

## Original Research Article

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## Induced Resistance in Potato against Early Blight Caused by *Alternaria solani* (Sorauer) Through Plant Extracts as Inducer

Virendra Kumar\*, S.K. Biswas, Kishan Lal, Devash Nagar and V. Tilak Chowdary

Department of Plant Pathology C.S.A.U.A. & T., Kanpur-208002, India

\*Corresponding author

### ABSTRACT

Pre-foliar spray with *Parthenium hysterophorus*, *Lantana camara*, *Physalis*, *Melilotus albus*, *Datura stramonium*, *Solanum nigrum*, *Achyranthus aspera*, *Salix* sp., *Thevetia peruviana*, *Durenta erecta* *Polyanthia longifolia* followed by challenge inoculation by *Alternaria solani* showed that among the treatment, *Lantana camara* treated plant representing minimum disease severity as 1.50, 5.33 and 8.12% at 2, 6 and 10 days of challenge inoculation. The potato plant treated with plant extracts as inducers sensitized to produce increased level of soluble protein and total phenol contents. The maximum increase of soluble protein content was found in *Lantana camara* treated potato leaves indicating 34.05, 36.65, 36.85 and 36.25 mg/g of fresh leaves against 18.33, 20.35 20.55 and 20.65 mg/g in case of control at 2, 4, 6 and 8 days of pathogen inoculation. Similarly, total phenol content was also found to be maximum in *Lantana camara* treated plant, representing 2.65 mg/g, 3.17 mg/g, 3.27 mg/g and 3.35 mg/g of fresh leaves at 2, 4, 6 and 8 days of pathogen inoculation. Correlation coefficient analysis revealed that there was negative correlation ( $r = -0.3074$ ,  $-0.2560$  and  $-0.2563$ ) between disease severity and soluble protein content at 2, 6 and 10 days of treatment. Similarly, total phenol content also showed negative correlation ( $r = -0.1591$ ,  $-0.5608$  and  $-0.5495$ ) with disease severity. It is therefore, suggested that, protection of potato plants against *A. solani* by Plant extracts as inducer might be due to stimulation of plants defense response.

#### Keywords

Induced resistance, *Alternaria solani*, Potato, *Lantana camara*, Plant extracts.

#### Article Info

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### Introduction

Early blight is caused by *Alternaria solani* (Sorauer) is the most destructive disease of potato in the tropical and subtropical regions with high temperature and alternating periods of dry weather and high humidity and/or irrigated potato soils, light-textured, sandy, low in organic matter (Gudmestad and Pasche, 2007). The disease can be very destructive if left uncontrolled, often resulting in complete defoliation of plants. In contrast to the name, it rarely develops early, but usually appears on mature foliage (Rowe *et al.*, Undated). Young and middle-aged plants have low susceptibility to infection being

disease influenced by the crop age. Young plants are relatively resistant, but the susceptibility increases gradually and continuously from the initiation of tuber formation so that mature plants are most susceptible to the disease (Campo *et al.*, 2001; Johnson and Teng, 1990; Rotem, 1981; Shtienberg *et al.*, 1996). Values in the literature for measured crop losses due to early blight vary enormously from 5 –78% (Waals *et al.*, 2004; Pasche *et al.*, 2004, 2005). The yield losses of up to 79% have been reported in the U.S., of which 20-40% is due to seedling losses (i.e., collar rot) in the

field. The disease can damage both potato foliage and tubers and can cause yield losses of 5-50%. The pathogen is perpetuated in infected leaf or stem tissues on or in the soil. This fungus is universally present in fields where these crops have been grown. It can also be carried on tomato seed and in potato tubers. Therefore, control of the pathogen is very difficult and is a challenging issue due to not only the endogenous growth of the pathogen, but also its ability to infect multiple hosts and survive for several years (Uppal *et al.*, 2008). Management strategies of the disease are mainly focused on the use of resistant hosts, chemical control and cultural practices, but are not always available or effective (Jahanshir Amini 2015, Thanassouloupoulos and Hooker, 1968). One of the potential methods of reduction of disease in plants against pathogens is the induction of plant resistance using biotics, abiotics, plant extracts and inducers (Kumar and Biswas, 2010, Kahkashan Arzoo *et al.*, 2012, Gozzo, 2003; Malolepsza, 2006).

Induced resistance is generally characterized by the increased synthesis of chemical compounds in a plant that is able to prevent the pathogen's growth and development. The compound may be *PR*-proteins, secondary metabolites in the form of alkaloid, phenol, flavonoids, glycoside, phytoalexin compounds and so on (Chairul, 2003). A resistant plant has a higher concentration of these chemical compounds (Agrios, 2005). Applying antagonistic fungus and bacteria in combination is one of the strategies to increase a plant's resistance through ISR (*Induced Systemic Resistance*) and there are not many researches on this strategy. Anis Rosyidah (2014) also reported that the influence of bio-control microbes like *T. viride*, *Streptomyces sp.* and *P. fluorescens* on the resistance of potato crop toward *Ralstonia solanacearum* disease and to get increased potato growth and yield (Anis Rosyidah, 2014). Keeping the

above points in view the present study was planned entitled "Induced resistance in potato against early blight caused by *Alternaria solani* (Sorauer) through plant extracts as inducer".

## **Materials and Methods**

The present investigation was undertaken during 2012 to 2014 at Department of Plant Pathology, C.S.A. University of Agriculture and Technology, Kanpur. The pathogen *A. solani* was isolated from diseased plant showing typical blight symptoms collected from Vegetable Research Farm, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India.

### **Isolation and purification of the pathogen**

A small piece of infected leaf from the border of a sporulating lesion along with some healthy green tissue was cut and dipped in a mercuric chloride solution (0.1%) for 30 seconds followed by rinsing in sterilized distilled water thrice and dried off with sterilized filter paper. The tissue pieces were placed at the center of a Petri plate which was previously filled with Potato Dextrose Agar medium. The plates were then incubated at  $18\pm 1^{\circ}\text{C}$ . The Petri plates were observed daily at every 24 hrs interval and noticed the presence of mycelium around the leaf bits. As soon as the mycelial growth is noticed around the bits, the pathogen was purified by hyphal tip culture method. The fungus was observed under a compound microscope and its identity was established on the basis of morphological and cultural characters.

### **Collection and preparation of plant extracts as inducers**

The plant extracts like *Parthenium hysterophorus*, *Lantana camara*, *Physalis*, *Melilotus albus*, *Datura stramonium*,

*Solanum nigrum*, *Achyranthus aspera*, *Salix* sp., *Thevetia peruviana*, *Durenta erecta* and *Polyanthia longifolia* were collected from Vegetable Research Farm, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur.

### **Preparation of pathogen inoculums**

The Petri plate containing 15 days old culture of the *A. solani* was taken and flooded with sterile water. The mycelia along with spores were scrapped off with the help of sterile forceps and collected in a beaker. The suspension was then sieved with the help of a strainer to remove media clods. The collected spore suspension was diluted with distilled water and required concentration of spore suspension was measured with the help of a Haemocytometer.

About 250 µl spore suspension was pipette into the counting chamber. The counting chamber of the Haemocytometer was covered with a cover slip. The Haemocytometer was further mounted over a compound microscope. Average number of spores per square was counted and the sporangial suspension was adjusted to  $4.5 \times 10^4$  sporangia ml<sup>-1</sup>.

### **Measurement of disease severity**

In order to ascertain the effect of inducing agents on disease development, an experiment was conducted in the wire house complex, Department of Plant Pathology with three replications for each treatment. Plants were sprayed with inducers separately at 48 hrs before foliar inoculation with pathogen. During the course of this experiment, two controls are kept; in one case, plants were sprayed with water (Check-1) and in second case, plants were inoculated using conidial suspension of *A. solani* @ ( $4.5 \times 10^4$  sporangia/ml) serve as (Check-2).

### **Artificial inoculation of pathogen**

After 48 hrs of spraying with inducers, plants were inoculated with spore suspension of pathogen. The concentration of spore was maintained at  $4.5 \times 10^4$  sporangia/ml. The conidial suspension was prepared from seven days old culture of the pathogen. The homogenized, suspension were inoculated on the foliage of each plant. The plants were then covered with polythene bags for 48hrs to provide suitable moisture and humidity for growth and development of the pathogen.

Observations for measuring the disease severity were taken after 2 days, 6 days and 10 days of pathogen inoculation. Disease severity was measured using a score chart consisting of 0-10 scale as described by D. K. Sahu, (2013). Ten leaves were randomly selected from the pot for measurement of disease severity.

The leaves with 1-10% infection received 1, 11-20% infection received 2, 21-30% infection received 3, 31-40% infection received 4, 41-50% infection received 5, 51-60% infection received 6, 61-70% infection received 7, 71-80% infection received 8, 81-90% infection received 9, 91-100% infection received 10. Per cent disease incidence (PDI) was calculated based on the following formula.

$$PDI = \frac{\text{Sum of all numerical Grade}}{\text{Total No. of leaves} \times \text{Maximum Grade}} \times 100$$

### **Biomolecules changes in potato due to effect of plant extracts as inducer during 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 2, 6, and 10 days after inoculation of the pathogen.

### **Estimation of soluble protein**

The method developed by Lowry *et al.*, (1951) was used with slight modification to estimate the total soluble protein content in the leaves of each treatment. The total soluble protein content was measured by double beam UV visible spectrophotometer at 660nm wave length. The content of soluble protein in leaves was express as mg/g of fresh leave.

Potato leaves from different treatments were harvested, washed with distilled water several times and blotter dried before protein extraction. A quality of 1.0 gm of each sample was cut into small pieces and grinded in pre-chilled pistil and mortar using 1:5 leaves: extraction buffer. The suspension was centrifuged at 10,000 rpm for 30 minutes at 4°C. 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 as 0.2, 0.4, 0.6, 0.8 and 1.0 ml and put into series of test tubes. A quantity of 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the sample extract was also pipette out and kept into other test tubes separately. Then volumes in all the tubes were made up to 1 ml with distilled 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 total phenols in potato plants after treatment with different plant

extracts as inducer, followed by inoculation of pathogen was estimated following procedure developed by Bray and Thrope (1954). In this method, the total phenol estimation was carried out with FCR, which was measured at 650 nm wave length calorimetrically. For estimations of phenol, 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 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, 0.4, 0.6, 0.8 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<sub>2</sub>CO<sub>3</sub> 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.

## **Results and Discussion**

### **Effect of plant extracts as inducer on severity of early blight of potato**

The effect of tuber treatment and foliar spray with plant extracts as inducers significantly reduced disease severity of early blight of potato as compared to control-1 and control-2 in glass house condition (Table 1). Among the treatment minimum disease severity with 1.50%, 5.33% and 8.12% were recorded where tuber treated and sprayed with *Lantana*

*camara* as inducers, followed by *Parthenium hysterophorus* as 5.50%, 9.55% and 12.0%, *Physalis* as 8.28%, 13.0% and 15.0% at 2, 6 and 10 days of pathogen inoculation, respectively. The *Melilotus albus* treated plants were showing 1.28%, 4.02% and 7.50% disease severity which are superior to control but inferior to *Lantana camara*, *Parthenium hysterophorus* and *Physalis* treated plant in respect to severity of disease at 2, 6 and 10 days of pathogen inoculation. From the table, it is also cleared, that all the inducers treated potato plants were showing comparatively low disease severity over control-1 and control-2. The decrease in disease severity might be due to activity of inducers which stimulate to synthesis of some defense related compounds in potato plant against *A. solani*.

The present finding was also supported by several workers as to application of biotics and abiotics inducers in induce resistance in many plants (Arzoo *et al.*, 2012; Kumar and Biswas, 2010, Girdhari *et. al.*, 2008). He *et al.*, (2002) found that reduced disease severity of *F. oxysporum* f.sp. *asparagi* in *Asparagus officinalis* due to pre inoculated with non-pathogenic strains of *F. oxysporum* as inducer.

Nashwa (2012) also found that plant extracts significantly reduced the early blight disease as well as increased the yield of tomato compared to the infected control under field conditions. Nashwa S.M.A. (2012).

### **Biomolecule changes in association with the effect of plant extracts as inducer during pathogenesis at different days**

Pre-inoculation with plant extracts change biomolecules like total soluble and protein total phenol content in plant due to effect of plant extracts as inducers were studied as pre inoculation method and changing bio-

molecules were estimate at 2, 4, 6, 8 and 10 days after inoculation. The results have given as following head:

### **Soluble protein**

The result presented in table 2 indicated that total soluble protein content in potato leaves due to application of Plant extracts as inducers ranges from 17.50 - 22.43mg/g of fresh leaves. The highest content of total soluble protein was recorded from *Lantana camara* treated potato leaves, indicating 34.05, 36.65, 36.85 and 36.25 mg/g of fresh leaves against 18.33, 20.35, 20.55 and 20.65 mg/g in case of control-1 and 20.30, 22.25, 22.35 and 21.00 mg/g of fresh leaves in case of control-2 at 2, 4, 6 and 8 days of pathogen inoculation. The plant extracts treated potato plant possess 43.03 and 42.06 per cent increased of total soluble protein over control-1 and over control-2 at 8 days of pathogen inoculation, respectively. The *Parthenium hysterophorus* treated potato plant showed 31.75, 33.45, 33.55 and 33.69mg/g of fresh leaves at 2, 4, 6 and 8 days of pathogen inoculation which is the second highest among the treatments, representing 38.70% over control-1 and 37.66% over control-2 at 8 days of pathogen inoculation. Among the treatment, the lowest quantity of soluble protein content was found in *Melilotus albus* treated potato leaves, showing 20.85, 22.30, 22.60 and 22.80 mg/g of fresh leave at 2, 4, 6 and 8 days of pathogen inoculation. From the table 2, it is also cleared that among the different days of interval, the maximum concentration of soluble protein was found at 8 days of pathogen inoculation in all the treatments, thereafter, it was declined gradually. The finding of the table showed that the application of inducing agent before application, the soluble protein content was increase in all the treatments. The highest 38.12% increased of soluble protein after application of inducing agent was recorded in

case of *Lantana camara* treated plant. The increased protein content in treated plants might be responsible for defense response in plant against *A. solani*. The result presented in table 3 shows that all the treatment significantly increased the total protein content as compared to control-1 and control-2 at 2, 4, 6 and 8 days of pathogen inoculation. Antoniew *et al.*, (1980) considered that pathogen related proteins (PR protein) are involved in plant defense response to pathogens. Boller (1985) was also opined that proteins are associated with defense in plants against fungi and bacteria. Metraux *et al.*, (1988) and Tuzun *et al.*,

(1989) also reported that proteins forms of chitinases and  $\beta$  -1, 3 glucanase may be involved in the defense of plants against fungi and bacteria by their action on the cell walls of invading pathogen. The productions of chitinase and  $\beta$ -1, 3 glucanase, which are pathogenesis related proteins (PR-proteins), have been studied most extensively. Biochemical change associated with induced resistance in crop plant against pathogens by non-conventional of chemical against have been reported by several workers (Adesh, 2008; Biswas *et al.*, 2003; Biswas *et al.*, 2012; Steiner and Schonbeack, 1995; Sindhan and Prashar, 1996).

**Table.1** Effect of plant extracts as inducer on disease severity of early blight of potato at Different days of interval

Name of inducer	Disease severity at different day's intervals after inoculation (%)		
	2 Days	6 Days	10 Days
<i>Parthenium hysterophorus</i>	5.50	9.55	12.0
<i>Lantana camara</i>	1.50	5.33	8.12
<i>Physalis</i>	8.28	13.0	15.00
<i>Melilotus albus</i>	1.28	4.02	7.50
<i>Datura stramonium</i>	13.55	19.10	22.43
<i>Solanum nigrum</i>	9.0	12.31	15.50
<i>Achyranthus aspera</i>	9.19	10.40	14.0
<i>Salix sp.</i>	9.2	13.10	17.25
<i>Thevetia peruviana</i>	8.35	11.24	15.13
<i>Durenta erecta</i>	8.38	12.23	16.15
<i>Polyanthia longifolia</i>	10.15	14.15	17.10
Control	36.35	42.50	55.25
C.D.P=(0.05)	33.12	46.12	57.60
S.E (m)	0.954	1.253	1.590
S.E (d)	0.326	0.429	0.544
C.V.	0.462	0.606	0.769

**Table.2** Effect of plant extracts as inducer on total soluble protein in potato leaves at different days of intervals after inoculation during pathogenesis (mg/g of fresh leaves)

Name of inducer	Soluble protein content at different days of interval (mg/g of fresh leaves)						Percent increased over, before application of inducers	Percent increased over control-1 (at 8 days)	Percent increased over control-2 (at 8 days)
	Before application of Inducers	2 Days	4 Days	6 Days	8 Days	10 Days			
<i>Parthenium hysterophorus</i>	21.20	31.75	33.45	33.55	33.69	32.17	37.07	38.70	37.66
<i>Lantana camara</i>	22.43	34.05	36.65	36.85	36.25	35.45	38.12	43.03	42.06
<i>Physalis</i>	21.10	31.10	32.65	32.26	32.55	31.32	35.17	36.55	35.48
<i>Melilotus albus</i>	16.68	20.85	22.30	22.60	22.80	21.11	26.84	9.42	7.89
<i>Datura stramonium</i>	18.65	20.90	22.65	22.85	22.95	21.30	18.73	10.02	8.49
<i>Solanum nigrum</i>	20.80	25.13	27.24	27.54	27.63	26.02	24.71	25.26	23.99
<i>Achyranthus aspera</i>	20.75	24.15	26.25	26.50	26.70	25.15	22.28	22.65	21.34
<i>Salix sp.</i>	20.70	23.99	25.33	25.56	25.75	24.33	19.61	19.80	18.44
<i>Thevetia peruviana</i>	18.68	22.85	24.30	24.60	24.80	23.11	24.67	16.73	15.32
<i>Durenta erecta</i>	20.85	26.65	28.10	28.41	28.63	27.42	27.17	27.87	26.65
<i>Polyanthia longifolia</i>	18.60	19.05	21.46	21.55	21.65	20.85	14.08	4.61	3.00
Control-1	17.56	18.33	20.35	20.55	20.65	19.10	14.96	-	-1.69
Control- 2	17.50	20.30	22.25	22.35	21.00	21.54	16.67	-1.66	-
<b>C.D.P=(0.05)</b>	<b>1.217</b>	<b>1.548</b>	<b>1.649</b>	<b>1.641</b>	<b>1.663</b>	<b>1.603</b>			
<b>S.E (m)</b>	<b>0.414</b>	<b>0.527</b>	<b>0.562</b>	<b>0.559</b>	<b>0.567</b>	<b>0.546</b>			
<b>S.E (d)</b>	<b>0.586</b>	<b>0.746</b>	<b>0.794</b>	<b>0.790</b>	<b>0.801</b>	<b>0.772</b>			
<b>C.V.</b>	<b>3.618</b>	<b>3.678</b>	<b>3.667</b>	<b>3.671</b>	<b>3.666</b>	<b>3.669</b>			

**Table.3** Effect of Plant extracts as inducer on total phenol in potato leaves at different days of intervals after inoculation during Pathogenesis (mg/g of fresh leaves)

Name of inducer	Total phenol content at different days of interval (mg/g of fresh leaves)						Percent increased over, before application of inducers	Percent increased over control-1 (at 8 days)	Percent increased over control-2 (at 8 days)
	Before application of Inducers	2 Days	4 Days	6 Days	8 Days	10 Days			
<i>Parthenium hysterophorus</i>	1.55	2.58	2.64	2.74	2.80	2.72	44.64	34.64	34.28
<i>Lantana camara</i>	1.60	2.65	3.17	3.27	3.35	3.29	52.23	45.37	45.07
<i>Physalis</i>	1.45	1.48	2.54	2.68	2.74	2.58	47.08	33.21	32.84
<i>Melilotus albus</i>	1.12	1.16	1.24	1.53	1.98	1.67	43.44	7.57	7.07
<i>Datura stramonium</i>	1.25	1.19	2.26	2.33	2.10	2.50	40.47	12.85	12.38
<i>Solanum nigrum</i>	1.40	1.35	2.42	2.50	2.63	2.53	46.76	30.41	30.03
<i>Achyranthus aspera</i>	1.36	1.26	2.36	2.40	2.43	2.49	44.03	24.69	24.27
<i>Salix sp.</i>	1.30	1.24	2.34	2.41	2.35	2.50	44.68	22.12	21.70
<i>Thevetia peruviana</i>	1.27	1.22	2.29	2.36	2.30	2.45	46.78	20.43	20.00
<i>Durenta erecta</i>	1.39	2.53	2.60	2.73	2.78	2.62	50.0	34.17	33.81
<i>Polyanthia longifolia</i>	1.34	1.20	2.27	2.38	2.42	2.36	44.62	24.38	23.96
Control-1	1.10	1.18	1.33	1.57	1.83	1.78	39.89	0.00	-0.54
Control- 2	1.11	1.19	1.34	1.58	1.84	1.73	39.67	-0.54	0.00
<b>C.D.P=(0.05)</b>	<b>0.083</b>	<b>0.103</b>	<b>0.139</b>	<b>0.143</b>	<b>0.149</b>	<b>0.150</b>			
<b>S.E (m)</b>	<b>0.028</b>	<b>0.035</b>	<b>0.047</b>	<b>0.049</b>	<b>0.051</b>	<b>0.051</b>			
<b>S.E (d)</b>	<b>0.040</b>	<b>0.050</b>	<b>0.067</b>	<b>0.069</b>	<b>0.072</b>	<b>0.072</b>			
<b>C.V.</b>	<b>3.626</b>	<b>3.847</b>	<b>3.744</b>	<b>3.715</b>	<b>3.683</b>	<b>3.664</b>			

**Table.4** 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	2 Days	-0.3074	$y = -0.2609x + 27.634$
	6 Days	-0.2560	$y = -0.1966x + 29.774$
	10 Days	-0.2563	$y = -0.1571x + 28.598$
Total phenol	2 Days	-0.1591	$y = -0.0221x + 1.818$
	6 Days	-0.5608	$y = -0.0296x + 2.8298$
	10 Days	-0.5495	$y = -0.0204x + 2.8307$

**Total phenol content**

The result presented in table 3 shows that all the treatments significantly increased the total phenol content as compared to control-1 and control-2 at 2, 4, 6 and 8 days of pathogen inoculation. The maximum total phenol content was found in *Lantana camara* treated potato leaves which were 2.65 mg/g, 3.17 mg/g, 3.27 mg/g and 3.35 mg/g of fresh leaves at 2, 4, 6 and 8 days of pathogen inoculation, respectively whereas, in case of control-1, the values are 1.18 mg/g, 1.33 mg/g, 1.57 and 1.83 mg/g of fresh leaf and for control-2, the value are 1.19 mg/g, 1.34 mg/g, 1.58 mg/g and 1.84 mg/g of fresh leaves. The *Lantana camara* treated potato leaves possess increased per cent of total soluble phenol as 45.37% over control-1 and 45.07% over control-2 at 8 days of pathogen inoculation. The second highest of total phenol content was found in *Parthenium hysterophorus* treated potato leaves which were 2.58 mg/g, 2.64 mg/g, 2.74 mg/g and 2.80 mg/g of fresh leaves at 2, 4, 6 and 8 days of pathogen inoculation. The per cent increased of total phenol content at 8 days of pathogen inoculation was of 34.64% over control-1 and 34.28% over control-2. The lowest quantity of soluble phenol was harvested in *Melilotus albus* treated potato leaves, indicating 1.16 mg/g, 1.24 mg/g, 1.53 and 1.98 mg/g of fresh leaf at 2, 4, 6 and 8 days. From the table 3, it is also showed that other treatments like *Physalis*, *Datura*

*stramonium*, *Solanum nigrum*, *Achyranthus aspera*, *Salix* sp., *Thevetia peruviana*, *Durenta erecta* and *Polyanthia longifolia* also increased total phenol content over control-1 and control-2. The total phenol content in potato leaves before and after application of inducing agents clearly indicated that inducing agents have ability to increased total phenol content, representing the value ranges from 17.56-22.43 mg/g of fresh leave as in before application and 20.65-36.25 mg/g of fresh leave as in after application at 8<sup>th</sup> days of pathogen inoculation. The highest per cent increased of total phenol before application of plant extracts inducers was found in *Lantana camara* treated potato leaves which were 52.23% at maximum 8 days. From the table, it is also cleared that the phenol content in all treated plants increased up to a certain period of time and thereafter, it was decreased gradually. The increased phenol content in treated plants might be due to defense response in plant against *A. solani*. Phenols are well known antifungal, antibacterial and antiviral compounds. The phytoalexins involved in disease resistance are phenolics in chemical constitution. Phenols are involved in disease resistance in many ways like hypersensitive cell death or lignifications of cell walls or increased content of phenol itself toxic also to pathogen (Nicholson and Hammerschimdt 1992). Arzoo *et al.*, (2012) reported that increase content of phenols is associated with defence response in tomato against Fusarium wilt induced by plant

extract. Girdhari *et al.*, (2008) also reported that increased total phenol content was found in rice leaves after treatment with biotic inducers.

Meena *et al.*, (2001) found that salicylic acid applied as pre-inoculation spray in groundnut plants challenge inoculated with *Cercosporidium personatum* resulted in three fold increases in phenol content on fourth day. Accumulation of phenolics at the site of infection is a general response of plants in many host- pathogen interactions and this accumulation is fostered by biotic and abiotic elicitors (Farkas and Kiraly, 1962). Retig and Chet (1974) also found a marked accumulation of total phenols when tomato plants were fed with catechol and resulted in suppression of symptom expression due to infection by *Fusarium oxysporum* f.sp. *lycopersici*. Therefore, increased content of phenol might be involved in the expression of disease resistance in plant. In the present findings also increased resistance is associated with increased content of soluble protein and total phenol content.

### **Correlation of disease severity with total soluble protein and total phenol content of potato leaves**

The results presented in table 4 revealed that the leaves treated with plant extracts as inducer decreases disease severity with increased level of soluble protein and total phenol content in potato leaves. The correlation regression equation showed that negative correlation (r) -0.3074, -0.2560, -0.2563 was found between total protein with disease severity at 2, 6, 10 days of pathogen inoculation, respectively. The corresponding simple regression equation also showed that increase level of soluble protein has negative role in increase disease development. Similarly, the correlation regression also equation showed that negative correlation (r) -

0.1591, -0.5608, -0.5495 between total phenol with disease severity at 2, 6, 10 days of pathogen inoculation, respectively.

The corresponding simple regression equation also showed that increase level of total phenol has negative role in increase disease development. Similar observation were also found in rice against brown leaf spot (Kumawat *et al.*, 2010), in tomato against *Fusarium* wilt (Kumar and Biswas, 2010, Kahkashan Arzoo *et al.*, 2012).

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