Journal of Scientific Research & Reports

16(1): 1-11, 2017; Article no.JSRR.36560
ISSN: 2320-0227

Induced Synthesis of Defense Enzymes during Induced Resistance against Early Blight of Potato Using Plant Extracts as Inducer

Virendra Kumar[1*], S. K. Biswas[1], V. Tilak Chowdary[1], Kishan Lal[1] and Prem Naresh[1]

[1]Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur- 208002, UP, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author VK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SKB and VTC managed the analyses of the study. Authors KL and PN managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JSRR/2017/36560
Editor(s):
(1) Tzasna Hernandez Delgado, Laboratory of Pharmacognosie, Biology Unit and Prototypes (UBIPRO). National Autonomous University of Mexico, Mexico.
Reviewers:
(1) Esraa Ashraf Ahmed ElHawary, Ain Shams University, Egypt.
(2) Aneta Popova, University of Food Technologies, Bulgaria.
Complete Peer review History: http://www.sciencedomain.org/review-history/21185

Original Research Article

Received 31st August 2017
Accepted 22nd September 2017
Published 1st October 2017

ABSTRACT

Aim: To find out the defense enzymes synthesis due to pre-application of plant extracts as inducers.
Study of Design: The experiment was conducted in glass house condition using three replications. The standard statistical analysis was done to find out the variance.
Place and Duration of Study: Department of Plant Pathology, CSA University of Agriculture & Technology, Kanpur Uttar Pradesh, India during 2012-2014.
Methodology: Standard methodology was followed to conduct the experiment.
Results: Among the 11 plants extracts viz. *Parthenium hysterophorus, Lantana camara, Physalis, Melilotus albus, Datura stromonium, Solanum nigrum, Achyranthus aspera, Salix* sp., *Thevetia peruviana*, *Duranta erecta, Polyalthia longifolia* as inducers, the maximum increased level of defense enzymes POD, PPO and PAL were found in *Lantana camara* treated potato leaves.

**Corresponding author: E-mail: virendra.csa11@gmail.com;*
E-mail: plantpathology9@gmail.com;

Conclusion: Pre treatment with plant extracts as inducer provided induced resistance in plant against *A. solani* resulting declined in disease severity and also increase the elevated level of defense related enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia lyase.

Keywords: Induced resistance; A. solani; plant extracts; potato; defense enzymes; disease severity.

ABBREVIATIONS

POD : Peroxidase
PPO : Polyphenol oxidase
PAL : Phenylalanine ammonia lyase

1. INTRODUCTION

Induced resistance is an innovative strategy to overcome production problems caused by plant diseases which is targeted due to stimulation of plant defense mechanisms that are inherent to plants [1]. It is a new area of extensive work aiming at developing new strategies for disease control meeting the requirements for safe application in greenhouse and fields conditions, namely: no direct toxicity to pathogens; no toxicity to plants and animals; no negative effects on plant growth, development and yield; broad spectrum of defense; low loading amount; long lasting protection; low economical cost for farmers; good profit for producer [2]. The plants possess inducible defense mechanisms that are activated upon contact with pathogenic or non-pathogenic micro-organisms, extracts of micro-organisms or chemicals, thus providing protection against a broad spectrum of pathogens. Most of the plants possess defense mechanisms against pathogen attack, which triggered by a stimulus prior to the pathogen attack, reduces the disease. The stimulus can increase the concentration of existing defense compounds that induce production a new defensive structures and chemicals [3]. The aqueous extracts of barley leaves induced oversized papillae formation in barley which in turn produces resistance against powdery mildew [4]. Pre-application of tomato seedling with non pathogenic strains of *Fusarium oxysporum* (Fo47), *Pseudomonas spp.* (Strain MF30), *Pseudomonas fluorescence* (Pf1) and spray of zoospores of *Phytophthora cryptogea* and conidial suspension of *Penicillium oxallicum* provided systemic induced resistance in plants [5-8]. Pre-foliar spray with plant extracts also sensitized to tomato plant to produced increased level of phenol, soluble protein and new proteins, involved in defense response in plant against Fusarium wilt [9]. [10] found that pre inoculation sprays of crude extract sensitized seedlings to produce elevated levels of protein, soluble protein and phenol contents. New proteins of different molecular weight (MW) that is, 110, 105, 38 and 32 KDa were measured by SDS-PAGE analysis. Keeping the above points in view the study was undertaken as “Induction of defense related enzymes during induced resistance against early blight of potato using plant extracts” as inducers in the present investigations.

2. MATERIALS AND METHODS

2.1 Isolation and Purification of *Alternaria solani*

The diseased plant showing typical early blight symptoms were collected from Vegetable Research Farm, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India for isolation of *Alternaria solani*.

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

2.2 Solution Preparation of Plant Extracts as Inducers

Extracts from leaves of eleven plants, namely *Parthenium hysterophorus, Lantana camara, Physalis, Melilotus albus, Datura stromonium,*

Solanum nigrum, Achyranthus aspera, Salix sp., *Thevetia peruviana*, *Duranta erecta, Polyalthia longifolia*, were collected from vicinity area of University campus. Ten grams of the fresh leaf material of each plant species were collected, washed with water and crushed in a mortar with pestle by adding sterile distilled water at the rate of 10 ml/g of plant tissue and the homogenates were centrifuged at 10000× *g* for 15 min at 4°C and the supernatant solutions were collected. The plant extract was further diluted to have 1% and 5% concentration (v/v) for present investigation.

2.3 Preparation of Pathogen Inoculums

The Petri plate containing 10 days old culture of the *A.solan i*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^{-1}.

2.4 Measurement of Disease Severity

The experiment was conducted at Wire house complex at Department of Plant Pathology during 2012-2014. Plants were sprayed with inducers separately at 45 days age. After 48 hrs of spraying with inducers, plants were inoculated with spore suspension of pathogen using hand atomizer. The plants were then covered with polythene bags for 48hrs to provide suitable moisture and humidity for growth and development of the 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.

Observations for measuring the disease severity were taken after 2 days, 6 days and 10 days of pathogen inoculation using a score chart consisting of 0-9 scale as described by [11]. Ten leaves were randomly selected from the each pot for measurement of disease severity. Fig. 1 depicts that 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.Per cent disease intensity (PDI) was calculated based on the following formula as described by [11].

$$\text{PDI} = \frac{sum\ of\ all\ numerical\ grades}{Total\ number\ of\ leaves\ counted \times maximum\ grade} \times 100$$

2.5 Induction of Defense Related Enzymes Due to Effect of Inducer during Pathogenesis

2.5.1 Estimation of peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PPL)

The activity of defense enzymes like peroxidase, PPO, PAL in potato plants after treatment with different plant extracts as inducers followed by inoculation of pathogen was assessed. The mature and fresh potato leaves were collected from different treatments and the changes in the activity of enzymes viz. peroxidase (POD), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) enzymes at 2, 4, 6, 8 and 10 days after pathogen inoculation was measured separately according to the procedure developed by [12,13].

2.6 Correlation Co-efficient and Regression Equation

The biomolecules analysis of potato leaves at different days of inoculation and disease severity of the corresponding days were also estimated. However, to determine the level of association, correlation coefficients (r) between defense enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia lyase and disease severity were calculated by standard statistical calculation. Simple regression equations (Y= a + bx) were also developed for all the variables (peroxidase, polyphenol oxidase and phenylalanine ammonia lyase) separately to understand their relation with disease severity.

3. RESULTS AND DISCUSSION

3.1 Effect of Plant Extracts as Inducer on Disease Severity and Induction of Defense Enzymes during Pathogenesis at Different Days

Induction of defense related enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia lyase due to effect of plant extracts as inducers were studied as pre inoculation method and changing defense enzymes were estimated at 2, 4, 6, 8 and 10 days after inoculation. The results have given as following head:

3.1.1 Effect of inducer on severity of early blight of potato

The data presented in the Table 1 showed that 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.

Among the different treatments, minimum disease severity with 1.50%, 5.33% and 8.12% were recorded in *Lantana camara* treated plant, followed by *Parthenium hysterophorus* as5.50%, 9.55% and 12.0%, *Physalis* as 8.28%, 13.0% and 15.0% at 2, 6 and 10 days of pathogen inoculation, respectively. It is also found that the *Melilotus albus* treated plant fully changed morphological characters of the plants and were also showing 1.28%, 4.02% and 7.50% disease severity which are superior to control but inferior to *Datura stromonium, Solanum nigrum, Achyranthus aspera, Salix* sp., *Thevetia peruviana*, *Duranta erecta, Polyalthia longifolia* treated plant. 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 the activity of inducers which stimulate the synthesis of some defense related compounds in potato plant against *A. solani.* [14] found that reduced disease severity of *F. oxysporum* f. sp. *asparagi* in *Asparagus officinalis* inoculated with non pathogenic strains of *F. oxysporum.*Various types of biological agents, virulent or avirulent strains of pathogens, plant extracts, crude extracts of bioagents and chemicals which are not considered as fungicides are used as inducers for induction of resistance in various crops [15-18]. The physical and biochemical changes associated with induction of resistance was reported by several workers [19-21]. There are also several reports indicating resistance due to activity of plant extracts as well as components of plant extracts, [22,23].

3.1.2 Induction of peroxidase due to effect of plant extracts as inducers

The result presented in Table 1 showed that there is a significant changed in activity of peroxidase at 2, 4, 6, 8 and 10 days after pathogen inoculation. The activity of peroxidase in potato leaves before application of inducers were ranges from 1.42-1.68 minute/g of fresh leaves whereas, in case post application method, the values were 1.78-2.37 min/g of fresh leaf, indicated that the inducing agent enhanced the peroxidase activity in potato leaf. The highest percent increased activity of peroxidase before and after application of inducers is ranges from 25.35-46.29. Among the treatments, the maximum activity was recorded in *Lantana camara* treated potato leaves with 2.48, 2.60, 2.61 and 2.66 min/g of fresh leaves at2, 4, 6 and 8 days, pathogen inoculation, respectively which is 33.83% increased over control-1 and 32.70% over control -2. The second highest activity of peroxidase was found in *Parthenium hysterophorus* treated plant indicating 2.57, 2.63, 2.64 and 2.69 minute/g of fresh leaves at 2, 4, 6 and 8 days of pathogen inoculation which is 34.57% increased over control-1 and 33.45% over control-2 at 8 days of pathogen inoculation. Among the treatment the lowest quantity of peroxidase was observed in *Polyalthia longiafolia* treated potato leaves which were 1.68, 1.73, 1.77 and 1.89minute/g at 2, 4, 6 and 8 days, respectively. It is also cleared from the Table 2 that all the treatments increased the activity of peroxidase at maximum of 8 days of pathogen inoculation and there after,it is was decreased gradually. The enzyme activity was increased significantly in diseased leaves as compared to healthy leaves in all treated plant. The increased activity of peroxidase in treated plants might be responsible for defense response in plant against *A. solani.* [24] reported that plant defense enzymes such as polyphenol oxidase (PPO), peroxidase (PO) and other enzymes such as chitinase, β-1, 3-glucanase and phenylalanine ammonia lyase (PAL) are involved in defense reactions against plant pathogens in many crops. [25] observed that the increase activities of polyphenol oxidase (PPO) and peroxidase (POD) in hot pepper (*Capsicum annuum* L.) pericarp which were evaluated using spectrophotometric method.

3.1.3 Effect of plant extracts as inducers on the activity of polyphenol oxidase

Polyphenol oxidase is another important defense enzyme, synthesized in plant due to effect of inducers or other environmental effect. The data presented in Table 2 showed that all the treatments significantly increased the activity of polyphenol oxidase as compared to control-1 and control-2 at 2, 4, 6 and 8 days of pathogen inoculation. As per concerned on application strategy of inducers, it is indicated that the range of polyphenol oxidase in potato leaves before application was 0.45-0.51 min/g of fresh leaves and after application the ranges is 1.28-1.75 min/g of fresh leave, indicating that plant extracts enhance the polyphenol oxidase activity in treated plant that provided defense response in plant.

Among the treatments, the maximum activity of polyphenol oxidase is found in *Lantana camara* treated potato leaves, representing the value of is 0.98, 1.85, 1.88 and 1.95 minute/g of fresh leaves against control-1 as 0.52, 1.22, 1.27, and 1.29 min/g and control-2, as 0.50, 1.19, 1.25, and 1.31 min/g of fresh leaves at 2, 4, 6 and 8 days of pathogen inoculation, respectively. The *Lantana camara* treated potato leaves posses increased per cent activity of polyphenol oxidase as 26.86 over control-1 and 25.71 over control-2 at 8 days of pathogen inoculation. The rest of the treatments were also showing increase content of polyphenol oxidase over control-1 and control-2. From the table, it is also cleared that among the different days of interval, maximum PPO activity was noticed at 8 days of pathogen inoculation, thereafter, it was decreased. The increased activity of polyphenol oxidase in treated plants might be responsible for defense response in plant against *A. solani.* [25]

3.1.4 Effect of plant extracts as inducer on the activity of Phenylalanine ammonia lyase (PAL)

The result presented in Table 3 showed that there is a significant changed in the activity of phenylalanine ammonia lyase before and after application of plant extracts. The ranges of activity of phenylalanine ammonia lyase found in potato leaves before and after application of plant extracts asinducers were 0.42-0.65 and 0.67 - 1.15 mg t-cinnamic acid produced/h/g of fresh leaves, indicating that the plant extracts have ability to increase PAL activity in potato leaves. Among the treatments, the maximum activity of phenyl alanine ammonia lyase was recorded in *Lantana camara* treated potato leaves with 0.85, 0.99, 1.06 and 1.19 mg t-cinnamic acid/h/g of fresh leaves at 2, 4, 6 and 8 days of pathogen inoculation, respectively, which is maximum 47.89% increased over control-1 and 47.05% over control-2. The second highest activity of phenylalanine ammonia lyase was found in *Parthenium hysterophorus* treated plant as 0.82, 0.88, 0.93 and 1.15 mg t-cinnamic acid produced/h/g of fresh leaves which is 46.08% over control-1 and 45.21% control-2 at 8 days of pathogen inoculation. From the Table 3, it is also indicated that all the treatments increased the activity of enzyme phenylalanine ammonia lyase at maximum at 8 days of pathogen inoculation, thereafter, it was declined gradually. The increased activity of enzyme in treated plants might be responsible for defense response in plant against *A. solani.* The increased in phenylalanine ammonia lyase activity in inoculated resistant and susceptible host were also reported by [26,27] also reported that the enzyme activities of PAL increased to almost 1.5-2 fold when seeds were treated previously with Aqueous leaf extract (ALE) of *O. sanctum* and increased to almost 2-3 fold when treated with Ethanolic leaf extract (ELE) of *O. sanctum.* According to them the highest enzyme activity appeared on day 4 and day 3 in case of ALE and ELE of *O. sanctum* respectively.

3.2 Correlation of Disease Severity with Defense Enzymes like Peroxidase, Polyphenol Oxidase and Phenylalanine Ammonia Lyase 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 accumulation of defense related enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in of potato leaves The correlation regression equation showed that negative correlation (r) -0.2972, -0.5004, and -0.2631 was found between defense related enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia lyase with disease severity at POD -0.5004, -0.2374, -0.2868, and PAL -0.2631, -1736, 0.1932, at 2days of pathogen inoculation. Similar observations as negative correlation (r) have also been found at 6 days and 10 days of pathogen inoculation. The corresponding simple regression equation also showed that increase level of defense related enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia lyase have negative

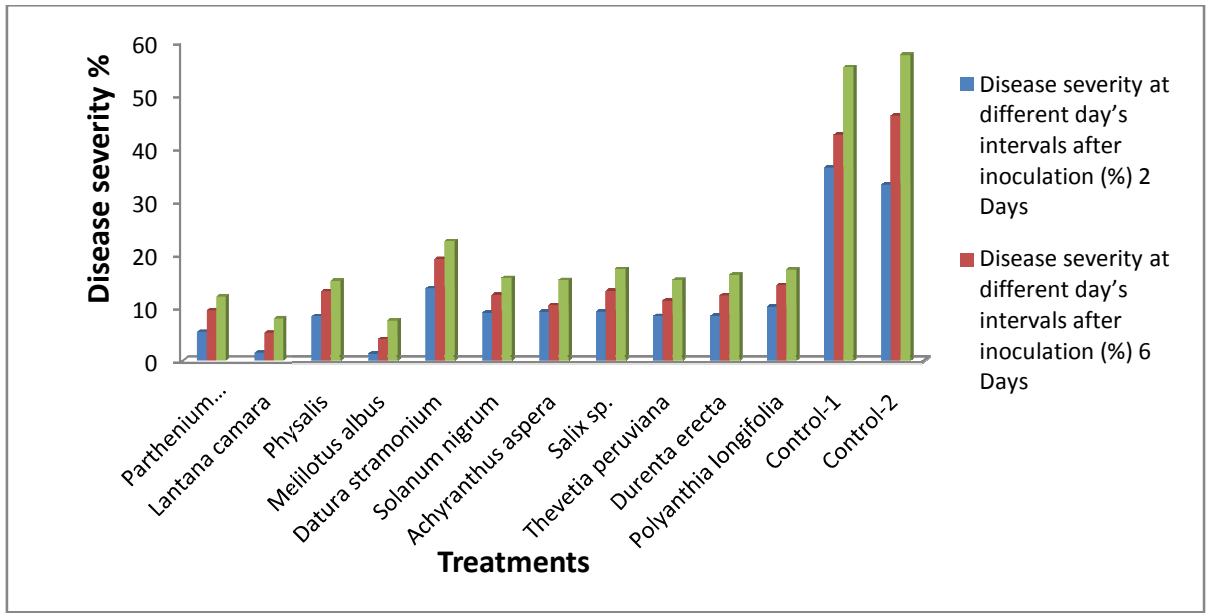

Fig. 1. Effect of plant extracts as inducer on disease severity of early blight of potato at different days

T_1= Parthenium hysterophorus, $T_{2=}$ Lantana camara T_3=Physalis T_4=Melilotus albus
T_5=Datura stramonium T_6= Solanum nigrum T_7= Achyranthus aspera T_8= Salix sp.
T_9= Thevetia peruviana T_{10}= Durenta erecta T_{11}=Polyanthia longifolia Control-1 &2

Table 1. Effect of plant extracts as inducer on activity of peroxidase in potato leaves at different days of intervals after pathogen inoculation

Name of inducer	Activity of peroxidase at different days of interval (minute/g of fresh leaves)						Per cent increased over, before application of inducers	Per cent increased over control-1 (at 8 days)	Per cent increased over control-2 (at 8 days)
	Before application of inducers	2 Days	4 Days	6 Days	8 Days	10 Days			
Parthenium hysterophorus	1.70	2.57	2.63	2.64	2.69	2.65	36.80	34.57	33.45
Lantana camara	1.68	2.48	2.60	2.61	2.66	2.62	36.84	33.83	32.70
Physalis	1.66	2.39	2.55	2.56	2.61	2.57	36.39	32.56	31.41
Melilotus albus	1.52	1.79	1.94	1.96	1.99	1.94	23.61	11.55	10.05
Datura stramonium	1.62	2.21	2.28	2.29	2.33	2.30	30.47	24.46	23.17
Solanum nigrum	1.60	2.14	2.24	2.27	2.29	2.28	30.13	23.14	21.83
Achyranthus aspera	1.58	1.87	2.16	2.19	2.24	2.21	29.46	21.42	20.08
Salix sp.	1.55	1.81	2.07	2.08	2.13	2.11	27.23	17.37	15.96
Thevetia peruviana	1.64	2.30	2.50	2.51	2.55	2.52	35.68	30.98	29.80
Durenta erecta	1.51	1.71	1.83	1.91	1.96	1.89	22.95	10.20	8.67
Polyalthia longifolia	1.46	1.68	1.73	1.77	1.89	1.81	22.75	6.87	5.29
Control-1	1.43	1.60	1.66	1.70	1.76	1.72	18.75	-	-1.70
Control- 2	1.42	1.62	1.69	1.73	1.79	1.82	20.67	1.67	-
C.D.P=(0.05)	**0.097**	**0.126**	**0.134**	**0.135**	**0.134**	**0.136**			
S.E (m)	**0.033**	**0.043**	**0.046**	**0.046**	**0.046**	**0.046**			
S.E (d)	**0.047**	**0.061**	**0.065**	**0.065**	**0.065**	**0.066**			
C.V.	**3.611**	**3.659**	**3.652**	**3.651**	**3.651**	**3.643**			

C.D.P =Critical difference point ; S.E(m) = Standard error mean ; S.E(d)= Standard error of difference; C.V = Coefficient of variation

Table 2. Effect of plant extracts as inducer on polyphenol oxidase activity in potato leaves at different days after pathogen inoculation

Name of inducer	Activity of polyphenol oxidase at different days of interval in (minute/g of fresh leaves)						Per cent increased over, before application of inducers	Per cent increased over control-1 (at 8 days)	Per cent increased over control-2 (at 8 days)
	Before application of inducers	2 Days	4 Days	6 Days	8 Days	10 Days			
Parthenium hysterophorus	0.53	0.96	1.67	1.69	1.76	1.72	69.88	26.70	25.56
Lantana camara	0.54	0.98	1.85	1.88	1.95	1.91	72.30	33.84	32.82
Physalis	0.52	0.94	1.64	1.66	1.67	1.64	68.86	22.75	21.55
Melilotus albus	0.46	0.75	1.31	1.38	1.43	1.40	67.83	9.79	8.39
Datura stramonium	0.50	0.90	1.60	1.62	1.63	1.61	69.32	20.85	19.63
Solanum nigrum	0.49	0.85	1.57	1.60	1.61	1.58	69.56	19.87	18.63
Achyranthus aspera	0.48	0.80	1.44	1.46	1.51	1.48	68.21	14.56	13.24
Salix sp.	0.47	0.78	1.34	1.41	1.46	1.43	67.80	11.64	10.27
Thevetia peruviana	0.51	0.92	1.62	1.64	1.65	1.63	69.09	21.81	20.60
Durenta erecta	0.45	0.66	1.23	1.28	1.35	1.32	66.66	4.45	2.96
Polyalthia longifolia	0.44	0.56	1.14	1.20	1.32	1.24	66.67	2.28	0.75
Control-1	0.45	0.52	1.22	1.27	1.29	1.28	65.11	-	-1.55
Control- 2	0.42	0.50	1.19	1.25	1.31	1.26	67.93	1.52	-
C.D.P=(0.05)	**0.030**	**0.050**	**0.087**	**0.092**	**0.093**	**0.094**			
S.E (m)	**0.010**	**0.017**	**0.030**	**0.031**	**0.032**	**0.032**			
S.E (d)	**0.014**	**0.024**	**0.042**	**0.044**	**0.045**	**0.045**			
C.V.	**3.613**	**3.683**	**3.769**	**3.644**	**3.645**	**3.633**			

C.D.P = Critical difference point; S.E(m) =Standard error mean ; S.E(d)= Standard error of difference; C.V = Coefficient of variation

Table 3. Effect of plant extracts as inducers on phenylalanine ammonia lyase activity in potato leaves at different days after pathogen inoculation

Name of inducer	Activity of enzyme *phenylalanine ammonia lyase* at different days of interval (minute/g of fresh leaves)						Per cent increased over, before application of inducers	Per cent increased over control-1 (at 8 days)	Per cent increased over control-2 (at 8 days)
	Before application of inducers	2 Days	4 Days	6 Days	8 Days	10 Days			
Parthenium hysterophorus	0.70	0.82	0.88	0.93	1.15	1.10	39.13	46.08	45.21
Lantana camara	0.75	0.85	0.99	1.06	1.19	1.15	36.97	47.89	47.05
Physalis	0.64	0.73	0.93	1.00	1.13	1.08	43.36	45.13	44.24
Melilotus albus	0.46	0.51	0.59	0.62	0.66	0.60	30.30	6.06	4.54
Datura stramonium	0.55	0.61	0.71	0.74	0.85	0.81	35.29	27.05	25.88
Solanum nigrum	0.53	0.57	0.64	0.66	0.77	0.73	31.16	19.48	18.18
Achyranthus aspera	0.51	0.55	0.62	0.65	0.73	0.68	30.13	15.06	13.69
Salix sp.	0.49	0.54	0.56	0.57	0.69	0.62	28.98	10.14	8.69
Thevetia peruviana	0.48	0.53	0.62	0.65	0.68	0.61	29.41	8.82	7.35
Durenta erecta	0.60	0.71	0.77	0.82	1.04	1.00	42.30	40.38	39.42
Polyalthia longifolia	0.44	0.52	0.57	0.60	0.64	0.59	31.25	3.12	1.56
Control-1	0.42	0.50	0.55	0.59	0.62	0.58	32.25	-	-1.61
Control- 2	0.40	0.46	0.52	0.56	0.63	0.57	36.50	1.58	-
C.D.P=(0.05)	**0.034**	**0.038**	**0.043**	**0.045**	**0.051**	**0.051**			
S.E (m)	**0.012**	**0.013**	**0.015**	**0.015**	**0.017**	**0.017**			
S.E (d)	**0.016**	**0.018**	**0.021**	**0.022**	**0.025**	**0.025**			
C.V.	**3.666**	**3.687**	**3.705**	**3.715**	**3.732**	**3.719**			

C.D.P = Critical difference point; S.E(m) = Standard error mean ; S.E(d)= Standard error of difference; C.V = Coefficient of variation

Table 4. Correlation of disease severity with peroxidase, polyphenol oxidase and phenylalanine ammonia lyase content of potato leaves

Biochemical parameters	Days after pathogen inoculation	Correlation coefficient (r) with disease severity	Regression equation
Peroxidase	2 Days	-0.2972	y = -0.0177x+2.2222
	6 Days	-0.3583	y = -0.0158x+2.4292
	10 Days	-0.3339	y = -0.0119x+2.4373
Polyphenol oxidase	2 Days	-0.5004	y = -0.0112x+09114
	6 Days	-0.2374	y = -0.0079x+1.6166
	10 Days	-0.2868	y = -0.0067x+1.6408
Phenylalanine ammonia lyase	2 Days	-0.2631	y = -0.0061x+1.6800
	6 Days	-0.1736	y = -0.0055x+0.8169
	10 Days	-0.1932	y = -0.0061x+0.9057

role in increase disease development. The negative correlation between disease severity with defense molecules like soluble protein, total phenol, PR proteins etc have also been reported by several workers [9].

4. CONCLUSION

It may be concluded from the present finding that pre treatment with plant extracts as inducer provided induced resistance in plant against *A.solani* resulting declined in disease severity. Prior application of inducers to challenge inoculation sensitized the plants to produce elevated level of defense related enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia lyase.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Khurana SMP. Potato diseases & crop protection: Changing scenario. J. Indian Potato Association. 1998;25:1-7.
2. Kuc J. Concepts and direction of induced systemic resistance in plants and its application. Eur. J. PI. Pathol. 2001;107:7-12.
3. Bailey BA, Bae H, Strem MD, Antunez de Mayolo G, Guiltinan MJ, Verica JA, Maximova SN, Bowers JH. Developmental expression of stress response genes in *Theobroma cacao*leaves and their response to Nep1 treatment and a compatible infection by *Phytophthora megakarya*. Plant Physiol. Biochem. 2005;43:611–622.
4. Ojha Suprakash, Narayan Chandra Chatterjee. Induction of resistance in tomato plants against *Fusarium oxysporum* f. Sp. Lycopersici mediated through salicylic acid and *Trichoderma harzianum*. J. Plant Protection Res. 2012;52(2):220-225.
5. Attitalla IH, Johnson P, Brishammar S, Quitanilla P. Systemic resistance to Fusarium wilt in tomato induced by *Phytopathora cryptogea*. J. Phytopath. 2001;149:373.
6. Fuchs JG, Moenne Loccuz Y, Defago G. Nonpathogenic *Fusarium oxysporum* (Fo 47) induces resistance to Fusarium wilt in tomato. Pl. Diseases. 1997;81:492.
7. Ramamorthy V, Ragucander T, Samiyappan T. Induction of defense related proteins in tomato roots treated with *Pseudomonas fluorescens* Pfl and *Fussarium oxysporum* f. sp. *lycopersici.* Plant Soil. 2002; 239(1):55.
8. Yokoyama K, Aist JR, Bayles CJ. A papilla regulating extract that induced resistance to barley powdery mildew. Physiol. Mol. Plant Pathol. 1991;39:71-78.
9. Arzoo K, Samir KB, Mohd R. Biochemical evidence of defence response in tomato against *Fusarim* wilt induced by plant extract. Plant Pathol. J. 2012;11(2):42-50.
10. Biswas SK, Srivastava KD, Aggarwal R, Praveen S, Singh DV. Biochemical changes in wheat induced by *Chaetomium globosum* against spot blotch pathogen. Indian Phytopathol. 2003;54:374-379.
11. Malcolmson JF. Assessment of field resistance to blight (*Phytophthora infestans*) in potatoes. Transactions of the British Mycolo. Society. 1976;67:321–325.
12. Hammerschmidt R, Nuckles E, Kuc J. Association of enhanced peroxidase activity with induced systemic resistance of

cucumber to *Colletotrichu mlagenarium*. Physiol. Plant Pathol. 1982;20:73–82.
13. Burrell MM, Rees TA. Metabolism of phenylalanine and tyrosine in rice leaves infected by *Pyricularia oryzae.* Physiol. Plant Pathol. 1974;4:497-508.
14. He CY, Hsiang T, Wolyn DJ. Induction of systemic disease resistance and pathogen defence responses in *Asparagus officinalis* inoculated with nonpathogenic strains of *Fusarium oxysporum.* Plant Pathology Journal. 2002;51(2):225-230.
15. Seleim MAA, Saead FA, AbdElMoneem KMH, AboElyousr KAM. Biological control of bacterial wilt of tomato by plant growth promoting rhizobacteria. Plant Pathol. J. 2011;10:146-153.
16. Metraux JP, Singer H, Rayals J, Ward E, WyssBenz M, et al. Increase in SA at the onset of SAR in cucumber. Sci. 1990;250: 1004-1006.
17. Cohen Y. 3aminobutyric acid induces systemic resistance against *Peronospore tabacina*. Physiol. Mol. Plant Pathol. 1994; 44:273-288.
18. De Cal A, Melgarejo PY. Repeated application of *Penicillium oxallicum* prolongs biocontrol of fusarium wilt of tomato plants. Eur. J. Plant Pathol. 2001; 107:805-811.
19. Kuc J. Phytoalexins, stress metabolism and disease resistance in plants. Annu. Rev. Phytopathol. 1995;33:275-297.
20. Benhamou N. Elicitor induced resistance in tomato plants against fungal pathogens: Ultrastructure and cytochemistry of induced response. Scanning Microscope. 1995;9:861-880.
21. Van Loon LC. The induction of pathogenesis related protein by pathogen and specific chemicals. Netherland J. Plant Pathol. 1983;85:265273.
22. Doubrava NS, Dean RA, Kuc J. Induction of systemic induced resistance to anthracrose caused by *Colletotrichum lagenarium* in cucumber by oxalate and extracts from spinach and rhubarb leaves. Physiol. Mol. Plant Pathol. 1988;33:69-79.
23. Singh UP, Ram D, Tewari UP. Induction of resistance in chickpea (*Cicer aeritinum*) by *Aegel marmelos* leaves against *Sclerotinia sclerotiorum*. Zeitschrift fur Planzen Krankheiten Pflanzenschutz. 1990;97:439-443.
24. Avdiushko SA, Kuc J. Detection of several enzymatic activities in leaf prints cucumber plants. Physiol. Mol. Plant Pathol. 1993; 42:441–454.
25. Arnnok P, Ruangviriyachai C, Mahachai R, Techawongstien S, Chanthai S. Optimization and determination of polyphenol oxidase and peroxidase activities in hot pepper (*Capsicum annuum* L.) pericarb. International Food Research Journa. 2010; 17:385-392.
26. Mandal, Itishree Kar, Arup K. Mukherjee, and Priyambada Acharya. Elicitor-induced defense responses in solanum lycopersicum against *Ralstonia solanacearum*. The Scientific World Journal. 2013;9:1-9.
27. Meena AK, Godara Gangopadhyay SL, Jeewa Ram S. Changes in biochemical Constituents on cluster bean due to *Alternaria cucumerina* var., *cyamopsidis.* Ann. Pl. Protec. Sci. 2012;20:386-388.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21185