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Induced resistance in potato against late blight caused by *Phytophthora infestans* (Mont.) de Bary through inorganic chemicals as inducer

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Abstract
Pre-inoculation spray with salicylic acid, indole acetic acid, di potassium hydrogen orthophosphate, hydrogen peroxide, calcium chloride, ferric chloride and metalaxyl followed by challenge inoculation by *Phytophthora infestans* showed that among the treatment, salicylic acid treated plant representing minimum disease severity as 18.55, 17.67 and 20.72% at 2, 6 and 10 days after challenge inoculation. The potato plant treated with inorganic chemical as inducers sensitized to produce increased level of soluble protein and total phenol contents. The maximum increase of soluble protein content was found in salicylic acid treated potato leaves indicating 33.05, 35.65, 35.85 and 35.95 mg/g of fresh leaves against 22.30, 24.25, 24.35 and 24.55 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 salicylic acid treated plant, representing 2.62, 3.15, 3.25 and 3.27 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 = -0.945, -0.996 and-0.969) between disease severity and soluble protein content at 2, 6 and 10 days of treatment. Similarly, total phenol content also showed negative correlation (r = -0.898, -0.887 and -0.864) with disease severity. Thus, the protection of potato plants against *P. infestans* by inorganic chemicals as inducer might be due to stimulation of plants defense response.

Keywords: *Phytophthora infestans*, potato, induced resistance, inorganic chemicals

Introduction
Late blight caused by *Phytophthora infestans* (Mont) de Bary is the most destructive disease of potato and was responsible for causing the Irish Famine in the middle of the19th century. It affects foliage including leaves, petiole and stem and also tubers. Under highly congenial conditions, late blight appears as epiphytotic form and kills the entire foliage within a few days. Yield losses due to late blight varies, depend on cultivars, area of cultivation and other environmental conditions. Binyam Tsedaley (2014) [3] reported that when the pathogen attacks both potato foliage in the field and tuber in the storage which can absolutely destroy a crop, producing a 100% crop loss. Tuber rot is comparatively less in blight resistant varieties as compared to susceptible ones. Tuber infection is also more in large and medium sized tubers and in low lying areas where drainage condition is poor. The pathogen is perpetuated in soil, tuber and crop residue through production of oospore. A number of management techniques of late blight have been developed and used thoughout the world. Host resistance to *P. infestans* is not stable due to development of new multi gene races of the pathogen. Therefore, fungicides play an important role in the management of the disease but frequent and excessive fungicide applications are followed by the farmer in the field that may result in damage to the environment. More recently, induced host resistance has emerged as an important tool for disease management with minimal negative impact on the environment. Sticher *et al.* (1995) [21] reported that induced systemic resistance or systemic acquired resistance (ISR or SAR) offers available alternative for eco-friendly management of plant diseases through stimulation of defense response in plants particularly where genetic resistance is not easily available or is insufficient.

Mayers *et al.*, (2005) [15] found that salicylic acid provided induce resistance in squash and tobacco against Cucumber mosaic virus (CMV). 2, 6-Dichloroisonicotinic acid (INA) was effective against a wide range of pathogens and was mediated by a salicylic acid-dependent process (Walters and Boyle, 2005) [26]. Biswas *et al.* (2012) [5] found that pre foliar spray with Indol acetic acid, Metalaxyl, Di- potassium hydrogen ortho phosphate, Hydrogen peroxide,

Calcium chloride, Salicylic acid and Ferric chloride as inducers provided induced resistance in plant against *Fusarium oxysporium.* f.Sp. *lycopersici* resulting declined disease incidence from 90.96 to 9.30 percent after 15 days of pathogen inoculation. Keeping the above points in view, the study was undertaken as "Induced resistance against *P. infestans* causing late blight of potato through inorganic chemicals as Inducer" in the present investigation.

Material and Methods

The present investigation was undertaken during 2013 to 2014 at Department of Plant Pathology, CSA University of Agriculture and Technology, Kanpur. The pathogen, *P. infestans* 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 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 plates which was previously filled with selective medium (tomato extract based media; media composition tomato juice 200ml, Calcium carbonate 0.04gm, Agar 20gm and Distilled water 800ml). The Petri plates were then incubated at 18 ± 1^0C. 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 (Rawat, 2012) [12].

Collection and preparation of inorganic chemicals as inducers

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

Preparation of pathogen inoculums and Artificial Inoculation of Pathogen

The Petri plate containing 15 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 per square was counted and the sporangial suspension was adjusted to 4.5×10^4 sporangia ml^{-1}.

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 and Complete Randomized Design (CRD) was followed. At the age of 45 days, 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 (Control-2) and in second case, plants were inoculated using sporangial suspension of *P. infestans* @ (4.5×10^4 sporangia/ml) serve as (Control-1).

After 48 hrs of spraying with inducers, plants were inoculated with spore suspension of pathogen. The concentration of sporangia was maintained at 4.5×10^4 sporangia/ml. The sporangial suspension was prepared from 15 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.

Measurement of disease severity

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 1-9 scale as described by Malcolmson, (1976) [14]. Scoring was based on Malcolmson's scale where the intensity of foliage blight caused by *P. infestans* is measured by asissing the overall amount of necrotic tissue per o\plant as follow: Score 1- percentage of necroti tissue > 90%. score 2; 81-90%, score 3; 71-80%, score 4; 61-70%, score 5; 41-60%, score 6; 26-40%, score 7; 11-25%, score 8; <10, score 9; complete immunity to the disease(no disease symptoms). Percent disease index (PDI) was calculated based on formula as described by Malcolmson, (1976) [14].

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

Biochemical changes in potato due to effect of inorganic chemicals 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, 4, 6, 8 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 leaf.

Estimation of total phenol

The accumulation of total phenols in potato plants after treatment with different inorganic chemicals as inducer, followed by inoculation of pathogen was estimated following procedure developed by Bray and Thrope (1954) [6].

Results

Effect of inorganic chemicals as inducer on severity of late blight of potato

The effect foliar spray with inorganic chemical as inducers significantly reduced disease severity of late blight of potato as compared to control-1 and control-2 in glass house condition (Table-1).

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

Name of inducers	Concentration	Disease severity(%) at different days of intervals after inoculation		
		2 Days	6 Days	10 Days
SA	10 mM	12.55	17.67	20.72
$CaCl_2$	10ppm	16.50	21.56	23.25
HP	1%	16.75	23.50	26.28
Metalaxyl	0.2%	22.24	27.65	40.15
DPHP	10mM	31.65	35.75	49.45
FC	5 mM	33.25	39.35	52.20
IAA	0.2%	36.85	43.10	54.50
Control-1		46.35	52.50	65.35
Control -2		37.85	45.65	56.75
C.D.P=(0.05)		1.871	2.215	2.823
S.E (m)		0.625	0.740	0.943
S.E (d)		0.884	1.046	1.334
C.V.		3.864	3.796	3.822

Among the treatment, minimum disease severity with 12.55%, 17.67% and 20.72% were recorded where treated plant sprayed with salicylic acid as inducers, followed by calcium chloride as16.50%, 21.56% and 23.25%, Hydrogen peroxide as 16.75%, 23.50% and 26.28% at 2, 6 and 10 days of pathogen inoculation, respectively. The metalaxyl treated plants were showing 22.24%, 27.65% and 40.15% disease severity which are superior to control but inferior to salicylic acid, calcium chloride and hydrogen peroxide treated plant in respect to severity of disease at 2, 6 and 10 days of pathogen inoculation. From the table-1, it is also cleared, that all the inducers treated potato plants were showing comparatively low disease severity over control-2 and control-1. 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 *P. infestans*.

Biochemical changes associated with the effect of inorganic chemicals as inducer.

Soluble protein

The results presented in Table-2 indicated that total soluble protein content in potato leaves due to application of inducers ranges from 20.58 - 21.42 mg/g of fresh leaves. The highest content of total soluble protein was recorded from salicylic acid treated potato leaves, indicating 33.05, 35.65, 35.85, 35.95 and 34.46 mg/g of fresh leaves against 22.30, 24.25, 24.35, 24.55 and 23.54 mg/g in case of control-1 and 23.45, 25.35, 25.55, 25.65 and 24.10 mg/g of fresh leaves in case of control-2 at 2, 4, 6, 8 and 10 days of pathogen inoculation. Among the treatment, the lowest quantity of soluble protein content was found in IAA treated potato leaves, showing 25.28, 27.25, 27.47, 27.67 and 26.53 mg/g of fresh leave at 2, 4, 6, 8 and 10 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 and after application, the soluble protein content was increase in all the treatments. The highest 40.40% increased of soluble protein after application of inducing agent was recorded in case of salicylic acid treated plant. The increased protein content in treated plants might be responsible for defense response in plant against P. infestans.

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, 8 and 10 days of pathogen inoculation. The maximum total phenol content was found in salicylic acid treated potato leaves which were 2.62 mg/g, 3.15 mg/g, 3.25 mg/g, 3.32mg/g and 3.27 mg/g of fresh leaves at 2, 4, 6, 8 and 10 days of pathogen inoculation, respectively whereas, in case of control-1, the values are 1.17, 1.25, 1.55, 1.80 and 169 mg/g of fresh leave and for control-2, the value are 1.20, 1.35, 1.60, 1.85 and 174 mg/g of fresh leaves. The salicylic acid treated potato leaves posses increased percent of total soluble phenol as 45.78% over control-1 and 44.28% over control-2 at 8 days of pathogen inoculation. The second highest of total phenol content was found in calcium chloride treated potato leaves which were 2.59, 2.65, 2.78, 2.83 and 2.76 mg/g of fresh leaves at 2, 4, 6, 8 and 10 days of pathogen inoculation. The percent increased of total phenol content at 8 days of pathogen inoculation was of 36.40% over control-1 and 34.63% over control-2.The lowest quantity of soluble phenol was harvested in IAA treated potato leaves, indicating 1.20, 2.26, 2.37, 2.42 and 2.36 mg/g of fresh leaves. From the table -3, it is also cleared that other treatments like hydrogen peroxide, metalaxyl, di potassium hydrogen orthophosphate and ferric chloride also increased total phenol content over control-1 and contol-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 20.58 - 21.42 mg/g of fresh leave as in before application and 24.55 - 34.46 mg/g of fresh leave as in after application at 8th days of pathogen inoculation. The highest percent increased of total phenol before and after application of inorganic inducers was found in salicylic acid treated potato leaves which was 53.01% at maximum 8 days. It is 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 *P. infestans*.

Table 2: Effect of inorganic chemicals 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 Inducers	Concentration	Soluble protein content (mg/g of fresh leaves) at different days after inoculation of pathogen.						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			
SA	10 mM	21.42	33.05	35.65	35.85	35.95	34.46	40.40	31.71	28.65
$CaCl_2$	10ppm	21.35	32.75	34.45	34.55	34.67	33.15	38.42	29.19	26.02
HP	1%	21.10	32.11	33.75	33.36	33.58	32.36	37.16	26.89	23.62
Metalaxyl	0.2%	20.98	30.27	32.65	32.76	32.88	31.56	36.19	25.33	21.99
DPHP	10mM	20.75	27.63	29.11	29.45	29.65	28.45	30.02	17.20	13.49
FC	5 mM	20.85	26.15	28.25	28.55	28.70	27.01	27.35	14.46	10.63
IAA	0.2%	20.72	25.28	27.25	27.47	27.67	26.53	25.12	11.28	7.30
Control-1		20.63	22.30	24.25	24.35	24.55	23.54	15.97	-	-4.48
Control -2		20.58	23.45	25.35	25.55	25.65	24.10	19.77	4.29	-
C.D.P=(0.05)		1.205	1.767	1.891	1.897	1.911	1.823			
S.E (m)		0.402	0.590	0.631	0.634	0.638	0.609			
S.E (d)		0.569	0.835	0.893	0.896	0.903	0.861			
C.V.		3.725	3.637	3.636	3.632	3.641	3.634			

Table 3: Effect of inorganic chemicals as inducer on total phenol in potato leaves at different days of intervals after inoculation during pathogenesis

Name of Inducers	Concentration	Total phenol content (mg/g of fresh leaves) at different days after inoculation of pathogen.						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			
SA	10 mM	1.56	2.62	3.15	3.25	3.32	3.27	53.01	45.78	44.28
$CaCl_2$	10ppm	1.40	2.59	2.65	2.78	2.83	2.76	50.53	36.40	34.63
HP	1%	1.38	2.54	2.61	2.72	2.77	2.61	50.18	35.02	33.21
Metalaxyl	0.2%	1.44	1.47	2.53	2.67	2.74	2.57	47.45	24.31	32.48
DPHP	10mM	1.40	1.36	2.43	2.51	2.65	2.54	47.17	32.08	30.19
FC	5 mM	1.35	1.25	2.35	2.42	2.48	2.41	45.56	27.42	25.40
IAA	0.2%	1.34	1.20	2.26	2.37	2.42	2.36	44.63	25.62	23.55
Control-1		1.12	1.17	1.25	1.55	1.80	1.69	37.78	-	-2.78
Control -2		1.10	1.20	1.35	1.60	1.85	1.74	40.54	2.70	-
C.D.P=(0.05)		0.057	0.084	0.108	0.115	0.119	0.117			
S.E (m)		0.019	0.028	0.036	0.038	0.040	0.039			
S.E (d)		0.027	0.040	0.051	0.054	0.056	0.055			
C.V.		2.640	2.842	2.728	2.723	2.673	2.729			

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 inorganic chemicals 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) -0.945, -0.996, -0.969 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) -0.898, -0.887, -0.864 between total phenol with disease severity at 2, 6, 10 days of pathogen inoculation, respectively.

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	-0.945	y = -2.808x + 107.1
	6 Days	-0.996	y = -2.935x + 122.7
	10 Days	-0.969	y = -3.926x + 157.1
Total phenol	2 Days	-0.898	y = -15.81x + 55.28
	6 Days	-0.887	y = -20.21x + 83.20
	10 Days	-0.864	y = -28.82x + 113.4

Discussion

Pre-inoculation spray with inorganic chemical as inducers significantly reduced disease severity of late blight of potato as compared to control-1 and control-2. Among the treatment, minimum disease severity with 12.55%, 17.67% and 20.72% were recorded where tuber treated and sprayed with salicylic acid as inducers at 2, 6 and 10 days of pathogen inoculation. The present finding were also supported by several workers as to application of biotics and abiotics inducers in induce resistance in many plants, potato and tomato etc. (Arzoo, *et al.*, 2012; Kumar and Biswas, 2010, Girdhari, *et al.*, 2008) [12, 10, 7]. The combined tuber treatment and spray application of salicylic acid resulted disease severity of 2.0% (Sudhir, *et al.*, 2010) [22]. Kuc (2001) [9] reported that SA was effective inducer of induce resistance against *P parasitica* var. *nicotianae.* Kone, *et al.*, (2009) [8] found that SA applied as a soil drench or foliar spray significantly reduced severity of Phytophthora blight squash caused by *Phytophthora capsici*,

as compared to control. Rajik *et al.* (2012) [12] also found that pre treatment with biotic inducers provided induced resistance in plant against *F. o.* f.sp. *lycopersici* resulting declined disease incidence from 100 to 7.69 percent.

Induction of resistance was found to be associated with some biochemical changes in plants by microbes, microbial metabolites, plant extract and chemical agents which are not considered as fungicides (Nicholson and Hammerschamidt, 1992) [19]. In present investigation also, total soluble protein and total phenol content was found to be influenced by inducers treatment. Total soluble protein content in potato leaves due to application of inorganic inducers ranges from 20.58-21.42 mg/g of fresh leaves. Antoniew, *et al.,* (1980) [1] considered that pathogen related proteins (PR protein) are involved in plant defense response to pathogens. Metraux, *et al.,* (1988) [16] and Tuzun, *et al.,* (1989) [24] also reported that proteins forms of chitinases and β -1, 3 glueanase 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 β-1, 3 glucanase, which are pathogenesis related proteins (PR-proteins), have been studied most extensively. Biochemical change associated with induced resistance in different crops against pathogens by non conventional of chemical against have been reported by several workers (Steiner and Schonbeack, 1995; Biswas *et al*,. 2012; Rajik *et al*, 2012; Surjeet, *et al.*, 2017) [21, 5, 18, 23].

The total phenol content also showed increased in all treated plants as compared to both the controls but the maximum in salicylic acid treated potato leaves. 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. Arzoo, *et al.,* (2012) [12] reported that increase content of phenols are associated with defence response in tomato against Fusarium wilt induced by plant extract. Girdhari, *et al.,* (2008) [7] 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. Vimala and Suriachandraselvan (2009) [25] noticed higher accumulation of phenolics in plants due to pre-treated with salicylic acid (1mM) resulted enhance in resistance against invasion of *Erysiphe cichoracearum* in bhindi. Lai *et al*., (2010) [12] also reported that pre-inoculation sprays of crude extract sensitized seedlings to produce elevated phenol contents. Kumar and Biswas (2010) [11] reported that increased total phenol was found in tomato leaves after treatment with inorganic chemicals.

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) [11], in tomato against Fusarium wilt (Kumar and Biswas, 2010; Arzoo, *et al.,* 2012) [12], in wheat against spot blotch (Mishra *et al.,* 2011) [17]. Surjit *et al.* (2017) [23] also found that Calcium chloride as inducer have ability to reduce disease severity of late blight of potato and also have potential to synthesize some defense compound like soluble protein total phenol etc.

Conclusion

Pre-inoculation and foliar spray with inorganic chemicals as inducer provided induced resistance in plant against *P. infestans* resulting declined disease severity. Prior application of inducers to challenge inoculation sensitized the seedling to produce increased level of soluble proteins and total phenol content. The both the factors (Phenol & protein) also showed negative co-relation with disease severity. Thus the inorganic chemicals can be used for plant disease management in near future.

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