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Eco-friendly management of *Phytophthora infestans* causing late blight of potato

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Abstract
The traditional defense of potato plant against potato late blight caused by *Phytophthora infestans* involved the frequent use of synthetic chemicals but due to development of resistance in most common pathogenic fungi against chemicals and also its residual effect on human health has given a push for obtaining alternatives strategies. Therefore, in the present study, five plant extracts were evaluated namely, *Azadirachta indica* (Neem), *Moringa oleifera* (Sohanjna), *Acacia nilotica* (Keekar), *E. camaldulensis* (Safeda) and *C. limon* to test antimicrobial activity against *P. infestans*. Experiment was arranged out in Completely Randomized Design (CRD) with three replications by poisoned food technique. Mycelial development and inhibition percentage of *P. infestans* were recorded following 3, 5 and 7 days post application. The result revealed that all the plant extracts significantly inhibited the mycelia growth of the pathogen when compared with control. However, among all plant extracts, *A. indica* (59.77%) was significantly superior over other treatments followed by *M. oleifera* (50.61%), *A. nilotica* (43.65%), *E. camaldulensis* (38%) and least inhibition was observed in *C. limon* (24.68).The result of present study suggested that neem extract was found most effective for controlling late blight of potato.

Keywords: potato, late blight, plant extracts, poison food technique, control

1. Introduction
Potato is essential vegetable crop consume worldwide in large quantity good yield potential and nutritional value [25]. Potato is a particular crop which fulfills the food needed for a country in a suitable manner. In Pakistan potato production is very low as compare to other developing countries. The main reasons of low productivity are diseases resembling, early blight, late blight, leaf spot, dry rot, charcoal rot, black scurf, common scab, soft rot, leaf roll etc. Among them, late blight caused by (*Phytophthora infestans*) (Mont.) de Bary is very destructive disease that had managed to most un-famous disaster in Ireland (England) during 1840–1845 [22]. In Pakistan, the occurrence of *P. infestans* was firstly reported from district Swat, a valley which provides ideal conditions for late blight pathogen [14]. The disease favors with low temperatures and high humidity and may result in the destruction of whole potato crop in the field within five days [5, 6,]. Late blight can cause 100% yield loss in Pakistan. *Phytophthora infestans* can infect all parts of the potato plant except roots [3]. Controls of pathogen mostly rely on use of fungicides but these are restricted due to the harmful effects on the environment by causing water and soil pollution and hence directly animals and humans are affected [30]. However, chemicals those are available to regulate plant diseases are declining due to swift development of resistance by plant pathogens. Therefore, plant pathologists are therefore left with no choice but to reconsider the available alternatives which are eco-friendly, biodegradable and less expensive [38]. Plant-derived products such as plant extracts are best alternative in late blight management [31]. These antimicrobial compounds are toxic to plant pathogens and eco-friendlyin nature [14]. Therefore, by keeping in mind of above, the present study was planned to test the efficacy of antifungal activity of different plant extracts at different concentrations by using poison food technique under laboratory conditions.

2. Materials and Methods
This study was conducted in laboratory of Plant Pathology, College of Agriculture, University of Sargodha (32° 7' 48" N and 72° 41' 8" E) during 2018-19. Infected potato leave samples were collected from Sargodha district and were brought to laboratory and stored at -4°C for further use.

2.1 Isolation of pathogen
The pathogen, *Phytophthora infestants* was isolated from infected potato plant samples. Infected leaves were cut into small pieces up to 1.5-2 cm and surface sterilized with 1% sodium hypochlorite (Bleach) for 1-2 minutes and washed three times with sterilized distilled water. Cut samples were dried between two layers of filter paper to remove excess water. The dried samples were placed on corn meal agar medium (17g corn meal agar dissolved in 1L of distilled water and then autoclaved. Ampicillin 0.25g was added in10ml of distilled water and added to prepared media) and incubated at 18 ±2°C for seven days for colony growth.

2.2 Preparation of plant extracts
Leaves of different plant e. g *Azadirachtin indica, Moringa oleifera, Acacia nilotica, Eucalyptus camaldulensis* and *Citrus limon* were washed with distilled water and then surface sterilized with 1% sodium hypochlorite solution. Leaves were grinded with pestle and mortar in sterile

distilled water at 1:1 (w/v) and filtered through a muslin cloth to produce a 100% crude extract and heated to avoid contamination for 15-20 minutes. Crude extracts were considered 100% and serial dilutions (5%, 10%, 15%) were made by using sterilized distilled water.

Table 1: Plants used for antifungal activity assay

Botanical name	Common name	Family
Azadirachta indica	Neem	Meliaceae
Moringa oleifera	Sohanjna	Moringaceae
Acacia nilotica	Keekar	Fabaceae
Eucalyptus camaldulensis	Safeda	Myrtaceae
Citrus limon	Lemon	Rutaceae

2.3 Preparation of extracts concentration

The required quantity of each plant extract was added separately so as to get a necessary concentration by using CMA (corn meal agar) which was used as nutrient medium. Plant extracts were mixed with growing medium and 15 ml poisoned medium was poured to petri dishes. After solidification the actively growing periphery of the seven days old culture of *P. infestants* was carefully cut using a cork borer and transferred aseptically to the center of each petri plate containing the poisoned medium. Corn Meal agar plates without the plant extracts used as control. The plates were incubated at 18±2°C for seven days and the mycelia growth was recorded [30].

2.4 Statistical Analysis

Data regarding plant extracts concentrations on pathogen growth were analyzed statistically by using Statistix (8.1) program at the 0.05% of the probability level [30]. Percent inhibition of mycelial growth compared to control was calculated. Percent inhibition over control calculated by formula:

$$\text{Inhibition percentage} = \frac{\text{control} - \text{treatment}}{\text{control}} \times 100$$ [23].

3. Results

Five plant extracts were evaluated at three different concentrations (5, 10 and 15%) through food poisoned technique to test antifungal activity in laboratory against, *Ph. infestans* causal agent of potato late blight. An insight into the experimental data revealed that there were significant differences among tested plants extract for inhibiting the mycelial growth of the pathogen.

The plant extracts evaluated against colony growth by taking average mycelial growth and inhibition percentage (Table 4). Results showed that all tested plant extracts were significantly reduced linear mycelial growth and increased inhibition percentage compared with control. Among tested plant extracts, *A. indica* was the most effective in decreasing the linear mycelial growth and increasing the inhibition percentage (33.24 mm and 59.77%) followed by *Moringa oleifera* (40.83 mm and 50.61%), *Acacia nilotica* (46.57 mm and 43.65%) and Eucalyptus camaldulensis (51.24 mm and 38%) respectively while, *Citrus limon* was the least effective extract (62.08 mm and 24.68%) to inhibit growth of pathogen. Among the different plant extracts tested, *A. indica* at 15 % concentration (59.77 %) was very effective to retarded growth of *P. infestans* followed by *Moringa oleifera*, *Acacia nilotica*, *Eucalyptus camaldulensis* and *Citrus limon* (50.61%, 43.65%, 38%, 24.68%) respectively. It has been observed that significant interaction between botanical and concentration was recorded while inhibition percentage was increasing by increasing concentration of plant extracts (Table 2-4).

Table 2: Mycelial growth and inhibition percentage after three days colony of *Phytophthora infestans* on corn meal agar

Plant Extract	Concentration %					
	Linear growth	Control	5%	10%	15%	Mean
Citrus limon	CG	27.66^{P}	22.67^{ST}	15^{ab}	11.33^{gh}	19.16
	IP	00	18.04	45.77	59.03	30.71
Eucalyptus camaldulensis	CG	27.66^{P}	12.66^{ef}	10.33^{ij}	6.33^{qr}	14.24
	IP	00	54.22	62.65	77.11	48.49
Acacia nilotica	CG	27.66^{P}	10.66^{hi}	8.33^{MN}	4.33^{tu}	12.74
	IP	00	61.46	69.88	84.34	53.92
Moringa olifera	CG	27.66^{P}	11^{hi}	8.66^{l-n}	5.66^{rs}	13.24
	IP	00	60.23	68.69	79.43	52.08
Azadirachta indica	CG	27.67^{P}	7.33^{op}	5^{st}	3^{vw}	10.75
	IP	00	73.50	81.92	89.15	61.14

CG= Colony growth IP=Inhibition percentage

Table 3: Mycelial growth and inhibition percentage after five days colony of *Phytophthora infestans* on corn meal agar

Plant Extract	Concentration %					
	Linear growth	Control	5%	10%	15%	Mean
Citrus limon	CG	55.67^{d}	48.66^{E}	33.66^{L}	27.33^{P}	41.33
	IP	00	12.59	39.53	50.90	25.75
Eucalyptus camaldulensis	CG	55.66^{d}	28.66^{O}	24.33^{R}	12.33^{f}	30.24
	IP	00	48.50	56.28	77.84	45.65
Acacia nilotica	CG	55.67^{d}	23.33^{S}	17.66^{WX}	9.33^{kl}	26.49
	IP	00	58.09	68.27	83.24	52.4
Moringa olifera	CG	55.67^{d}	21.33^{U}	18.33^{VW}	14.33^{bc}	27.41
	IP	00	61.88	67.07	74.25	50.80
Azadirachta indica	CG	55.66^{d}	14^{cd}	10.33^{IJ}	7.33^{op}	21.83
	IP	00	74.84	81.44	86.63	60.72

CG= Colony growth IP=Inhibition percentage

Table 4: Mycelial growth and inhibition percentage after seven days colony of *Phytophthora infestans* on corn meal agar

Plant Extract	Concentration percentage					
	Linear growth	Control	5%	10%	15%	Mean
Citrus limon	CG	82.33[a]	68.66[B]	56[d]	41.33[HI]	62.08
	IP	00	16.60	31.98	49.79	24.68
Eucalyptus camaldulensis	CG	82.66[A]	55.33[D]	43.66[G]	23.33[S]	51.24
	IP	00	33.06	47.18	71.77	38
Acacia nilotica	CG	82.66[A]	45.33[F]	39.66[J]	18.66[V]	46.57
	IP	00	45.16	52.02	77.42	43.65
Moringa olifera	CG	82.67[A]	32.66[M]	27.33[P]	20.66[U]	40.83
	IP	00	60.50	66.94	75	50.61
Azadirachta indica	CG	82.67[A]	20.66[U]	16.33[Z]	13.33[de]	33.24
	IP	00	75	80.24	83.87	59.77

CG= Colony growth IP=Inhibition percentage

4. Discussion

Excessive application of chemicals is not eco-friendly and economical while use of plant extracts and biologically active products present in plants are more beneficial for protection against plant pathogens [30]. Such alternatives could be valuable as bio-pesticides for controlling plant diseases because they are biodegradable and selective in their activities. Therefore, in present study five plants extracts (*A. indica, M. olifera, A. nilotica, E. camaldulensis and C. limon*) were evaluated against late blight disease of potato. Among all plant extracts tested, *Azadirachta indica* showed maximum disease incidence (59.77%), followed by *Moringa olifera* (50.61%), *Acacia nilotica* (43.65%), *Eucalyptus camaldulensis* (38%) and *Citrus limon* (24.68%) as compared to control. The results of present studies are in line with those of [1] who observed that *Azadirachta indica* expressed inhibitory effects against *Phytophthora infestans*. Extract of *Azadirachta indica* contain a chemical, azadirachtin which is very effective against fungal pathogens particularly late blight disease of potato[34,17]used leaf extract of neem against late blight disease and reported its effectiveness as it inhibit the mycelia growth of *Phytophthora infestans* [12]. Our results are in the line of [29] who reported that the extract of *Azadirachta indica* has good inhibitory effect against *P. infestans* spore germination in concentration dependent manner. Plant extracts are not only effective against phytopathogens but also effective against human pathogens [35]. Efficacy of plant extracts to control plant pathogens have been demonstrated by several researchers [24, 28, 32]. Medicinal plants showed more aggressiveness in controlling plant diseases [5]. Similar findings have been documented by several researchers who found antifungal activity of moringa against plant pathogens [2, 3, 7, 8, 20,]. The fungicidal effect of Moringa extracts on different soil-borne fungi such as *Rhizoctonia, Pythium* and *Fusarium* was recorded [2, 5, 7, 8, 11, 20, 27]. Therefore, from the prior argument it may be accomplished that biochemical substances present in different plants are environment friendly and economical to use against plant pathogens [6, 36, 19].

5. Conclusion

Late blight of potato is an economically important disease in Punjab, Pakistan caused by *Phytophthora infestans*. The disease was well controlled by plant extracts under laboratory conditions. These need to be further evaluated the eco-friendly approaches in field conditions so as to make minimum use of chemicals.

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7. Conflict of interest

"The authors declare that there is no conflict of interest to publish the article"

8. References

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