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Research Article
Evaluation of Inducers in Systemic Acquired Resistance for Management of Brinjal Phomopsis Blight

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Abstract

Background and Objective: The plant posses inducible biochemical defense response that is activated upon contact with some biotic and abiotic inducers in systemic acquired resistance. Therefore, the study was undertaken to evaluate inducers in induced resistance for management of phomopsis blight of brinjal. **Materials and Methods:** The experiment was conducted by using biotic and abiotic inducers in induced resistance against the phomopsis blight in brinjal. The defense molecules like soluble protein, total phenol content and defense enzymes in brinjal plant after treatment with different inducers followed by inoculation of pathogen was measured at 2, 6 and 10 days separately accounting to the standard procedures developed by various scientists. Correlation-coefficient (r) between soluble protein, total phenol content and defense enzymes with disease incidence were calculated by standard statistical calculation. **Results:** All the treatments were able to reduce the disease severity from 22.90-2.02% at 2 days, 33.92-4.05% at 6 days and 34.78-5.00% at 10 days after inoculation with the minimum in salicylic acid treated plants. The treated plants sensitize to produce increased level of soluble protein, total phenol and defense enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia lyase contents. The correlation regression equation showed that negative correlation (r) -0.920, -0.555, -0.835, -0.888 and -0.885 was found between total protein, total phenol and defense related enzymes with disease severity at 2 days of pathogen inoculation respectively. Similar observations as negative correlation (r) have also been found at 6 days and 10 days of pathogen inoculation. **Conclusion:** Prior to pathogenic inoculation, foliar sprayed with salicylic acid sensitize the plant to produced increase level of total phenol, soluble protein and defense enzymes and can be recommended for management of phomopsis blight of brinjal in near future.

Key words: Phomopsis blight, inducers, soluble protein, total phenol, defense enzyme, correlation regression

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Eggplant (*Solanum melongena* L.) commonly known as brinjal is a typical and well known vegetable, grown particularly in the sub-tropical and tropical areas of the world. The crop is suffered by number of diseases caused by fungi, bacteria, virus, nematodes, MLO etc. Among these, leaf blight and fruit rot caused by *Phomopsis vexans* (Sacc. and Syd.) is a major concerned in all brinjal growing areas of India as well as in the world. The pathogen is perpetuated in seed and infected crop residue, present in the soil. Management of the disease can be done through cultural, chemicals, biological and use of resistant varieties. But all these conventional methods are trend to direct control of pests and diseases by their elimination. Moreover, in some cases these practice raised problems due to development of resistant strains among the pathogens, they are also hazarded to environment. Therefore, there is a search for new strategy which can supplement all traditional system of plant disease management. In this context, induce resistance a new and innovative technique search out to overcome production problems of the most serious and destructive phomopsis blight disease in brinjal caused by *Phomopsis vexans*. Several bio-agents, inorganic chemicals, a virulent race of pathogen, secondary metabolites, plant extracts, chemicals which are not considered as fungicides were used as inducers in induced resistance in plant through activation of a plant's defense response conveyed by many researchers[1-5].

A numbers of induced resistance inducers have been evaluated for management of several diseases like bio-agents in tomato against Fusarium wilt[6], in rice against brown leaf spot[7], avirulent races in wheat against spot blotch[8], secondary metabolites of *Chaetomium globosum* in wheat against spot blotch[3], inorganic chemical in tomato against Fusarium wilt[9], in potato against late blight[10], plant extract in tomato against Fusarium wilt[11], salicylic acid in squash against Phytophthora blight[12] and in squash and tobacco against cucumber mosaic virus (CMV)[13].

Biochemical changes associated with indication of resistance due to increase the activity of peroxidase, phenylalanine ammonia lyase, chalcone isomerase and reactive oxygen species and also increase levels of soluble proteins and total phenol content are reported by Malolepsza[2], Biswas *et al.*[14], Kumar *et al.*[15] and Buzi *et al.*[16]. Biswas *et al.*[3]. found that a new protein of different molecular weight was synthesized due to pre-application of crude extract of *C. globosum* against *Drechslera sorokiniana.* Bokshi *et al.*[17] indicated that dichloroisonicotinic acid increased chitinase and peroxidase activities and reduced powdery mildew and downy mildew on leaves of melons. Enhanced resistance in tomato against *P. infestans* was correlated with the accumulation of pathogenesis related proteins such as PR-1 chitinase, Beta-1, 3-glucanase[18,19]. Keeping the above fact in view, it is considered imperative to carry out the study entitled "Evaluation of inducers in systemic acquired resistance for management of brinjal Phomopsis blight".

MATERIALS AND METHODS

Isolation, purification and identification of the pathogen: Brinjal plants showing typical blight symptoms were first identified and then collected from Vegetable Research Farm, Chandra Shekhar Azad University of Agriculture and Technology, Kalyanpur (Kanpur), Uttar Pradesh, India during May-June in 2016-17. 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 blotter paper. The tissue pieces were placed at the center of Petri plate which was previously filled with PDA medium. The plates were then incubated at 26±2°C. The Petri plates were observed daily at 24 h 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.

Collection and preparation of inorganic chemicals as inducer: The inorganic chemicals were collected from laboratory of the Department of Plant Pathology and some are purchases from local market. The chemical inducers used in the present study, different concentrations of inducers were prepared by weighing required quantity of inducers separately and placed in conical flask containing 100 mL of sterilized water. Each conical flask was shaken until they are dissolved completely to prepare the required concentration of solutions (Table 1).

Collection of bio agents and plant extracts: The bio-agents like *Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were collected from Department of Plant Pathology, CSAUA and T, Kanpur and plant parts were collected around the vicinity area of this University.

Table 1: Details of concentrations of inducers

Sl. No.	Treatments	Concentrations seedling treatments and foliar spray
T_1	Salicylic acid	10 Mm
T_2	Calcium chloride	10 ppm
T_3	Di-potassium hydrogen orthophosphate	10 Mm
T_4	Hydrogen peroxide	1.0%
T_5	Ferric chloride	5 Mm
T_6	Indole-3 acetic acid	0.2%
T_7	Metalaxyl	0.2%
T_8	Ascorbic acid	0.2%
T_9	*Trichoderma viride*	10^8 CFU mL^{-1}
T_{10}	*T. harzianum*	10^8 CFU mL^{-1}
T_{11}	*Bacillus subtilis*	10^8 CFU mL^{-1}
T_{12}	*P. fluorescens*	10^8 CFU mL^{-1}
T_{13}	Neem leaf extract	5 mL L^{-1}
T_{14}	Datura leaf extract	5 mL L^{-1}
T_{15}	Untreated control	-

Preparation of solution of bio-agents and pathogen: The Petri plate containing 10 days old culture of bio-agent and *Phomopsis vexans* 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 using 250 µL spore suspension which 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 conidial suspension was adjusted to 4.5×0^4 conidia mL^{-1}.

Procedure of solution preparation: One millimolar solution preparation can be calculated as:

$$EV = \frac{N}{M}\times 1000$$

where, E is the equivalent/molecular weight of particular chemical, V is the required volume needed, N/M is the normality/molarities of concentration of particular chemical needed.

Weight calculated amount of chemical inducers by electronic weighing scale. Dissolved weighted quantity of chemical inducers in 500 mL of distilled water in a 500 mL of beaker and shake well until all the required quantity of chemical has dissolved. Add this solution in 1000 mL of measuring cylinder and make the final volume, 1000 mL by adding distilled water for final concentration of required chemical inducers (mM L^{-1}).

PPM solution preparation: One ppm is equivalent to 1 mg of something L^{-1} of water (mg L^{-1}) or 1 mg of something kg^{-1} soil (mg kg^{-1}).

Effect of different inducers on disease severity of phomopsis blight and biochemical changes in brinjal: The experiment was conducted in the wire house complex of Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur during 2016-17. Seedlings were transplanted after giving treatments with different inducers separately. At 55 days after transplanting, plants were sprayed with different inducers separately (Table 1) and after 48 h, plants were inoculated with spore suspension of test pathogen @4.5×10^4 spore mL^{-1}. 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 only (Check-1) and in second case; plants were inoculated using spore suspension of *P. vexans* (Check-2). Three replications were kept for each treatment. The observations were taken on severity of disease and change defense molecules. All treatments were applied as seedling and foliar application on brinjal plants twice at 15 days interval.

Measurement of disease severity: The severity on leaf blight was scored on 0-5 point scale Horsfall and Baratt[20] which is given as under:

Grades	Leaf blight (%)
0	Free from infection
1	1-10% are affected
2	10.1-25% are affected
3	25.1-50% are affected
4	50.1-75% are affected
5	>75% are affected

$$\text{Disease severity (PDS \%)} = \frac{\text{Sum of all numerical value}}{\text{Total number of leaves examined}}\times 100 \times \text{Maximum disease grade}$$

Biomolecules changes in brinjal plant

Estimation of soluble protein: The method developed by Lowry *et al.*[21] was used with slight modification to estimate the total soluble protein content in the leaves of each treatment. Brinjal leaves from different treatments were harvested, washed with distilled water several times and blotter dried before protein extraction. A quality of 1.0 g of

each sample was cut into small pieces and grinded in pre-chilled pistil and mortar using 1:5 leaves: extraction buffer. The suspension was centrifuged at 10,000 rpm for 30 min at 4°C. The supernatant was collected. A quantity of 7.5 mL of the supernatant was transferred in a tube and mixed with 2.5 mL of sample buffer and used for protein estimation. The working standard solution was pipette out (0.2, 0.4, 0.6, 0.8 and 1.0 mL), was put into series of test tubes. A quantity of 0.2, 0.4, 0.6, 0.8 and 1.0 mL of the sample extract was also pipette out and kept into other test tubes separately. Then volumes in all the tubes were made up to 1 mL with distilled water. A tube with 1 mL of water served as a blank. Later on, 5 mL of solution C was added in each test tube and incubated at room temperature for 10 min. Thereafter, 0.5 mL of FCR was mixed well immediately and incubated at room temperature for 30 min in dark place. The total soluble protein content was measured by Double beam UV visible spectrophotometer at 660 nm wave length. The content of soluble protein in leaves was express as mg g^{-1} of fresh leave.

Estimation of total phenol contents: The accumulation of total phenols in brinjal plants after treatment with different inducers and followed by inoculation of pathogen was estimated Bray and Thrope[22].

For estimations of phenol, 1.0 g of leaf sample of brinjal 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 min at room temperature. Supernatant was separated and re-extracted for 5 times with required volume of 80% ethanol, centrifuged and the supernatant were pooled. It was then evaporated to dryness and residues were dissolved in 5 mL of distilled water. Different aliquots (0.2, 0.4, 0.6, 0.8 and 1.0 mL) were pipette out into test tubes and the volume in each tube was made to 3 mL with water. Subsequently, 0.5 mL of FCR was added and after 3 min, 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) spectrophotometer and the standard curve using different concentration of phenols was prepared. From the standard curve, the concentration of phenols in the test sample was determined and expressed as mg phenols per gm of sample materials.

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

Estimation of defense enzymes during pathogenesis: The activity of defense enzymes like peroxidase, PPO and PAL in brinjal plants after treatment with different inducers, followed by inoculation with test pathogen was assessed. The 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, 6 and 10 days after pathogen inoculation was measured separately as per procedure developed by Hammerschmidt *et al.*[23], Malik and Singh[24] and Burrell and Ress[25] for Peroxidase (PO), Polyphenol oxidase (PPO) and Phenylalanine ammonia lyase (PPL) respectively.

Statistical analysis: All statistical analysis was done by using SPSS ver. 16. The results are the mean of the three replicates of each treatment. The data was statistically analyzed by using one-way analysis of variance (ANOVA) and mean separations were compared with Duncan's multiple range tests at the $p \leq 0.05$ significance level. Differences at $p \leq 0.05$ were considered to be significant.

RESULTS

Influence of inducers on severity of phomopsis blight in brinjal: Pre-inoculation with inorganic chemicals, bio control agents and plant extracts as inducers proved effective in reducing the disease severity as compared to control (Fig. 1). Salicylic acid as inducers has found most effective to minimize disease severity as 2.02, 4.05 and 5.0% at 2, 6 and 10 days of pathogen inoculation, respectively, followed by calcium chloride as 2.15, 4.95 and 5.10%. The Ferric chloride treated plants were showing 6.10, 6.90 and 10.20% disease severity at 2, 6 and 10 days of pathogen inoculation which are superior to control but inferior to calcium chloride and hydrogen peroxide treated plant. From the table, it is also cleared, that all the inducers treated brinjal plants were showing comparatively low disease severity as compare to control-1 and control-2. The decrease in disease severity might be due to the activity of inducers which stimulate to synthesis of some defense related compounds in brinjal plant against *P. vexans*.

Influence of inducers on synthesis of defense molecules in brinjal leaves at different days of intervals during pathogenesis

Soluble protein: Protein is an important molecules synthesized in plant due to effect of inducers which act as a defense molecules in plant against several diseases. The data presented in Table 2 showed that total soluble protein content in brinjal leaves ranges from 17.70-20.40 mg g^{-1} of fresh leaves in case of before application whereas, in case of after

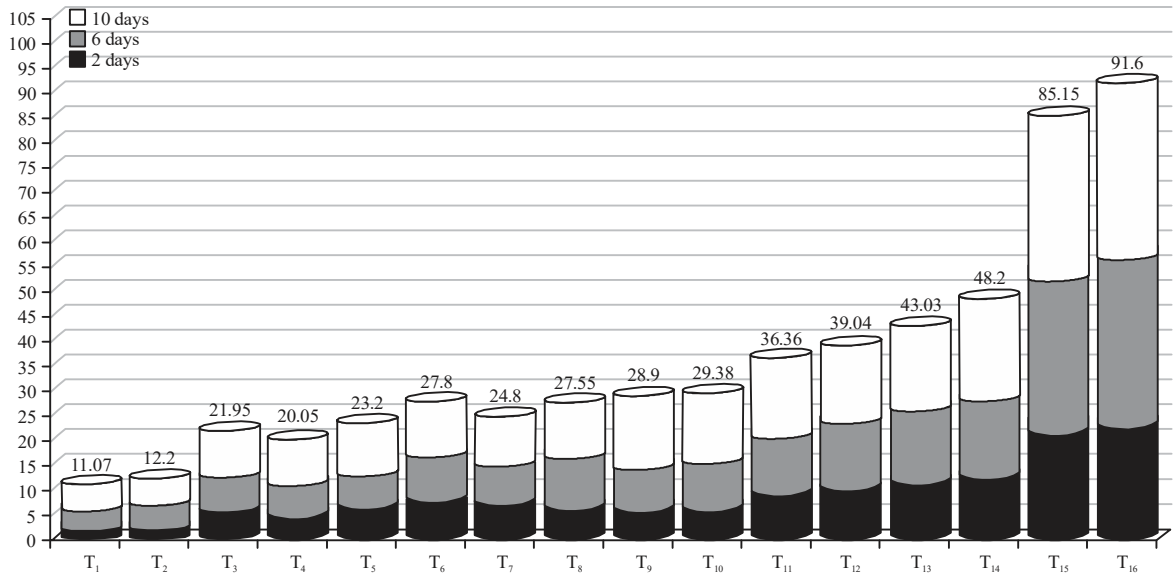

Fig. 1: Effect of biotic and abiotic inducers on disease severity of phomopsis blight of brinjal in leaves

T_1: Salicylic acid, T_2: Calcium chloride, T_3: Di-potassium hydrogen ortho phosphate, T_4: Hydrogen peroxide, T_5: Ferric chloride, T_6: Indole-3 acetic acid, T_7: Metalaxyl, T_8: Ascorbic acid, T_9: *T. viride*, T_{10}: *T. harzianum*, T_{11}: *Bacillus subtilis*, T_{12}: *P. fluorescens*, T_{13}: Neem leaf extract, T_{14}: Datura leaf extract, T_{15}: Control-1, T_{16}: Control-2

Table 2: Influence of inducers on activity of soluble protein content in brinjal leaves at different days of intervals during pathogenesis (mg g^{-1} of fresh leaves)

	Soluble protein content at different days of interval (mg g^{-1} of fresh leaves)				Increase (%) over,	Increase (%)	Increase (%)
Treatments	Before application of inducers	2 days	6 days	10 days	before application of inducers	over control-1 (at 6 days)	over control-2 (at 6 days)
T_1	20.40	31.56	36.06	35.76	43.42	29.89	30.69
T_2	20.05	30.82	34.78	34.56	42.35	27.31	28.14
T_3	20.00	29.45	33.69	33.86	40.63	24.96	25.82
T_4	19.80	29.06	32.99	32.76	39.98	23.37	24.24
T_5	19.79	28.15	32.87	32.65	39.79	23.09	23.97
T_6	19.77	28.10	32.82	32.60	39.76	22.97	23.85
T_7	19.75	27.18	32.77	32.55	39.73	22.85	23.74
T_8	19.73	27.00	32.75	32.50	39.75	22.80	23.69
T_9	19.02	26.90	31.10	32.45	38.84	18.71	19.64
T_{10}	18.81	26.85	30.50	31.25	38.32	17.11	18.06
T_{11}	18.79	26.50	29.76	29.22	36.86	15.05	16.02
T_{12}	18.75	26.28	28.81	28.36	34.91	12.25	13.25
T_{13}	18.60	26.10	27.78	27.36	33.04	08.99	10.04
T_{14}	18.10	23.50	26.78	26.15	32.41	05.60	06.68
T_{15}	17.75	21.20	25.28	25.75	29.78	-	01.14
T_{16}	17.70	22.50	24.99	23.23	29.17	-0.01	-
CDP = (5)	1.054	1.063	1.048	1.535	-	-	-
SE (m)	0.562	0.510	0.512	0.750	-	-	-
SE (d)	0.334	0.342	0.362	0.531	-	-	-
CV	2.015	2.165	2.018	2.987	-	-	-

T_1: Salicylic acid, T_2: Calcium chloride, T_3: Di-potassium hydrogen ortho phosphate, T_4: Hydrogen peroxide, T_5: Ferric chloride, T_6: Indole-3 acetic acid, T_7: Metalaxyl, T_8: Ascorbic acid, T_9: *T. viride*, T_{10}: *T. harzianum*, T_{11}: *Bacillus subtilis*, T_{12}: *P. fluorescens*, T_{13}: Neem leaf extract, T_{14}: Datura leaf extract, T_{15}: Control-1, T_{16}: Control-2

application it ranges from 21.20-31.56, 24.99-36.06 and 23.23-35.76 mg g^{-1} of fresh leaves at 2, 6 and 10 days of inoculation, respectively. The highest content of total soluble protein was recorded from salicylic acid treated brinjal leaves, indicating 31.56, 36.06 and 35.76 mg g^{-1} of fresh leaves against 21.20, 25.28 and 25.75 mg g^{-1} in case of control-1 and 22.50, 24.99 and 23.23 mg g^{-1} of fresh leaves in case of control-2 at 2, 6 and 10 days of pathogen inoculation. The salicylic acid treated brinjal plants possess 29.89 and 30.69% increase of total soluble protein over control-1 and over control-2 at 6 days of pathogen inoculation, respectively.

Table 3: Consequence of biotic and abiotic inducers on activity of total phenol content in brinjal leaves at different days of intervals during pathogenesis (mg g^{-1} of fresh leaves)

	Total phenol content at different days of interval (mg g^{-1})				Increase (%) over, before application	Increase (%) over control-1	Increase (%) over control-2
Treatments	Before application of inducers	2 days	6 days	10 days	of inducers	(at 6 days)	(at 6 days)
T_1	1.57	2.63	3.37	3.19	53.41	64.39	67.35
T_2	1.43	2.58	2.89	2.69	51.52	59.30	62.71
T_3	1.41	2.55	2.95	2.65	51.21	58.47	61.93
T_4	1.47	1.44	2.81	2.57	47.68	57.29	60.85
T_5	1.42	1.39	2.68	2.47	47.01	55.22	58.95
T_6	1.36	1.35	2.40	2.39	43.33	50.00	54.16
T_7	1.31	1.25	2.35	2.30	44.25	48.93	53.19
T_8	1.30	1.22	2.32	2.29	43.96	48.27	52.58
T_9	1.28	1.20	2.28	2.26	43.85	47.36	51.75
T_{10}	1.28	1.19	2.25	2.25	43.11	46.66	51.11
T_{11}	1.27	1.20	2.16	2.23	41.20	44.44	49.07
T_{12}	1.26	1.17	2.11	2.22	40.28	43.10	47.86
T_{13}	1.25	1.15	2.08	2.19	42.39	44.70	49.30
T_{14}	1.24	1.11	2.17	2.15	40.38	42.30	47.11
T_{15}	1.08	1.10	1.20	1.25	10.00	-	08.33
T_{16}	1.05	1.09	1.31	1.18	04.00	-0.09	0
CDP = (5)	0.086	0.089	0.095	0.10	-	-	-
SE (m)	0.035	0.042	0.046	0.05	-	-	-
SE (d)	0.024	0.032	0.033	0.035	-	-	-
CV	2.350	2.391	2.430	2.718	-	-	-

T_1: Salicylic acid, T_2: Calcium chloride, T_3: Di-potassium hydrogen ortho phosphate, T_4: Hydrogen peroxide, T_5: Ferric chloride, T_6: Indole-3 acetic acid, T_7: Metalaxyl, T_8: Ascorbic acid, T_9: *T. viride*, T_{10}: *T. harzianum*, T_{11}: *Bacillus subtilis*, T_{12}: *P. fluorescens*, T_{13}: Neem leaf extract, T_{14}: Datura leaf extract, T_{15}: Control-1, T_{16}: Control-2

From the Table 2, it is also cleared that among the different days of interval, the maximum concentration of soluble protein was found at 6 days of pathogen inoculation in all the treatments, thereafter, it was declined gradually.

Total phenol content: Phenol has important antifungal, anti-bacterial and anti-viral properties and is an important defense compound synthesized in plant due to effect of inducers. The result presented in Table 3 shows that all the treatment significantly increased the total phenol content as compared to control-1 and control-2 at 2, 6 and 10 days of pathogen inoculation. The maximum total phenol content was found in salicylic acid treated brinjal leaves which were 2.63, 3.37 and 3.19 mg g^{-1} of fresh leaves at 2, 6 and 10 days of pathogen inoculation, respectively whereas, in case of control-1, the values are 1.10, 1.20 and 1.25 mg g^{-1} and for control-2, the value are 1.09, 1.31, 1.18 mg g^{-1} of fresh leaves. The salicylic acid treated brinjal leaves possess increased percent of total phenol as 64.39% over control-1 and 67.35% over control-2 at 6 days of pathogen inoculation. The bio-agents treated plant also showing increase content of total phenol over both the controls but inferior to salicylic acid and calcium chloride treated plants. From the table it is also cleared that the total phenol content was increase from 2-6 days and thereafter decline. Comparing among inducers viz. inorganic chemicals, bio-agents and botanicals, the maximum content of phenol was found in case of inorganic chemical treated plants.

Peroxidase activity: The results presented in Table 4 indicated that the activity of peroxidase was significantly increased in all treated leaves of brinjal as compare to controls. The highest per cent increased activity of peroxidase before and after application of inorganic inducers is found in salicylic acid treated brinjal leaf which was 44.26% at maximum 6 days of pathogen inoculation. The salicylic acid treated brinjal leaves showed maximum with 2.30, 2.44 and 2.38 min g^{-1} of fresh leaves at 2, 6 and 10 days, pathogen inoculation respectively, which is 40.57% increase over control-1 and 44.26% over control -2 at 6 days of pathogen inoculation. The calcium chloride treated plant indicating 2.20, 2.39 and 2.34 min g^{-1} of fresh leaves at 2, 6 and 10 days of pathogen inoculation which is 39.33% increase over control-1 and 43.09% over control-2 at 6 days of pathogen inoculation. The bio-agents and botanicals treated plants were also showing increase activity of peroxidase but inferior to inorganic chemical (salicylic acid) treated plants. The increased activity of peroxidase in treated plants might be responsible for defense response in plant against Phomopsis blight.

Polyphenol oxidase: Polyphenol oxidase is another important defense molecule, synthesized in plant due to effect of inducers in plant. The data presented in Table 5 showed that among the treatments, the maximum activity of polyphenol oxidase is found in salicylic acid treated brinjal leaves, representing the value of is 0.95, 1.90 and 1.71 min g^{-1} of fresh leaves against control-1 as 0.50, 1.15 and 1.25 mg g^{-1}

Table 4: Influenced of inducers on synthesis of peroxidase in brinjal leaves at different days of intervals during pathogenesis (min g^{-1} of fresh leaves)

	Activity of enzyme peroxidase at different days of inoculation				Increase (%) over, before application	Increase (%) over control-1	Increase (%) over control-2
Treatments	Before application of inducers	2 days	6 days	10 days	of inducers	(at 6 days)	(at 6 days)
T_1	1.65	2.30	2.44	2.38	32.37	40.57	44.26
T_2	1.64	2.20	2.39	2.34	31.38	39.33	43.09
T_3	1.63	1.93	2.32	2.25	29.74	37.50	41.37
T_4	1.62	1.89	2.22	2.16	27.02	34.68	38.73
T_5	1.60	1.90	2.07	2.02	22.70	29.95	34.29
T_6	1.59	1.80	2.04	1.92	22.05	28.92	33.33
T_7	1.57	1.76	1.97	1.82	20.30	26.39	30.96
T_8	1.56	1.72	1.95	1.80	20.00	25.64	30.25
T_9	1.55	1.67	1.90	1.69	18.42	23.68	28.42
T_{10}	1.53	1.63	1.85	1.66	17.29	21.62	26.48
T_{11}	1.52	1.60	1.72	1.65	11.62	15.69	20.93
T_{12}	1.51	1.58	1.68	1.64	10.11	13.69	19.04
T_{13}	1.50	1.55	1.66	1.62	09.63	12.65	18.07
T_{14}	1.49	1.45	1.64	1.58	09.15	11.58	17.07
T_{15}	1.35	1.37	1.45	1.38	06.89	0	06.20
T_{16}	1.30	1.32	1.360	1.34	04.41	-0.066	-
CDP = (5)	0.102	0.104	0.106	0.084	-	-	-
SE (m)	0.053	0.050	0.052	0.041	-	-	-
SE (d)	0.035	0.034	0.037	0.029	-	-	-
CV	3.154	3.024	3.295	2.745	-	-	-

T_1: Salicylic acid, T_2: Calcium chloride, T_3: Di-potassium hydrogen ortho phosphate, T_4: Hydrogen peroxide, T_5: Ferric chloride, T_6: Indole-3 acetic acid, T_7: Metalaxyl, T_8: Ascorbic acid, T_9: *T. viride*, T_{10}: *T. harzianum*, T_{11}: *Bacillus subtilis*, T_{12}: *P. fluorescens*, T_{13}: Neem leaf extract, T_{14}: Datura leaf extract, T_{15}: Control-1, T_{16}: Control-2

Table 5: Consequence of inducers on activity of Polyphenol oxidase in brinjal leaves at different days after pathogen inoculation during pathogenesis (min g^{-1} of fresh leaves)

	Activity of enzyme polyphenol oxidase at different days of interval in (min g^{-1}) of fresh leaves				Increase (%) over, before application	Increase (%) over control-1	Increase (%) over control-2
Inducers	Before application of inducers	2 days	6 days	10 days	of inducers	(at 6 days)	(at 6 days)
T_1	0.63	0.95	1.90	1.71	66.84	39.47	44.73
T_2	0.62	0.93	1.78	1.68	65.16	35.39	41.01
T_3	0.61	0.91	1.73	1.65	64.73	33.52	39.30
T_4	0.60	0.87	1.70	1.62	64.70	32.35	38.23
T_5	0.59	0.88	1.65	1.58	64.24	30.30	36.36
T_6	0.58	0.85	1.62	1.55	64.19	29.01	35.18
T_7	0.57	0.82	1.58	1.51	63.92	27.21	33.54
T_8	0.55	0.79	1.50	1.48	63.33	23.33	30.00
T_9	0.54	0.75	1.48	1.42	63.51	22.29	29.05
T_{10}	0.53	0.71	1.42	138.00	62.67	19.01	26.05
T_{11}	0.51	0.67	1.36	1.36	60.25	15.44	22.79
T_{12}	0.50	0.65	1.30	1.35	61.53	11.53	19.23
T_{13}	0.48	0.59	1.25	1.31	61.60	0.80	16.00
T_{14}	0.47	0.56	1.20	1.28	60.83	04.16	12.50
T_{15}	0.46	0.50	1.15	1.25	60.00	0.00	08.69
T_{16}	0.44	0.47	1.05	1.22	58.09	-0.095	0.00
CDP = (5)	0.051	0.062	0.069	0.065	-	-	-
SE (m)	0.025	0.030	0.034	0.032	-	-	-
SE (d)	0.018	0.21	0.024	0.022	-		-
CV	2.321	2.490	2.623	2.747	-	-	-

T_1: Salicylic acid, T_2: Calcium chloride, T_3: Di-potassium hydrogen ortho phosphate, T_4: Hydrogen peroxide, T_5: Ferric chloride, T_6: Indole-3 acetic acid, T_7: Metalaxyl, T_8: Ascorbic acid, T_9: *T. viride*, T_{10}: *T. harzianum*, T_{11}: *Bacillus subtilis*, T_{12}: *P. fluorescens*, T_{13}: Neem leaf extract, T_{14}: Datura leaf extract, T_{15}: Control-1, T_{16}: Control-2

and control-2, as 0.47, 1.05 and 1.22 mg g^{-1} of fresh leaves at 2, 6 and 10 days of pathogen inoculation respectively. From the table, it is also cleared that the activity of polyphenol oxidase in all the treated plants expanded up to a specific period of time and then declined. The expanded activity of polyphenol oxidase in treated plants may be in charge of barrier reaction in plant against *P. vexans*.

Table 6: Consequence of inducers on activity of phenylalanine ammonia lyase in brinjal leaves at different days of intervals during pathogenesis (mg t-cinnamic acid produced/h/g of fresh leaves)

Inducers	Activity of enzyme phenylalanine ammonia lyase at different days of interval (min g^{-1})				Increase (%) over, before application of inducers	Increase (%) over control-1 (at 6 days)	Increase (%) over control-2 (at 6 days)
	Before application of inducers	2 days	6 days	10 days			
T_1	0.82	0.87	1.09	1.05	21.90	37.14	40.00
T_2	0.81	0.83	1.06	0.99	18.18	3333	36.36
T_3	0.79	0.82	0.99	0.96	17.70	3125	34.37
T_4	0.77	0.80	0.97	0.92	16.30	2826	31.52
T_5	0.75	0.78	0.94	0.89	15.73	2584	29.21
T_6	0.73	0.77	0.89	0.86	15.11	2325	26.74
T_7	0.71	0.74	0.86	0.84	15.47	2142	25.00
T_8	0.70	0.73	0.81	0.80	12.50	175	21.25
T_9	0.68	0.69	0.78	0.77	11.68	1428	18.18
T_{10}	0.67	0.68	0.75	0.74	09.45	1081	14.86
T_{11}	0.66	0.67	0.73	0.72	08.33	0833	12.50
T_{12}	0.64	0.66	0.72	0.71	08.45	0704	11.26
T_{13}	0.63	0.64	0.71	0.69	07.24	0434	08.69
T_{14}	0.60	0.61	0.70	0.68	05.88	0294	07.35
T_{15}	0.57	0.58	0.67	0.66	04.54	0	04.54
T_{16}	0.52	0.53	0.63	0.61	01.58	-0.0476	0
CDP = (5)	0.029	0.030	0.033	0.040	-	-	-
SE (m)	0.014	0.015	1.09	0.019	-	-	-
SE (d)	0.010	0.010	1.06	0.014	-	-	-
CV	2.572	2.508	0.99	2.729	-	-	-

T_1: Salicylic acid, T_2: Calcium chloride, T_3: Di-potassium hydrogen ortho phosphate, T_4: Hydrogen peroxide, T_5: Ferric chloride, T_6: Indole-3 acetic acid, T_7: Metalaxyl, T_8: Ascorbic acid, T_9: *T. viride*, T_{10}: *T. harzianum*, T_{11}: *Bacillus subtilis*, T_{12}: *P. fluorescens*, T_{13}: Neem leaf extract, T_{14}: Datura leaf extract, T_{15}: Control-1, T_{16}: Control-2

Table 7: Correlation of disease severity with total soluble protein, total phenol peroxidase, polyphenol oxidase and phenylalanine ammonia lyase content of brinjal leaves

Biochemical parameters	Days after pathogen inoculation	Correlation coefficient (r) with disease severity	Regression equation
Total soluble protein	2	-0.920	y = -0.4288x+30.696
	6	-0.712	y = -0.2826x+34.546
	10	-0.908	y = -0.4009x+36.765
Total phenol	2	-0.555	y = -0.0522x+1.9325
	6	-0.907	y = -0.0589x+3.0521
	10	-0.957	y = -0.0547x+3.0718
Peroxidase	2	-0.835	y = -0.0381x+2.0624
	6	-0.851	y = -0.0321x+2.3087
	10	-0.797	y = -0.0280x+2.2620
Polyphenol oxidase	2	-0.888	y = -0.0233x+0.9473
	6	-0.853	y = -0.0246x+1.7796
	10	-0.022	y = -0.0904x+11.327
Phenylalanine ammonia lyase	2	-0.885	y = -0.0221x+0.8255
	6	-0.737	y = -0.0139x+1.029
	10	-0.879	y = -0.0124x+0.9359

Phenylalanine ammonia lyase (PAL): Inorganic chemicals, bio-agents and botanicals as inducers have ability to increase Phenylalanine ammonia lyase (PAL) activities in treated plants. The data presented in the Table 6 showed that the maximum activity of phenylalanine ammonia lyase was recorded in salicylic acid treated brinjal leaves with the value of 0.87, 1.09 and 1.05 mg t-cinnamic acid produced/h/g of fresh leaves at 2, 6 and 10 days, of pathogen inoculation respectively, which is increased as 37.14% over control-1 and 40.00% over control-2 which was followed by calcium chloride treated plant as 0.83, 0.99 and 1.06 mg t-cinnamic acid produced/h/g of fresh leaves. The lowest quantity of enzyme phenylalanine ammonia lyase was observed in datura leaf extract treated brinjal leaves as 0.61, 0.68 and 0.67 mg t-cinnamic acid produced/h/g fresh leaves of brinjal at 2, 6 and 10 days, respectively. From the table, it is cleared that all treatments increase the activity of the phenylalanine ammonium lyase at 6 days of pathogen inoculation it was gradually decrease. Enhanced activity of the PAL in the treated plants may be responsible for the defensive response in the plant against *P. vexans*.

Correlation of disease severity with induced defense molecules in brinjal leaves: The results presented in Table 7 revealed that the leaves treated with inorganic chemicals, bio-agents and botanicals as inducer decreases disease

severity with increased level of soluble protein, total phenol and accumulation of defense related enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in of brinjal leaves. The correlation regression equation showed that negative correlation as (r) -0.920, -0.555, -0.835, -0.888 and -0.885 between total protein, total phenol, 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 soluble protein, total phenol and defense related enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia lyase have negative role in increase disease development.

DISCUSSION

Induced resistance is a new, eco-friendly non-conventional method of plant disease management which can enhance defense response in plant has received great attention in recent years. In the present study, seedling treatment and foliar spray with inorganic chemicals, bio control agents and botanicals as inducers significantly reduced disease severity of Phomopsis blight of brinjal. Mosa[26] reported that abiotic agents, viz. hydrogen peroxide, di-potassium hydrogen phosphate, salicylic acid and benzothiadiazole (BTH) and biotic agent, *P. fluorescens* provided induce resistance in rice against blast caused by *Pyricularia grisea*. Ata *et al.*[27], also used five chemical compounds, i.e., hydrogen peroxide (H_2O_2) at 0.25, 0.50 and 1.0%, salicylic acid (SA), mono and di-basic potassium phosphate (KH_2PO_4) and Bion [acibenzolar-S-methyl] (BTH) at 2, 4 and 8 mM, were applied as a foliar spray to evaluate their capabilities to induce resistance against rust disease of sugar beet caused by *Uromyces betae* in green house and field in Egypt.

High content of soluble protein which is an indicator of first stage of defense mechanism was also found increased in inducers treated brinjal plants against *P. vexans*. Antoniw *et al.*[4] considered that pathogen related proteins (PR protein) are involved in plant defense response to pathogens. Boller[28] was also opined that proteins are associated with defense in plants against fungi and bacteria. Metraux *et al.*[29] and Tuzun *et al.*[30], also reported that proteins forms of chitinases and β-1, 3 glucanase may be involved in the defense of plants against fungi and bacteria by their action on the cell walls of invading pathogen. Pre-application of abiotic inducers sensitized the seedling to produce increased level of soluble protein in many plants[3,6,9].

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 to pathogen[31]. In the present study also, total phenol content was increased in all the inducers treated plants. Vimala and Suriachandraselvan[32] noticed higher accumulation of phenolics in plants due to pre-treated with salicylic acid (1 mM) resulted enhance in resistance against invasion of *Erysiphe cichoracearum* in bhendi. Kumawat *et al.*[33] reported that the accumulation of phenol in plants is fostered by biotic and abiotic elicitors. Meena *et al.*[34] found that pre-application of salicylic acid on ground nut plant increases 3-4 times phenol content at 4th days of inoculation against *Cercospora personata*. Mishra *et al.*[35] also reported that increased total phenol content was found on varieties of wheat showing resistance to spot blotch. Biswas *et al.*[36] reported that pre-application of plant extracts as inducers provided protection of tomato plants and reduced wilt incidence from 84.46-8.40% and increase soluble protein and total phenol content with the minimum in garlic extract treated seedlings.

Pre application of inducers increases the activity of peroxidase was significantly increased in all treated leaves of brinjal as compare to controls. Mandal *et al.*[37] found that induced resistance in hydroponic tomato against *Ralstonia solanecearum* using the elicitors like chitosan, salicylic acid and jasmonic acid resulting increased in phenolic compound, lignin and peroxidase activities in treated plants. Li *et al.*[38] reported pre-treatments of salicylic acid (SA), hydrogen peroxide (H_2O_2) and calcium chloride ($CaCl_2$) induced H_2O_2 accumulation, with H_2O_2+$CaCl_2$ being most efficient, but the effect was transient. Kumar *et al.*[39] reported that hydrogen peroxide is most stable molecule among reactive oxygen species, which play a vital role in growth and development of plant as signaling molecule at low concentration in response to various abiotic and biotic stresses. Kumar *et al.*[10] found that inducing agent enhanced the peroxidase activity in potato leave with the highest in salicylic acid treated plants.

Polyphenol oxidase is another important defense molecule, synthesized in plant due to effect of inducers in plant. It has been found that all the treatments significantly increased the activity of polyphenol oxidase as compared to healthy and infected plant (controls) at 2, 6 and 10 days of pathogen inoculation. Mandal *et al.*[37] reported that polyphenol oxidase activities increased several folds by elicitors like chitosan, salicylic acid and jasmonic acid. Chitosan treatment induced a significant increase in the activities of PPO and POD and enhanced the content of phenolic compounds in tomato fruits providing protection against gray mould and blue mould disease[40]. Mandal *et al.*[41] found that

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. Kumar *et al*.[5] found that the maximum activity of polyphenol oxidase is found in *Lantana camara* treated potato leaves, provided induced resistance in potato against early blight.

Pre application of inducers increases the PAL activities in all treated plants. It has been found that PAL activities ranges from 0.52-0.82 min g^{-1} in before application and 0.63-1.09 min g^{-1} after application indicating inducers have ability to increase PAL activity in brinjal plant. Conrath *et al*.[42] demonstrated that pre-treatment of salicylic acid or benzothiadiazole leads direct activation of certain defence related gene including those encoding phenyl alanine ammonia lyase. Anil and Pankaj[43] found that PAL activity was increased in inoculated leaves. Phenylalanine ammonia lyase activity was also found at increase level in resistant cultivars as compare to susceptible host[44]. Devi and Marimuthu[45] also reported that maximum PAL activity was observed (48.70 OD min g^{-1}) on fourth day of inoculation of pathogen in *Polygonum minus*. Enhancement of PAL activities was reported in response to *R. solani* inoculation in cowpea pre-treated with salicylic acid[46].

Brinjal leaves treated with inorganic chemicals, crude extract of bio agents and plant extracts as inducers, decreases disease severity with increased level of soluble protein, total phenol and accumulation of defense related enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia lyase. The correlation regression equation showed that negative correlation (r) -0.920, -0.555, -0.835, -0.888 and -0.885 was found between total protein, total phenol, defense related enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia lyase with disease severity at 2 days of pathogen inoculation respectively. Similar observations were also found in rice against brown leaf spot[7], in tomato against Fusarium wilt[11,47] in wheat against spot blotch[35]. Bariya *et al*.[48] also reported correlation between defense related enzymes activity after treatment of biotic and abiotic inducers to brinjal plants.

CONCLUSION

In conclusion, inducers seem to be an efficient activator of several plant defense mechanisms. Considering that inducers-dependent resistance does not appear to result from an antimicrobial effect of the compound, inducing agents could be a useful tool for investigating induced resistance in brinjal. Along with conventional fungicides, biocontrol agents and improved seed varieties, inducers may be helpful in preventing phomopsis blight of brinjal.

SIGNIFICANCE STATEMENT

This study discovered that salicylic acid can be used as inducer in induced resistance for management of phomopsis blight of brinjal in near future of agriculture which can also be helpful to minimize indiscriminate used of fungicides for the benefit of human health and environment. The study will help the researchers to uncover the critical areas of innovative ideas of disease management approaches that many researchers were not able to explore. Thus, a new theory on innovative disease management ideas may be arrived in the field of Plant Pathology.

REFERENCES

1. Achuo, E.A., K. Audenaert, H. Meziana and M. Hofte, 2004. The salicylic acid-dependent defence pathway is effective against different pathogens in tomato and tobacco. Plant Pathol., 53: 65-72.
2. Malolepsza, U., 2006. Induction of disease resistance by acibenzolar-*S*-methyl and *o*-hydroxyethylorutin against *Botrytis cinerea* in tomato plants. Crop Protect., 25: 956-962.
3. Biswas, S.K., K.D. Srivastava, R. Aggarwal, S. Praveen and D.V. Singh, 2003. Biochemical changes in wheat induced by *Chaetomium globosum* against spot blotch pathogen. Indian Phytopathol., 54: 374-379.
4. Antoniw, J.F., C.E. Ritter, W.S. Pierpoint and L.C. van Loon, 1980. Comparison of three pathogenesis-related proteins from plants of two cultivars of tobacco infected with TMV. J. Gen. Virol., 47: 79-87.
5. Kumar, V., S.K. Biswas, V.T. Chowdary, K. Lal and P. Naresh, 2017. Induced synthesis of defense enzymes during induced resistance against early blight of potato using plant extracts as inducer. J. Scient. Res. Rep., 16: 1-11.
6. Rajik, M., S.K. Biswas and S. Shakti, 2012. Biochemical basis of defense response in plant against *Fusarium* wilt through bio-agents as an inducers. Afr. J. Agric. Res., 7: 5849-5857.
7. Kumawat, G.L., S.K. Biswas and S.S.L. Srivastava, 2008. Biochemical evidence of defense response in paddy induced by bio-agents against brown leaf spot pathogen. Indian Phytopathol., 61: 197-203.
8. Biswas, S.K., C. Biswas and K.D. Srivastava, 2012. Resistance to wheat spot blotch induced by crude extract of *Chaetomium globosum* and mild strain of *Drechslera sorokiniana*. J. Mycopathol. Res., 50: 267-271.

9. Biswas, S.K., N.K. Pandey and M. Rajik, 2012. Inductions of defense response in tomato against Fusarium wilt through inorganic chemicals as inducers. J. Plant Pathol. Microbiol., Vol. 3, No. 4. 10.4172/2157-7471.1000128.
10. Kumar, R., S.K. Biswas, V. Kumar, K. Lal, U. Kumar and V.T. Chowdary, 2017. Synthesis of defense enzymes in potato in induced resistance against late blight using inorganic chemicals as inducer. Plant Pathol. J., 16: 130-137.
11. Arzoo, K., S.K. Biswas and M. Rajik, 2012. Biochemical evidences of defence response in tomato against *Fusarium* wilt induced by plant extracts. Plant Pathol. J., 11: 42-50.
12. Kone, D., A.S. Csinos, K.L. Jackson and P. Ji, 2009. Evaluation of systemic acquired resistance inducers for control of *Phytophthora capsici* on squash. Crop Protect., 28: 533-538.
13. Mayers, C.N., K.C. Lee, C.A. Moore, S.M. Wong and J.P. Carr, 2005. Salicylic acid-induced resistance to *Cucumber mosaic* virus in squash and *Arabidopsis thaliana*: Contrasting mechanisms of induction and antiviral action. Mol. Plant-Microbe Interact., 18: 428-434.
14. Biswas, C., S.S.L. Srivastava and S.K. Biswas, 2010. Biochemical changes associated with induction of resistance by *Trichoderma* spp. in paddy against brown spot disease. Indian Phytopath., 63: 269-272.
15. Kumar, S., S.K. Biswas, V. Kumar, K. Lal, A. Bansal and V.T. Chowdary, 2017. Induced resistance in potato against late blight caused by *Phytophthora infestans* (Mont.) De Bary, through calcium chloride. Int. J. Curr. Microbiol. Applied Sci., 6: 410-417.
16. Buzi, A., G. Chilosi, D. De Sillo and P. Magro, 2004. Induction of resistance in melon to *Didymella bryoniae* and *Sclerotinia sclerotiorum* by seed treatments with acibenzolar-*S*-methyl and methyl jasmonate but not with salicylic acid. J. Phytopathol., 152: 34-42.
17. Bokshi, A.I., S.C. Morris, R.M. McConchie and B.J. Deverall, 2006. Pre-harvest application of 2, 6-dichloroisonicotinic acid, -aminobutyric acid or benzothiadiazole to control post-harvest storage diseases of melons by inducing Systemic Acquired Resistance (SAR). J. Hortic. Sci. Biotechnol., 81: 700-706.
18. Cohen, Y., T. Niderman, E. Mosinger and R. Fluhr, 1994. β-Aminobutyric acid induces the accumulation of pathogenesis-related proteins in tomato (*Lycopersicon esculentum* L.) plants and resistance to late blight infection caused by *Phytophthora infestans*. Plant Physiol., 104: 59-66.
19. Jeun, Y.C. and H. Buchenauer, 2001. Infection structures and localization of the pathogenesis-related protein AP24 in leaves of tomato plants exhibiting systemic acquired resistance against *Phytophthora infestans* after pre-treatment with 3-aminobutyric acid or tobacco necrosis virus. J. Phytopathol., 149: 141-153.
20. Horsfall, J.G. and R.W. Barratt, 1945. An improved grading system for measuring plant diseases. Phytopathology, 35: 655-655.
21. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
22. Bray, H.G. and W.V. Thorpe, 1954. Analysis of phenolic compounds of interest in metabolism. Methods Biochem. Anal., 1: 27-52.
23. Hammerschmidt, R., E.M. Nuckles and J. Kuc, 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol. Plant Pathol., 20: 73-82.
24. Malik, C.P. and M.B. Singh, 1980. Extraction and Estimation of Amino Acids and Keto Acids. In: Plant Enzymology and Histo-Enzymology, Malik, C.P. and M.B. Singh (Eds.). Kalyani Publishers, New Delhi, India, pp: 257-262.
25. Burrell, M.M. and T.A. Rees, 1974. Metabolism of phenylalanine and tyrosine by rice leaves infected by *Piricularia oryzae*. Physiol. Plant Pathol., 4: 497-508.
26. Mosa, A.A., 2002. Induced resistance in rice against blast disease using abiotic and biotic agents. Ann. Agric. Sci. Ain-Shams Univ., 47: 993-1008.
27. Ata, A.A., M.G. El-Samman, M.A. Moursy and M.H. Mostafa, 2005. Effect of rust infection on sugar beet yield components. Egypt. J. Agric. Res., 83: 11-19.
28. Boller, T., 1985. Induction of Hydrolases as a Defense Reaction against Pathogens. In: Cellular and Molecular Biology of Plant Stress, Key, J.L. and T. Kosuge (Eds.). Alan R. Liss Publisher, New York, ISBN-13: 9780845126219, pp: 247-262.
29. Metraux, J.P., L. Streit and T. Staub, 1988. A pathogenesis-related protein in cucumber is a chitinase. Physiol. Mol. Plant Pathol., 33: 1-9.
30. Tuzun, S., M.N. Rao, U. Vogeli, C.H. Schardl and J. Kuc, 1989. Induced systemic resistance to blue mold: Early induction and accumulation of β-1,3-glucanases, chitinases and other pathogenesis-related proteins (b-Proteins) in immunized tobacco. Phytopathology, 79: 979-983.
31. Nicholson, R.L. and R. Hammerschmidt, 1992. Phenolic compounds and their role in disease resistance. Annu. Rev. Phytopathol., 30: 369-389.
32. Vimala, R. and M. Suriachandraselvan, 2009. Induced resistance in bhendi against powdery mildew by foliar application of salicylic acid. J. Biopestic., 2: 111-114.
33. Kumawat, G.L., S.K. Biswas and M. Rajik, 2010. Antagonistic evaluation of *Trichoderma* spp. and their effect on seed germination and growth of paddy seedling. J. Plant Dis. Sci., 5: 203-207.
34. Meena, B., T. Marimuthu and R. Velazhahan, 2001. Salicylic acid induces systemic resistance in groundnut against late leaf spot caused by *Cercosporidium personatum*. J. Mycol. Plant Pathol., 31: 139-145.

35. Mishra, V.K., S.K. Biswas and M. Rajik, 2011. Biochemical mechanism of resistance to alternaria blight by different varieties of wheat. Int. J. Plant Pathol., 2: 72-80.
36. Biswas, S.K., H.G. Prakesh, R. Palat and J. Bahar, 2019. Induced synthesis of defense molecules in tomato (*Solamum lycopercicum* L.) against Fusarium wilt through plant extracts. Bangladesh J. Bot., 48: 169-175.
37. Mandal, S., I. Kar, A.K. Mukherjee and P. Acharya, 2013. Elicitor-induced defense responses in *Solanum lycopersicum* against *Ralstonia solanacearum*. Scient. World J., Vol. 2013. 10.1155/2013/561056.
38. Li, B., Q. Chen, X. Lv, C. Lan, J. Zhao, R. Qiu and Q. Weng, 2009. Phenotypic and genotypic characterization of *Phytophthora infestans* isolates from China. J. Phytopathol., 157: 558-567.
39. Kumar, S., T.S. Thind, A. Bala and A.K. Gupta, 2010. Induced resistance in potato against *Phytophthora infestans* using chemicals and bio-agents. Plant Dis. Res., 25: 12-18.
40. Liu, J., S.P. Tian, X. Meng and Y. Xu, 2007. Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit. Postharvest Biol. Technol., 44: 300-306.
41. Mandal, S., N. Mallick and A. Mitra, 2009. Salicylic acid-induced resistance to *Fusarium oxysporum* f. sp. *lycopersici* in tomato. Plant Physiol. Biochem., 47: 642-649.
42. Conrath, U., C.M.J. Pieterse and B. Mauch-Mani, 2002. Priming in plant-pathogen interactions. Trends Plant Sci., 7: 210-216.
43. Anil, S. and Pankaj, 2001. Effect of *Rotylenchulus reniformis* on phenylalanin ammonia lyase and peroxidase activity in cotton. Ann. Plant Prot. Sci., 9: 341-345.
44. Meena, A.K., S.L. Godara, S. Gangopadhyay and J. Ram, 2012. Changes in biochemical constituents in Cluster bean due to *Alternaria cucumerina* var. *cyamopsidis*. Ann. Plant Prot. Sci., 20: 386-388.
45. Devi, R.P. and P. Marimuthu, 2011. Effect of botanical formulation of *Polygonum minus* (P-40) on control of *Alternaria solani*. J. Plant Pathol. Microbiol., 2: 1-4.
46. Chandra, A., R. Saxena, A. Dubey and P. Saxena, 2007. Change in phenylalanine ammonia lyase activity and isozyme patterns of polyphenol oxidase and peroxidase by salicylic acid leading to enhance resistance in cowpea against *Rhizoctonia solani*. Acta Physiol. Plant., 29: 361-367.
47. Kumar, A. and S.K. Biswas, 2010. Biochemical evidences of induced resistance in tomato plant against *Fusarium* with through inorganic chemicals. J. Mycopathol. Res., 48: 213-219.
48. Bariya, H.S., V.R. Thakkar, A.N. Thakkar and R.B. Subramanian, 2011. Induction of systemic resistance in different varieties of *Solanum tuberosum* by pure and crude elicitor treatment. Indian J. Exp. Biol., 49: 151-162.