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DISEASE MANAGEMENT OF LATE BLIGHT POTATO WITH PLANT EXTRACTS

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ABSTRACT
Plant extracts viz. *Salix sp., Achyranthusaspera, Solanumnigrum, Partheniumhysterophorus, Daturastramonium, Melilotusalbus, Convolvulus arvensis and Lantana camara*astuber treatment and foliar spray significantly increased seed germination from 71 – 86 % and plant height with the highest is noted in case of *Lantana camara*treated plant showing 86.34% and 20.2 cm against 71.0 % and 10.4 cm respectively in case of control. Biochemical analysis of treated plant showed that there was increase content of soluble protein and total phenol content.The maximum soluble protein content was found in *Lantana camera* treated plant showing 32.62 mg/g of fresh leaves against 21.57 mg/g of fresh leaves in case of control. Similarly, high content of phenols was also recorded in *Partheniumhysterophorus* treated plant representing 2.28 mg/g of fresh leaves against 1.52 in control at 15 days of inoculation. The disease severity showed negative correlation with soluble protein (r=-0.5486) and total phenol (r=-0.3225) at 15 days of pathogenic inoculation. Thus the plant extracts have ability not only to increase seed tuber germination and plant growth promotion but also synthesized defense molecules (Protein & Phenol) in plant resulted decline disease severity from 96.12 – 8.93 per cent.

INTRODUCTION

Potatois the world's fourth-largest food crop, following maize, wheat and rice and considered as "King of vegetables".Potato is rich source of carbohydrates, vitamins and minerals and is used as staple food in many countries, especially in England. The worldwide production of potato in 2010 was about 324 million tonnes.(F.A.O 2011), but in India, production was 453.44 lakh tonnes from 1922.2 ha. of land which is 21.6 percent of total area under vegetables. Uttar Pradeshranked first in potato production in India but at per concerned on productivity, the state is far behind those other countries like Europe and America. The main reasons of low productivity are diseases like, 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 *(Phytophthorainfestans*) (Mont.) deBary is most destructive disease that had led to most un-famous catastrophe in Ireland (England) during 1840-1845.The management of the disease can be done through host resistance, cultural adjustments, biological and use of fungicides (Sahu, *et al*., 2013, Kumar and Srivastava 2013, Prasad and Naik, 2003). But, there is no doubt that use of fungicides is the best strategy for management of the disease.But indiscriminate use of synthetic pesticides, plant pathogens are gaining resistance against them and also creating environmental and toxicological problems to our ecosystem. In this context, the popularity of botanical pesticides once again increasing and some plant products are being used for sustainable disease management were reported by several workers (Enyiukwu *et al*., 2014,Gurjar *et al.,* 2012, Arzoo *et al.,* 2012. The presence of antifungal compounds in higher plants has long been recognized as an important factor for disease control (Mahadevan, 1982). The pesticidal compounds of plant origin are most effective and have little or no side effects in human beings in comparison to synthetic compounds (Kumar *et al.,* 1995). Therefore, the study was undertaken on disease management of late blight of potato with plant extractsin the present investigation

MATERIALS AND METHODS

Collection of plant materials

The commonly available plants like *Salix sp., Achyrant husaspera, Solanumnigrum, Parthenium hysterophorus, Daturastramonium, Melilotusalba, Convolvulus arvensis, Lantana camara, and Achyranthusaspera* were collected from Students Research Farm Chandra Shekhar Azad University of Agriculture and Technology, Kanpur. The extracts of these plants were used to evaluate antifungal activity.

Preparation of plant extracts

The fresh and mature leaves of the common plants viz. *Salix sp., Achyranthusaspera, Solanumnigrum, Partheniumhy sterophorus, Daturafastusa, Melilotisalba, Lantana camara, and Achyranthusaspera,* were selected for preparation of plant extracts. The collected leaves were thoroughly washed under running clean tap water to remove dust and other foreign matter from the leaf surface. The extract was obtained from individual plant material as described byKahkashanArzoo, (2010). The extract was then diluted by mixing with water at

10 percent concentration.

Seed tuber treatment with extracts

Truly labeled potato seed tubers of variety 'KufriSindhuri' were collected from Vegetable Research Farm, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur to conduct the experiment. Seed tubers were placed in each jar containing 10% solution of each extract for five hours. It was then removed from the jar and shade dry for half an hour and used for sowing in pots.

Effect of plant extracts on germination and growth parameters of potato

The experiment was conducted at the Glasshouse complex, Department of Plant Pathology, C.S.A. University of Agriculture and Technology, Kanpur. The seed tubers of potato variety 'Kufri Sindhuri' were treated with plant extracts separately and sown in 30cm earthen pots, which were previously filled with a mixture of sterilized sandy loam and farm yard manure in the ratio of 2:1. In each pot 2 seed tubers were sown and wateredas per need basis. Three replications per treatment and three pots were sown with untreated seed tubers served as control. Observations pertaining to effect of different treatments on germination and growth of plants were recorded. The observation on germination of tuber was taken at every 24 hours upto 10 days. Germination percentage was calculated by use of following formula:-

Germination % = Number of germinated seed tubers / Number of total seeds X100

On the other hand,growth of plants was recorded at every 24 hrsinterval up to 30 days age of plants.

Effect of plant extracts on tuber yield

To explore the possible effect of the plant extracts on tuber yield was observed and data were taken on the weight of the total tubers per treatment and number of large, medium and small tubers.

Effect of plant extracts on disease severity

Spraying of plant extracts

In order to determine the efficacy of plant extracts against disease development, the plants were sprayed with different plant extracts solutions separately after 48 hours of pathogenic inoculation. For preparation of plant extracts solution, 5gm of each plant were taken and crushed in mortar and pestle along with 25 ml of distilled water. It was later filtered with muslin cloth and pure extracts were prepared. At the time of spraying, the extracts were diluted in 225 ml of distilled water to make final solution of 250 ml.

Measurement of disease severity

The disease severity was measured after 5 days, 10 days and 15 days of pathogen inoculation. Disease severity was recorded using a score chart consisting of 0-9 scale as described by Malcolimson (1976). 20 leaves were randomly selected from the pot for measurement of disease severity. The leaves with 1-9% infection received 1, 10% infection received 2, 11-25% infection received 3, 26-40% infection received 4, 41-60% infection received 5, 61-70% infection received 6, 71-80% infection received 7, 81-90% infection received 8, 91-100% infection received 9 (Malcolimson, 1976). The disease severity of individual plants was calculated by following formula:-

$$\text{Diseaseseverity(PDI)} = \frac{\text{Class rating x class frequently}}{\text{Total no of leaves x maximum class rating}} \text{x100}$$

Biochemical changes in potato due to effect of plant extracts and pathogenesis

The mature and fresh potato leaves were collected from different treatments and the changes in the content of soluble protein and total phenol in leaves were estimated at 5, 10, and 15 days after inoculation of the pathogen.

Estimation of soluble protein

The method developed by Lowry *et al*. (1951) was used with slight modification to determine the total soluble protein content.Potato leaves from different treatments were harvested, washed with distilled water several times and blotter dried before protein extraction. A quantity of 1.0 gm of each sample cut into small pieces and grinded in pre-chilled pistil and mortar using 15 leaves in extraction buffer. The suspension was centrifuged at 10,000 rpm for 30 minutes at 4ºC. The supernatant was collected. A quantity of 7.5 ml of the supernatant was transferred in a tube and mixed with 2.5 ml of sample buffer and used for protein estimation. The working standard solution was pipette out and 0.2, 0.6 and 1.0 mL of the solution was put into series of test tubes. A quantity of 0.2 ml, 0.6 ml and 1.0 of the sample extract was also pipette out and kept into other test tubes separately. Then the volume in all tubes were made upto 1 ml with water. A tube with 1 ml of water served as a blank. Later on, 5 ml of solution C was added in each test tube and incubated at room temperature for 10 min. Thereafter, 0.5 ml of FCR was mixed well immediately and incubated at room temperature for 30 min in dark place. The absorbance at 660 nm against the blank was read and standard graph was drown to calculate the amount of soluble protein in sample and represented as mg/ g of fresh sample.

Estimation of total phenol

The accumulation of phenols in potato plants after treatment with different plant extracts followed by inoculation of pathogen was estimated following procedure developed by Bray and Thrope (1954) with slight modification. For estimations, 1.0 gm of leaf sample of potato was ground in a pestle and mortar in 10 times volume of 80% ethanol. It was then centrifuged to homogenate the suspension at 10,000 rpm for 30 minutes at room temperature. Supernatant was separated and re-extracted for 5 times with required volume of 80% ethanol, centrifuged and the supernatant were pooled. It was then evaporated to dryness and residues were dissolved in 5 ml of distilled water. Different aliquots (0.2, 0.6 and 1.0 ml) were pipette out into test tubes and the volume in each tube was made to 3 ml with water. Subsequently 0.5 ml of FCR was added and after three minutes, 2 ml of 20% Na_2CO_3 solution in each tube was thoroughly mixed. Then absorbance at 650 nm against blank was measured using Ultra Violet Visible (UV-VIS) spectrophotometer and the standard curve using different concentration of phenols was prepared. From the standard curve the concentration of phenols in the test sample was determined and expressed as mg phenols per gm of sample materials.

Correlation coefficient and regression equation

The biochemical analysis of potato leaves at different growth stages and disease severity of the corresponding growthstages showed that reduced disease severity was associated with increased soluble protein and total phenol content. However, to determine the level of association correlation coefficients (r) between soluble protein and disease incidence as well as between total phenol and disease severity were calculated by standard statistical calculation. Simple regression equations (Y = a + bx) were also developed for both the variables (protein and phenol) separately to understand their relation with disease severity as described by Biswas *et al*. (2010).

RESULTS AND DISCUSSION

Germination and growth parameters of potato plants

Germination percentage : The result in Table 1 indicated that the highest germination percentage is noted in *Lantana camara* treatment (86.34%) followed by *Solanumnigrum* (83.34%), *Convolvulus arvensis* (80.00%). From the table, it is also cleared that all the treatments showed a significant increase in germination percentage over control.Arzoo*et al*. *(*2013) reported that significant increase in seed germination of tomato with the use of different plant extracts. NajatMaraiki(2013) also reported that tomato seed germination was enhanced by 67% and 40% when seeds were the treated with extract of *Artemisia absinthium*and *Ocimumbasilicum*, respectively. Roy *et al*. (2012) also observed significant increase in seed germination of okra when treated with bohera (*Terminalia belirica*) extract.

Plant height

The observations on plant height were taken at 7days, 14days, 21 days and 30 days after sowing. The data presented in Table 1 shows that the plant height of potato was maximum in *Lantana* treated seed tubers which is 20.2 cm against 10.4 cm in case of control. The seed tuber treated with *Solanum* extracts registered second highest in case of plant height with the value of 17.5 cm. Among the treatments minimum plant height was recorded in case of *Melilotusalbus* extract treated plants with the value of 7.0cm at 30 days age of plant which is much below than control (10.4cm). It is also found that the treatment T_6 (*Melilotusalbus)* completely changed the morphology of plant. Roy *et al* (2012) also reported enhanced the growth of shoot length of swamp cabbage (*Impoeaaquatica*) and okra (*Hibiscus esculentus*) by aquous extract of *Terminalia belirica* whereas the aqueous extract of horitoki significantly reduced and delayed germination, growth of shoot length of swamp cabbage seeds compared with control.

Tuber size and yield

The effect of seed treatment and foliar spray with plant extracts on tuber size and yield was studied after harvesting. Tubers were graded as large (more than 50 gm), medium (25 gm – 49.5 gm) and small (less than 25 gm). Highest yield (386.08gm) was recorded from *Lantana camara* treatment followed by *Pathenium* (352.92gm), *Convolvulus* (315gm) and *Solanum* (307.28gm) (Table 2). Lowest yield was recorded from the treatment of *Melilotusalbus* with the value of 26.50gm only, which is below even control. From the table, it is cleared that large size (> 50 gm) was obtained only from

Table 1: Effect of plant extracts on germination and growth parameters of potato at different days of interval (Glass house condition)

Treatment	Plant height at different days after sowing				Germination	%increase
	7days	14days	21days	30days	%	over control
Salix sp.	2.13	3.6	5.2	12.3	77.00	17.84
Achyranthusaspera	1.86	2.53	3.9	10.2	70.34	7.65
Solanumnigrum	1.63	3.43	7.4	17.5	83.34	27.54
Partheniumhysterophorus	1.5	1.86	3.5	10.2	73.34	12.54
Daturastramonium	2.5	4.96	6.5	11.8	76.64	12.24
Melilotusalbus	0.96	1.3	2.2	7.0	71.67	17.29
Convolvulus arvensis	1.86	3.03	4.6	12.9	80.00	9.68
Lantana camara	7.00	8.46	10.9	20.2	86.34	22.43
Control	2.23	4.7	6.3	10.4	65.34	
S.E.	0.658	0.7872	0.9486	1.4919	2.4449	
C.D. (P=0.05)	1.3526	1.6182	1.9503	3.0667	5.0256	

Table 2: Effect of plant extracts on tuber size and yield (Glass house conditions).

Treatment	Large(>50 gm)		Medium(25-49.5gm)		Small (<25gm)		Yield
Salix sp.	0.0	0.0	0.0	0.0	43	272.38	272.38
Achyranthusaspera	0.0	0.0	1	25.86	35	299.14	325
Solanumnigrum	1	56.66	2	55.78	19	194.14	372.8
Partheniumhysterophorus	0.0	0.0	4	130.30	17	222.62	352.92
Daturastramonium	1	54.14	1	26.72	15	146.64	227.5
Melilotusalbus	0.0	0.0	0.0	0.0	6	26.50	26.50
Convolvulus arvensis	0.0	0.0	4	121.34	23	194.36	315.00
Lantana camara	1	62.12	3	88.32	23	235.64	386.08
Control	0.0	0.0	2	53.82	15	123.24	177.06
S.E.	0.2720	0.8165	0.2720	3.0429	1.547	5.5468	
C.D. (P=0.05)	0.5719	1.7158	0.5719	6.3941	2.4264	11.655	

Table 3: Effect of plant extracts on total soluble protein content in potato leaves after 5days,10days and 15 days of pathogen inoculation.

Treatment	Total soluble protein mg/g fresh leaves			% increase over control
	5 days	10 days	15 days	
Salix sp.	25.85	27.63	26.45	22.62
Achyranthusaspera	25.37	26.58	25.57	18.54
Solanumnigrum	29.49	30.67	29.16	35.18
Partheniumhysterophorus	30.39	32.75	32.25	49.51
Daturastramonium	23.40	25.40	23.38	8.39
Melilotusalbus	24.14	26.58	24.63	14.18
Convolvulus arvensis	30.19	32.11	31.55	46.26
Lantana camara	30.51	33.24	32.62	51.22
Control	20.94	22.49	21.57	
S.E.	0.8165	1.0887	0.9815	
C.D. (P=0.05)	1.7160	2.2876	2.0621	

Table 4: Effect of plant extracts on total phenol content of potato leaves after 5 days, 10 days and 15 days of pathogen inoculation

Treatment	Total phenol mg/g fresh leaves			% increase over control
	5 days	10 days	15 days	
Salix sp.	1.606	1.78	1.75	15.13
Achyranthusaspera	1.58	1.74	1.68	10.52
Solanumnigrum	1.78	1.92	1.91	25.65
Partheniumhysterophorus	2.12	2.37	2.28	50.00
Daturastramonium	1.52	1.62	1.54	1.31
Melilotusalbus	1.58	1.74	1.68	10.52
Convolvulus arvensis	2.03	2.27	2.22	46.05
Lantana camara	2.09	2.31	2.24	47.36
Control	1.48	1.59	1.52	
S.E.	0.1653	0.2129	0.2033	
C.D. (P= 0.05)	0.3466	0.4461	0.2033	

Table 5: Effect of plant extracts on disease severity of late blight of potato

Treatment	Disease severity (%)		
	5 days	10 days	15 days
Salix sp.	9.0	13.09	16.25
Achyranhusaspera	6.20	10.31	13.56
Solanumnigrum	9.32	13.32	16.92
Partheniumhysterophorus	5.28	9.48	12.53
Daturastramonium	14.21	19.14	22.23
Melilotusalbus	1.32	4.32	7.84
Convolvulus arvensis	9.04	14.14	16.41
Lantana camara	1.75	5.62	8.93
Control	62.11	80.92	96.12
S.E.	1.9876	1.74528	1.6330
C.D. (P= 0.05)	4.1766	3.6621	3.4315

*Solanumnigrum*and*Daturastramonium*treated plants whereas, maximum medium (25- 49.5 gm) of potato tuber were obtained from *Partheniumhy sterophorus and Convolvulus arvensis*treated plants. It is also cleared from the table showed that total minimum number of potato was obtained from *Melilotusalbus*treated plants which is only 6 against 43 in case of *Salix sp.* treated plants which is the highest in number. Higher numbers of tubers in *Salix sp.* treated plants might be due to growth promoting effect of root. The finding of the present investigation was supported by FaragHanaa *et al.* (2011,). Majeed *et al.* (2011) found better yield of potato with application of plant extracts of *Podophyllumhexandrum*, *Withaniasomnifera* and *Xanthium strumarium*. Culver *et al.,* (2012) also reported increased fruit weight and number of stem branches of tomato plants increased when plants were treated with *Moringaoleifera* leaf extract.

Soluble proteins

The data presented in Table-3 showed that the soluble protein contents in *Lantana camara* treated leaves were 30.51 mg/gm, 33.24 mg/gm and 32.62 mg/gm of fresh leaves at 5, 10 and 15 days of pathogen inoculation which is the highest among the treatments. In case of control, the soluble protein content was 20.94mg/gm, 22.49mg/gm and 21.57 mg/gm at 5, 10 and 15 days after pathogen inoculation. After 15 days of pathogen inoculation *Lantana camara* treated leaves showed 32.62mg/g of soluble proteins content, which was 51.22% higher over control. Other treatments like *Partheniumhy sterophorus* (32.25 mg/g), *Convolvulus arvensis* (31.55 mg/g), *Solanumnigrum* (29.16 mg/g) and *Salix sp.*(26.45 mg/g) increased the protein content by 49.51%, 46.26%, 35.18% and 22.62%, respectively over control. From the table it is also cleared that all treatments increased protein content to a maximum at 10th day of pathogen inoculation, thereafter it decrease gradually from 10 to 15 days. The decrease protein content indiseased plants than healthy may be due to utilization of some protein by the pathogen.The present result matches with the result obtained by Singh and Prithviraj (1977) that neemazol, a product of neem increased protein pea leaves while to *Erysiphepisi*.

Arzoo *et al.* (2013) also reported increased levels of total soluble protein in plant extract treated tomato seedlings.

Table 6: Correlation of disease severity with total soluble protein and total phenol content of potato leaves

Biochemical Parameters	Days after pathogen inoculation	Correlation coefficient (r) with disease severity	Regression equation
Total soluble protein	5 days	-0.5224	Y = 31.2234-0.2142 X
	10 days	-0.5842	Y = 30.7855-0.1361 X
	15 days	-0.5486	Y = 30.6707-0.1185 X
Total phenol	5 days	-0.5360	Y = 2.1431-0.0100 X
	10 days	-0.5656	Y = 2.3013-0.0098 X
	15 days	-0.3225	Y = 2.2223-0.0086 X

Chandrasekaran and Rajappan, (2001) found the alteration in protein and sugar content of soya bean plants as induced by plant extract, antagonists and chemicals. Biswas *et al*. (2003) also estimated elevated level of total soluble protein in wheat when treated with crude extracts of *Chaetomiumg lobosum* against *Bipolarissorokiniana*.

Total phenol

The results presented in Table 5 shows that all the treatment significantly increased the total phenol content as compared to control at 5, 10 and 15 days after pathogen inoculation. The maximum amount of phenol content was found in*Partheniumhy sterophorus* extracted treatment with a value of 2.12mg/g, 2.37mg/g and 2.28mg/g of leaves against 1.48mg/g, 1.59mg/g and 1.52mg/g, respectively in control at 5, 10 and 15 days. The percent increase in phenol content in *Partheniumhysterophorus* treated leaves were 50.00% higher than control at 15th day of pathogen inoculation. The others treatments, *Latanacamara*, *Convolvulus arvensis, and Melilotusalbus* were also showed increased content of total phenol content over control.Matern and Kneusal (1988) suggested that the first stage of defense mechanism involve a rapid accumulation of phenol at the infection site which restricts or slows the growth of the pathogen. Disease inhibitory effect of *Lantana camara* leaf extracts due to the presence of sesquiterpenes, mainly â- ß-caryophyllene, zingiberene, -humulene, arcurcumene, gemacrene-D and bisabolene as major leaf and flower essential oil constituents (Singh *et al*., 1991, 2002, Nagassoum *et al*., 1999, Khan *et al*., 2002, Andersson and Dobson 2003).*Daeyt et al*. (2000) reported induction of phenolic compounds in two cultivars of cucumber by treatment with extract of *Renoutriasachalinensis*.

Disease severity

The effect of tuber treatment and foliar spray with plant extracts on severity of disease revealed that there is a decline in late blight severity due to various treatments (Table-5). The susceptible variety Kufri Sindhuri of potato showed a 100% severity in case of *P. infestans*treated plants. The minimum late blight severity was recorded in *M.albus*treated plants which was 1.32% followed by *L.camara, P.hysterophorus*and *A.aspera*treated plants where disease severity were 1.75%, 5.28% and 6.20%, respectively. The decrease in disease severity might be the activity of plant extracts which stimulate to synthesis of some defense molecules in potato against *P. infestans*. Majeed *et al*. (2011) found that the efficacy of aqueous leaf extracts of three medicinal plants (*Podophyllum hexandrum, Withaniasomnifera and Xanthium strumarium)* against late blight of potato caused by *P.infestans*.Moushib *et al*. (2013) reported that sugar beet extract (SBE) induce defense responses in potato plants to combat *P.infestans*. Mharajan *et al*. (2010) also found the antifungal activity of ethanol extracts of five different plant materials viz. *Brassica nigra*, *Cinnamomumcamphora*, *Eupatorium adenophorum*, *L.camara*and *M. azedarach* against of *P. infestans* .According to, Varaprasad Bobbarala (2012) caumarins and saponins are the main antimicrobial active molecules in *M. albus*.

Correlation coefficient and regression equation

The leaves treated with plant extracts showed decreased disease incidence with increased level of soluble protein. A negative correlation (r) -0.5224, -0.5842 and -0.5486 was found between disease severity and soluble protein content (Table 6). Similarly, correlation between disease severity and total phenol content also showed negative correlation. The corresponding simple regression equation also showed the negative relation between total protein and disease severity as well as total phenol and disease severity.

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