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Inorganic chemical as inducer in induced resistance against Alternaria blight of potato

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Abstract
Tuber treatment and foliar spray with inorganic chemicals as inducers significantly reduced disease severity of early blight of potato as compared to control 1. The minimum disease severity with 12.40%, 17.60% and 20.65% were recorded in case of both tuber treatment and foliar spray with salicyclic acid as inducer at 2, 6 and 10 days of pathogen inoculation, respectively. Biochemical changes are associated with induced resistance showed that total soluble protein content in potato leaves before application of inducers ranges from 20.59 -21.44 mg/g of fresh leaves. Whereas after application ranges from 22.38 to 33.09 at 2 days, 24.36 to 35.39 at 4 days, 24.46 to 35.75 at 6 days, 24.66 to 35.98 and 23.62 to 34.58 mg/g of fresh leaves as 10 days of inoculation, indicating soluble protein content increase in all the treatments. The highest content of soluble protein was recorded from salicylic acid treated potato leaves, indicating 33.07, 35.27, 35.67, 35.87 and 34.48 mg/g of fresh leaves. Similarly, the maximum total phenol content was found in salicylic acid treated potato leaves which were 2.64, 3.17, 3.27, 3.34 and 3.29 mg/g of fresh leaves at 2, 4, 6, 8 and 10 days of pathogen inoculation, respectively.
The correlation regression equation showed negative correlation (r) -0.723 and -0.850, between disease severity with total protein and total phenol at 2 days of pathogen inoculation, respectively. Similarly, negative correlation had also been found -0.957 and -0.823, at 4 days, -0.615 and -0.141, at 6 days, -0.943 and -0.916 at 8 days and -0.695 and -0.777 at 10 days of pathogen inoculation.

Keywords: resistance, Alternaria, Inorganic, significantly

1. Introduction
The potato (*Solanum tuberosum* L.) is a starchy, tuber crop from the perennial nightshade and considered as a "King of Vegetable". The crop is suffered by numbers of diseases like, early blight, late blight, leaf spot, dry rot, charcoal rot, black scurf, common scab, soft rot, leaf roll etc. Among them, early blight caused by *Alternaria solani* (Sorauer) is the most destructive disease of potato in all potato growing areas of the world as well as in India. The disease may appear from seedling to harvesting stage of crop and even in storage condition as dry rot of tuber. The disease can be very destructive if left uncontrolled, often resulting in complete defoliation of plants. The losses caused by the disease ranges from 5–78 % have been reported in Uttar Pradesh depending on the variety and plant protection manures adopted (Deptt. of Sci. & Tech., 2016). Every 1% increase in intensity can reduce yield by 1.36%, and complete crop failure can also occur when the disease is most severe. Yield losses up to 79% have been reported in the United State, of which 20-40% is due to seedling losses (i.e., collar rot) in the field, 20-30% as foliar damage. In storage, *A. solani* can cause dry rot of tubers and may also reduce storage length, both of which diminish the quantity and quality of marketable tubers. (www. wikipedia encyclopedia).
The management of the disease is mostly done through conventional methods like cultural, chemical, biological and use of resistant varieties. The efficacies of these current strategies for management of diseases have some limitations. Moreover, most of the conventional chemical, biological and use of resistant variety tend towards the direct methods of plant disease management. But in case of monocotyledonous and dicotyledonous crops, these practices raised problem due to development of resistant strain among the pathogen, which are also hazardous to our environment. Considering the destructive nature of pathogen and lack of efficient control measures, development of alternative or complementary approaches for management of this disease is highly desirable. A control practice that has shown promise for plant disease management is the use of systemically induced plant resistance. Sticher *et al.,* (1997) [22] reported that induced systemic resistance or systemic acquired resistance (ISR or SAR) offers available alternative for eco-friendly management of plant diseases. Biswas *et al.* 2011 found that pre-application of crude extract of *Chaetomium globosum* and avirulent races of *Drechslera sorokiniana* provided induced resistance in wheat against spot blotch. Surjeet *et al.* 2017 [25] found that seedling dip and foliar sprayed with calcium chloride provide protection of tomato plant against late blight of potato. Biswas *et al*. 2019 reported that pre-treatment with plant extract provided induced resistance in tomato against Fusarium wilt. Rajik *et al.* (2012) reported that pre-treatment with different isolates of bioagents collected from different places of India provided induced resistance in plant against *F. o.* f.sp. *lycopersici*, resulting declined disease. Biochemical changes associated with induced resistance in crop plant against pathogens by non-conventional chemicals, bio-agents, avirulent races, plant extracts etc. have been reported by several workers (Kumar, and Biswas, 2010; Biswas, *et al*. 2012, Rakesh *et al*.2017, Surender *et al*. 2020 [24].
The potentiality of different plant extracts as inducers were assessed on physiological and biochemical activities in tomato against Fusarim wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* and the results showed that pre application of inducers provided protection to the tomato plant and

reduced the disease intensity (Arzoo *et al.*, 2012, Biswas *et al*. 2019) [14]. Biswas *et al.*, (2003) also reported that some new proteins were associated with induced resistance to *Bipolaris sorokiniana* induced by crude extracts of *Chaetomium globosum*.

Keeping the above points in view, the study entitled "Evaluation of inorganic chemicals as inducer in systemic acquired resistance for management of Alternaria blight of potato " has been taken in the present investigations.

Materials and Methods

Isolation, purification and identification of *Alternaria solani*

Alternaria blight symptoms were collected from Vegetable Research Farm, Chandra Shekhar Azad University of Agriculture and Technology, Kalyanpur (Kanpur). Infected leaf from border of the lesion along with some healthy green tissue was cut and dipped in mercuric chloride solution (0.1%) for 30 seconds which was followed by three times rinsed in sterilized distilled water. The pieces were then dried off with sterilized filter paper. The sterilized pieces were placed at the center of Petriplate which was previously filled with PDA medium. The plates were then incubated at 25±1^{0}C. The Petri plates were observed daily at 24 hrs interval and noticed the presence of mycelium growth 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 isolated pathogen was identified on the basis of its morphological and cultural characters and pathogenic behavior towards the host.

Preparation of spore suspension of pathogen

The Petri plate containing 10-15 days old culture of *Alternaria 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 concentration of spore suspension was measured with the help of a Haemocytometer as 10^5 conidia/ml.

Collection and preparation of inorganic chemicals as inducer

The inorganic chemicals as in mentioned Table- 1 were collected from laboratory of the Department of Plant Pathology and some of them are purchases from local market. Different concentrations of inducers were prepared by weighing required quantity of inducers separately and placed in conical flask by adding 100 ml of sterilized water for each conical flask and shaken until they get dissolved completely to attain the required concentration of solutions (Table- 1).

Table 1: List of inorganic chemicals and their concentration used to conduct the experiment.

Treatment	Concentration
Salicylic acid (SA)	10mM
Calcium chloride ($CaCl_2$)	10mM
Hydrogen peroxide (HP)	10 ppm
Metalaxyl	0.1%
Di-potassium hydrogen Orthophosphate (DPHP)	0.2%
Ferric chloride ($FeCl_3$)	5mM
Copper sulphate	10mM
Indole acetic acid (IAA)	1%
Copper chloride	10mM
Control-1 (Healthy)	-
Control-2 (Infected)	-

Effect of inorganic chemicals as inducer on disease severity of early blight of potato

The experiment was conducted at the Glass house complex, Department of Plant Pathology, C.S.A. University of Agriculture and Technology, Kanpur. The seed tubers of potato variety '*Kufri Sindhuri*' were treated with inducers separately and sown in 30cm earthen pots, which were previously filled with a mixture of sterilized sandy loam and Farm yard manure in the ratio of 2:1. In each pot, two seed tubers were sown and watered as per needed.

In order to ascertain the effect of inducing agents on disease development, plants were sprayed with inducers separately at 48 hrs before foliar inoculation with 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 with conidial suspension of *A. solani* (Check-2). The observations on disease severity were taken at 2 days, 6 days and 10 days of inoculations.

Measurement of disease severity

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 Sahu, (2013). Ten leaves were randomly selected from each 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.

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

Biomolecule changes in potato due to effect of inorganic chemicals as inducers

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 leave.

Reagents Needed

Solution A: 20% sodium carbonate in 0.1 N NaOH); Solution B: 0.5% copper sulphate $CuSo_4.5H_2O$ in sodium potassium tartrate); Solution C: Alkaline copper solution prepared by mixing 50 ml of solution A with 1 ml of solution B just prior to use); Folin- Ciocalteu Reagent (FCR); stock standard protein solution (prepared by dissolving 50 mg of Bovine serum albumin /50 ml of water) and working standard solution (prepared by diluting 10 ml of the stock solution to 50 ml with water to obtain 200 micro gram protein /ml).

Procedure

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 pestle and mortar using 1:5 leaves: extraction buffer. The suspension was centrifuged at 10,000 rpm for 30 minutes at 4^0C. The supernatant was collected. A quantity of 7.5 ml of the supernatant was transferred in a tube and mixed with 2.5 ml of sample buffer and used for protein estimation. The working standard solution was pipette out and 02, 0.6 and 1.0 ml and was put into series of test tubes. A quantity of 0.2 ml, 0.6 ml and 1.0 of the sample extract was also pipette out and kept into other test tubes separately. Then 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 phenols in potato plants after treatment with different inorganic chemicals as inducers as 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 radiation calorimetrically.

Reagents Needed

Ethanol 80%, FCR, Na_2CO_3 20% and standard (100 mg catechol in 100 ml of water, which was diluted 10 times for a working standard).

Procedure

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, 0.6 and 1.0 ml) were pipette out into test tubes and the volume in each tube was made to 3 ml with water. Subsequently, 0.5 ml of FCR was added and after three minutes, 2 ml of 20% Na_2CO_3 solution in each tube was thoroughly mixed. Then absorbance at 650 nm against blank was measured using Ultra Violet Visible (UV-VIS) sphectrophotometer and the standard curve using different concentration of phenols was prepared. From the standard curve, the concentration of phenols in the test sample was determined and expressed as mg phenols per gm of sample materials.

Correlation 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 soluble protein and total phenol content. However, to determine the level of association, correlation coefficients (r) between disease severity with soluble protein and total phenol content were calculated by standard statistical calculation. Simple regression equations (Y= a + bx) were also developed for soluble protein and total phenol separately to understand their relation with disease severity.

Results and discussion

Effect of inorganic chemicals as inducer on severity of early blight of potato.

The effect of tuber treatment and foliar spray with inorganic chemicals as inducers significantly reduced disease severity of early blight of potato as compared to control 1 (Pathogen inoculated) under wire house condition. Among the treatments, minimum disease severity with 12.40%, 17.60% and 20.65% were recorded in case of both tuber treatment and foliar spray with salicyclic acid as inducer followed by calcium chloride as 15.92%, 21.06% and 22.74%, hydrogen peroxide as 16.67%, 23.75% and 27.10% at 2, 6 and 10 days of pathogen inoculation, respectively (Table 2). The control plants were showing 46.9, 53.25 and 67.30 per cent disease severity at 2, 6 and 10 days of pathogen inoculation. From the table, it is cleared that among all inorganic chemicals, cuprous chloride tested plant found least effective in minimizing severity of early blight, representing 39.32, 48.10 and 58.25 per cent disease severity at 2, 6 and 10 days of pathogen inoculation. Surjeet *et al*. (2017) [25] found that tuber treatment and foliar spray with calcium chloride as inducers significantly reduced disease severity of late blight of potato as compared to control. Biswas *et al.* 2019 found that pre-inoculation spray of neem leaf extracts on tomato plants significantly reduced the wilt incidence in tomato at 5, 10 and 15 days after pathogen inoculation. Surender *et al.* (2020) [24] reported that salicylic acid treated brinjal leaves as inducer showed decrease disease severity of phomopsis blight at 2, 6 and 10 days, pathogen inoculation.

Table 2: Effect of inorganic chemicals as inducer on disease severity of early blight of potato

Name of inducers	Concentration	Disease severity at different days intervals after inoculation (%) 2015-16		
		2 Days	6 Days	10 Days
SA	10 mM	12.40	17.60	20.65
CaCl2	10mM	15.92	21.06	22.74
HP	10 ppm	16.67	23.75	27.10
Metalaxyl	0.1%	22.96	28.50	40.28
DPHP	0.2%	32.15	36.45	48.42
FeCl3	5mM	36.75	44.22	53.80
CuSO4	10mM	37.40	46.35	55.25
IAA	1%	38.88	47.64	57.95
CuCl2	10mM	39.32	48.10	58.25
Control-1		46.90	53.25	67.30
C.D.P=(0.05)		1.980	2.292	2.792
S.E (m)		0.667	0.771	0.940
S.E (d)		0.943	1.091	1.329
C.V.		3.857	3.780	3.789

Effect of inorganic chemicals as inducer on biochemical changes in potato plants

Soluble protein

Biochemical changes are associated with induced resistance. The result presented in Table 3 indicated that total soluble protein content in potato leaves before application of inducers ranges from 20.59 -21.44 mg/g of fresh leaves. Whereas after application ranges from 22.38 to 33.09 at 2 days, 24.36 to 35.39 at 4 days, 24.46 to 35.75 at 6 days, 24.66 to 35.98 and 23.62 to 34.58 mg/g of fresh leaves as 10 days of inoculation, indicating soluble protein content increase in all the treatments. The highest content of soluble protein was recorded from salicylic acid treated potato leaves, indicating 33.07, 35.27, 35.67, 35.87 and 34.48 mg/g of fresh leaves against 22.32, 24.27, 24.37, 24.57 and 23.56 mg/g in case of control-1 and 23.47, 25.37, 25.57, 25.67 and 24.12 mg/g of fresh leaves in case of control-2 at 2, 4, 6, 8 and 10 days of pathogen inoculation. The salicylic acid treated potato plant posses 31.50 and 28.43 per cent increased of total soluble protein over control 1 (Inoculated) and over control 2 (Uninoculated) at 8 days of pathogen inoculation, respectively. The calcium chloride treated potato plant showed 32.77, 34.47, 34.57, 34.69 and 33.15 mg/g of fresh leaves at 2, 4, 6, 8 and 10 days of pathogen inoculation which is the second highest among the treatments, representing 29.17% higher over control 1 and 26.00% over control 2 at 8 days of inoculation.

From the table, it is also cleared that among the different days, the maximum concentration of soluble protein was found at 8 days of inoculation in all the treatments, thereafter, it declined gradually. Biswas *et al*. (2012) found that the induction of defense response in tomato plant due to increase content of soluble protein content after application of calcium chloride as inducer at 5, 10 and 15 days of pathogen inoculation. Rakesh *et al*. 2018 reported that 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. Biswas *et al.*, (2003) also reported that some new proteins were associated with induced resistance to *Bipolaris sorokiniana* induced by crude extracts of *Chaetomium globosum*. He also found that six metabolites are involved in induction of induced resistance in plant.

Total phenol

Phenol has antifungal, bacterial and antiviral properties synthesized in plant against pathogenic agents or other external stimulating agents. The data presented in Table-4 shows that all the treatments significantly increased the total phenol content as compared to control 1 (Inoculated) and control 2 (Uninoculated) 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 2.64, 3.17, 3.27, 3.34 and 3.29 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, 1.27, 1.57, 1.82 and 1.71 mg/g of fresh leave and for control 2, the value are 1.19, 1.37, 1.62, 1.87 and 1.76 mg/g of fresh leaves. The salicylic acid treated potato leaves posses increased per cent of total phenol as 45.50% over control-1 and 44.01% 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 2.61, 2.67, 2.87, 3.00 and 2.78 mg/g of fresh leaves at 2, 4, 6, 8 and 10 days of pathogen inoculation.

From the table, it is clear that the total phenol content in potato leaves before and after application of inducers clearly indicated that inorganic chemicals have the ability to increase total phenol content, representing the value ranges from 1.14 - 1.57-mg/g of fresh leave as in before application and 1.87 - 3.34 mg/g of fresh leave as in after application at 8th days of pathogen inoculation. The highest per cent increased of total phenol before and after application of inorganic chemicals was found in salicylic acid treated potato leaves which was 53.00% at 8 days of pathogen inoculation. The increased phenol content in treated plants might be due to defense response in plant against *A. solani.* Arzoo, *et al.*, (2012) [14] 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. Kumar and Biswas (2010) reported that increased total phenol was found in tomato leaves after treatment with inorganic chemicals. 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; Rajik *et al*, 2012; Surjeet, *et al.*, 2017) [25].

Correlation between disease severity with total soluble protein and total phenol 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 soluble protein and total phenol content in of potato leaves. The correlation regression equation showed negative correlation (r) -0.723 and -0.850, between disease severity with total protein and total phenol at 2 days of pathogen inoculation, respectively. Similarly, negative correlation had also been found -0.957 and -0.823, at 4 days, -0.615 and -0.141, at 6 days, -0.943 and -0.916 at 8 days and -0.695 and -0.777 at 10 days of pathogen inoculation. The corresponding simple regression equation also showed that increase level of soluble protein

and total phenol content have negative role in increase disease development. Surdeep *et al*. (2018) found that negative correlation between total phenol and soluble protein with disease severity at different stages of plant growth and in different germplasms. Similar observation were also found in rice against brown leaf spot (Kumawat, *et al.*, 2010), in tomato against Fusarium wilt (Kumar and Biswas, 2010; Arzoo, *et al.*, 2012) [14], in wheat against spot blotch (Mishra *et al.*, 2011).

Table 5: 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	R^2= -0.723	Y= -0.267x +36.81
	4 Days	R^2 = -0.957	Y = -1.093x + 36.90
	6 Days	R^2= -0.615	Y = -0.233x +39.45
	8 Days	R^2 = -0.943	Y = -1.099x + 37.44
	10 Days	R^2= -0.695	Y= -0.184x +38.12
Total phenol	2 Days	R^2= -0.850	Y= -0.049x +3.155
	4 Days	R^2 = -0.823	Y = -0.150x + 3.194
	6 Days	R^2= -0.140	Y= -0.025x +3.274
	8 Days	R^2 = -0.916	Y = -0.128x + 3.318
	10 Days	R^2= -0.777	Y = -0.023x +3.516

Table 3: Effect of inorganic chemicals as inducer on total soluble protein in potato leaves at different days of intervals

Name of Inducers	Soluble protein content at different days of interval (mg/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	After application							
		2 Days	4 Days	6 Days	8 Days	10 Days			
SA @ 10 mM	21.44	33.07	35.27	35.66	35.87	34.48	39.21	31.50	28.43
$CaCl_2$@ 10 mM	21.37	32.77	34.47	34.57	34.69	33.15	38.39	29.17	26.00
HP @10ppm	21.30	32.13	33.38	33.77	34.00	32.38	37.35	27.73	24.50
Metalaxyl @ 0.1%	21.20	30.29	32.67	32.78	32.90	31.58	35.56	25.32	21.97
DPHP @0.2%	21.12	30.20	32.00	32.50	32.70	31.50	34.00	24.86	21.49
$FeCl_{3@}$ 5mM	21.00	30.10	31.70	32.58	32.60	31.40	33.75	24.63	21.25
$CuSO_4$ @10mM	20.87	27.65	29.13	29.47	29.67	28.47	29.65	17.18	13.48
IAA @1%	20.77	26.17	28.27	28.57	28.72	27.03	27.68	14.44	10.61
$CuCl_{2\ @}$10mM	20.74	26.00	27.27	27.49	27.96	26.55	25.82	12.12	8.19
Control-1	20.65	22.32	24.27	24.37	24.57	23.56	15.95	-	-4.47
Control-2	20.59	23.47	25.37	25.57	25.67	24.12	19.78	4.28	-
C.D.P=(0.05)	1.311	1.801	1.873	1.895	1.881	1.884			
S.E (m)	0.444	0.606	0.630	0.638	0.633	0.634			
S.E (d)	0.628	0.858	0.892	0.902	0.896	0.897			
C.V.	3.610	3.634	3.644	3.633	3.628	3.628			

Table 4: Effect of inorganic chemicals as inducer on total phenol in potato leaves at different days of intervals

Name of Inducer	Total phenol content at different days of interval (mg/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	After application							
		2 Days	4 Days	6 Days	8 Days	10 Days			
SA @ 10 mM	1.57	2.64	3.17	3.27	3.34	3.29	53.00	45.50	44.01
$CaCl_2$@ 10 mM	1.46	2.61	2.67	2.87	3.00	2.78	49.12	39.33	37.66
HP @10ppm	1.43	2.56	2.63	2.74	2.79	2.63	48.74	34.76	32.97
Metalaxyl @ 0.1%	1.42	1.49	2.53	2.69	2.76	2.59	48.55	34.05	32.24
DPHP @0.2%	1.39	1.37	2.45	2.53	2.67	2.56	47.94	31.83	29.96
$FeCl_{3@}$ 5mM	1.37	1.27	2.37	2.44	2.49	2.43	44.97	26.90	24.89
$CuSO_4$ @10mM	1.36	1.22	2.28	2.39	2.46	2.38	44.71	26.01	23.98
IAA @1%	1.34	1.21	2.25	2.36	2.41	2.35	44.39	24.48	22.40
$CuCl_{2\ @}$10mM	1.34	1.20	2.23	2.34	2.39	2.31	43.93	23.84	21.75
Control-1	1.12	1.17	1.27	1.57	1.82	1.71	38.46	-	-2.74
Control-2	1.14	1.19	1.37	1.62	1.87	1.76	39.03	2.67	-
C.D.P=(0.05)	0.083	0.107	0.145	0.153	0.158	0.152			
S.E (m)	0.028	0.036	0.049	0.052	0.053	0.051			
S.E (d)	0.040	0.051	0.069	0.074	0.075	0.073			
C.V.	3.621	3.840	3.689	3.676	3.653	3.659			

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