

Article

Identification of potential chemical fungicides with diverse groups of active ingredient for controlling late blight of potato in Bangladesh

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Abstract: Potato is the third important food crop in Bangladesh and Bangladesh ranked as third and seventh in Asia and in the world, respectively. But potato production greatly affected by the late blight of potato caused by an oomycete, *Phytophthora infestans* (Mont.) de Bary worldwide. Application of need-based fungicides is the key factor to control this disease and to avoid development of fungicide resistance in *P. infestans*. Experiments were conducted in both *in vitro* and in field condition to evaluate some selected chemical fungicides to identify the best one to control the late blight disease of potato. The results of *in vitro* test revealed that all the fungicides inhibited the growth of *P. infestans* by 94% over control. But the results of field experiment conceded that the lowest late blight severity was found in T₁₀ (Curzate M8) (0.49%) followed by T₆ (AcrobateMz) (0.52%), T₈ (Micra) (0.59%), T₇ (Xtramil) (0.61%), T₃ (Daconyl) (0.84%), T₅ (Secure) (1.28%), T₉ (Sanoxanyl) (1.43%), T₁ (Unilax) (1.83%), T₂ (TemperM) (2.58%) and T₄ (Amiscore) (6.34%) at 71 DAP while the maximum late blight severity (95.33%) was observed in T₀ (water as control). The highest yield was obtained in T₁₀ (Curzate M8) (25.94 t/ha) followed by T₆ (AcrobateMz) (25.67 t/ha), T₅ (Secure) (25.22 t/ha), T₇ (Xtramil) (24.61 t/ha), T₈ (Micra) (24.61 t/ha) and T₉ (Sanoxanyl) (23.67 t/ha) which were statistically similar and the lowest (8.39 t/ha) yield was observed in T₀ (water as control). The highest Benefit Cost Ratio (BCR) was obtained from when potato plants were sprayed with T₁₀ (Curzate M8) (1.02) following by T₇ (Xtramil) (0.99), T₈ (Micra) (0.99), T₆ (AcrobateMz) (0.92), T₉ (Sanoxanyl) (0.90), T₅ (Secure) (0.89), T₁ (Unilax) (0.80), T₃ (Daconil) (0.74), T₄ (Amiscore) (0.71) and T₂ (TemperM) (0.44) compared to control T₀ (water as control) (-0.30). The results indicated that application of these fungicides yielded a benefit of Tk. ranged by 0.44 to 1.02 over the investment of Tk. 1.00. Therefore, identification of a number of potential chemical fungicides might be useful in the alternate use of these fungicides against late blight of potato in the field.

Keywords: late blight; oomycete; *Phytophthora infestans*; chemical fungicides; Curzate M8

1. Introduction

Potato is the third important food crop in Bangladesh and it is the third largest potato producer in Asia (after china and India) which standing seventh globally (FAOSTAT, 2021). It is truly a global crop. Presently potato consumption has been steadily increasing in the developing countries while it has been declining in Europe, and

North America. Recently, more potato production has been observed in developing countries than in the developed world and it has an essential role in the food security of developing countries (Wijesinha-Bettoni and Mouillé, 2019). But late blight of potato caused by an oomycete, *Phytophthora infestans* (Mont.) de Bary is one of the major reasons for affecting yields in the world. According to the study report of Guenther *et al.* (2001), the economic losses of late blight to US potato growers (cost of spraying plus losses from disease) averaged more than US \$500/ha, making late blight one of the most economically important disease of potato. The cost of *P. infestans* to the potato alone is about to US \$6.7 billion annually (USA Blight, 2012). According to Haverkort *et al.* (2009) the crop losses and estimated to cause approximately US\$ 14 billion per annum worldwide, of which around US\$ 12 billion per annum is from developing countries thus, it has been considered as a global threat in potato production. In Bangladesh, the yield losses have been estimated approximately by 25-57% due to late blight disease of potato (Ali and Dey, 1994). In 2019, Bangladesh produced 9.7 million tonnes on 0.5 million ha, representing 2.6 % of world production (FAOSTAT, 2021). The average yield of potatoes in Bangladesh in 2019, as calculated by the FAO (FAOSTAT, 2021), was 20.6 t/ha, which is lower as compared to the potential yield and the result of other potato growing countries of the world. For example, in Ireland, Netherlands and, USA, potato yield is 44.1, 42.0, and 50.3 t/ha in 2019, respectively (FAOSTAT, 2021). Moreover, late blight is considered one of the five highest ranking priorities for potato research in Asia, Africa and Latin America (Fuglie, 2007).

The pathogen infects the entire plant and spreads its sporangia rapidly by the wind if the conditions are favorable. If the crop is not protected with fungicides with different modes of action, resistance to some chemicals may occur and the destruction of potato crops is a matter of weeks (Abad and Ochoa, 1995; Díaz-de la Cruz *et al.*, 2014). Nevertheless, the best management measure has still been the use of fungicides, but the populations of *P. infestans* have developed resistance to the fungicides, favored by the continuous application of a single fungicide (Damicone, 2004). Application of need-based effective fungicides for the better management of late blight of potato is the key activity to control the late blight of potato (Singh and Bhat, 2003). Outbreak of Late blight of potato appears suddenly within 2-3 days of prevailing favorable condition in Bangladesh and experts advises preventive sprays to farmers when a favorable late blight infection period is predicted to occur. Time to time several fungicides including contact, systemic and translaminar have been evaluated; however, the pathogen has shown a remarkable capacity for change with respect to host genotype and fungicides. As a result, disease control requires regular application of fungicides at high rates and short intervals throughout the growing season (Lal *et al.*, 2015). Fungicide mixtures, containing two or more fungicides with different modes of action, have been developed with the twin objectives of broadening the activity spectrum against plant diseases and to check the development of resistance in the target pathogens (Thind, 2012). Hossain *et al.* (2009) showed that metalaxyl resistance was present in *P. infestans* isolates collected in 1995-1996 from Bangladesh. Recently, from a metalaxyl sensitivity test considering both 2018 and 2019 *P. infestans* isolates, the results indicated that 38% of the total *P. infestans* isolates tested were intermediate and 62% of the total *P. infestans* isolates were metalaxyl resistant (Islam *et al.* unpublished data). Moreover, development of metalaxyl resistance in *P. infestans* races globally had made this systemic fungicide redundant and so far, farmers are waiting for its apt replacement.

As resistant varieties are not available, so chemical control is playing a major role in potato production in Bangladesh. Most of the farmers or potato producing companies are trying to control this late blight disease traditionally by using only mancozeb or combined with carbendazim. They failed to control this disease in most of the cases by this traditional method. Although the Mancozeb gave good control of late blight of potato but when the environment is in disfavor its controlling power gradually reduce and farmers face great difficulties (Siddique *et al.*, 2016). Farmers use a particular chemical fungicide repeatedly in controlling late blight of potato in Bangladesh. As a consequence, fungicide resistance in *P. infestans* population is developed and this fungicide resistance cannot be compromised by increasing dose and frequency of chemical fungicides spray. The possible mechanisms are summarized as i) migration and mixed occurrence of diverse genotypes of *P. infestans* in a given agricultural ecosystem arises from migration, genetic recombination or evolution, ii) elimination of fungicides-sensitive strains due to selection pressure as a result of continuous application of fungicides and increases the frequency of resistant genotypes in a population and iii) emergence of resistant genotypes of *P. infestans* due to mutation by UV radiation in potato fields located at high altitudes or due to some of the fungicides containing mutagenic chemicals and iv) evolution of fungicide resistance in *P. infestans* due to natural mutations in the course of gamete formation (Clayton and Shattock, 1995; Colon *et al.* 1995; Delen 2016; Vincelli and Dixon, 2002 and Majeed *et al.* 2015). Therefore, in the present study some potential chemical fungicides containing diverse groups of active ingredients were identified for their alternate use instead of the repeated use of a particular fungicide.

2. Materials and Methods

2.1. Experimental location and design

The efficacy of some selected chemical fungicides was evaluated in both *in vitro* in the laboratory and in the field condition against late blight of potato in 2017-2018. Field experiment was conducted in the farmer's field, Sutia Khali, Mymensingh Sadar, Mymensingh. Field experiment was conducted following Randomized Complete Block Design (RCBD) with three replications. Plot size for field experiment was 3 x 2 m². Row to row distance was 60 cm while plant to plant distance was 25 cm.

2.2. Treatments

The following treatments were used for the field experiment; T₀ = Water (control), T₁ = Foliar spray of Unilax (Metalaxyl + Mancozeb) 2 gm/L, T₂ = Foliar spray of TemperM (Propeneb + Cymoxanil) 2gm/L, T₃ = Foliar spray of Daconil (Clorothalonil) 1.5 ml/L, T₄ = Foliar spray of Amiscore (Azoxystrobin + Difenconazole) 1ml/L, T₅ = Foliar spray of Secure (Fenamedon + Mancozeb) 2gm/L, T₆ = Foliar spray of AcrobateMz (Dimethomorph + Mancozeb) 4 gm/L, T₇ = Foliar spray of Xtramil (Cymoxanil + Mancozeb) 2gm/L, T₈ = Foliar spray of Micra (Cymoxanil + Mancozeb) 2gm/L, T₉ = Foliar spray of Sanoxanyl (Cymoxanil + Mancozeb) 2gm/L and T₁₀ = Foliar spray of Curzate M8 (Cymoxanil + Mancozeb) 2gm/L.

2.3. Growing potato for field experiments

Land was prepared by ploughing and cross ploughing with a power tiller. Before the final land preparation Cowdung (7.5 t/ha), DAP (260kg/ha), MOP (260kg/ha), Gypsum (120kg/ha), Zinc (7.5kg/ha), Boron (7.5kg/ha), Magnesium (45 kg/ha), Furadan (7.5kg/ha) and Urea (120kg/ha) were applied. Then experimental Layout was prepared based on the total number of plots required. Apparently, disease free and uniform tubers of a popular potato cultivar (Diamant, a variety showing susceptibility under severe outbreak) were cut into pieces with at least one bud and were left for 24 h for suberization. Then, the suberized tuber pieces were treated by drenching with the selected fungicides (at above mentioned dose) solution by spraying and the treated tubers were left for at least 1 h for adherence. Treated and non-treated tuber pieces were planted in experimental plots. Two top dressings of urea (120 kg/ha) were applied at 33 and 60 DAP along with two irrigations at 27 and 60 DAP. Weeding was performed at 25 DAP followed by earthen up at 33 and 43 DAP.

2.4. Foliar application of the fungicides

In field experiment, the chemical fungicide(s) were sprayed at 34, 41, 48, 54, 57, 62, 69 and 75 DAP (at above mentioned dose) to evaluate the performance of the chemical fungicides against late blight of potato.

2.5. Data collection

Ten potato plants were randomly selected to collect data in the field. The plants were then tagged for confirmation. After application of the treatments, the following data were collected to compare the treatments performance.

(a) Incidence and severity: Data on the late blight incidence and severity were recorded at 48, 59 and 71 DAP according to the formula and the scales mentioned below, respectively.

$$\text{Late blight incidence (\%)} = \frac{\text{Number of late blight infected plants}}{\text{Total number of plants examined}} \times 100$$

Severity scale to assess the late blight disease severity developed by James (1971). Briefly, 1 = 0% blight (no disease observed), 2 = 0.1% blight (a few scattered plants blighted; no more than 1 or 2 spots in 12-yard radius), 3 = 1% blight (up to 10 spots per plant; or general light infection), 4 = 5% blight (about 50 spots per plant; up to 1 in 10 leaflets infected), 5 = 25% blight (nearly every leaflet infected, but plants retain normal form; plants may smell of blight; field looks green although every plant is affected), 6 = 50% blight (every plant affected and about 50% of leaf area destroyed), 7 = 75% blight (about 75% of leaf area destroyed; field appears neither predominantly brown or green), 8 = 95% blight (only a few leaves on plants, but stems green), and 9 = 100% blight (all leaves dead, stems dead or dying).

(b) Growth and yield parameters: The following growth and yield parameters were considered for field experiments: i) Plant height was recorded at 34, 52 and 71 DAP. ii) Number of plants per hill was recorded at the time of harvest. iii) Number of tubers per plant was recorded at the time of harvest. iv) Yield was recorded at the time of harvest.

2.6. Economic analyses

The benefit-cost ratio (BCR) was calculated for each treatment according to method of Mondal *et al.* (1994). The cost-benefit analysis was done based on gross returns and cost of each treatment to compare the profitability among the treatments. The gross return and net return were calculated for each treatment as follows: Gross return (TK/ha) = Fruit Yield (kg/ha) × Price (TK/kg), Net return (TK/ha) = Gross return (TK/ha) - Cost of production plus treatment cost (TK/ha), The BCR was calculated as shown below:

$$\text{BCR} = \frac{AXC - B}{B}$$

Where, A = Selling price (Tk./kg), B = Cost of cultivation + Treatment cost (Tk./ha), C = Yield (kg/ha)

2.7. Statistical analysis

Data were analyzed using MStatC statistical program. Means were compared using Duncan's Multiple Range Test (DMRT).

3. Results

3.1. Identification of potential fungicide(s) against late blight of potato both in *in vitro* and field condition

To identify the best fungicide(s) against late blight of potato, some selected fungicides available in the market were evaluated both in *in vitro* and in field condition in 2017-2018.

3.2. *In vitro* growth inhibition of *P. infestans* by some selected commercially available fungicides

The effects of selected fungicides were evaluated in the *in vitro* growth inhibition of *P. infestans* on pea agar medium. The results revealed that all of the fungicides inhibited the growth of *P. infestans* by 94% over control (Figure 1 A, B & C).

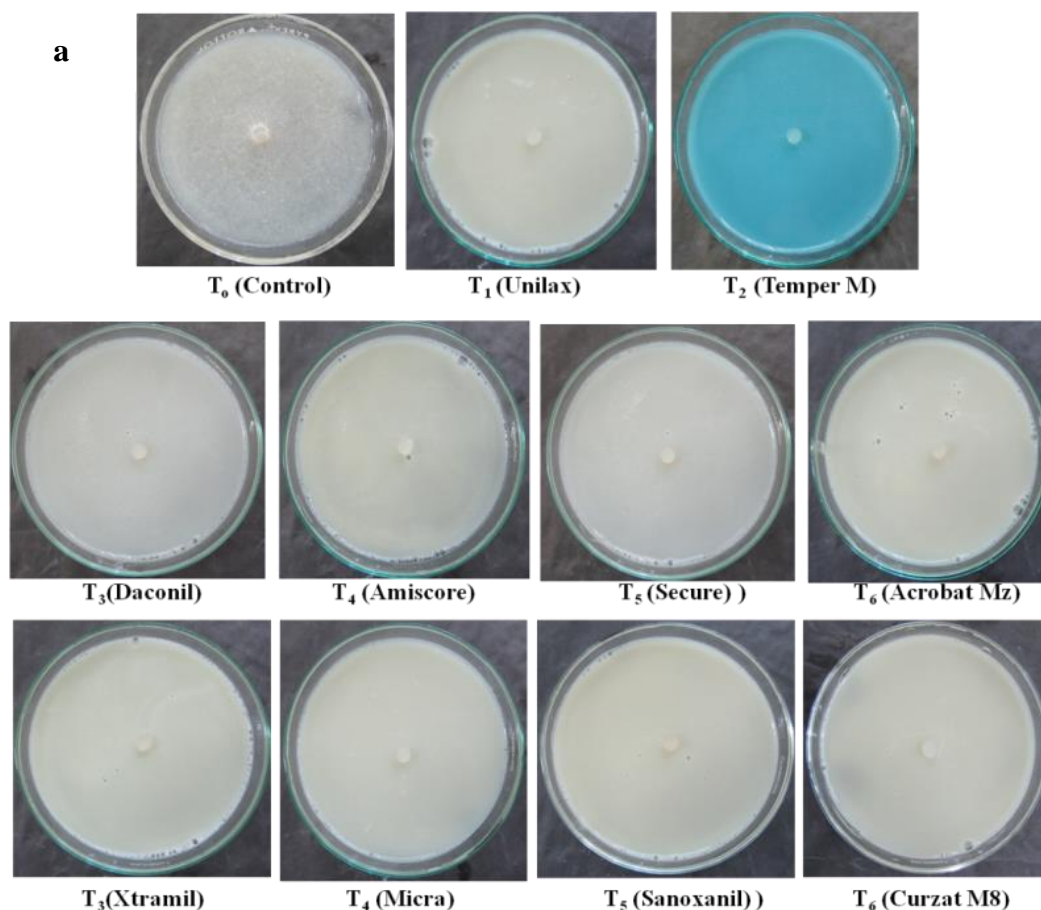


Figure 1a. *In vitro* growth inhibition of *P. infestans* by some selected commercially available fungicides on pea agar medium amended with the fungicides.

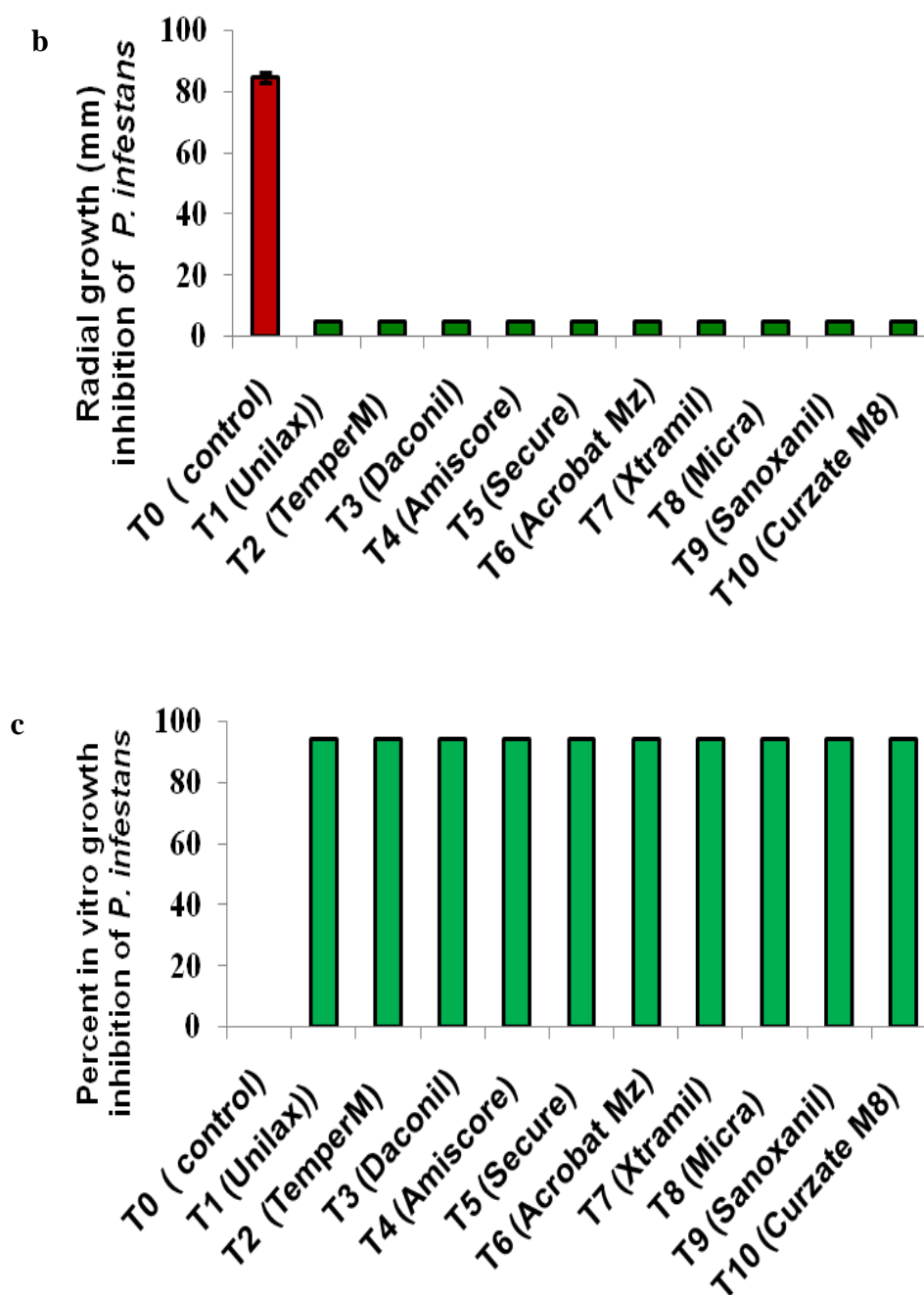


Figure 1b. Radial growth inhibition (mm) of *P. infestans* by some selected fungicides and **1c.** Percent *in vitro* growth inhibition of *P. infestans* by some selected fungicides over control.

3.3. Comparative efficacy of different potential fungicides in controlling late blight of potato

Comparative efficacy of different potential fungicides against late blight of potato available in the market of Bangladesh in 2017-2018 was studied on different parameters of potato cultivation. Results revealed that incase of late blight incidence after 59 DAP the highest late blight incidence (100%) was found in T₀ (water as control) while the lowest late blight incidence (9.05%) was found in T₁₀ (Curzate M8) (9.05%) followed by T₆ (AcrobateMz) (9.84%). Statistically similar results were found in T₃ (Daconyl) (27.41%), T₉ (Sanoxanyl) (30.33%), T₇ (Xtramil) (33.19%), T₈ (Micra) (34.17%), T₅ (Secure) (36.99%), T₁ (Unilax) (42.20%), T₄ (Amiscore) (45.40%) and T₂ (TemperM) (52.87%). At 71 DAP the maximum late blight incidence (100%) was found in T₀ (water as control) (100%), T₁ (Unilax) (100%), T₂ (TemperM) (100%), T₄ (Amiscore) (100%), T₅ (Secure) (100%) and T₉ (Sanoxanyl) (100%) whereas the minimum late blight incidence (97.44%) was observed

in T₁₀ (Curzate M8) (97.44%) followed by T₃ (Daconyl) (97.50%), T₇ (Xtramyl) (97.52%), T₆ (AcrobateMz) (97.54%) and T₈ (Micra) (97.59%) (Table 1).

Table 1. Comparative efficacy of different potential fungicides in controlling late blight of potato.

Treatment	% Late blight incidence		% Late blight severity	
	Days after planting		Days after planting	
	59	71	59	71
T ₀	100.00±0.00 ^a	100.00	6.27±4.81 ^a	95.33±2.68 ^a
T ₁	42.20±19.45 ^b	100.00	0.04±0.02 ^b	1.83±0.55 ^b
T ₂	52.87±25.18 ^b	100.00	0.05±0.03 ^b	2.58±1.12 ^b
T ₃	27.41±15.50 ^b	97.50	0.027±0.01 ^b	0.84±0.37 ^b
T ₄	45.40±17.00 ^b	100.00	0.06±0.02 ^b	6.34±5.39 ^b
T ₅	36.99±14.61 ^b	100.00	0.053±0.02 ^b	1.28±0.67 ^b
T ₆	9.84±1.41 ^b	97.54	0.01±0.01 ^b	0.52±0.03 ^b
T ₇	33.19±7.58 ^b	97.52	0.04±0.01 ^b	0.61±0.12 ^b
T ₈	34.17±13.44 ^b	97.59	0.04±0.01 ^b	0.59±0.42 ^b
T ₉	30.33±13.38 ^b	100.00	0.04±0.02 ^b	1.43±0.34 ^b
T ₁₀	9.05±2.14 ^b	97.44	0.007±0.00 ^b	0.49±0.17 ^b
Level of significance	*	NS	*	*
CV (%)	66.08	2.32	417.53	28.67

Data are the averages of three replications. Values with same letters in the same column are statistically similar. NS = Non-significant and * indicates the means were significant at 5% level of probability.

T₀ = Water (control), T₁ = Foliar spray of Unilax (Metalaxyl + Mancozeb) 2 gm/L, T₂ = Foliar spray of TemperM (Propeneb + Cymoxanyl) 2gm/L, T₃ = Foliar spray of Daconyl (Clorothalonil) 1.5 ml/L, T₄ = Foliar spray of Amiscore (Azoxystrobin + Difenconazol) 1ml/L, T₅ = Foliar spray of Secure (Fenamedon + Mancozeb) 2gm/L, T₆ = Foliar spray of AcrobateMz (Dimethomorf + Mancozeb) 4 gm/L, T₇ = Foliar spray of Xtramyl (Cymoxanil + Mancozeb) 2gm/L, T₈ = Foliar spray of Micra (Cymoxanyl + Mancozeb) 2gm/L, T₉ = Foliar spray of Sanoxanyl (Cymoxanyl + Mancozeb) 2gm/L and T₁₀ = Foliar spray of Curzate M8 (Cymoxanyl + Mancozeb) 2gm/L.

Considering % late blight severity at 59 DAP the highest severity of late blight (6.27%) was observed in T₀ (water as control) and the lowest was in T₁₀ (Curzate M8) (0.007%). Other treatments showed the statistically similar results such as T₆ (AcrobateMz) (0.01%), T₃ (Daconyl) (0.027%), T₁ (Unilax) (0.04%), T₇ (Xtramyl) (0.04%), T₈ (Micra) (0.04%), T₉ (Sanoxanyl) (0.04%), T₂ (TemperM) (0.05%), T₅ (Secure) (0.053%) and T₄ (Amiscore) (0.06%) (Table 1).

At 71 DAP the maximum severity of late blight (95.33%) was resulted in T₀ (water as control) (95.33%) and the minimum (0.49%) severity of late blight was found in T₁₀ (Curzate M8) (0.49%). Statistically similar results were obtained from T₆ (AcrobateMz) (0.52%), T₈ (Micra) (0.59%), T₇ (Xtramyl) (0.61%), T₃ (Daconyl) (0.84%), T₅ (Secure) (1.28%), T₉ (Sanoxanyl) (1.43%), T₁ (Unilax) (1.83%), T₂ (TemperM) (2.58%) and T₄ (Amiscore) (6.34%) (Table 1 and Figure 2).

The results of percent reduction of late blight severity over control at 71 DAP, revealed that the highest reduction (99.48%) was estimated in T₁₀ (Curzate M8) (Positive control) (99.48) followed by T₆ (AcrobateMz) (99.45), T₇ (Xtramyl) (99.36), T₈ (Micra) (99.35), T₃ (Daconyl) (99.13), T₅ (Secure) (98.67), T₉ (Sanoxanyl) (98.52), T₁ (Unilax) (98.09), T₂ (TemperM) (97.23), T₄ (Amiscore) (93.02) and the lowest (0.00) reduction was found in T₀ (negative control) (0.00) (Figure 3).

3.4. Comparative efficacy of different potential fungicides in increasing growth and yield contributing parameters of potato

Comparative efficacy of different potential fungicides in increasing growth and yield contributing parameters of potato such as germination (%), Plant height (cm), No. of plantlets/hill, No. of tubers/plant and Yield (t/ha), were evaluated in 2017-2018. The results of % germination showed that the lowest germination was observed in T₁ (Unilax) (95.24%) and the highest was observed in T₃ (Daconyl) (97.62%), T₅ (Secure) (97.62%), T₇ (Xtramyl) (97.62%) and T₈ (Micra) (97.62%) followed by T₂ (TemperM) (96.83%), T₄ (Amiscore) (96.83%), T₆ (AcrobateMz) (96.83%), T₉ (Sanoxanyl) (96.83%), T₁₀ (Curzate M8) (96.03%) and T₀ (water as control) (96.03%) (Table 2).

Data on the Plant height (cm) were collected at 34, 52 and 71 DAP. At 34 DAP the highest plant height (28.00 cm) was observed in T₇ (Xtramyl) (28.00 cm) and lowest plant height was in T₀ (water as control) (22.23 cm)

which was statistically similar with T₈ (Micra) (23.10 cm) followed by T₆ (AcrobateMz) (24.17 cm), T₂ (TemperM) (24.60 cm), T₁ (Unilax) (25.03 cm), T₃ (Daconyl) (25.03 cm), T₉ (Sanoxanyl) (25.37 cm), T₄ (Amiscore) (26.10 cm), T₁₀ (Curzate M8) (26.10 cm) and T₅ (Secure) (26.13 cm). Data collected at 52 DAP showed that the maximum plant height was found in T₇ (Xtramyl) (61.40 cm) which was statistically identical with T₅ (Secure) (60.17 cm) and the minimum plant height (46.63 cm) was observed in T₁ (Unilax) (46.63). Statistically similar results were found in T₆ (AcrobateMz) (55.90 cm), T₉ (Sanoxanyl) (54.87 cm) and T₈ (Micra) (54.50 cm) which were higher than T₂ (TemperM) (49.40 cm), T₀ (water as control) (53.00 cm) but lower than T₃ (Daconyl) (57.17 cm), T₁₀ (Curzate M8) (58.37 cm) and T₄ (Amiscore) (59.17 cm). In case of the data at 71 DAP the highest plant height was obtained in T₂ (TemperM) (89.30 cm) and the lowest plant height (71.23 cm) was calculated in T₀ (water as control) (71.23 cm). Statistically similar results were counted in T₉ (Sanoxanyl) (82.07 cm), T₁ (Unilax) (82.17 cm), T₁₀ (Curzate M8) (82.63 cm), T₅ (Secure) (83.37 cm) and T₆ (AcrobateMz) (83.50 cm) which were higher than T₃ (Daconyl) (75.27 cm), T₇ (Xtramyl) (75.70 cm) and T₈ (Micra) (76.10 cm) but lower than T₄ (Amiscore) (86.13 cm) (Table 2).

Table 2. Comparative efficacy of different potential fungicides in increasing growth and yield contributing parameters of potato.

Treatments	Germination (%)	Plant height (cm)			No. of plantlets/hill	No. of tubers/plant	Yield (t/ha)
		Days after planting (DAP)					
		34	52	71			
T ₀	96.03	22.27±0.23 ^e	53.00±0.40 ^g	71.23±0.38 ^e	1.73	4.467	8.39±1.31 ^d
T ₁	95.24	25.03±0.44 ^{bc}	46.63±0.93 ⁱ	82.17±0.12 ^c	2.33	6.733	22.44±1.48 ^{abc}
T ₂	96.83	24.60±1.05 ^c	49.40±0.85 ^h	89.30±0.15 ^a	1.73	5.000	18.11±2.95 ^c
T ₃	97.62	25.03±0.39 ^{bc}	57.17±0.64 ^{de}	75.27±0.27 ^d	2.00	5.600	21.72±2.36 ^{bc}
T ₄	96.83	26.10±0.29 ^b	59.17±0.15 ^{bc}	86.13±0.33 ^b	1.87	6.200	21.67±0.48 ^{bc}
T ₅	97.62	26.13±0.66 ^b	60.17±0.07 ^{ab}	83.37±0.43 ^c	2.20	5.933	25.22±0.62 ^{ab}
T ₆	96.83	24.17±0.70 ^{cd}	55.90±0.20 ^{ef}	83.50±0.87 ^c	2.07	5.867	25.67±0.96 ^{ab}
T ₇	97.62	28.00±0.61 ^a	61.40±0.17 ^a	75.70±0.92 ^d	1.73	4.800	24.61±1.11 ^{ab}
T ₈	97.62	23.10±0.58 ^{de}	54.50±0.06 ^f	76.10±0.92 ^d	2.27	6.067	24.61±2.42 ^{ab}
T ₉	96.83	25.37±0.49 ^{bc}	54.87±0.27 ^f	82.07±0.22 ^c	2.13	5.933	23.67±0.33 ^{ab}
T ₁₀	96.03	26.10±0.55 ^b	58.37±0.69 ^{cd}	82.63±0.27 ^c	2.47	6.133	25.94±1.18 ^a
Level of significance	NS	*	*	*	NS	NS	*
CV (%)	2.35	2.68	1.48	1.17	19.09	22.54	10.58

Data are the averages of three replications. Values with same letters in the same column are statistically similar. NS = Non-significant and * indicates the means were significant at 5% level of probability.

T₀ = Water (control), T₁ = Foliar spray of Unilax (Metalaxyl + Mancozeb) 2 gm/L, T₂ = Foliar spray of TemperM (Propeneb + Cymoxanyl) 2gm/L, T₃ = Foliar spray of Daconyl (Clorothalonyl) 1.5 ml/L, T₄ = Foliar spray of Amiscore (Azoxyastrobin + Difenconazole) 1ml/L, T₅ = Foliar spray of Secure (Fenamedon + Mancozeb) 2gm/L, T₆ = Foliar spray of AcrobateMz (Dimethomorf + Mancozeb) 4 gm/L, T₇ = Foliar spray of Xtramyl (Cymoxanyl + Mancozeb) 2gm/L, T₈ = Foliar spray of Micra (Cymoxanyl + Mancozeb) 2gm/L, T₉ = Foliar spray of Sanoxanyl (Cymoxanyl + Mancozeb) 2gm/L and T₁₀ = Foliar spray of Curzate M8 (Cymoxanyl + Mancozeb) 2gm/L.

Number of plantlets per hill were recorded for all the treatments and presented in the Table 2. Results showed that the highest number of plantlets per hill was recorded in T₁₀ (Curzate M8) (2.47) and lowest was in T₀ (water as control) (1.73), T₂ (TemperM) (1.73) and T₇ (Xtramyl) (1.73). Other treatments showed the results as T₄ (Amiscore) (1.87), T₃ (Daconyl) (2.00), T₆ (AcrobateMz) (2.07), T₉ (Sanoxanyl) (2.13), T₅ (Secure) (2.20), T₈ (Micra) (2.27) and T₁ (Unilax) (2.33) which were lower than T₁₀ (Curzate M8) (2.47) but higher than T₀ (water as control) (1.73) (Table 2).

The results of the number of tubers per plant revealed that the maximum (6.733) number of tubers per plant were found in T₁ (Unilax) (6.733). The minimum (4.467) number of tubers were observed in T₀ (water as control) (4.467) followed by T₇ (Xtramyl) (4.800), T₂ (TemperM) (5.000), T₃ (Daconyl) (5.600), T₆ (AcrobateMz) (5.86), T₅ (Secure) (5.933), T₉ (Sanoxanyl) (5.933), T₈ (Micra) (6.067), T₁₀ (Curzate M8) (6.133) and T₄ (Amiscore) (6.200) (Table 2).

Treatments had the significant effects on the yield of potato. From Table 2, results revealed that the highest yield was obtained in T₁₀ (Curzate M8) (25.94) followed by T₆ (AcrobateMz) (25.67), T₅ (Secure) (25.22), T₇ (Xtramyl) (24.61), T₈ (Micra) (24.61) and T₉ (Sanoxanyl) (23.67) which were statistically similar. The lowest

(8.39) yield was observed in T₀ (water as control). Statistically similar results were obtained in T₂ (TemperM) (18.11), T₄ (Amiscore) (21.67), T₃ (Daconil) (21.72) and T₁ (Unilax) (22.44) (Table 2).

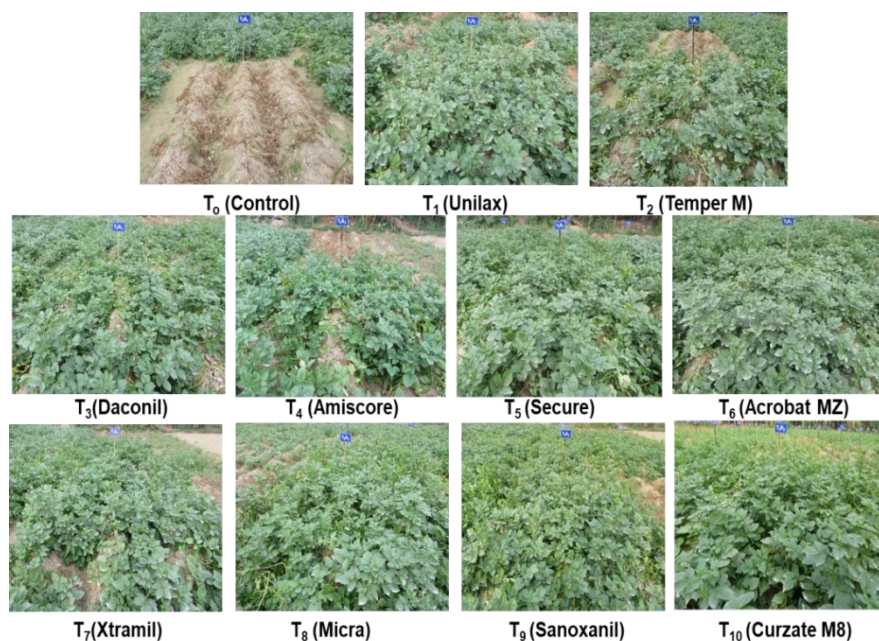


Figure 2. Effect of different fungicides in reducing late blight severity of potato under field condition. Photographs were taken at 71 DAP. T₀ = Water (control), T₁ = Foliar spray of Unilax (Metalaxyl + Mancozeb) 2 gm/L, T₂ = Foliar spray of TemperM (Propeneb + Cymoxanyl) 2 gm/L, T₃ = Foliar spray of Daconyl (Clorothalonyl) 1.5 ml/L, T₄ = Foliar spray of Amiscore (Azoxytrobilin + Difenconazol) 1ml/L, T₅ = Foliar spray of Secure (Fenamedon + Mancozeb) 2gm/L, T₆ = Foliar spray of AcrobatMz (Dimethomorf + Mancozeb) 4 gm/L, T₇ = Foliar spray of Xtramyl (Cymoxanil + Mancozeb) 2gm/L, T₈ = Foliar spray of Micra (Cymoxanyl + Mancozeb) 2gm/L, T₉ = Foliar spray of Sanoxanyl (Cymoxanyl + Mancozeb) 2gm/L and T₁₀ = Foliar spray of Curzate M8 (Cymoxanyl + Mancozeb) 2gm/L.

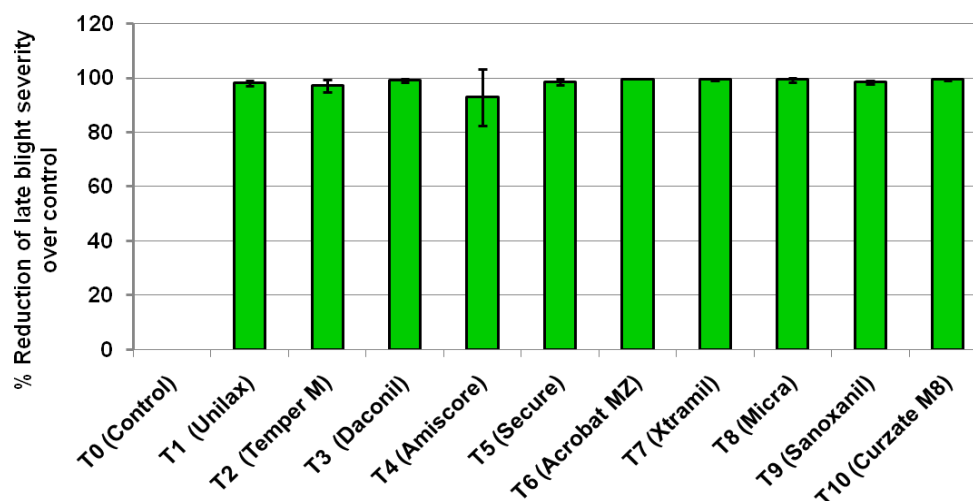


Figure 3. Effect of different fungicides in reducing percent reduction of late blight severity of potato under field condition at 71 DAP. T₀ = Water (control), T₁ = Foliar spray of Unilax (Metalaxyl + Mancozeb) 2 gm/L, T₂ = Foliar spray of TemperM (Propeneb + Cymoxanyl) 2 gm/L, T₃ = Foliar spray of Daconyl (Clorothalonyl) 1.5 ml/L, T₄ = Foliar spray of Amiscore (Azoxytrobilin + Difenconazol) 1ml/L, T₅ = Foliar spray of Secure (Fenamedon + Mancozeb) 2gm/L, T₆ = Foliar spray of AcrobatMz (Dimethomorf + Mancozeb) 4 gm/L, T₇ = Foliar spray of Xtramyl (Cymoxanil + Mancozeb) 2gm/L, T₈ = Foliar spray of Micra (Cymoxanyl + Mancozeb) 2gm/L, T₉ = Foliar spray of Sanoxanyl (Cymoxanyl + Mancozeb) 2gm/L and T₁₀ = Foliar spray of Curzate M8 (Cymoxanyl + Mancozeb) 2gm/L.

3.5. Cost-benefit analyses of different fungicides used for controlling late blight of potato

Using the data of Comparative efficacy of different potential fungicides in controlling late blight and increasing yield of potato during 2017-2018, Benefit Cost Ratio (BCR) was calculated in each of the treatments shown in Table 3. The results from the table of cost benefit analysis revealed that the maximum (Tk.415111.11) gross return was obtained from the treatment T₁₀ (Curzate M8) and the net return was 209611.11Tk./ha for the same treatment. Thus, the highest Benefit Cost Ratio, (BCR) 1.02 was obtained from when potato plants were sprayed with T₁₀ (Curzate M8) (1.02) following by T₇ (Xtramyl) (0.99), T₈ (Micra) (0.99), T₆ (AcrobateMz) (0.92), T₉ (Sanoxanil) (0.90), T₅ (Secure) (0.89), T₁ (Unilax) (0.80), T₃ (Daconil) (0.74), T₄ (Amiscore) (0.71) and T₂ (TemperM) (0.44). The results indicated that application of these fungicides yielded a benefit of Tk. ranged by 0.44 to 1.02 over the investment of Tk. 1.00. The lowest (-0.30) Benefit Cost Ratio was obtained in control treatment T₀ (water as control) (-0.30) that is an investment of Tk. 1.00 resulted a loss of Tk. 0.30 (Table 3).

Table 3. Cost-benefit analyses of different fungicides used for controlling late blight of potato.

Treatments	Yield (t/ha)	Gross return (Tk./ha)	Production cost (Tk./ha)	Cost of the treatment (Tk/ha)	Total production cost with treatment (Tk/ha)	Net return (Tk./ha)	BCR
T ₀	8.39	134222.22	192500	0	192500	-58277.78	-0.30
T ₁	22.44	359111.11	192500	7200	199700	159411.11	0.80
T ₂	18.11	289777.78	192500	8160	200660	89117.78	0.44
T ₃	21.72	347555.56	192500	7376	199876	147679.56	0.74
T ₄	21.67	346666.67	192500	10560	203060	143606.67	0.71
T ₅	25.22	403555.56	192500	20800	213300	190255.56	0.89
T ₆	25.67	410666.67	192500	20960	213460	197206.67	0.92
T ₇	24.61	393777.78	192500	5360	197860	195917.78	0.99
T ₈	24.61	393777.78	192500	5600	198100	195677.78	0.99
T ₉	23.67	378666.67	192500	6720	199220	179446.67	0.90
T ₁₀	25.94	415111.11	192500	13000	205500	209611.11	1.02

Price: Potato Tk. 16.00/kg, Fungicide Tk. 670-2600/kg & Tk.1230- 2640/L, Dose: Fungicide 8-16 kg/ha (on an average) and 4-6 L/ha. (on an average).

T₀ = Water (control), T₁ = Foliar spray of Unilax (Metalaxyl + Mancozeb) 2 gm/L, T₂ = Foliar spray of TemperM (Propeneb + Cymoxanil) 2gm/L, T₃ = Foliar spray of Daconyl (Clorothalonil) 1.5 ml/L, T₄ = Foliar spray of Amiscore (Azoxystrobin + Difenoconazol) 1ml/L, T₅ = Foliar spray of Secure (Fenamedon + Mancozeb) 2gm/L, T₆ = Foliar spray of AcrobateMz (Dimethomorf + Mancozeb) 4 gm/L, T₇ = Foliar spray of Xtramyl(Cymoxanil + Mancozeb) 2gm/L, T₈ = Foliar spray of Micra (Cymoxanil + Mancozeb) 2gm/L, T₉ = Foliar spray of Sanoxanil (Cymoxanil + Mancozeb) 2gm/L and T₁₀ = Foliar spray of Curzate M8 (Cymoxanil + Mancozeb) 2gm/L.

4. Discussion

For successful management of late blight of potato chemical fungicides are the most used tool till the date. However, the efficacy of fungicide likes metalaxil declining due to developing resistance by the pathogen. Hence monitoring of the efficacy of commonly available fungicides is essential. The present study evaluated the effectiveness and identified the best potential fungicide(s) from the common fungicides used by Bangladeshi farmer. The oomycetes like *P. infestans* are not true fungi, but use the same mechanisms to infect plants. Oomycete's cell walls contain cellulose instead of chitin and ergosterol is not a main sterol in the Oomycete cell membrane. So, fungicides targeting chitin and ergosterol synthesis are generally not effective against these pathogens. So, it is a bit more difficult to control late blight of potato. Hence in the present study we focused on the fungicide's mode of action to select fungicides. All the treatments showed significantly better foliage controlled as well as tuber yield over non- treated control. In the present investigation the highest percent reduction of late blight severity was estimated in T₁₀ (Curzate M8 = Cymoxanil 8% + Mancozeb 64%) (Positive control) (99.48) followed by T₆ (AcrobateMz = Dimethomorf + Mancozeb) (99.45), T₇ (Xtramyl = Cymoxanil 8% + Mancozeb 64%) (99.36), T₈ (Micra=Cymoxanil 8% + Mancozeb 64%) (99.35), T₃ (Daconyl = Clorothalonil) (99.13) compared to untreated control. It was noticed that Cymoxanil 8% + Mancozeb 64% resulted as the best fungicide in reducing the late blight of potato. The results were in accordance with the findings of Grayson *et al.* (1995) that the Cymoxanil 8%+ Mancozeb 64% and Dimethomorph 50% were effective oomycetes fungicide useful for the control of late blight on potatoes by preventive (prophylactic) as well as moderate curative (therapeutic) sprays. Similar results were obtained by Johnson *et al.* (2000) who

reported that Cymoxanil 8% + Mancozeb 64% had some activity after infection. Dimethomorph plus Mancozeb and Cymoxanil plus Mancozeb reduced sporulation more consistently (Johnson *et al.*, 2000) to control the late blight disease compared to others. Mhatre *et al.* (2020) reported that two fungicide treatments were found more promising *viz.*, mancozeb- cymoxanil + mancozeb and chlorothalonil-ametocetradin + dimethomorph to reduce the disease severity and increase the potato yield. Chakraborty and Mazumdar (2012) 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. Sharma and Saikia (2013) found that the prophylactic application of either Cymoxanil 8% + Mancozeb 64% or Dimethomorph 50% with two additional sprays of the respective fungicides at 10 days interval gave 100% control of the disease in field condition at 74DAS. Lal *et al.* (2017) observed that the late blight severity controlled 74.45 % by mancozeb 75% WP (0.2%, before appearance) followed by two more spray with mancozeb 75% WP (0.2%) +dimethomorph 50% WP (0.2%) at 7-10 days intervals and mancozeb75% WP (0.2%, before appearance) followed by two more spray with cymoxanil 8% +mancozeb 64 % WP (0.3%) at 7-10 days intervals controlled 71.29%. In the present investigation it was observed that the spray with Metalaxyl 8% + Mancozeb 64% was not found more effective. It might be due to continuous and increased use of Metalaxyl 8% + Mancozeb 64% may lead to the development of resistant strain of *P. infestans* and it was supported by other workers Ali and Dey (1999), Singh *et al.* (2005). Siddique *et al.* (2016) found that the lowest (75.68) percentage of disease control and the lowest yield (15.67 ton/ha) were recorded on fungicides containing 2.0 mg/L Ridomil MZ 72 (Metalaxyl 8% + Mancozeb 64%) with 1.0 ml/L Autostin 50 WDG (Carbondaxim 50%) during 2014-2015.

Fungicides can be categorized as preventive and curative. Preventive products contain active ingredients like Chlorothalonil or Mancozeb and should spray on the leaf before spore reach to the surface of leaf. The fungicide layer on the top of the leaf inhibits the germination of the spores and prevents the disease. The curative products contain active ingredients like cymoxanil, dimethomorph and can be sprayed after spore has germinated and penetrated the leaf and act by killing the fungal structure at this stage. Fungicides mode of action are classified in following three major group: i) Inhibitors of sterol synthesis, ii) Inhibitors of mitochondrial electron transport (respiration inhibitors) and iii) Multi-site enzyme inhibitors, nucleic acid and protein synthesis inhibitors. Mancozeb is a multisite protectant fungicide with more than 60 years of use, without a known history of pathogen resistance and good efficacy against early and late blight (Serge & Daniele, 2015). Propineb is a non-specific, multi-site fungicide with protective action against germinating conidia. It works as a good curative and anti-sporulant on disease causing pathogens. Cymoxanil is a penetrant fungicide with protectant and curative activity. Its post-infection activity stops the development of the fungus during the incubation. It is able to penetrate the crop leaf and improves the effectiveness of companion fungicides, especially during periods of intensive disease pressure. Alone, it has a very short period of activity: two days at most. Therefore, it is used in two-, or in three-way mixtures with protectants and/or systemic fungicides. It inhibits different metabolic processes and promotes the natural defenses of the plant. Cymoxanil 8% + Mancozeb 64% WP, it is a mixture of two fungicides- Mancozeb and Cymoxanil. The partner Mancozeb acts by its contact action. The mancozeb is fungitoxic when exposed to air. It is converted to an isothiocyanate, which inactivates the Sulphahydral (SH) groups in enzymes of fungi. Sometimes the metals are exchanged between mancozeb and enzymes of fungi, thus causing disturbance in fungal enzyme functioning. Other partner Cymoxanil has contact and locally systemic activity. It inhibits Sporulation in fungi.

Dimethomorph is an inhibitor of cell-wall synthesis and has a moderate amount of translaminar and acropetal systemicity, xylem-translocated systemic fungicide with curative, protectant and antispore activity and able to disrupts the asexual life cycle stages of *P. infestans* (Cohen *et al.*, 1995; Kuhn *et al.*, 1991), while cymoxanil is a penetrant fungicide with protectant and curative activity and it was reported that *P. infestans* colony growth and germ tube emergence of sporangia and encysted zoospores were highly sensitive to cymoxanil (Ziogas & Davidse, 1987). Chlorothalonil is a protectant fungicide and, when used alone (Grünwald, Rubio- Covarrubias, & Fry, 2000; Nowicki *et al.*, 2011) or in combination with other fungicides such as propamocarb + chlorothalonil, metalaxyl + chlorothalonil (Johnson *et al.*, 1997), was reported effective in reducing late blight severity. In many countries, the systemic fungicides (dimethomorph, ametocetradin, famoxadone, etc.) are often used in combination with contact fungicides (mancozeb, chlorothalonil, etc.) to reduce the potential risk of development of fungicide resistance in *P. infestans* (Banerjee *et al.*, 2017; Cooke *et al.*, 2011; Nowicki *et al.*, 2011). Furthermore, fungicides with single active ingredient are less effective than that contain more than one active ingredient. It is found that from the field experiment fungicides contain mixtures of active ingredients were better over fungicides which contain only one active ingredient (Khalifa, 2017). Therefore, in the present study, the best fungicide(s) was identified with more than one active ingredient to

reduce risk to development of fungicide resistance in *P. infestans* and for better management of late blight of potato.

Regarding yield parameters, all treatments gave higher yield in comparison to control treatment. The highest tuber yield (25.94t/ha) was observed in T₁₀ (Curzate M8 = Cymoxanil 8% + Mancozeb 64%) (Positive control) (25.94t/ha) followed by 25.67 t/ha in T₆ (AcrobateMz = Dimethomorf + Mancozeb) (99.45) and 25.22 t/ha in T₅ (Secure= Fenamedon + Mancozeb). These results were similar to the results of Siddique (2019). He observed that the highest (99.70) percentage of disease control and the highest yield (26.68 MT/ha) were recorded on fungicides containing 3.5 mg/L Sunoxanil 72 WP (Cymoxanil 8% + Mancozeb 64%) with 3.0 ml/L Contaf 5 EC (Hexaconazole 5%) during 2015-2016. It was concluded that Sunoxanil 72 WP (Cymoxanil 8% + Mancozeb 64%) acted as the best fungicide when applied as prophylactic measures. The highest Benefit Cost Ratio, (BCR) 1.02 was obtained from when potato plants were sprayed with T₁₀ (Curzate M8 = Cymoxanil 8% + Mancozeb 64%) (1.02) following by T₇ (Xtramil = Cymoxanil 8% + Mancozeb 64%) (0.99), T₈ (Micra =Cymoxanil 8%+Mancozeb 64%) (0.99), T₆ (AcrobateMz = Dimethomorf + Mancozeb) (0.92), T₉ (Sanoxanil = Cymoxanil 8%+Mancozeb 64%) (0.90). The results indicated that application of these fungicides yielded a benefit of Tk. ranged by 0.90 to 1.02 over the investment of Tk. 1.00. This result was in agreement with Mhatre *et al.* (2020). They reported that based on financial analysis the fungicidal scheduling based on mancozeb-cymoxanil+ mancozeb or chlorothalonil-ametoctradin + dimethomorph can be followed to obtain the maximum BC ratio over control with effective management of late blight at the southern hills of India. Therefore, from this present experiment it is recommended that Curzate M8 (Cymoxanil 8% + Mancozeb 64%) @ 2 g/L application should be done for the control and management of late blight disease of Potato.

5. Conclusions

From the present study, it is obvious that application of Curzate M8 (Cymoxanil + Mancozeb), AcrobateMz (Dimethomorph + Mancozeb), Micra (Cymoxanil + Mancozeb), Xtramil (Cymoxanil + Mancozeb), Daconyl (Clorothalonil) had lesser disease severity with higher yield than untreated ones. These results collectively conceded that these potential fungicides identified in the present study may open up the avenues for alternate use of these fungicides instead of the repeated use of a particular fungicide against late blight of potato and thus avoiding the development of fungicide resistance in *P. infestans* field population in the country.

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Data availability

All relevant data are within the manuscript.

Conflict of interest

None to declare.

Authors' contribution

Conceptualization, Md. Rashidul Islam; Methodology, Md. Rashidul Islam, Md. Huzzatul Islam, Shafiqul Islam, Md. Mostafa Masud; Software, Md. Huzzatul Islam, Md. Mostafa Masud; Validation, Md. Rashidul Islam and Md. Huzzatul Islam; Formal Analysis, Md. Huzzatul Islam, Md. Mostafa Masud; Investigation, Md. Rashidul Islam and Md. Huzzatul Islam; Resources, Md. Rashidul Islam; data curation, Md. Huzzatul Islam, Safiqul Islam, Md. Mostafa Masud, Md. Shariful Islam; Mamuna Mahjabin Mita; Writing—original draft preparation, Md. Huzzatul Islam, Shafiqul Islam, Md. Mostafa Masud, Writing—review and editing, Md. Rashidul Islam; Md. Huzzatul Islam, Md. Mostafa Masud; Md. Shariful Islam; Mamuna Mahjabin Mita; Visualization, Md. Rashidul Islam; Md. Huzzatul Islam, Md. Mostafa Masud, Shafiqul Islam; Supervision, Md. Rashidul Islam; Project administration, Md. Rashidul Islam; Funding acquisition, Md. Rashidul Islam; All authors have read and agreed to the published version of the manuscript.

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