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RESEARCH ARTICLE

EFFECT OF ABIOTIC FACTORS ON SURVIVAL OF *Phytophthora infestans* AND MANAGEMENT OF LATE BLIGHT OF POTATO THROUGH CHEMICALS

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Abstract

In vitro and *in vivo* trials were conducted to check the effect of temperature, photoperiod and soil temperature and also the efficacy of systemic and contact fungicides for controlling the late blight of potato, *Phytophthora infestan.* The maximum growth of zoospore germtube of all the *P. infestans* isolates was achieved at temperature 24°C with 16h of photoperiod and minimum at temperature 30°C. The rest of temperature *viz*., 18, 21, 27°C were not suitable in enhancing germtube length. Maximum recovery of pathogen from the potato field in the presence of soil, naturally infected with *P. infestans* was recorded at temperature ranges of 15-30°C. Radial growth of *P. infestans* was greatest at 25°C. Soil temperatures that were recorded during certain times of the years in potato field were found to be inhibitory to sporulation and growth of the pathogen. Wet foliage was best in inducing the disease with artificial spray of the spores of pathogen @ 10^5 spores/ml. Systemic fungicide Ridomil @0.25% was found to be superior compared to Dithane M-45 (@ 0.25%) . The percent late blight incidence in potato crop sprayed with dithane M-45 ranged between 0- 6.2%, while with ridomil, sprayed potato crop showed almost complete control of the disease and its incidence ranged between 0-1.8%.

INTRODUCTION

Potato is an important crop grown in India and world wide.The main problem of growing potato (*Solanum tuberosum*) worldwide are economic losses due to late blight, which is caused by *Phytophthora infestans* which can destroy all parts of potato plants (*Solanum tuberosum* L.) within two weeks in wet conditions (Hooker, 1981; Fry *et al*., 1993; van der Zaag, 1996). In India, potato is grown in all the states producing 25 million tons from total area of 1.4 million ha. The various factors limiting yield of potato include lack of HYV, inadequate supply of healthy seed tubers and high incidence of disease and pest. The pathogen however, invades and infects potato plants in the field via zoosporangia which disperse via soil water, rain splash and wind (van der Zaag, 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. Potato plants infected with *Phytophthora 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 der Zaag, 1996).

The objective of the study was to examine the influence of temperature on growth and sporulation of *P. infestans* and development of the disease on inoculation with the pathogens. Seasonal changes in soil temperature within the potato field were recorded for determining the periods when temperature may be inhibitory to the pathogen development. Among the fungicides, Ridomil and Dithane M-45 were evaluated against *P. infestans.* Both the fungicides have already been used in different parts of the country for management of late blight of potato. However, it is quite essential to evaluate their efficacy against the disease under the climatic condition of Meerut prior to their introduction in to the state. Therefore, an experiment has been carried out to investigate the performances of Ridomil and Dithane M-45 under Uttar Pradesh condition both in pot and field conditions.

METHODS AND MATERIALS

Isolation of *P. infestans*

Isolates of *P. infestans* were recovered from infected potato leaves collected from farmer's fields (2009-2010) location in and around Meerut. Isolation was done by soaking the infected lesion in 70% ethanol for 15 second, later surface sterilization in 0.525% sodium hypochlorite for 2 min, then washing in sterile distilled water and finally plating onto Rye agar medium amended with antibiotics, rifampicin (0.02g/l), polymixin B sulphate (0.05 g/l), ampicillin (0.10 g/l) and ancomycin (0.05 g/l) (Goodwin, *et al.* 1992) and incubated at 24°C in a BOD incubator. All the isolates were subsequently maintained on Rye agar medium slants at 24^{o}C till further use.

Effect of temperature on zoospore germination

Germinated zoosporangia were used for testing the effects of temperature on the spore germination at 18-30°C. The zoosporangial suspension was incubated at different temperatures for five hours after which observations of change in germ tube length were taken. At the end of the experiment, a drop of cotton blue was added to arrest the further growth of germ tube length which was recorded using a haemocytometer.

Mycelial growth of *P. infestans* isolates at 24°C originally incubated at 10-30°C

Mycelial growth of various isolates of *P. infestans* originally, incubated at various temperature regime of 10-30°C was tested at 24°C to check whether changing temperatures from 10-30°C would make the isolates to regain their growth.

Mycelial growth on agar

Each isolate was grown in three replicate petri dishes containing Rye agar medium. Radial growth (in mm) was measured after 4 days of incubation at different temperature conditions.

Effect of photoperiod on sporangial germination

6 plantlets inoculated with *P. infestans* were grown in sterile pots and placed in temperature controlled environment chambers which were grown for 32 days in independent growth chambers under 2 combinations of one constant temperature (24° C) with varying photoperiod of 12 and 16 hours day length respectively (three replications). The relative humidity was programmed in the chambers at 80% and illumination was provided by 500W Philips lamps. The fourth fully expanded compound leaves counted from the apex of main stem were detached 32 days after plants were incubated at photoperiod treatments.

Lesion development on potato stem

Various inoculum sizes in terms of number of sporangia/ml were used for studying the minimum concentration of spores in inducing the late blight in potato tubers. Spore concentration of 10^2-10^3, 10^3-10^4, 10^4-10^5,

10^5-10^6, 10^6-10^7 and 10^7-10^8 sporangia/ml were sprayed on the different potato tubers respectively to check the induction of disease.

In another experiment time of spray and foliage condition was studied to check the intensity of development of infection. In this first spray was carried out during the dusk or after wetting the foliar and contrary to this the another spray inoculation was done during noon when the leaves were dry using hand spray pump.

In another experiment the location of incoulation was studied for lesion development. Two filter-paper discs soaked in inoculum were placed on stems: one on lower part (L), 4 to 5 cm above roots, and one on upper part (U), 13 to 14 cm above roots. Inoculation was made on plants that have not yet reached flowering to allow time for disease development.

The disease severity was observed using the scale shown below

0 or necrosis: 9, 11–20 mm: 6, 41–50 mm: 3,
1–3 mm: 8, 21–30 mm: 5, 51–60 mm: 2,
4–10 mm: 7, 31–40 mm: 4, > 60 mm: 1.

Effect of soil temperature:

Soil temperatures at 8 a.m. and 5p.m. were recorded daily from January 2008-December 2009 at the 10cm depth in potato field using soil thermometer at Central Potato Research Institute Campus Modipuram, Meerut.

In vivo* effect of contact & systemic fungicide in controlling *P. infestans.

Commonly used fungicide namely Dithan M-45 (a contact) and Ridomil (a systemic fungicide) were tested for their protective effects against late blight at five different locations. The crop was sprayed @ 0.25% conc. of each fungicide after 15 days interval in each location with five replication for each fungicide treatment. At the end of field experiment, the average incidence of late blight diseases was assessed by taking five observations in each field covering five corners & a centre of host field. Average late blight incidence was calculated by adding disease incidence of all the five observations.

Results and Discussion

Sporangium formation:

P. infestans pathogen is highly variable and changes with slight change in climatic conditions. There is a possibility that the temperature requirement of *P. infestans* has widened and may be able to cause late blight even during the warmer periods. It is with this context that 5 isolates collected from plains were observed for their response to different temperature regimes.

The results revealed that zoospore germtube length of *P. infestans* isolates was maximum at 24°C and minimum at 30°C (Table 1). The similar trend of germtube length was in the remaining isolates of *P. infestans* collected from Mawana, Lawad, Muzaffarnagar, Meerut and Bijnor. The germtube length under remaining temperature *viz*., 18, 21, 27, 30°C ranged between 34 to 68 micrometer. The germtube length of Meerut isolates ranged between 34-70, Mawana isolates ranged between 42 to 68 micrometer, Lawad isolates ranged between 43 to 73 micrometer , Muzaffarnagar isolates ranged between 45 to 76 micrometer and that of Bijnor ranged between 34 to 69 micrometer at a wide range of temperature (18 to 30^{o}C).

These results suggest that maximum growth of zoospore germtube of all the *P. infestans* isolates has been achieved at temperature 24°C and minimum at temperature 30°C. The rest of temperature *viz*., 18, 21, 27°C were not suitable as suitable in enhancing germtube length as was in 24 ^{o}C.

Covarrubias *et al.*, 2010 also observed that at 24°Cmaximum extension in zoospore germ tube length. Also, working on the citrus canker Matheron *et al*., 1992 found that in *P. citrophthora* and *P. parasitica* abundant production of sporangia was at 20-25°C.

Table 1: Effect of temperature on zoospore germ tube length in micrometer of *P. infestans*

S.No.	Isolates	Germination of germ tube in micrometer (µm) at different temperature				
		18 ^{0}C	21 ^{0}C	25 ^{0}C	27 ^{0}C	30 ^{0}C
1	Meerut	62±2.0	68±3.0	70±1.2	63±2.2	34±0.5
2	Mawana	58±1.0	65±1.0	68±2.3	65±1.3	42±1.3
3	Lawad	60±1.5	64±0.5	73±3.3	61±2.1	43±0.6
4	M.Nagar	65±2.1	67±2.5	76±2.1	63±2.2	45±0.8
5	Bijnor	61±2.0	68±1.3	69±1.6	66±2.5	34±0.6

***All the values given in the table are the mean standard deviations (±) of the triplicate experiments.**

Table 2: Effect of temperature on *p. infestans* mycelium growth in milimeter (mm)

S.No.	Isolates	Mycelium growth *P. Infestans* in milimeter different after 48 hour				
		18^{o}C	21^{o}C	25^{o}C	27^{o}C	30^{o}C
1	C.C.S. University	5.50±0.50	11.5±1.20	28.50±3.50	18.50±1.30	4.9±0.25
2	Mawana	7.50±0.25	10.0±2.50	28.00±2.50	15.00±1.10	6.8±0.75
3	Lawad	7.00±1.30	16.5±1.50	27.50±1.20	14.50±2.00	4.8±0.45
4	Bavle Khas	9.50±2.50	19.0±0.50	25.50±2.50	12.50±0.75	9.8±0.56

***All the values given in the table are the mean standard deviations (±) of the triplicate experiments.**

Table 3: Mycelial growth of *P. infestans* isolates at 24°C originally incubated at 18-30°C

S.NO	Initial temperature (°C)	Final temperature (°C)	Initial mycelial radial growth (in mm)	Final mycelial radial growth (in mm)	Net increase in growth (%)
1	18	24	9.5±1.3	10.2±0.5	7.3±0.8
2	21		11.5±2.4	18.8±1.3	63.4±5.6
3	27		18.5±3.6	24.6±4.3	32.9±2.9
4	30		9.8±1.5	10.3±0.6	5.1±0.4

***All the values given in the table are the mean standard deviations (±) of the triplicate experiments.**

Table 4: Optimization of spore concentration for artificially inducing the Late blight of potato in potato cultivars

Spore concentration/ml	Disease score*
10^2-10^3	9
10^3-10^4	8
10^4-10^5	3
10^5-10^6	2
10^6-10^7	2
10^7-10^8	1

*** As per the disease score chart given in materials and methods**

Table 5: Evaluation of systemic and contact fungicides in controlling Late blight of potato

S.No.	Isolates	Avg. Late blight incidence (%)				
		Control	Disease incidence in Dithane M-45	% Disease Control	Disease incidence in Ridomil	% Disease Control
1	Modipuram	2.50	Nil	100	Nil	100
2	C.C.S. University	30.50	6.2	79.67	1.8	94.09
3	Lawad	25.50	5.0	80.39	1.0	96.07
4	Mawana	20.20	4.0	80.19	0.5	97.52
5	Bavle Khas	5.60	1.0	82.14	Nil	100

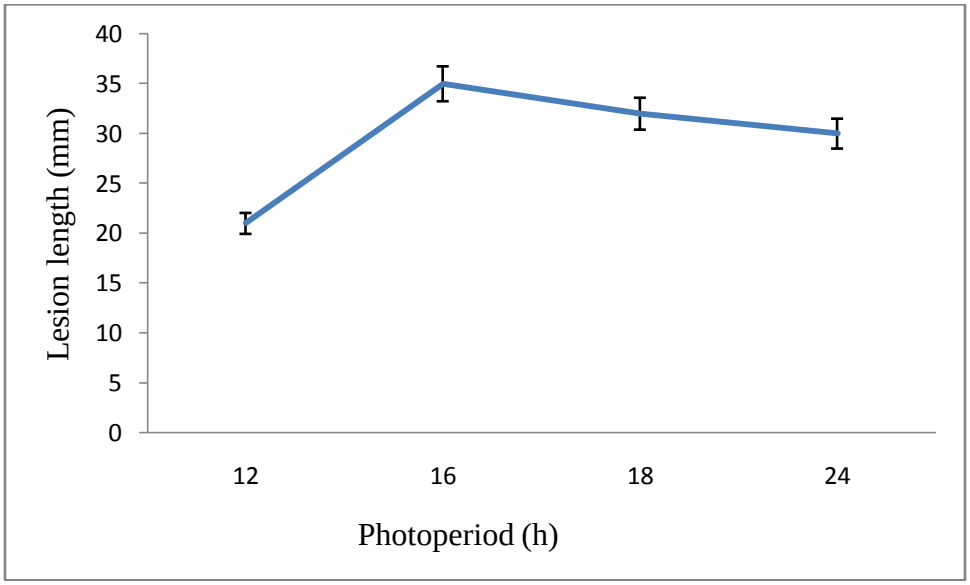

Figure 1: Effect of photoperiod on the growth of *Phytophthora infestans*

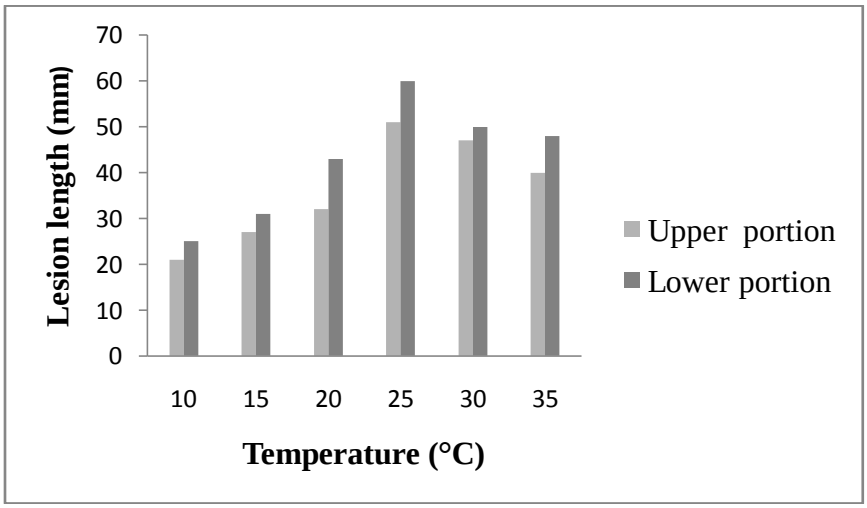

Figure 2: Evaluation of different spots for artificial inoculation of *P. infestans*

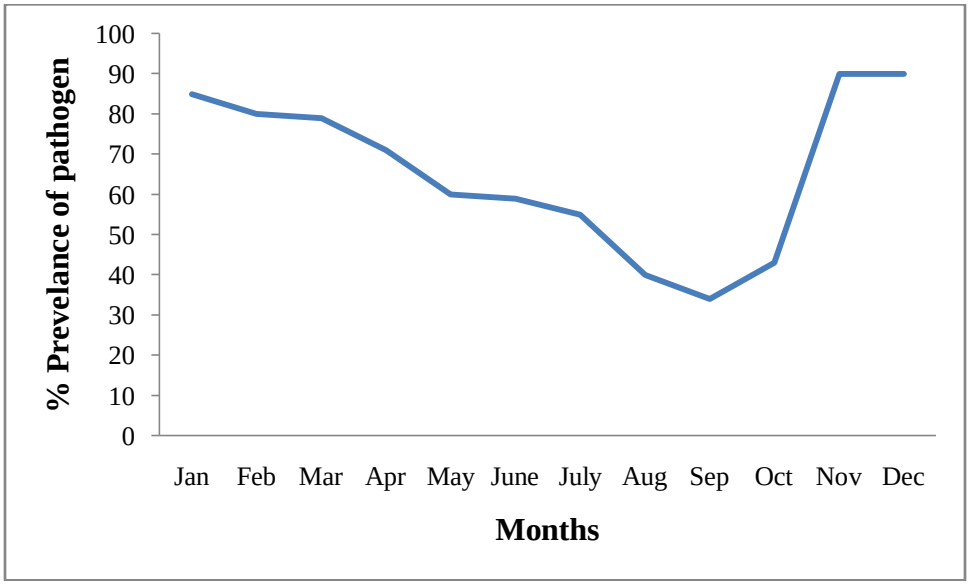

Figure 3: Evaluation of soil temperature during different months of years for prevelance of pathogen in the soil

Mycelial growth on agar

In vitro different temperature conditions (18, 21, 25, 27 and 30°C) were tried to find out their impact on mycelium growth of *P. infestans.* 25°C was found the best with highest recorded mycelium growth (25.50-28.5 mm) amongst all four isolates. This temperature was next followed by 21 and 27°C, where the mycelial growth of all four isolates ranged between 11.5 to 19.0 mm and 12.5 to 18.5 mm radial growth respectively. Mycelial growth was lowest at 18°C and 30°C temperature and it ranged between 5.50 to 9.50 mm and 4.9-9.8mm respectively (Table 2). 18°C temperature was not all good for *P. infestans* mycelial growth while temperature 25°C is the best and showed optimum growth of *P. infestans.*

Otaye *et al*., 2010 found that *P. parasitica* had greater growth at 25°C (15.4mm) while, *P. citrophthora* showed maximum growth at 20°C (20.1mm).

In vitro late blight incidence data collected from five different location in Meerut and around exhibited that average percent late blight disease incidence was minimum (2.5%) in farmers fields of Modipuram and highest (30.5%) around farmers field C.C.S. University. The other three location namely Lawad, Mawana, & Bavle Khas had disease incidence ranging between 5.6 to 25.8% in unsprayed field where potato crop was not given any chemical treatment.

Mycelial growth of *P. infestans* isolates at 24°C originally incubated at 18-30°C

It was found that amongst the isolates grown at different temperatures only the isolates grown at 21 and 27°C revived properly. The one previously grown at 18°C and 30°C failed. The reason could be the denaturation of the growth enzymes at lower temperatures (Table 3).

The results of this study demonstrated that temperature is an important environmental factor with differential effects on each *Phytophthora* growth stage and could have important implications for disease and disease management. Our results confirmed the influence of temperature on mycelial mat growth, sporangia production and zoospore cyst germination for different *Phytophthora* spp.

Sporangia production is an important stage in the life cycle of *Phytophthora* spp., providing the opportunity for increase in infective units and increased disease (Matheron and Matejka 1992). Timmer *et al*., 2000 found that *Phytophthora* sporangia can germinate either indirectly by releasing zoospores or directly by growing germ tubes and thus enhancing plant infection. Results obtained in our studies showed that sporangia production in *Phytophthora* was influenced differently by temperature. The results on the effect of temperature on *Phytophthora* sporangia production were very similar to those previously reported by Mizubuti and Fry (1998). The temperature range over which the tested isolates were able to produce abundant sporangia appeared to be narrow, suggesting that temperature as a factor may confine the pathogenic activities of *Phytophthora* to certain geographic locations and seasons of the year.

Effect of photoperiod on sporangial germination

Photoperiod only had an observable effect on the increment of sporangia density under 16h of day length which did not affect the final infected leaf area. The effect of photoperiod was not tested statistically, but it was evident that higher sporangial density was at 16h than at other photoperiods at 24°C. Photoperiod, light quality and blight intensity as well as the duration and intensity of near UV and IR radiations have a direct effect on pathogen development and host susceptibility. Bright sunshine reduced the ambient relative humidity around the foliage (Harrison and Lowe, 1989). Krug1965 and Steward *et al*., 1981 observed lower temperature and the shorter photoperiod induced earliness in all the tested potato cultivars.

Lesion development on potato stem:

Before carrying out this experiment the isolates were reproduced several times on leaves of a potato cultivar susceptible to *P. infestans*. Inoculum consisted of a sporangial suspension that was prepared from sporulating lesions of potato leaflets and adjusted (with haemocytometer) to different concentrations. It was found that at the concentration of 10^4 -10^5 sporangia per 1 ml disease symptoms appeared more frequently and intensely than at the concentrations below it. Hence, it could be said that for minimum disease to occur the spore concentration should be nominally 10^5 spores/ml of the suspension (Table 4).

It was also observed that the inoculum sprayed on wet leaves or during dusk resulted in more disease compared to the one sprayed in the noon. *P. infestans* for inoculation to work, the inoculum can not dry out. Thus, prior to inoculation, the field were sprayed with overhead irrigation for a sufficiently long period to be sure all foliage were wet. It was considered best to inoculate plants at dusk so that the inoculum won't dry and to protect zoospores and sporangia from direct sunlight. The inoculum was applied as evenly as possible in each plot with a hand-held, manually pumped sprayer (approximately 20 ml per plant).

Working out on .position of inoculation it was found that in the upper portion of the excised stem the disease score was 2 (51-60mm lesion) while in the lower portion it was found to be 4 (31-40mm lesion) (Fig 2). These results were contrary to the one found by Matheron *et al.*, 1992. He found that the maximum Citrus canker lesion was observed in the root excised portion than found on the stem excised portion.

Soil temperature:

Soil temperature was recorded 10cm below the soil surface for two years were similar to the one which suppresses sporangium formation by *P. infestans*. The mean temperature of the soil ranged from 12.3°C in January to a maximum of 39°C in June. During last one decade, *P. infestans* caused frequent epidemics particularly in subtropical plains. They appeared even during the early part of the season i.e. when the temperature is warmer during the month of October and November (Fig 3).

One concerns about use of soil temperature in predicting activity of soil borne pathogens is that this parameters changes with distance from the soil (Matheron *et al*., 1992). The soil temperature at 10cm depth during the summer may inhibit sporulation of *P. infestans* and resultant infection but the temperature at 30-90 cm depth may be conducive to sporulation and disease development. The utility of soil temperature as an indicator of pathogen activity of potential development of late blight could be enhanced by vertical distribution of the pathogen as well as temperature at various depths and surface of the soil.

To facilitate timely application of the fungicides, more in depth information is needed on the effect of an alternate periods of favorable and un-favorable temperatures on subsequent sporulation and disease development. Population and growth of a pathogen are related more to the relative extent of favorable and un-favorable temperatures than to the mean daily temperatures (37°C).

Management of Disease using chemicals:

Spray of systemic fungicide Ridomil @0.25% conc. on potato crop was found to be superior compared to the other contact fungicide i.e Dithan M-45 @ 0.25% . The percent late blight incidence in potato crop sprayed with Dithane M-45 ranged between 0- 6.2% whereas Ridomil sprayed potato crop showed almost complete control of the disease and its incidence in five location ranged between 0-1.8% (Table 5). These results confirm the previous findings. Where it has been reported that Ridomil is superior to Dithan M-45 in controlling late blight incidence. All the treatments showed significantly better foliage controlled as well as tuber yield over non- treated control. The results of this study were consistent with previous studies and indicated that the application of protective fungicides could reduce foliar late blight to acceptable levels (Clayton and Shattock, 1995; Fontem, 2001; Kassa and Buyene, 2001; Kirk *et al.*, 2001; Oliambo *et al.*, 2001).

Conclusion:

From this study it can be concluded that the environmental factors can play an important role in minimizing the production losses caused to the crop due to the pathogen attack. As *Phytophthora infestans* is more prevelant during the month of Nov-Dec, so early sowing of the crop can be practised to overcome it. Moreover, knowledge of these factors help in timely spray of the fungicide depending upon the intensity of occurrence of pathogen under natural conditions or can help in just need based application of the fungicides.

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