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Research Article
Synthesis of Defense Enzymes in Potato in Induced Resistance against Late Blight using Inorganic Chemicals as Inducer

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Abstract

Background and Objective: Induced resistance is an innovative strategy to overcome production problems of the most serious and destructive late blight [*Phytophthora infestans*] (*P. infestans*) diseases in potato, causes great Irish famine in 1845. Biochemical changes are associated with induced resistance. Therefore, the study was undertaken to find out the induction of defense enzymes using inorganic chemicals as inducer in induced resistance against late blight of potato. **Materials and Methods:** The experiment was conducted at Wire house complex at Department of Plant Pathology during 2012-2015 using inorganic chemicals viz. salicylic acid, indole acetic acid, di-potassium hydrogen orthophosphate, hydrogen peroxide, calcium chloride, ferric chloride and metalaxyl as inducers in induced resistance against the disease. The activity of defense enzymes like peroxidase (POD), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) in potato plants after treatment with different inorganic chemical as inducers followed by inoculation of pathogen was measured at 2, 4, 6, 8 and 10 days separately according to the standard procedure developed by various scientists. Correlation coefficients (r) between defense enzymes and disease severity were calculated by standard statistical calculation. **Results:** All the treatments were able to reduce the disease severity with the lowest in salicylic acid treated plant, representing, the value 12.55-20.72% against 46.35-65.35% in case of control. Biochemical analysis revealed that increased content of defense enzymes like POD, PPO and PAL were found in all treated plants but the maximum in salicylic acid treated leaves at different days of interval. Correlation coefficient analysis also revealed that there was negative correlation r = (-0.886, -0.986 and -0.985),(-0.936, -0.985 and -0.986),(-0.945, -0.872 and -0.972) between disease severity and defense enzymes POD, PPO and PAL respectively at 2, 6 and 10 days of treatments. **Conclusion:** Pre-foliar sprayed with inorganic chemicals stimulate to synthesize the increase content of peroxidase, PPO and PAL which might be provided protection of potato plants against *P. infestans.* Salicylic acid can be used as inducers in induced resistance in potato against late blight.

Key words: Inducers, inorganic chemicals, defense enzymes, disease severity, negative correlation

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Induced resistance is an innovative strategy to overcome production problems caused by plant diseases, is the targeted use of disease defense mechanisms that are inherent to plants[1]. Most of the plants possess defense mechanisms against pathogen attack, which triggered by a stimulus prior to the pathogen attack and reduces the disease. The stimulus can increase the concentration of existing defense compounds that induce production of a new defensive structures and chemicals[2]. Besides preformed barriers and constitutively expressed antimicrobials, 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. 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 64 Aglika Edreva farmers and good profit for producers[3].

The mechanism of resistance was found in association of some defense compounds like soluble protein, total phenol and synthesis of some new proteins due to the effect of biotics and abitics inducers[4,5]. Pre-application of crude extract of *Chaetomium globosum* provided induced resistance in wheat against spot blotch and also sensitized the plant to produce evaluated level of total phenol, soluble protein and some new proteins of different molecular weight (MW) that is, 110, 105, 38 and 32 KDa were measured by SDS-PAGE analysis[2,6]. Similarly, 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[7]. Aqueous extracts of barley leaves induced oversized papillae formation in barley which in turn produces resistance against powdery mildew[8]. 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[9-13]. Keeping the above points in view the study was undertaken in the present investigation to find out the induction of defense enzymes due to application of inorganic chemicals as inducer in induced resistance in potato against late blight.

MATERIALS AND METHODS

The materials and methods adopted in the present investigation to find out the induction of defense enzymes using inorganic chemicals as inducer in induced resistance against late blight of potato based on laboratory (*in vitro*) as well as wire house experiments (*in vivo*) at Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology Kanpur during 2011-2013. The procedure and techniques applied during the course of investigation were elucidated as below:

Isolation and purification of *Phytophthora infestans* : The diseased plant showing typical blight symptoms were collected from Vegetable Research Farm, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India for isolation of *Phytophthora infestans*.

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 sec 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 selective medium (tomato extract based media). The plates were then incubated at 18±1°C. The petri plates were observed daily at every 24 h 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 identification of the pathogen was established on the basis of morphological and cultural characters.

Solution preparation of inorganic chemicals as inducers: The inorganic chemical like salicylic acid (SA), indole acetic acid (IAA), di potassium hydrogen orthophosphate (DPHP), hydrogen peroxide (H_2O_2), calcium chloride ($CaCl_2$), ferric chloride ($FeCl_2$) and metalaxyl were collected from laboratory of the Department of Plant Pathology and some of them also purchases from local market. Different concentrations were prepared by weighing required quantity of inorganic inducers and dissolved in require amount of distilled water separately. The concentration of inorganic chemicals are prepared as SA (10 mM), DPHP (10 mM), $CaCl_2$ (10 ppm), H_2O_2 (1%), metalaxyl (0.2%), $FeCl_2$ (5 mM) and IAA (0.2%).

Preparation of pathogen inoculums: The petri plate containing 10 days old culture of the *P. infestans* 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/square was counted and the sporangial suspension was adjusted to 4.5×0^4 sporangia mL^{-1}.

Measurement of disease severity: The experiment was conducted at Wire house complex at Department of Plant Pathology during 2012-2015. Plants were sprayed with inducers separately at 45 days age. After 48 h of spraying with inducers, plants were inoculated with spore suspension of pathogen using hand atomizer. The plants were then covered with polythene bags for 48 h 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 sporangial suspension of *P. infestans* @ (4.5×0^4 sporangia mL^{-1}) serve as check-2.

Observations for measuring the disease severity were taken after 2, 6 and 10 days of pathogen inoculation using a score chart consisting of 0-9 scale as described by Malcolmson[14]. Ten leaves were randomly selected from the each 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 and 91-100% infection received 9. Percent disease intensity (PDI) was calculated based on the following formula as described by Malcolmson[14]:

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

Induction of defense related enzymes due to effect of inducer during pathogenesis

Estimation of peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL): The activity of defense enzymes like peroxidase, PPO, PAL in potato plants after treatment with different inorganic chemical 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 Hammerschmidt *et al.*[15] and Burrell and Ress[2].

Correlation co-efficient and regression equation: The biochemical analysis of potato leaves at different days of inoculation and disease severity of the corresponding days showed that reduced disease severity was associated with increased level of defense enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia lyase content[15]. 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[16]. 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[16].

RESULTS AND DISCUSSION

Effect of inorganic chemicals 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 inorganic chemical 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.

Effect of inducer on severity of late blight of potato: The effect of tuber treatment and foliar spray with inorganic chemical as inducers significantly reduced disease severity from 65.35-20.72% at 10 days after inoculation as compared to control-1 and control-2 in glass house condition. Among the treatments, minimum disease severity were recorded in salicylic acid treated plant, followed by calcium chloride, hydrogen peroxide at 2, 6 and 10 days of pathogen inoculation, respectively. The metalaxyl treated plants were superior to control but inferior to calcium chloride and hydrogen peroxide treated plant in respect to severity of disease.

Table 1: Effect of inorganic chemicals as inducer on disease severity of late blight of potato

		Disease severity at different days intervals after inoculation (%)		
Name of inducers	Concentration	2 days	6 days	10 days
SA	10 mM	12.55	17.67	20.72
$CaCl_2$	10 mM	16.50	21.56	23.25
HP	10 ppm	16.75	23.50	26.28
Metalaxyl	1%	22.24	27.65	40.15
DPHP	0.20%	31.65	35.75	49.45
FC	5 mM	33.25	39.35	52.20
IAA	0.20%	36.85	43.10	54.50
Control-1		46.35	52.50	65.35
Control-2		37.85	45.65	56.75
CD (p = 0.05)		1.871	2.215	2.823
SE (m)		0.625	0.740	0.943
SE (d)		0.884	1.046	1.334
CV		3.864	3.796	3.822

Table 2: Effect of inorganic chemicals as inducer on activity of peroxidase in potato leaves at different days of intervals after pathogen inoculation (min g^{-1} of fresh leaves)

		Activity of enzyme peroxidase at different days of interval (min g^{-1} of fresh leaves)								
Name of inducers	Concentration	Before application of inducers	2 days	4 days	6 days	8 days	10 days	Increased over, before application of inducers (%)	Increased over control-1 (%) 8 days	Increased over control-2 (%) 8 days
SA	10 mM	1.62	2.25	2.31	2.32	2.37	2.33	31.65	24.89	20.68
$CaCl_2$	10mM	1.60	2.16	2.27	2.30	2.32	2.29	31.03	23.28	18.97
HP	10 ppm	1.57	1.89	2.18	2.21	2.26	2.23	30.53	21.24	16.81
Metalaxyl	1%	1.54	1.83	2.09	2.10	2.15	2.13	28.37	17.21	12.56
DPHP	0.20%	1.51	1.80	1.95	1.97	2.00	1.95	24.50	11.00	6.00
FC	5 mM	1.50	1.73	1.85	1.92	1.97	1.92	23.86	9.64	4.57
IAA	0.20%	1.47	1.69	1.74	1.78	1.90	1.82	22.63	6.32	1.05
Control-1		1.43	1.64	1.68	1.72	1.78	1.74	19.66	-	-5.62
Control-2		1.42	1.69	1.76	1.82	1.88	1.77	24.47	5.32	-
CD (p = 0.05)		0.095	0.117	0.124	0.125	0.129	0.125			
SE (m)		0.032	0.039	0.042	0.042	0.043	0.042			
SE (d)		0.045	0.055	0.059	0.059	0.061	0.059			
CV		3.633	3.657	3.633	3.601	3.617	3.599			

From the Table 1, 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 to synthesis of some defense related compounds in potato plant against *P. infestans*. Plant spraying with salicylic acid completely controlled late blight of potato showing 2% disease severity after 7 days of pathogen inoculation[17,18]. Reduced disease severity of *F. oxysporum* f.sp. *asparagi* in *Asparagus officinalis* inoculated with non pathogenic strains of *F. oxysporum*[16]. Evaluation of inducers viz. ascorbic acid (AA), dichloro-isonicotinic acid (INA), ethylene diamine tetra acetic acid (EDTA) and calcium chloride against late and early blight diseases under greenhouse and field conditions[1]. SA was effective inducer of induced resistance against *P. parasitica* var. *nicotianae*[6]. Application of SA as soil drench or foliar spray significantly reduced severity of Phytophthora blight of squash caused by *Phytophthora capsici*, as compared to control[19]. Similar, effects were observed by pre-foliar spray with indole acetic acid, metalaxyl, di potassium hydrogen orthophosphate, hydrogen peroxide, calcium chloride, salicylic acid and ferric chloride as inducers provided induced resistance in plant against *F. oxysporum* f. sp. *lycopersici,* resulting decline in the disease incidence from 90.96-9.30% after 15 days of pathogen inoculation. The minimum disease incidence (9.30%) was reported from calcium chloride treated plants[4].

Induction of peroxidase due to effect of inorganic chemical as inducers: The result presented in Table 2 showed that there is a significant change varies from 1.42-1.62 as before application to 1.74-2.33 as post application in activity of peroxidase at 10 days after pathogen inoculation. The activity of peroxidase in potato leaves before application of inducers were ranges from 1.42-1.62 min g^{-1} of fresh leaves whereas, in case post application method, the values were

Table 3: Effect of inorganic chemicals as inducer on activity of polyphenol oxidase activity in potato leaves at different days after pathogen inoculation (min g^{-1} of fresh leaves)

		Activity of enzyme polyphenol oxidase at different days of interval (min g^{-1} of fresh leaves)								
Name of inducers	Concentration	Before application of inducers	2 days	4 days	6 days	8 days	10 days	Increased over, before application of inducers (%)	Increased over control-1 (%) 8 days	Increased over control-2 (%) 8 days
SA	10 mM	0.51	0.95	1.65	1.68	1.75	1.71	70.86	26.86	25.71
$CaCl_2$	10 mM	0.50	0.92	1.62	1.64	1.65	1.63	69.70	22.42	21.21
HP	10 ppm	0.51	0.87	1.59	1.62	1.62	1.60	68.52	20.99	19.75
Metalaxyl	1%	0.50	0.80	1.45	1.47	1.52	1.49	67.11	15.79	14.47
DPHP	0.20%	0.49	0.77	1.33	1.40	1.45	1.42	66.21	11.72	10.34
FC	5 mM	0.48	0.71	1.28	1.35	1.40	1.37	65.71	8.57	7.34
IAA	0.20%	0.47	0.66	1.23	1.28	1.35	1.32	65.19	5.19	3.70
Control-1		0.45	0.57	1.15	1.21	1.28	1.25	65.84	-	-1.56
Control-2		0.47	0.51	1.20	1.26	1.30	1.27	63.85	1.54	-
CD (p = 0.05)		0.031	0.051	0.093	0.096	0.098	0.095			
SE (m)		0.010	0.017	0.031	0.032	0.033	0.032			
SE (d)		0.015	0.024	0.044	0.045	0.046	0.045			
CV		3.795	3.635	3.656	3.646	3.673	3.611			

1.78-2.37min g^{-1} of fresh leave, indicated that the inducing agent enhanced the peroxidase activity in potato leave. 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 salicylic acid treated potato fresh leaves at 2, 4, 6 and 8 days, pathogen inoculation, respectively which increased over control-1 and control-2. The second highest activity of peroxidase was found in calcium chloride treated plant fresh leaves at 2, 4, 6 and 8 days of pathogen inoculation which over control-1 and control-2 at 8 days of pathogen inoculation. The lowest quantity of peroxidase was observed in IAA treated potato leaves 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 thereafter, it is decreased gradually. The enzyme activity was increased significantly by 5.32% 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 *P. infestans.* 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[20]. In the present study also the increased content of defense enzymes have also been noticed in all inducers treated plant. The increase activities of polyphenol oxidase (PPO) and peroxidase (POD) in hot pepper (*Capsicum annuum* L.) pericarp were evaluated using spectrophotometric method[21]. Similar, result was found that increase in total phenols, o-dihydroxy phenols, defense related enzymes (PAL, PPO, PO) in salicylic acid treated plants after challenge inoculations with *P. infestans* indicating (52.4%) increased in peroxidase and chitinase activity over control[21,18].

Effect of inorganic chemical 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 provided defense response in plant. The data presented in Table 3 showed that all the treatments significantly increased the activity of polyphenol oxidase from 5.19-26.86 over control-1 and -1.56-25.71 over control-2 at 8 days of pathogen inoculation. The maximum increased of polyphenol oxidase before application of inorganic inducers was found in salicyclic acid treated potato leaves with 70.86% at maximum 8 days of pathogen inoculation.

Among the treatments, the maximum activity of polyphenol oxidase is found in salicylic acid treated fresh leaves against control-1 and control-2 of fresh leaves at 2, 4, 6 and 8 days of pathogen inoculation, respectively. The salicylic acid treated potato leaves posses increased percent activity of polyphenol oxidase over control-1 and 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. The calcium chloride treated potato fresh leaves of polyphenol oxidase at 2, 4, 6 and 8 days of pathogen inoculation which is the second highest among the treatment. 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 *P. infestans*.

Table 4: Effect of inorganic chemicals as inducers on activity of enzyme phenylalanine ammonia lyase activity in potato leaves at different days after pathogen inoculation (mg t-cinnamic acid produced h^{-1} g^{-1} of fresh leaves)

		Activity of enzyme polyphenol oxidase at different days of interval (min g^{-1} of fresh leaves)								
Name of inducers	Concentration	Before application of inducers	2 days	4 days	6 days	8 days	10 days	Increased over, before application of inducers (%)	Increased over control-1 (%) 8 days	Increased over control-2 (%) 8 days
SA	10 mM	0.65	0.75	0.95	1.02	1.15	1.10	34.78	41.74	40.87
$CaCl_2$	10 mM	0.60	0.72	0.78	0.83	1.05	1.00	31.43	36.19	35.24
HP	10 ppm	0.56	0.67	0.76	0.78	0.95	0.91	29.47	29.47	28.42
Metalaxyl	1%	0.54	0.62	0.72	0.75	0.86	0.82	27.91	22.09	20.93
DPHP	0.20%	0.53	0.58	0.65	0.67	0.78	0.74	25.64	14.10	12.82
FC	5 mM	0.52	0.56	0.63	0.65	0.74	0.69	24.32	9.46	8.11
IAA	0.20%	0.50	0.55	0.58	0.60	0.71	0.65	22.54	5.63	4.23
Control-1		0.46	0.53	0.61	0.64	0.67	0.62	20.90	-	-1.49
Control-2		0.42	0.52	0.58	0.62	0.68	0.63	23.53	1.47	-
CD (p = 0.05)		0.034	0.038	0.043	0.044	0.048	0.044			
SE (m)		0.011	0.013	0.014	0.015	0.016	0.015			
SE (d)		0.016	0.018	0.020	0.021	0.023	0.021			
CV		3.743	3.874	3.807	3.685	3.784	3.736			

Plant treated with salicylic acid as foliar spray provided induced resistance in tomato against *Fusarium oxysporum* f. sp. *lycopersici* and also increased the appreciable amount of PAL and polyphenol oxidase activity in treated plant[14]. The severity of black scurf and stem canker was reduced by foliar treatment with salicylic acid (60 mg L^{-1}) which result from the increase activity of peroxidase and chitinase accompanied by a significant increased in the lignin and suberin content[22,5]. Among the different concentrations of SA (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mM), 0.5 mm showed significant increases in the activities of both peroxidase and polyphenol oxidase and also lowering down the *F. oxysporum* infection in tomato[23].

Effect of inorganic chemicals as inducer on the activity of phenylalanine ammonia lyase (PAL): The result presented in Table 4 showed that there is a significant changed in the activity of phenylalanine ammonia lyase ranges from 0.42-0.65 in before application to 0.67-1.15 mg t-cinnamic acid produced h^{-1} g^{-1} of fresh leaves after application of inorganic chemicals. The ranges of activity of phenylalanine ammonia lyase found in potato leaves before and after application of inorganic inducers were 0.42-0.65 and 0.67-1.15 mg t-cinnamic acid produced h^{-1} g^{-1} of fresh leaves, indicating that the inorganic chemicals have ability to increase PAL activity in potato leaves. The highest percent increased activity of PAL before and after application of inorganic inducers is found in salicylic acid treated potato leaves which were 34.78%. Among the treatments, the maximum activity of phenylalanine ammonia lyase was recorded in salicylic acid fresh leaves at 2, 4, 6 and 8 days of pathogen inoculation, respectively, which is maximum increased over control-1 and control-2. The second highest activity of phenylalanine ammonia lyase was found in calcium chloride treated plant fresh leaves over control-1 and control-2 at 8 days of pathogen inoculation. The lowest quantity of phenylalanine ammonia lyase was observed in IAA treated potato fresh leaves of potato at 2, 4, 6 and 8 days, respectively.

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 *P. infestans.* Similar trend in PAL activity and recorded that the activity of PAL increased in inoculated leaves[24,25]. The increased in phenylalanine ammonia lyase activity in inoculated resistant and susceptible host[26]. 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*[27]. The highest enzyme activity appeared on days 4 and 3 in case of ALE and ELE of *O. sanctum,* respectively. The maximum PAL activity was observed (48.70 OD min g^{-1}) with T4 (2.0% P-40) on 4th day of inoculation of pathogen, *Polygonum minus*[28].

Correlation of disease severity with defense enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia lyase content of potato leaves: The results presented in Table 5 revealed that the leaves treated with inorganic chemicals 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

Table 5: 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	-0.886	y = -47.93x+117.0
	6	-0.986	y = -52.50x+139.9
	10	-0.985	y = -69.90x+184.4
Polyphenol oxidase	2	-0.936	y = -71.47x+81.90
	6	-0.985	y = -67.04x+130.2
	10	-0.986	y = -96.70x+183.5
Phenylalanine ammonia lyase	2	-0.945	y = -130.4x+107.9
	6	-0.872	y = -78.37x+91.21
	10	-0.972	y = -91.00x+115.5

correlation regression analysis showed that negative correlation (r) -0.886, -0.936 and -0.945 was found between defense related enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia lyase with disease severity at 2 days of pathogen inoculation, respectively. Similar observations as negative correlation (r) have also been found at 6 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 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[7,29].

CONCLUSION AND FUTURE RECOMMENDATION

It may be concluded from the present finding that pre treatment with inorganic chemicals as inducer provided induced resistance in plant against *P. infestans* resulting declined disease incidence. 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.

Thus a new theory on induced resistance may be arrived for management of plant diseases in near future and may also help the researcher to uncover the critical areas of induced resistance.

SIGNIFICANCE STATEMENTS

Pre-inoculation with salicylic acid provided induced resistance in potato plant, results in disease severity decline tremendously. Biochemical analysis of the treated leaves revealed that increased content of defense enzymes like POD, PPO and PAL. The study was confirmed by correlation coefficient analysis showing negative correlation between disease severity with changes bio-molecules. This study will help the researcher to uncover the critical areas of induced resistance that many researchers were not able to explore. Thus a new theory on induced resistance may be arrived for management of plant diseases in near future.

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