MANAGEMENT OF LATE BLIGHT DISEASE OF POTATO THROUGH SELECTED BOTANICALS AND CHEMICAL FUNGICIDES

BY

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CERTIFICATE

This is to certify that the thesis entitled, **" MANAGEMENT OF LATE BLIGHT DISEASE OF POTATO THROUGH SELECTED BOTANICALS AND CHEMICAL FUNGICIDES "** submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN PLANT PATHOLOGY,** embodies the result of a piece of bonafide research work carried out by **Registration No. 15-06882** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

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Dedicated to
My
Beloved Parents

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The Author

MANAGEMENT OF LATE BLIGHT DISEASE OF POTATO THROUGH SELECTED BOTANICALS AND CHEMICAL FUNGICIDES

ABSTRACT

Four selected plant extracts viz Papaya leaf extract (1:3), Neem leaf extract (1:3), Marigold leaf extract (1:3), Mahogany seed extract (1:6) and three fungicides viz Ridomil Gold (0.5%), Dithane M 45 (0.45%) and Topgan (0.7%) were evaluated aganist *Phytophthora infestans* causing late blight disease of potato. The experiment was carried out in the Plant Pathology Laboratory and the Central farm of Sher-e-Bangla Agricultural University. Ridomil Gold and Dithane M 45 showed promising performance in controlling the disease while the lowest disease incidence and severity were recorded irrespective of different days after planting (DAP) of potato seed tubers. Potato plant showed the lowest plant infection (75%) and leaf infection (34.92 %) at 80 DAP in Ridomil Gold treated plot followed by Dithane M 45 treated plot. The lowest disease severity (17.35%) was recorded in case of Ridomil Gold at 80 DAP while it was 19.35 % in case of Dithane M 45 and 24.66 % in case of Topgan. The remarkable yield increase 254.52 % was noticed in case of Ridomil Gold, followed by Dithane M 45 (239.94 %) and Topgan (220.69 %). The performances of the plant extracts used in this experiment was not upto the mark in controlling the disease incidence and severity (PDI) and increasing yield comparison to the chemical fungicides but better than control.

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CHAPTER 1

INTRODUCTION

Potato (*Solanum tuberosum* **L.) is one of the most important food and cash crop belonging to the family Solanaceae. The crop has enough potential to increase agricultural production in our country.** Potato can play an important role in supplying vegetable throughout the year and can solve the nutritional problems to a great extent for the lower income group. The area under this crop was increasing rapidly and the farmers are gradually adopting it as a cash crop. According to Bureau of Statistics (BBS, 2014) during 2013-2014, the production of potato was 8.95 million metric tons from 0.462 million hectare of land in Bangladesh. Tuber yield was only 19.371 t/ha in the country which is lower as compared to other potato growing countries of the world (Tuber yield in Netherland is 45 t/ha, tuber yield in Japan is 41 t/ha etc.) (www.fao.org/potato-2008). Potato is one of the important crops in whole world due to its high value for human nutrition (Desjardins *et al*., 1995; FAO, 2010). It is the fourth most imported crop in the world and is planted in 18.2 million hectare of land with a total yield reaching 314.1 million tone (FAO, 2010). The major constraints in potato production have been the incidence of wide range of pests and diseases, difficulties in the production and distribution of disease free seeds, lack of HYV, inadequate supply of healthy seed tubers and high incidence of diseases and pests, inadequacies of cold storage facilities resulting in rotting and sprouting and violent price fluctuations. Of them diseases play an important role for lower yield in the country. In Bangladesh a total of 54 diseases (biotic and abiotic) of potato have so far been recorded (Dey and Ali, 1994). Among the diseases, late blight caused by *Phytophthora infestans* is serious one posing a potential threat to the potato crop, accounting for significant annual losses world-wide.

It causes 25-57 % yield losses in potato. The disease can destroy the entire foliage quickly causing reduced tuber yields. Sporangia released from infected plants are known to be capable of wind borne migration for over several kilometres. The disease is of common occurrence in Bangladesh for over 30 years and causes considerable yield losses.

Late blight is a devastating disease of potato, reducing crop quality and quantity (Fry *et al*., 1993). Many varieties used today are moderately or highly susceptible to late blight. Fungicide application is a widely implemented strategy to control the disease. However, the chemical control of the disease has several drawbacks. During the late 1980s and 1990s, introduction of new clonal lineages of *Phytophthora infestans* to potato growing areas of the world led to severe late blight outbreaks (Fry and Goodwin, 1993). These new clonal lineages caused a new disease management challenge because many were resistant to the fungicide metalaxyl, which had become an integral tool for foliar late blight suppression (Daayf *et al*., 2003) and increased the costs of crop production. Moreover, the public has expressed concern about the heavy reliance on chemicals in plant protection strategies. Therefore, developing a new control strategy to prevent development of new clonal lineages and to meet public demand in reduction of pesticide use is need to be addressed.

Phytophthora infestans (Mont) de Bary, the causal agent of late blight, is the most devastating pathogen in potatoes and tomatoes worldwide. *P. infestans* left its footprint in human history in the 19th century while it was responsible for the Irish potato famine (Erwin and Ribeiro, 1996). This pathogen causes defoliation and huge blighting of potato leaves and tomato fruits. Epidemics of late blight happen with high intensity, practically in all the areas where potato and tomato are grown, especially during the winter season and rainy weather. *P.*

infestans usually requires low temperatures for development, the optimum temperature and relative humidity being 18–22^0 C and almost 100%, respectively (Erwin and Ribeiro, 1996). Temperatures remain generally low in tropical areas. Yield losses caused by late blight can be very high when control measures are not appropriately adopted. Losses have been estimated to be as high as 71 % in potato and 100% in tomato (Fontem *et al*., 2005).

Potato plants (*Solanum tuberosum* L.) may be totally destroyed by *P. infestans* within two weeks in wet conditions (Hooker, 1981; Fry *et al*., 1993; Van Derzaag, 1996). *P. infestans* can survive under adverse conditions and over winter in the form of oospores. The pathogen however, invades and infects potato plants in the field via zoosporangia or zoospores which disperse via soil water, rain splash and wind (Van Derzaag, 1996). The zoosporangia may directly germinate on potato organs or produce zoospores in sporangium, which are motile and disperse, following encystment, germination and host penetration within 2-3 hours under favorable conditions of high relative humidity, rain or sprinkler irrigation. Infection occurs when leaves are moist for at least 5 hours at 15-20^0 C. Spore germination results in colonization and infection causing symptoms on leaves, stem or tubers and production of new spores within 4-5 days (Rich, 1983). Potato plants infected with *P. infestans* may also show wilt symptoms which start in younger leaves leading to stunted plants and leaf chlorosis. If the tuber seed potatoes are infected, the emerging seedlings wilt after emergence, becoming infected through the vascular tissue and finally gummosis occurs from the tuber buds after harvest (Van Derzaag, 1996). Late blight disease has been controlled using chemical fungicides at seed dressing and from interval spraying until harvest. Metalaxyl (systemic fungicide), Fostyl A-1, Mancozeb, Fentin-acetate phosphate, Chlorotalonyl and Captafol are the commonly used chemical

fungicides (Milgroom and Fry, 1988; Samoucha and Cohen, 1986). Though the use of chemical fungicides has resulted in an increased degree of pathogen resistance (Levy *et al*., 1983).

About 25.5 to 57.25 % yield loss occurs due to late blight depending on degree of susceptibility of the cultivar, time of appearance and age of plant infection. Indiscriminate use of systemic fungicides especially metalaxyl (Ridomil) provides chance to develop resistant strain of the fungus has been reported from home and abroad (Ali and Dey, 1999; Gupta *et al.*, 1999; Singh, 2000). Comprehensive studies on late blight of potato are limited in Bangladesh (Ali and Dey 1999; Islam *et al.*, 2002). Epidemiological studies indicated that the disease is devastating at 12-25^0 C with relative humidity more than 85%. The disease is currently controlled by growing resistant varieties or by spraying copper fungicides (Cao *et al*., 2001a). However, resistant varieties are rather rare and consumers often refuse to use them because of the poor yield.

Copper fungicides contain copper, a heavy metal that has a wide range of side effects. Therefore, the use of copper in organic agriculture in the European Union is rather restricted and will be gradually banned in the near future. The use of natural products for the control of fungal diseases in plants is considered as an interesting alternative to synthetic fungicides due to their less negative impacts on the environment (Cao and Forrer, 2001b). According to Bradshaw (1992) metalaxyl + Mancozeb delayed disease progress more efficiently than mancozeb alone. Thind *et al.* (1989) claimed that only Ridomil controlled *P. infestans* when applied after infection. Indiscriminate use of Ridomil induce the development of *P. infestans* resistant strain of late blight throughout the world. In Bangladesh the resistant strain of *P. infestans* has also been identified (Dey and Ali, 1994). So, Ridomil Mz-72 has been withdrawn from the country and new formulation of

metalaxyl like Ridomil Gold, Metaril, Coromil, Vitamyl, Unilax and Zhemetalax are under process for introductions in the country whose resistant strain of *P. infestans* not yet developed in abroad. For overcoming this alarming situation mixture or alternate use of metalaxyl and mancozeb has been suggested in many countries (Gerasimova *et al.,* 1994; Singh *et al.,* 1994). Apaydin *et al.* (1999) suggested use of propineb to control late blight of potato effectively by reducing disease incidence and increased yield. Dozens of plant extracts or plant essential oils have been tested against *P. infestans in vitro* for the inhibitory effect and the control efficacy under greenhouse conditions. Some plant materials, e.g., *Potentilla erecta* and *Salviae officinalis* showed a promising effect against potato late blight (Quintanilla *et al*., 2002; Blaeser and Steiner, 1999). Plant extracts from several Chinese traditional medicinal herbs were tested for controlling effects against potato late blight on detached potato leaves, seedlings and potato slices in the hope of finding such an alternative. At present no resistant source of the potato is available in the country. Metalaxyl resistant strain of *P. infestans* has also been reported in the country **(**Dey and Ali, 1994). Research on this disease is going on at Tuber Crops Research Centre (TCRC), BARI, SAU over several years. Therefore, the present study was undertaken to investigate the effectiveness of botanicals and fungicides spraying for the management of *p. infestans* of potato.

On the basis of above facts, the present investigation was undertaken to achieve the following objectives.

- To isolate and identify *Phytophthora infestans* causing late blight of potato.
- To evaluate the efficacy of some selected chemicals and botanicals for the management of late blight of potato.

CHAPTER 2

REVIEW OF LITERATURE

Phytophthora infestans is a soil and air borne pathogen causing serious blight disease especially in potato and tomato throughout the world. It is a zoospore producing fungus attacking foliage part of the plant. The infection is very speedy when favoured by low temperature and high humidly. Literature related to *P. infestans* management of late blight of potato have been presented in this chapter.

2.1 Pathogen(s) and pathogenesis

Castro (1963) reported that *P. infestans* produces a variety of characteristics structures most of which are mycelium containing cellulose and sporangia of various shapes and sizes depending on species or isolates, germinating by germ tube or production of biflagellate zoospores. Zoospores are pre dominantly uni-nucleate with only a few bi-nucleate.

Erwin *et al.* (1963) studied the genus *P. infestans*, including nearly 70 described species of plant pathogens of world wide distribution . They have found that *P. infestans* was unique among non-obligate parasites, all species are pathogens of higher plants. They obtained that is this group of pathogens the subject of variability is of vital importance for a comprehensive understanding of pathogenesis and the problems involved in control through host resistance.

Stams (1985) described that sporangiophores of *P. infestans* are erect, branching compound, sympodial with a small swelling at the base of each branch. Sporangia are abundant on host, ellipsoid, semipapillate. Ogonia rare in host of single culture. Antheridia amphigynous, elongated cylindrical.

Ahmed (1999) studied *P. infestans* that was isolated from diseased leaf, stem and fruit. He placed surface sterilized samples on moist blotter paper in petridish with sterile forceps and incubated at 20 ± 2^0 C to allow the pathogen to grow. After incubation period the fungus was identified under stereoscopic and compound microscopes. The pathogen produced branched sporangiophores. Lemon shaped papillate sporangia were produced at the tips of the branches of the sporangiophores. Sporangia were pointed at the end.

Van Derzaag (1996) reported that *P. infestans* can survive under adverse conditions and over winter in the form of oospores. The pathogen however, invades and infects potato plants in the field via zoosporangia or zoospores which disperse via soil water, rain splash and wind.

Islam *et al*. (2002) reported that at 7 days of incubation *P. infestans* produced white mycelial growth on PDA plate then its colour gradually turned into grayish white. The pathogen produced branched sporangiophores. Lemon shaped papillate sporangia were produced at the tips of the branches of the sporangiophores . Sporangia were pointed at end. At the places where sporangia were produced the sporangiophores formed swelling , characteristics of *P. infestans.* Zoospores of P. infestans were liberated during the incubation period at 12 ± 1^0 C for 24 hours. Zoospores were basically ellipsoid ovoid in shape and possessed a groove that ran longitudinally along the zoospore. After competition of swimming period the zoospores encyst. Single sporangium also germinated by sending out germ tube.

Cao and Forrer (2001) reported that the use of natural products for the control of fungal diseases in plants is considered as an interesting alternative to synthetic fungicides due to their less negative impacts on the environment.

Rich (1983) reported that the zoosporangia of *P. infestans* may directly germinate on potato organs or produce zoospores in sporangium, which are motile and disperse, following encystment, rain or sprinkler irrigation. Infection occurs when leaves are moist for at least 5 hours at $15\text{-}20^0$ C. Spore germination results in

colonization and infection causing symptoms on leaves, stem or tubers and production of new spores within 4-5 days.

Hooker (1981) and Fry *et al*.(1993) reported that potato plants (*Solanum tuberosum* L.) may be totally destroyed by *P. infestans* within two weeks in wet conditions.

Erwin and Ribeiro (1996) reported that epidemics of late blight happen with high intensity, practically in all the areas where potato and tomato are grown, especially during the winter season and rainy weather. *P. infestans* usually requires low temperatures for development, the optimum temperature and relative humidity being 18–22^0 C and 100%, respectively.

2.2 Disease symptoms

Thompson and Kell (1957) described that the first symptoms of late blight were irregular, greenish-black, water soaked spots on the leaves. These spots enlarged rapidly in moist weather producing sometimes snow white downy growth on the lower surfaces. The stems often showed spots turned dark-green colour blotched area which then turned brown as the fruit become older. The fruit surface was firm and had a wrinkled appearance.

Kalloo (1986) observed the symptoms of late blight on fruit that was olivaceous, greasy and water-soaked spots. The stem-end portion of the fruit was affected and the water-soaked grey green spots of every size were discernable.

Watterson (1986) observed that late blight affected leaves had irregular dark lesions around which a fine white moulded ring that developed during wet weather. Affected fruit had firm large irregular brownish-green blotches. The fruit surfaces appeared rough.

Agrios (1988) described the symptoms caused by *P. infestans* that appear at first as circular or irregular spots usually the tips or edges of the lower leaves. Then spots enlarge rapidly and form brown, blighed area with indefinite borders. A zone of white, downly fungus growth appears at the border of the lesions on

the undersides of the leaves resulting the entire leaflet infected which soon die and become limp.

2.3 Effect of weather conditions and culture media on development of *P. infestans*

Singh (1978) reported that the sporangia are multinucleate (7-30 nuclei). The optimum for germination of the sporangia by zoospores is 12-13^0 C and by a germ tube at 24^0 C. The minimum temperature for sporangial germination was as low as 2^0 C and 3^0 C, while the maximum may go up to 24-30^0 C with excessive humidity (above 90% R.H). Four important conditions for the development of late blight in severe form have been suggested

i. Night temperature below the dew point for at least 4 hours.
ii. Minimum temperature of 10^0 C or slightly above .
iii. Clouds on the next day and

Rainfall during the next 24 hour of at least 0.1mm

Singh (1978) reported that the method of germination of the sporangia is largely governed by the temperature. Low temperature favours zoospores formation while at higher temperatures the sporangia germinate by germ tubes. A relative humidity of above 90 percent is necessary for germination of sporangia.

Mehrotra (1980) reported that zoospore production is favoured by the temperature of 9 to 15^0 C.

Bartkaite (1985) observed that *Alternaria solani* and *P. infestans* did not interact in pure culture or on tomato. They can therefore, be used for complex inoculation.

Bedlan (1987) reported that wet weather increased the occurrence of *P. infestans.*

Phukan and Barvah (1989) reported that a temperature of 20^0 C and 100% R.H. were suitable for the growth of *P. infestans* .

Zahid *et al* (1993) noted that low temp (12-15^0 C, high humidity 90%), drizzle, foggy and cloudy weather were favourable for development of late blight.

Ayub *et al.* (1997) investigated the effect of culture media, temperature and light on sporangial production of *P. infestans* . They reported that rye seed agar and chickpea sucrose agar gave maximum vegetative growth and scanty sporangial production while fungus produced abundant sporangia on tuber and tomato slices. Highest sporangial production was recorded at 20±1^0 C under 12 hours in continuous light plus 12 hours in continuous darkness.

Apaydin *et al.* (1999) reported that the disease progress was observed when the total daily average temperature for 15 successive days were 12^0 C or over and the average daily relative humidity was 70 percent or over the disease would be visible in 7-15 days.

2.4 Management through fungicides

Gross *et al*. (1982) repoted that sparying potato tops 3-5 times with 25% Ridomil (Metalaxy) was highly effective aganist late blight (*P. infestans*).

Piekiewicz (1983) tested effectiveness of systemic fungicides aganist late blight of potato and tomato (*P. infestans*) during 1977-1982. He found that these fungicides limited the rate of disease spreading and owing to the increased tuber yield by 24.5% in the mean and tuber infestation was smaller by 73.2%. He also said that the fungus was somewhat resistant aganist Metalaxyl Ridomil 25WP.

Bradshaw (1992) reported that the use of fungicides for control of potato late blight (*Phytophthora infestans).* He described that metalaxyl + Mancozeb delayed disease progress more efficiently than mancozeb alone.

Milgroom and Fry (1988) reported that late blight disease was controlled using chemical fungicides at seed dressing and from interval spraying until harvest.

Metalaxyl (systemic fungicide), Fostyl A-1, Mancozeb, Fentin-acetate phosphate, Chlorotalonyl and Captafol were the commonly used chemical fungicides.

Samoucha and Cohen (1986) reported that Melody Duo (Propined) was effective in reducing late blight incidence and increased yield.

Quintanilla *et al*. (2002) reported that some plant materials, e.g., *Potentilla erecta* and *Salviae officinalis* showed a promising effect against potato late blight.

Gerasimova *et al.* (1994) reported that for controlling late blight disease, mixture or alternate use of metalaxyl and mancozeb has been suggested in many countries.

Thind *et al*. (1989) tested 6 fungicides under laboratory pot house and field conditions. They observed that only Ridomil (Metalaxyl) controlled (*P. infestans*) when applied after infection.

Alam *et al*. (1991) reported that they applied 8 fungicides for controlling *P. infestans* on cv. Kufri sindhuri under field conditions during 1989-90. It was found that Ridomol MZ-72 (Mancozel+Metalaxy) gave the best result followed by Dithane M-45 (Mancozeb). These treatments gave yields of 27.15 and 25.45 t/ha , respectively, compared to 16.95 t/ha in the untreated (control).

Sharma (1993) conducted an experiment for two years at CPRS, Jalandahor, Punjub, India to evolve effective fungicidal spary schedule aganist *P.infestans*. He claimed that one spray of Ridomil MZ-72 WP @ 0.25% alternated with two sprays of Dithane M-45 reduced foliage blight from 99.75% to 11.65%. The spray schedule was as much effective in checking disease as application of two sprays of Ridomil MZ-72 WP and was superior to Dithane M-45 (41.53%)

Vanitha and Ramacandram (1985) observed that application of @ 0.1% Ridomil (Metalaxyl) or chlorothalonil @ 0.1% or thiram @ 0.3% at the first sign of *P. parasitica* (*P. nicotiana var. parasitica*) infection and 15 days later another application of Ridomil @ 0.1%, or chlorothalonil @ 0.1%, plus nochi leaf extract gave the lowest disease incidence.

2.5 Combined effect of sanitation with fungicide

Cohen (1987) reported that the disease caused by *P. infestans* was the most serious one affecting the potato crop in Israel. He also observed that the maintenance of field sanitation was crucial for avoiding the appearance of primary foci in the crop. He also reported that preventive sprays with Mancozeb (or similar surface fungicides) were useful when Metalaxyl tolerant populations of the fungus appear, it was recommended to use cymoxanil mixtures such as Mancur or sandocur-M (once every 2 weeks). To prevent tuber infection at harvest, it was essential to destroy the foliage and wait for 2 weeks for tubers to produce a hard periderm.

Fontem (2001) evaluated the effect of crop sanitation and reduced sprays with 12% metalaxyl + 60% cuprous oxide (as Ridomil plus) on late blight of potato (*P. infestans*) in 2 field trials in 1993 in Dashing, Cameroon. In the 1st trials, sanitation (weekly removals of blighted leaves) and 2 fungicidal treatments were applied starting from the appearance of first disease symptoms, In the 2nd trial only fungicides were applied at varying rates for which marketable yields increased by 94%. It was concluded that *P. infestans* could be controlled by removal of diseased leaves and application of reduced fungicides doses.

Begum (2001) in her experimental results reported that the sanitation and fungicide (Ridomil @ 0.2%) spray reduced the late blight disease incidence and severity as compared to other single treatments.

2.6 Effect on diseased and yield components

Ahmed (1999) observed that the use of single effect of sanitation, fungicide and garlic extract reduced late blight incidence and severity and increased 36.55%, 22.12% and 36.17% fruits yields over control, respectively. The combined applications of sanitation, fungicide and garlic extract increased only 23.76% fruit yield over control.

Fontem *et al*. (2005) reported that temperatures remain generally low in tropical areas. Yield losses caused by late blight can be very high when control measures are not appropriately adopted. Losses have been estimated to be as high as 71% in potato and 100% in tomato.

CHAPTER 2

MATERIALS AND METHODS

3.1 Experimental site

The experiment was conducted in the Plant Pathology Laboratory and Central Farm field of Sher-e-Bangla Agricultural University (SAU), Sher-e- Bangla Nagar, Dhaka-1207.

3.2 Experimental period

The experiments was conducted in the winter season started from November 2015 to May 2016.

3.3 Laboratory experiment

3.3.1 Collection of diseased specimens

Diseased samples of potato (*Solanum tuberosum*) were collected from the Farm field of Sher-e-Bangla Agricultural University, Sher-e Bangla Nagar, Dhaka-1207. Collected samples were put in polyethylene bags immediate after collection to avoid drying.

3.3.2 Sterilization of materials and equipments

For surface sterilization, 0.1 % sodium hypochlorite (NaOCl) was used for plant materials such as leaf, stem etc. and other equipment's like inoculation-needles, inoculation chamber, forceps, hands etc. were sterilized with the help of rectified spirit.

3.3.3 Identification of *Phytophthora infestans* observing under microscope

Diseased leaf samples were brought to the laboratory. Sample surface was sterilized by dipping in 0.1 % NaOCl solution for 30 second and rinsed in

sterile water. Leaves were placed on moist blotter paper on petridish, incubated at 20 ± 2^0 C for 2 days in 12 hours with alternate light and darkness. For sporulation, the inocula was placed on potato slices and incubated for 20 days at 20 ± 2^0 C in the normal lab condition. After incubation when the whitish growth of fungus was observed on the potato slices. Temporary slides were prepared for identification under compound microscope. The incubated potato slices were also observed under stereoscopic microscope. The *Phytophthora infestans* was identified following the key out lined by Alexopoulos (1996) and Ingram and Williams (1991).

Figure 1. Symptom of late blight of potato leaf caused by *Phytophthora infestans*

Figure 2. Collection of diseased leaves caused by *Phytophthora infestans*

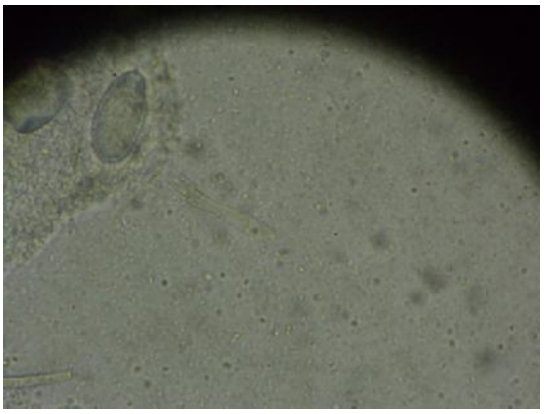

Figure 3. Sporangium of *Phytophthora infestans* under compound microscope

3.4 Field experiment

3.4.1 Climate

The experimental area was under the sub-tropical climate which characterized by the comparatively low rainfall, low humidity, low temperature, relatively short day during November to May and high rainfall, high humidity, high temperature and long day period during April to September.

The annual precipitation and potential evapotranspiration of the site were 2152 mm and 1297 mm, respectively. The average maximum and minimum temperature was 30.34^0 C and 21.21^0 C, respectively with mean temperature of 25.17^0 C. (AppendixII) Temperature during the cropping period ranged from 12.2 ^{0} C to 31.2^0 C. The humidity varied from 73.52% to 81.2%. The day length ranged from 10.5-11.0 hours only and there was no rainfall during the experimental period.

3.4.2 Soil type

The soil of the experimental site belongs to the Agro-Ecological Region of "Madhupur Tract" (AEZ No. 28). It was Deep Red Brown Terrace soil and belongs to "Nodda" cultivated series. The top soil is slightly clay loam in texture. Organic matter content was very low (0.82%) and soil pH varied from 5.47-5.63. The information about AEZ 28 is given below:

Characteristics of AEZ-28

Land type	Medium high land
General soil type	Non-Calcareous Dark gray floodplain soil
Soil series	Tejgaon
Topography	Upland
Elevation	8.45
Location	SAU Farm, Dhaka
Field Level	Above flood level
Drainage	Fairly good
Firmness (consistency)	Compact to friable when dry

3.4.3 Fertility status of the field soil:

The soil of experimental site was analyzed in Soil Resource Development Institute (SRDI), Dhaka and found as loamy soil which contains total Nitrogen 0.061(%),

Phosphorus 35022 microgram per gram of soil, Sulphur 22.60 microgram per gram of soil, Potassium 0.030 miliequivalent per 100 gram soil and Calcium 2.67 miliequivalent per 100 gram soil.

Physical and chemical properties of the experimental soil

Soil properties	**Value**
Soil texture	clay loam
Soil pH	5.8
Organic matter (%)	1.35
Total N (%)	0.08
C : N ratio	10 : 1
Available P (ppm)	35
Exchangeable K (me/100g soil)	0.18
Available S (ppm)	40

3.4.4 Variety

Diamond variety was used for the present experiment.

3.4. 5 Design of the experiment

The experiment was carried out in Randomized Complete Block Design (RCBD) with three replications.

3.4.6 Land preparation

A piece of medium high land with well drainage system was selected. The experimental field was first ploughed on 18 November 2016. The

land was ploughed thoroughly with a power tiller and then laddering was done to obtain a desirable tilt. The clods of the land were hammered to make the soil into small pieces. Weeds, stubbles and crop residues were cleaned from the land. The final plugging and land preparation was done on 30 November 2015.

3.4.7 Layout

The field layout was done as per experimental design on 1 December, 2015. The field was divided into three blocks each of which representing a replication. The unit plot size was 1m × 4 m and plot to plot distance was 0.4 m and block to block distance was 0.75 meter.

3.4.8 Planting potato tubers

Selected healthy and disease free potato seeds were planted in the experimental field. Planting was done with the help of *khurpi* (a hand operated implement). For planting, a hole was made with *khurpi*, so that the seed of potato was dipped in soil, but must be touching with surface soil. The hole was completely covered with the help of thumb finger. This planted potato seeds were watered after seven days with the help of watering cane.

3.4.9 Treatments

T_1 = Mahogany leaf extract @ 1:6 (w/v)

T_2 = Neem leaf extract @ 1:3 (w/v)

T_3 = Papaya leaf extract @ 1:3 (w/v)

T_4 = Marigold leaf extract @ 1:3 (w/v)

T_5 = Ridomil Gold @ 0.5 %

T_6 = Topgan @ 0.7 %

T_7 = Dithane M 45 @ 0.45 %

T_8 = Control

Figure 4: Data collection in the experimental field.

Figure 5: Spraying fungicides in the experimental field.

3.4.10 Intercultural Operation

3.4.10.1 Plant protection

The crop was protected from the attack of insect-pest by spraying insecticide Ektara. The insecticide spraying was done as required according to the recommended doses.

3.4.10. 2 Gap filling

After plantation of potato seeds in the field it was noted that some gaps had been found either for missing plantation or drying out of the germinated seedlings. For maintaining optimum number of plant population gaps filling were done properly.

3.4.10. 3 Irrigation

Irrigation was done at 10-15 days interval as per necessity.

3.4.10. 4 Weeding

Weeding was done fourth time in the experimental period starting from 20 days after planting, 40 days after planting, 55 days after planting and 70 days after planting.

3.4.11 Collection of fungicides and plant extracts

Three fungicides namely Ridomil gold, Topgan and Dithane M 45 were collected from local market. Seeds of mahogany and leaf of neem and papaya and marigold were collected from Sher-e -Bangla Agricultural University campus. Poultry manure was collected from the Agargoan nursery, Sher-e- Bangla Nagar, Dhaka-1207.

A

B

C

D

Plate 1. Preparation of different plant extracts

A. Neem leaf & it's extract B. Papaya leaf & it's extract C. Mahogany seed & it's extract D. Marigold leaf & it's extract.

E

F

G

Plate 2. Preparation of solutions of different chemical fungicides

E. Ridomil Gold F. Dithane M 45 G. Topgan

Table 1. The particulars of fungicides used in this study against *Phytophthora infestans.*

Trade name	Common name	Active ingredient	Conc. Used
Ridomil gold	Metalaxyl+Mancozeb	68% Metalaxyl	0.5%
Dithane M 45	Manganous ethylene bisdithiocarbamate-ion	80% Mancozeb	0.45%
Topgan	Copper-oxychloride	50% Copper oxychloride	0.7%

Table 2. The particulars of plant extracts used in this study

Common name	Scientific name	Plant parts	Concentration
Neem	*Azadirachta indica*	Leaf	1:3
Papaya	Carica papaya	Leaf	1:3
Marigold	*Calendula officinalis*	Leaf	1:3
Mahogany	*Swietenia mahagoni*	Seed	1:6

3.4.12 Preparation of fungicidal suspension

Recommended doses of fungicidal solution were prepared by mixing thoroughly with requisite quantity of fungicide and normal water. It was required 7 gm/liter of Topgan, 4.5 gm/liter of Dithane M 45, 0.5 gm/liter of Ridomil Gold for preparation of solution for recommended concentration

3.4.13 Preparation of plant extracts

The plant extracts were prepared by using the method exercised by Ashrafuzzaman and Hossain (1991). For preparation of extracts, collected leaves and seeds were weighed in an electric balance and then washed in water. After washing the big leaves were cut into small pieces. For getting extract, weighed plant parts were blended in a mortar & pastel and then distilled water was added into the mortar. The pulverized mass was squeezed through 3 folds of fine cotton cloth. For getting 1:3 (w/v) ratio 300 ml of distilled water was added with 100g plant parts. The particulars of the botanicals used for the experiment are listed in Table 3.

3.4.14 Application of fertilizers and manures

The following dose of fertilizers and manures were applied for the potato cultivation.

Table 3. Doses of fertilizers and manures applied in the field experiment

Fertilizers / Manures	Dose /ha
Urea	300 kg
TSP	150 kg
MOP	250 kg
Gypsum	40 kg
Cow dung	10 tons

The 1/3rd urea and whole amount of other fertilizers were applied as basal dose and rest 2/3rd urea was applied at 30 DAP and 50 DAP followed by an irrigation.

3.4.15 Experimental design

The experimental plots were arranged in Randomized Complete Block Design (RCBD) with three (3) replications (Appendix-I). The experiment details were presented bellow:

- Total area : 183.75 m^2
- No. of plot : 30
- Plot size : (1 ×4) m^2
- Block to block distance : 0.75m
- Plot to border distance : 0.75 m
- Plot to plot distance (Length wise) : 0.4 m
- Plot to plot distance (Breath wise) : .0.4 m
- Plant to plant spacing : 15 cm
- Row to row spacing : 20 cm

3.4.16 Application of fungicides and plant extracts

At recommended doses, the suspension/solution of fungicides were prepared by mixing thoroughly with requisite quantity of normal plain water. Spraying was started from one month after transplanting. Totally 7 spraying were done with 7 days intervals with a hand sprayer. To avoid the drifting of the fungicides during application, spraying was done very carefully, specially observing air motion. A control treatment was maintained in each block where spraying was done with plain water only. The fungicides and plant extracts were applied to the foliar part of plants of potato plants by hand sprayer with 7 days

interval. Precautions were taken to avoid drifting of spray materials from plant to neighboring plants.

3.4.17 Data collection

The data were recorded on the following parameters at an interval of 30 days.

1. Disease Incidence (% plant infection & % leaf infection)
2. Disease Severity (% leaf area diseased)
3. Yield (ton/ha)

Calculation of disease incidence of different treatment

Percent disease incidence was calculated using the following formula:

$$\text{(\%) disease Incidence} = \frac{\text{Number of diseased plant/leaf}}{\text{Number of total plants/leaves inspected}} \times 100$$

3.4. 18 Estimation of PDI

Leaf area diseased of the ten selected plants in each plot against each treatment were measured and recorded by eye estimation. Mean percentage of leaf area

diseased was calculated by dividing number of total observation and used for PDI (percent disease index) estimation. The disease scoring scale (0-5) was used to estimate the disease severity (PDI) of late blight of potato for each unit plot under each treatment. The scale is presented below:

0 = No disease symptoms.

1 = A few spots towards the tip, covering less than 10 % leaf area.

2 = Several dark purplish brown patches covering 10 % to less than 20 % leaf area.

3 = Several patches with paler outer zone, covering 20 % to 40 % leaf area.

4 = Long streaks covering 40% to 75% leaf area .

5 = Complete drying of the leaves / stems or breaking of the leaves/stems. from the base.

The percent disease index (PDI) was calculated using the following formula (Islam, 2002)**:**

$$PDI = \frac{\text{Total sum of numerical ratings}}{\text{Number of observation X Maximum grade in the scale}} \times 100$$

3.4.19 Harvesting

Potato tuber were harvested on 3rd May 2016, at which the plants have been showing the sign of drying out of most leaves. Potato tuber were carefully lifted

with the help of khurpy. To avoid injury, proper care was taken during harvesting the tuber by khurpy.

3.4.20 Weight of tuber per plot

Weight of potato tuber per plot was recorded individually for each treatment. Yield was converted into ton/ha.

3.4.21 Storing of the tuber

After harvesting, curing and sun drying, the potato tuber were stored at room temperature for the period of May to August, on the floor of a pakka room keeping good ventilation.

3.4.22 Statistical analysis

Randomized Completely Block Design (RCBD) was followed for field experiments. The data were statistically analyzed by using computer package program MSTAT-C.