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MANAGEMENT OF LATE BLIGHT OF POTATO CAUSED BY PHYTOPHTHORA INFESTANS THROUGH BOTANICAL AQUEOUS EXTRACTS

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ABSTRACT

Late blight caused by *Phytophthora infestans* (Mont.) de Bary is most devastating pathogen of potato crop. Disease caused Ireland's worst devastation during 1840–1845 and since then, it has caused considerable yield losses globally. The repeated and injudicious synthetic fungicide applications against late blight developed fungicide resistance in *P. infestans* have given a push to finding out alternate ways to control the disease. The study was carried out to evaluate the efficacy of garlic (*Allium sativum*), neem (*Azadirachta indica*), turmeric (*Curcuma longa*), mint (*Mentha*) at 5, 10 and 15% concentrations as bio-fungicides against late blight of potato. *In-vitro* effect of aqueous plant extracts was evaluated on percent inhibition and radial growth of pathogen. In comparison to the control, *A. sativum* and *A. indica* at 15% concentration were found to be more effective in inhibiting *P. infestans* mycelial growth by 58.4% and 43.9%, respectively. In the greenhouse trials, overall potato late blight disease incidence was minimum (5.81%) where *A. sativum* extract was used followed by *A. indica* (8.45%) at 15% concentration as compared to control (61.18%). Similarly, 15% aqueous extracts of *A. sativum* was found highly effective with 15.4% disease severity, as compared to control (54.13%). The use of *A. sativum* and *A. indica* aqueous plant extracts at a concentration of 30% was found to be the most promising and effective measure against the late blight pathogen. Study provides a comparison of environment friendly botanical extract based disease control for organic vegetable production opportunity inspite of health hazardous synthetic agro-chemicals.

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important vegetable crop that is widely consumed around the world due to its high yield potential and nutritional value (Poehlm and Slepper, 1995). It belongs to the family Solanaceae originated from South America (Spooner *et al.*, 2005). In Pakistan, the potato is grown on 57838 hectares with a total production of 594210 tons and yield 10.27 tons/ha (FAO, 2020). In Pakistan, an average of 1.3 million ha out

of the area of 24 million ha under cultivation is apportioned to potato crop producing 20 million tons per hectare (Sajid and Aftab, 2012). The average yield of potato in Pakistan is far less compared to other potato growing countries in the world. This yield gap may be attributed to various biotic and a biotic factors. Among biotic factors, late blight disease caused by *Phytophthora infestans* contributed a lot in the reduction of potato yield (Berhan, 2021; Raza *et al.*, 2019; Majeed and Muhammad, 2018).

Late blight of potato is most damaging disease among various diseases of potato (Moumene *et al.*, 2015). It is a destructive disease in potato growing areas in Rawalakot that causes severe losses in the potato season. *P. infestans* belongs to the group oomycetes, is measured to be foremost constraint for potato production all over the globe (Adl *et al.*, 2005). Contrasting the other *Phytophthora* species that causes soilborne or root rotting diseases in various vegetable crops, *P. infestans* primarily causing foliage diseases targeting stem, leaves, fruits and tubers in potato and tomato crops. Disease is airborne that spread through asexual spores during its growing season (Shattock, 2002). Numerous applications of fungicides keep elevated problems of resistance in pathogen, markedly to metalaxyl (Phenylamide) (Kato *et al.*, 1997).

Because of the undesirable properties of synthetic agrochemicals and the growing threat to the environment, one possible alternative is to use naturally occurring biologically safe control agents for disease suppression.

In this context, plant extracts (Krebs *et al.*, 2006), and biological antagonists (Ghorbani *et al.*, 2005; Hyder *et al.*, 2020; Bashir *et al.*, 2020) may perhaps be second as essential alternatives to synthetic fungicides for calculating late blight. Active compounds of plant extracts are phenols, flavonoids, alkaloids, quinones, saponines, tannins and sterols (Halama and Van Haluwin, 2004).

Extracts of many plants have been recorded to have antibacterial, antifungal, and insecticidal properties under lab., greenhouse and field conditions. Different plant extracts (Krebs *et al.*, 2006; Bashir *et al.*, 2020; Gopi *et al.*, 2020; Rogozhin *et al.*, 2020) been successfully used to control potato diseases and may be future alternatives to synthetic fungicides for controlling late blight. Wang *et al.* (2004) reported 90% restriction of some fungal diseases and late blight of potato by foliar application of 1% (w/v) acetone and n-hexane or aqueous leaf extracts of *Inula viscose.* Numerous studies have recorded the influence of plant-derived products on *P. infestans* growth (Olanya and Larkin, 2006; Stephan *et al.*, 2005; Raza *et al.*, 2019). Botanicals are less toxic to human and other organisms, cost effective and often have a narrow range of activity (Aslam *et al.*, 2010).

The present studies have been designed to explore the alternative means for managing late light of potato using plant extracts.

MATERIALS AND METHODS

Pathogen Isolation and Identification

Rye agar medium was used to isolate and purify the pathogen detached from diseased symptomatic potato leaves having late blight infection (Thomas and Naik, 2017). After thoroughly washing and sterilizing symptomatic leaf pieces with 0.1% sodium hypochlorite solution for 2 min, the specimens were plated on rye agar medium followed by incubation at 27 °C for 7-10 days. Purification was done using a single spore culture technique.

Plant Materials

Four different medicinal plants, including garlic (*Allium sativum*), neem (*Azadirachta indica*), turmeric (*Curcuma longa*) and mint (*Mentha*), were collected for the preparation of aqueous plant extracts. The plants were selected based on traditional medicinal values and earlier studies that demonstrated antifungal properties.

Preparation of Crude Extracts

All the plant materials were air-dried and powdered. The extracts were acquired by distillation of 100 g preserved plant powder in 1L distilled water followed by heating the autoclave at 100 °C for 30 minutes using closed cap vials. The extracts were filtered through 3 to 4 layers of muslin cloth and then through Whatman No.1 filter paper. The stock aqueous solutions were then kept in the refrigerator at 4 °C until they were needed again.

In-vitro testing of Plant Extracts

A 9 cm Petri plate was divided into four sections for the in-vitro antifungal assay. The direct contact method was used for mycelial growth inhibition. The minimum inhibitory concentration (MIC) of the aqueous extract and microbiological procedures were determined according to Paranagama *et al.* (2003). Using micropipettes, 5 ml of plant extract was poured into Petri dishes of a similar diameter for each treatment. The Petri plates with the rye agar medium and plant extract were kept in a 28°C incubator. Treatments were replicated thrice for each *P.*

infestans isolate. Calculating the average of two diameters, deliberating on two, and drawing a corner axis on the edge of the plated Petri dishes were used to record the data.

Different concentrations of the extracts were prepared by diluting the crude extracts to prepare 5, 10, and 15% concentrations. The research was organized in completely randomized design (CRD) with three replications. Measurements were recorded as soon as the growth in the control plate reached maximum, and the diameter of the fungal colony was measured using a procedure determined by Sallam and Abo-ElyouSr (2013). Fungal-toxicity of test extracts was calculated according to the formula in terms of percentage growth inhibition (Taskeen-un-Nisa and Mir, 2010);

$$PGI = \frac{\mathrm{DC} - \mathrm{DT}}{\mathrm{DT}} \times 100$$

Where: PGI = Percentage growth inhibition; DC = Regular increase diameter of fungal colonies obtained from limitation plates; DT = Regular progression diameter of fungal colonies obtained from treated plates.

Pathogenicity Test of *Phytophthora infestans*

Isolates of *P. infestans* were tested for their virulence and aggressiveness determination through detached leaflets of 4-week-old seedlings of potato. Aggressiveness was determined by assessing the lesion length and width (mm) of diseased leaves from which lesion sizes were calculated (Fontem *et al.*, 2005). The most aggressive isolate was used for further lab based studies.

***In-vivo* testing of Plant Extracts**

The result of aqueous extracts of four botanicals on late blight infected potato was investigated in the greenhouse. In addition, an examination was performed on the diseases incidence and severity of potato late blight disease. A potato variety "cardinal", collected from National Agricultural Research Center (NARC) Islamabad, was used in the greenhouse experiment. The plant extracts were applied with a concentration of 5%, 10% and 15% as foliar applications at 30 ml/plant. In control, the culture of distilled water against *P. infestans* was applied. Finally, pots were ordered using a completely random design (CRD) with three replications in a greenhouse.

The first foliar spray of the plant extracts was done on seventh weeks (45-days) old potato plants and the second one was done after 15 days of the first spray. After two days of the second foliar application of plant extracts (62 days after planting), the potato plants were inoculated with 20ml of *P. infestans* suspension containing 5×10^4 spores/ml using an atomizer. After inoculation, plants were enclosed with polyethylene bags for forty-eight hours to continue high moisture conditions. The bags were uncovered after 48 hours, and plants were kept under greenhouse conditions. Disease severity was recorded in each treatment by using disease estimation scale (Ghazanfar *et al.*, 2010).

Incidence of late blight was assessed by counting the number of diseased leaves, and percentage was calculated from the total six leaves observed. The observations started from the appearance of the first symptom on leaves until the downfall of observed leaves. To check the disease severity, six leaves of each plant were used.

The percent incidence was calculated as;

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased leaves}}{\text{Total no. of leaves observed}} \times 100$$

RESULTS

Isolates of *P. infestans* from natural late blight infections were obtained from the foremost potato growing areas of Rawalakot, AJK. The isolates were screened during the potato budding season. The shattered potato leaves, with sporulating lesions of *P. infestans*, were collected from fields that were approximately 5–10 kilometres apart from each other. Identification was done by observing sporangia of the fungus under a microscope. Sporangia were colour-less and produced free-floating zoospores with two flagella, one tinsel, and one flagellate. The mycelium of fungus noted as white, ranging from creamy to white. Likewise, colony colours vary, ranging from white to creamy (Table 1). The sporangiophores of the purified isolates were non-septate, short, straight, and white to creamy in color, ellipsoid to oblong in shape, with a long beak. The average length and width of sporangia were noted as 29-36x19-22 µm. Septa are formed at the time of reproduction or maturity.

Pathogenicity Test

A pathogenicity test was carried out to confirm pathogen associated with the host. After one week of inoculation with spore suspension of *P. infestans*, symptoms of late blight started appearing on the potato leaves. These symptoms include development of irregularly shaped covered water filled lesions on young foliage with whitish sporulation of the pathogen around the margin of lesions. Diseased leaves were detached and used to re-isolate the pathogen that showed the same morphological and cultural characteristics, confirming Koch's postulate (Figure 1).

Table 1. Cultural characteristics of purified isolates obtained.

Structure	Characteristics
Colony	White, fluffy in color, slow growing or sometimes lumpy in appearance.
Mycelium	Non-Septate, Branched, white or Creamy in color.
Growth	Slow, takes 15 to 20 days to fill the petri plate. Colony diameter is 8-9 cm.
Margin	Smooth or wavy.

Figure 1. Replicate representing R1, R2, R3 showing diseased is the confirmation of pathogen virulence.

***In-vitro* Evaluation of Plant Extracts against Pathogenic Isolates**

All four aqueous plant extracts, viz., garlic (*Allium sativum*), neem (*Azadirachta indica*), turmeric (*Curcuma longa*), and mint (Mentha), showed antifungal activity against *P. infestans.* Means of radial mycelial growth (RMG) showed a decrease in the growth of fungus with an increase in the concentration of botanicals from 5-15%. Among botanicals, minimum mycelial growth was recorded by *A. sativum* among all tested concentrations compared to control. *A. sativum* showed 30.25, 26.25 and 18.25 mm growth, at 5, 10, and 15% concentrations, respectively. Following *A. sativum* results, *A. indica* showed more mycelial growth at 31.5, 30.41, and 26.83 mm at 5, 10, and 15% concentrations, respectively**.** *C. longa* was least effective against *P. infestans,* which showed 30.58 mm radial mycelial growth at 15% concentration. Maximum percentage inhibition was shown by *A. sativum* 58.4% using 15% concentration. *A. indica* showed 31.5, 30.4, and 26.8% mycelial growth of *Phytophthora infestans* with an application of 5, 10 and 15% concentrations, respectively (Table 2). *Mentha* showed less inhibition percentage; 33.75, 31, and 28% mycelial growth at 5, 10 and 15% concentrations, following *C. longa* 5% resulted in 35.9% mycelial growth, 43.9% colony growth where no treatment was incorporated (Figure 2).

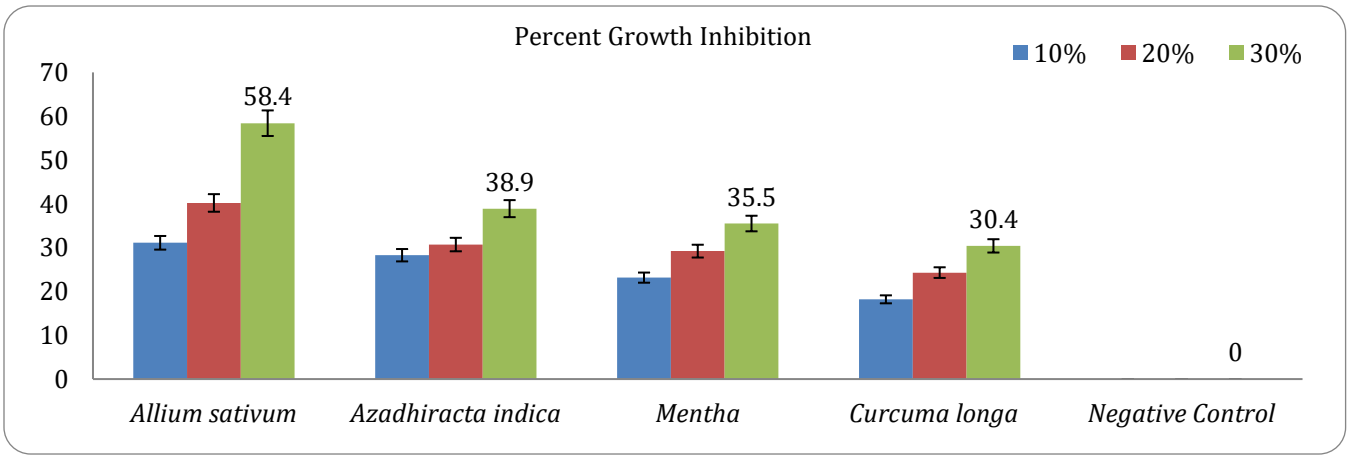

Figure 2. Percentage inhibition (0-80) of *Phytophthora infestans* colony *in-vitro* against plant extracts.

Table 2. Botanical extracts impact of 3 levels of concentrations on radial mycelial growth of *Phytophthora infestans.*

Treatments	Radial Mycelial Growth (mm) Concentrations applications		
	5%	10%	15%
Allium sativum	**30.250±0.1f*	26.250±0.1i	18.250±0.1 l
Azadirachta indica	31.500±0.1 d	30.417±0.1f	26.8333±0.0 h
Mentha	33.750±0.1 c	31.083±0.0de	28.0333±0.1 g
Curcuma longa	35.917±0.0 b	33.250±0.0 c	30.583±0.0 ef
Positive control	2.917±0.0 n	2.917±0.0 n	2.917±0.0 n
Negative Control	43.917±0.0 a	43.917±0.0 a	43.917±0.0 a

*Figures in the column followed by the same letter are not significantly different according to the LSD test at 0.05.
**Mean± Standard error of mean

Greenhouse Evaluation of Plant Extracts on Disease Development

All four plant extracts viz., *Mentha, Allium sativum*, *Azadirachta indica* and *Curcuma longa* reduced incidence of late blight under greenhouse with considerable variations. *Allium sativum* extract at 15% concentration exhibited the best results in reducing the incidence of late blight (5.81%) followed by *Mentha* (9.5%) and *Curcuma longa* (10.43%) as compared to control.

Effect of Plant Extracts on Potato Late Blight Disease Severity

Late blight infection was much less common in greenhouse conditions with entire handlings and plant extracts than in controlled conditions. Botanical extracts at 15% concentration level gave valuable reduction in disease infection with reduce infection and infestation level. Lowest disease incidence (5.82) was observed where *A. sativum* was applied while maximum incidence was observed in control where 61% plants were found infested (Table 3). Botanical extracts exhibited significant differences in reducing disease severity. In greenhouse conditions, *A. sativum*, *A. indica*, water and acetone extracts reduced late blight effectively. *Allium sativum* application at 15% gave maximum disease reduction and disease severity was 02 on rating scale and 15.4% coverage, followed by *A. indica* with 16.8% and 19.66% by *Curcuma longa*. Mentha was found least effective with 4 rating scale of late blight severity (Table 4) with 27% disease coverage.

Table 3. Impact of botanical Extracts on incidence of late blight on potato crop in greenhouse conditions.

Treatments	Concentrations		
	5%	10%	15%
Mentha	31.009 c	22. 701 d	9.5800 f
Allium sativum	29.649 c	19.430 e	5.8193 g
Azadirachta indica	30.635 c	21.885 de	8.4543 fg
Curcuma longa	33.746 b	23.232 d	10.43 5f
Control	61.180 a	61.180 a	61.180 a

*Figures in the column followed by the same letter are not significantly different according to the LSD test at 0.05. CV= 3.77; LSD=0.7282

Table 4. Efficiency of different Plant Extracts on late blight disease severity (Saleem *et al*., 2016).

Plant Extract	Concentration			Rating (15% conc.)	Response
	5%	10%	15%		
Mentha	41.700 bc	34.200 f	26.933 gh	4	MS
Allium sativum	38.700 d	27.700 f	15.400 h	2	R
Azadirachta indica	40.167 cd	28.667 f	16.800 gh	2	R
Curcuma longa	43.467 b	33.300 e	19.660 g	2	R
Control	54.133 a	54.133 a	54.133 a	9	S-HS

*Figures in the column followed by the same letter are not significantly different according to the LSD test at 0.05. CV=1.52; LSD=0.7232

DISCUSSION

Late blight of potatoes is an important biotic constraint, having the potential to damage its production at all stages. Due to its epidemic nature, disease control is imperative to raise a healthy potato crop. It provides a backbone to the potato farmer's based agricultural economy by saving the crop. The pathogen is mostly managed extensively by using fungicides, including protectants and systemics, to avoid and eradicate the pathogen, first to protect the crop from further invasion and then to eradicate the pathogen from the diseased plant. The appearance of the symptoms makes the farmer panic, and he rushes for fungicidal spray whether it is needed or not. Because of the nature of pathogen spread to potato or tomato crops, new pathogen strains have been developed. It makes pathogen control difficult for farmers and scientists alike, and it jeopardizes food security concerns. Plant extracts have long been used by humans to treat a variety of ailments in humans, animals, and plants. Due to their crude nature, they equally provide supplement nutrients, which not only minimize the pathogen efficiency but improve the plant health, making the plant resistant while attacked by the pathogen. In this study, late blight (*P. infestans*) isolates from various sites in Rawalakot were used. Aqueous extracts of *Azadirachta indica, Mentha, Allium sativum,* and *Curcuma longa* were tested against late blight of potato infection under *in vivo* and controlled conditions. Results revealed that aqueous extracts of *Allium A. sativum* showed maximum (66%) mycelial growth inhibition of *P. infestans*, while least (36%) by *C. longa*. In greenhouse conditions, 15% concentrations of the aqueous extracts of both; *A. sativum* with the reduced 5.8% and 8.5% disease incidence, gave maximum control of *P. infestans* as compared to control. This has confirmed the antifungal potential of these extracts against *P. infestans* infection on potato. The antifungal effects of plant extracts further suggest that these plants are carriers of chemicals that are effective against plant pathogens. They also provide nutrients to the host plant, making it resistant and reducing disease severity, even after the disease has invaded and the environment is favourable. The aqueous extract of *Mentha* was found to have unique characteristics of detoxifying the fungal metabolites, resulting in inhibition of fungal growth. This has already been confirmed by the findings of many scientific studies (França *et al.*, 2018; Alhoot *et al.*, 2019) Aqueous extracts of garlic are known to have volatile compounds like linear chain aldehydes, allyl sulfides, and disulfides, which are stable compounds. These compounds have been proven effective against different types of fungal mycelial growth (Bianchi *et al.*, 1997).

The inhibitory effect of botanical extracts on *Phytophthora infestans in-vivo* has shown a reduction in fungal mycelial growth. A variety of primary and secondary metabolites are reported from these tested plants as having the potential to reduce fungal growth. These metabolites play a very significant role in protection against insects, herbivores, and microorganisms. These secondary metabolites reduce the development of disease either by acting directly on pathogens or by inducing some kind of resistance mechanism (Kagale *et al.*, 2004). Several workers have reported similar effects of different plant extracts against *P. infestans.* Aqueous leaf extracts of *A. indica* showed antifungal properties against *A. alternate* from tomato fruits with 85% control of fruit rot *in vivo* (Bashir *et al.*, 2020), and *P. infestans* infection on potato both under in-*vitro* and greenhouse conditions (Dahlin *et al.*, 2017).

Results further revealed that different plant extracts varied in their efficacy against tested fungi. This might be due to the presence of variation in the components of antifungal chemicals in different plant species and even in one species in different areas. Each compound of a plant extract shows their contribution to the extract's biological activity (Derbalah *et al.*, 2011). *Azadirachta indica* antifungal activity was due to the presence of Azadirachtin, which belongs to the class of terpenoids and is effective against bacteria and fungi (Gurjar *et al.*, 2012). Garlic is historically used as a preservative and as a drug against various diseases of animals and humans as it has antimicrobial properties due to allicin (S-allylcystein-S-OXIDE) and a lot of other fungi (Gurjar *et al.*, 2012). The current study allows scientists to further evaluate and standardize the botanical extract against late blight of potato for effective, long-term, and environmentally friendly control.

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CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally to the research and compiling the data as well as editing the manuscript.