymoxanil + mancozeb 64WP and difenaconazole at 500 µg/ml yielding spore germination of 6.66-9.66 per cent compared to 80.66 per cent observed in control. All the test fungicides at the lowest test concentration of 10 mg/ml and metalaxyl 32WP at 50 mg/ml concentration as well proved least inhibitory allowing 65.66-70.66 spore germination

compared to check 80.60 percent. The insight of the data (Fig. 3), further reveals maximum antisporeulant activity of dimethomorph yielding more than 90 per cent inhibition of sporulation at 200 µg/ml compared to control since no mycelia growth was allowed at 500 µg, so antisporeulant activity of the fungicide could not be ascertained. All other fungicides, caused reduction in sporulation at the test concentrations viz., 10 to 500 µg/ml.

The results on the efficacy of protectant fungicides (Table-4) reveal that all the fungicides significantly reduced the germinability of the sporangia at all the test concentrations. On an overall basis, mancozeb proved most effective allowing sporangia germination to the extent of only 35.67 per cent followed by propineb and chlorothalonil with sporangia germination of 38.86-39-86 per cent as compared to 80.66 per cent germination observed in un-amended check plates. On an average, there also existed a gradual decrease in spore germination with every increase in fungicide concentration such that the germination was maximum 72.91 per cent at the lowest test concentration (100 µg/ml) and minimum 24.91 per cent, at the highest test concentration of 2000 µg/ml. A significant interaction between fungicides and their concentrations also existed. Mancozeb proved most antisporeulant and restricted the sporangial germination at 2000 µg/ml concentration followed by propineb and chlorothalonil at 2000 µg/ml yielding spore germination of 8.00 per cent compared to 80.66 per cent observed in control. All the test fungicides at the lowest test concentration of 100 and at 500 µg/ml concentration proved least inhibitory allowing 65.00-71.00 spore germination compared to check 80.66 per cent. The insight of the data (Fig. 4) further reveals that Mancozeb again proved effective antisporeulant activity providing the 90 per cent reduction in sporangial germination at 2000 µg/ml. Propineb and chlorothalonil were the next best fungicides at all the test concentration.

Table 3: Germination of *Phytophthora infestans* sporangia obtained on V-8 medium amended separately with different concentrations of systemic/translaminar fungicides

Fungicide	Sporangia Germination (%) at fungicide concentrations					
	10 $\mu\text{g}^{-\text{ml}}$	50 $\mu\text{g}^{-\text{ml}}$	100 $\mu\text{g}^{-\text{ml}}$	200 $\mu\text{g}^{-\text{ml}}$	500 $\mu\text{g}^{-\text{ml}}$	Mean
Cymoxanil 50 WP	69.00 (56.23)	64.00 (53.15)	43.66 (41.35)	10.33 (18.68)	10.00 (18.34)	39.40 (37.55)
Cymoxanil 8+Mancozeb 72 WP	70.66 (57.24)	63.00 (52.54)	35.66 (37.65)	7.33 (15.65)	6.66 (14.92)	36.67 (35.60)
Cymoxanil 8+ mancozeb 64 WP	69.00 (56.30)	63.00 (53.29)	37.00 (37.35)	11.66 (19.94)	7.66 (16.02)	37.86 (36.60)
Matalaxyl 8+ Mancozeb 64 WP	68.33 (55.89)	62.00 (52.78)	35.00 (36.25)	9.33 (17.52)	8.00 (16.34)	36.53 (35.65)
Metalaxyl 32 WP	70.33 (57.02)	65.66 (54.15)	33.33 (35.25)	14.66 (22.50)	13.33 (21.39)	39.46 (38.06)
Fenamidone10 + Mancozeb 50 WP	68.66 (55.98)	63.33 (52.78)	43.66 (41.35)	9.33 (17.52)	9.00 (17.41)	38.73 (36.98)
Difenaconazole 25 EC	68.66 (55.97)	64.33 (53.35)	38.66 (38.43)	11.00 (19.32)	9.66 (18.07)	38.46 (37.03)
Dimethomorph 50 WP	67.00 (54.94)	61.00 (51.37)	31.00 (33.33)	-	-	31.80 (28.02)
Control	80.66 (63.92)	80.66 (63.92)	80.66 (63.92)	80.66 (63.92)	80.66 (63.92)	80.66 (63.92)
Mean	70.25 (57.05)	65.37 (54.06)	42.25 (40.61)	17.11 (63.92)	16.11 (20.71)	-

CD (0.05%)

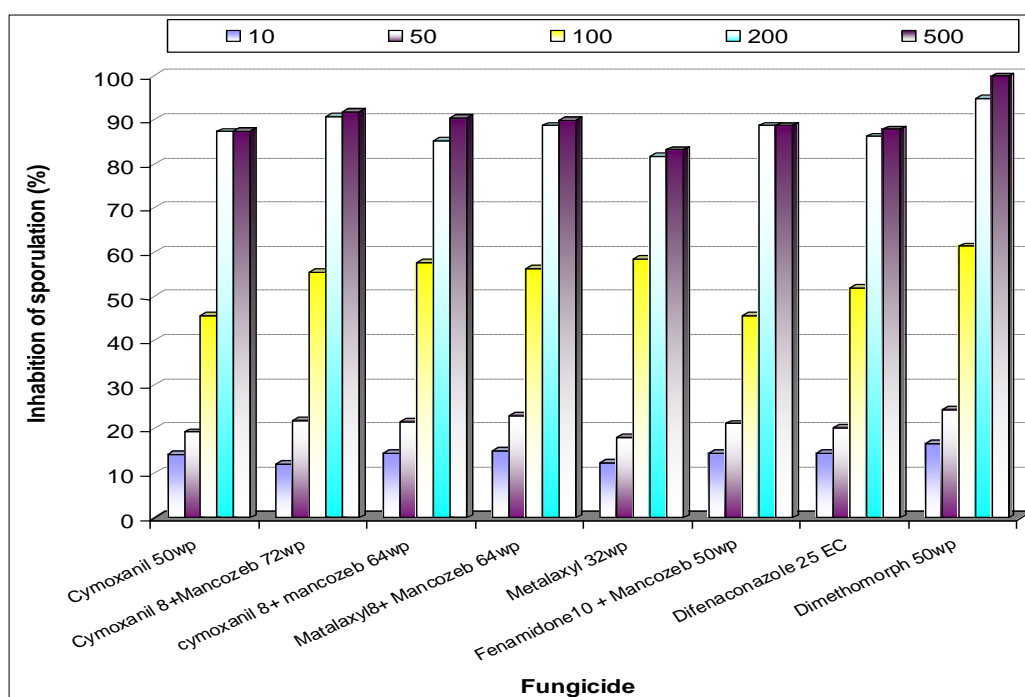
Fungicide = 0.74

Concnetration = 0.41

Fungicide x Concentration = 3.70

Data is mean of three replications

Figures in parenthesis are arc sine transformed values

**Fig 3:** Inhibition of sporulation % of *P. infestans* by different concentrations of systemic/translaminar fungicides**Table 4:** Germination of *Phytophthora infestans* sporangia obtained on V-8 medium amended separately with different concentrations of protectant fungicides

Fungicide	Sporangia Germination (%) at fungicide concentrations					Mean
	100 $\mu\text{g}^{-\text{ml}}$	500 $\mu\text{g}^{-\text{ml}}$	1000 $\mu\text{g}^{-\text{ml}}$	2000 $\mu\text{g}^{-\text{ml}}$	5000 $\mu\text{g}^{-\text{ml}}$	
Propineb 70 WP	70.00 (56.84)	68.1 (56.18)	30.00 (33.20)	17.33 (24.56)	8.00 (16.41)	38.86 (37.44)
Mancozeb 75 WP	70.00 (56.84)	65.00 (53.73)	25 (29.99)	12.33 (20.54)	3.00 (9.88)	35.67 (34.19)
Chlorothalonil 75 WP	71.00 (57.48)	69.00 (56.17)	32.00 (34.42)	19.33 (25.96)	8.00 (16.36)	39.86 (38.08)
Control	80.66 (63.92)	80.66 (63.92)	80.66 (63.92)	80.66 (63.92)	80.66 (63.92)	80.66 (63.92)
Mean	72.91 (58.76)	70.91 (57.50)	41.91 (40.38)	32.41 (33.75)	24.91 (26.65)	-

CD (0.05%)

Fungicide = 0.34

Concentration = 0.42

Fungicide x Concentration = 1.70

Figure in parenthesis are arc sine transformed values

Data are mean of three replications

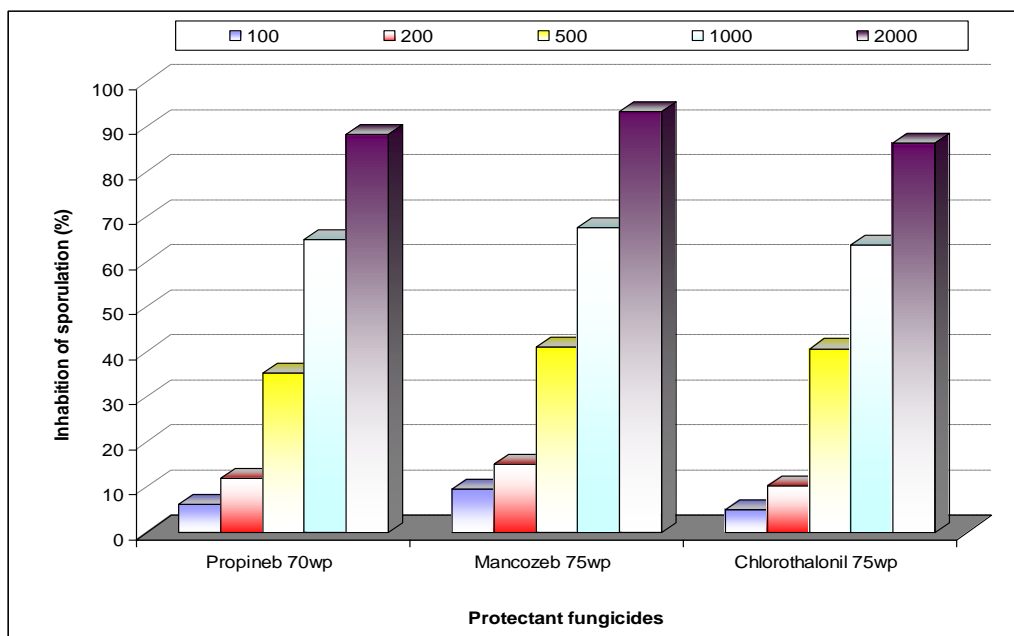


Fig 4: Inhibition of sporulation % of *P. infestans* by different concentrations of protectant fungicides

The use of fungicides for controlling plant diseases caused by fungi are well documented (Nene and Thapliyal, 2002) [7]; however, the control of diseases such as that of potato late blight caused by Phycomycetous fungi, by the use of traditional protectant fungicide has not been so successful necessitating to evaluate newer molecules for its control. During the present studies, dimethomorph 50WP at 200-500µg/ml was most inhibitory to *P. infestans* mycelia growth followed by cymoxanil 8+mancozeb 64WP among the systemic/translaminar fungicides. Among protectants, however, mancozeb was the most inhibitory to mycelial growth followed by propineb and chlorothalonil. The spore production and spore germinability was also accordingly reduced. The *in vitro* evaluations indicate that these chemicals could be used for the control of late blight disease as protectants or therapeutants. The application of fungicides that would help to inhibit mycelial growth and act as antisporegents are likely to control the spread of this disease (Johnson *et al.*, 2000; Matheron and Porchas, 2000) [3, 6]. The present studies are in accordance with those of many researchers (Rani *et al.*, 2009) [8]. The efficacy of mancozeb or of prolineb and chlorothalonil against many foliar disease pathogens such as *P. infestans* has long been reported (Johnson *et al.*, 2000) [3]. However, dimethomorph, cymoxanil and metaxyl have shown better mycelial inhibition against the pathogen (Rani *et al.*, 2009; Khan *et al.*, 2003) [8, 5]. Chakraborty and Mazumdar, 2012 [2] reported that prophylactic sprays of chlorothalonil/mancozeb followed by systemic/translaminar fungicides were found effective than postsymptomatic sprays. They also reported that the severe late blight can be effectively managed with prophylactic spray of mancozeb @0.25% followed by cymoxanil + mancozeb or dimethomorph + mancozeb @0.3% at the onset of disease and one more spray of mancozeb@0.25% seven days after application of systemic fungicides. Khadka *et al.*, 2016 [4] also reported that spraying with Dimethomorph and fenamidol+mancozeb showed less disease against late blight of potato. Siddique *et al.*, 2016 [9] reported that the highest percentage of disease control and the highest yield were recorded with Cymoxanil 8%+Mancozeb 64% fungicide which are in consonance with our results.

So, from this present study it can be concluded that chemical fungicides were highly effective in the management of late blight disease of potato and they can also be combined with other cultural practices by integrating with them to prevent the development of new races of the pathogen due to the chance of resistance development against the available fungicides. With the present study it is evident that proper combination of both systemic and contact fungicides and at optimum concentrations can help to prevent resistant development of the pathogen. And also based on their inhibitory activities of sporulation and sporangial germination of the pathogen, some of them can be applied as prophylactic (before symptoms appear) sprays.

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