



# Evaluation of Abiotic and Biotic Elicitors for Induction of Defense Related Enzymes in Pea against *U. Viciae-Fabae* (Pers.) J. Schrot

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### Abstract

Total of fifteen elicitors tested alone/or in combination for induction of defense related enzymes in pea against *U. viciae-fabae* (Pers.) J. Schrot results in significant induction of total phenols, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in all the treatment as compare to control. Salicylic acid, *Pseudomonas fluorescens*, salicylic acid + *Pseudomonas fluorescens* were found most effective in induction of total phenols and peroxidase at 72 hrs after spray of elicitors. Polyphenol oxidase induction was found significantly high in oxalic acid, *Pseudomonas fluorescens* + *Trichoderma harzianum* and chitosan + *Pseudomonas fluorescens* at 72 hrs after spray of elicitors. Among all the treatments, maximum induction of Phenylalanine ammonia lyase activity was found in oxalic acid, *Trichoderma harzianum* + *Pseudomonas fluorescens* and isonicotinic acid + *Trichoderma harzianum* after 48hrs of spray of elicitors. Effect of different elicitors on percent disease index (PDI) 20 days after inoculation with uredospores of *U. viciae-fabae* showed least PDI in salicylic acid, *Trichoderma harzianum* + *Pseudomonas fluorescens* and chitosan + *Pseudomonas fluorescens* treated plants.

### Keywords

Elicitors *U. viciae-fabae*; *Pseudomonas fluorescens*; *Trichoderma harzianum*; Percent disease index

## Introduction

A large proportion of Indian population is vegetarian and pulses are the main source of protein for them. The protein content in pulses is about 18-25 per cent. This makes pulse one of the cheapest source of protein for human consumption [1].

India is the largest producer, consumer and importer of pulses in the world. In India pulses are grown about 24-26 million hectares of area producing 17-19 million tonnes of pulses annually. India accounts for over one third of the total world area and over 20 per cent of total world pulse production. Consequently per capita production and availability of pulses in the country has witnessed sharp decline. Per capita net pulse availability has declined from around 60 grams per day in the 1950s to 40 grams in the 1980s and further to around

35 grams per day in 2000s. However, in the past four years, there has been significant increase in consumption averaging around 50 grams due to higher production, under owing to National Food Security Mission (NFSM), with major emphasis on pulses and their imports, mostly of dry peas from Canada and Australia [2].

Major pulses grown in India include chickpea or bengal gram (*Cicer arietinum*), pigeonpea or red gram (*Cajanus cajan*), lentil (*Lens culinaris*), urdbean or black gram (*Vigna mungo*), mungbean or green gram (*Vigna radiata*), lablab bean (*Lablab purpureus*), moth bean (*Vigna aconitifolia*), horse gram (*Dolichos uniflorus*), pea (*Pisum sativum* L.), grass pea or khesari (*Lathyrus sativus*), cowpea (*Vigna unguiculata*), and broad bean or faba bean (*Vicia faba*).

During 2012-13, field pea (*Pisum sativum* L.) occupies an area of 0.76 million hectares with a production 0.84 million tonnes and productivity of 1100 kg/ha in our country. In Uttarakhand, area, production and productivity of pea during 2012-13 was 61.0 thousand hectares, 51.3 thousand tones and 841 kg/ha, respectively [3].

Pea is affected by a number of fungal (rust, powdery mildew, downy mildew, root rot, alternaria blight, aschochyta blight, wilt, anthracnose, cercospora leaf spot, damping off, seedling rot etc.), bacterial (bacterial blight and brown spot), nematode (cyst nematode, lesion nematode and root-knot nematode) and viral diseases (cucumber mosaic virus, pea early browning virus, pea enation mosaic, pea mosaic, pea seed borne mosaic, pea streak and pea stunt). These diseases, under the right conditions, can significantly decrease both yield and quality. Among these, the rust of pea caused by *Uromyces viciae-fabae* [4], *Uromyces fabae* [5] is considered the most important under warm and humid conditions [6]. It has been reported from different parts of the country including eastern India [7,8], central India [9], southern parts of India [10,11] and from Himalayan region of Uttarakhand and Himachal Pradesh [12,13]. In the last few years, disease has been observed in almost epiphytotic form and could cause up to 20-100% losses in yield [13,14].

Plants can be sensitized for a more rapid or more intense mobilization of defence responses leading to enhanced resistance to biotic or abiotic stresses [15]. Many factors such as prior pathogen attack and various chemical and environmental stimuli may act on plants to induce systemic acquired resistance (SAR) to subsequent pathogen attack [5,12,16,17]. SAR has been reported to be effective against a broad spectrum of pathogens including viruses, fungi, bacteria, nematodes and parasitic weeds [15]. Induction of systemic resistance is associated with gene induction, the activation of a wide range of resistance mechanisms and the production of a wide range of defence compounds. It is race non-specific and is often effective against a broad spectrum of pathogenic agents [18,19]. Thus, study on induction of host defence through biotic and abiotic elicitors can be one of the effective sustainable approaches in disease management.

## Materials and Methods

Present investigation was carried out both in glass house and Bio-control Laboratory of Department of Plant Pathology in 2014-15 at G.B. Pant University of Agriculture and Technology, Pantnagar. Soil was collected from the upper 0-15 cm layer from NEBCRC and was sterilized by autoclaving at 21lb (121.6°C) for one hour on three

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consecutive days. The sterilized soil was filled in 5 kg capacity plastic pots and kept in glasshouse. Pots were watered and left for two days to maintain appropriate moisture for proper seed germination. Seeds of highly susceptible pea cultivar 'HFP-4' were washed thoroughly with sterilized distilled water and then treated with sodium hypochlorite for 60-90 seconds and then washed 2-3 times in sterilized distilled water under aseptic condition. Seven seeds were sown in each pot. Three healthy seedlings were maintained in each pot. Pots were watered regularly as and when required to maintain optimum moisture. Experiments were laid out in a completely randomized design with three replications. Recommended concentrations of biotic and abiotic elicitor alone and/or in combination (Table 1) were prepared in sterile water to which Tween-20 (0.03%, v/v) was added. Control plants were treated with sterile water plus Tween-20. Solutions were applied with the help of a paint-brush on 70 days old pea plants. After 24 hours, the top five leaves were collected from all the treatments. Then, on the same day plants were inoculated by spraying with a solution of  $1.5 \times 10^6$  spores/ml of the local aggressive isolate *U. viciae fabae*. After 24, 46 and 72 hours of inoculation, the top five leaves per treatments were harvested and brought to the laboratory in an ice box. Fresh leaves were weighed and used for determination of activities of enzymes. Disease scoring was also recorded for different treatments on 20 days after inoculation (DAI) using 0-9 rating scale [20] and then disease score data were converted into Per cent Disease Index (PDI) [21].

**Per cent disease index** = Sum of all numerical rating  $\times$  100

Number of leaves examined  $\times$  Maximum grade

The experimental results were then analyzed statistically. The following enzymatic analysis has been carried out:

### Peroxidase (PO) activity

Assay of peroxidase (PO) activity was carried out as per the procedure described by Hammerschmidt et al. [22]. Enzyme extract was prepared by homogenizing one gram of leaf samples in 0.1M sodium phosphate buffer (pH 6.0). It was then centrifuged at 10,000 rpm for 20 min. The reaction mixture consisted of 2.5 ml of a mixture

containing 0.25 per cent (v/v) guaiacol in 0.01 M sodium phosphate buffer, pH 6.0 and 0.1 M hydrogen peroxide. Enzyme extract (0.1 ml) was added to initiate the reaction, which was followed colorimetrically at 480 nm. The boiled enzyme preparation served as blank. Activity was expressed as the increase in absorbance at  $480 \text{ nm min}^{-1} \text{ mg}^{-1}$  leaf sample.

### Polyphenol oxidase (PPO) activity

PPO activity was determined as per the procedure given by Mayer et al. [23]. Leaf samples (1 g) were homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used as the enzyme source. The reaction mixture consisted of 200  $\mu\text{L}$  of the enzyme extract and 1.5 mL of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction, 200  $\mu\text{L}$  of 0.01M catechol was added and the activity was expressed as changes in absorbance at  $495 \text{ nm min}^{-1} \text{ mg}^{-1}$  leaf sample.

### Phenylalanine ammonia lyase (PAL) activity

Enzyme extracted in 0.1 M sodium phosphate buffer (pH 7.0) was used out as per the method described by Ross and Sederoff [24]. About 1 g of leaf sample was homogenized with 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4°C. The homogenate was centrifuged for 2.0 min at 10,000 rpm. The supernatant was used as a crude extract for enzyme activity. The assay mixture containing 100  $\mu\text{L}$  of enzyme, 500  $\mu\text{L}$  of 50 mM Tris HCl (pH 8.8) and 600  $\mu\text{L}$  of 1 mM L-phenylalanine was incubated for 60 min and the reaction was arrested by adding, 2 N HCl. Later 1.5 ml of toluene was added, vortexed for 30 sec, centrifuged (1000 rpm, 5 min) and toluene fraction containing trans- cinnamic acid was separated. The toluene phase was measured at 290 nm against the blank of toluene. Standard curve was drawn with graded amounts of cinnamic acid in toluene. The enzyme activity was expressed in  $\mu\text{ moles of cinnamic acid min}^{-1} \text{ mg}^{-1}$  of protein.

### Total phenolics

Total phenolics content was determined by following the method of Swain and Hills [25]. One gram leaves were homogenized in 10

**Table 1:** List of elicitors alone and in combination used for evaluation of defence related enzymes induction in pea against *U. viciae fabae* (Pers.) J. Schrot.

Treatments	Name (source)	Concentration
<b>Abiotic elicitors</b>		
1.	Salicylic acid (Sigma-Aldrich)	5 mM
2.	Chitosan (Sigma-Aldrich)	1 g/100 ml (1%)
3.	Oxalic acid (Sigma-Aldrich)	20 mM
4.	Isonicotinic acid (Sigma-Aldrich)	100 $\mu\text{g/ml}$
<b>Biotic elicitors</b>		
5.	<i>Pseudomonas fluorescens</i> (GBPUAT, Pantnagar)	$10^8$ cfu/ml
6.	<i>Trichoderma harzianum</i> (GBPUAT, Pantnagar)	$10^8$ cfu/ml
7.	<i>Trichoderma harzianum</i> + <i>Pseudomonas fluorescens</i>	$10^8$ cfu/ml+ $10^8$ cfu/ml
<b>Combinations</b>		
8.	Salicylic acid + <i>Trichoderma harzianum</i>	2.5 mM + $10^8$ cfu/ml
9.	Salicylic acid + <i>Pseudomonas fluorescens</i>	2.5 mM + $10^8$ cfu/ml
10.	Chitosan + <i>Trichoderma harzianum</i>	0.5 g/100 ml+ $10^8$ cfu/ml
11.	Chitosan + <i>Pseudomonas fluorescens</i>	0.5 g/100 ml+ $10^8$ cfu/ml
12.	Oxalic acid + <i>Trichoderma harzianum</i>	10 mM + $10^8$ cfu/ml
13.	Oxalic acid + <i>Pseudomonas fluorescens</i>	10 mM + $10^8$ cfu/ml
14.	Isonicotinic acid + <i>Trichoderma harzianum</i>	50 $\mu\text{g/ml}$ + $10^8$ cfu/ml
15.	Isonicotinic acid + <i>Pseudomonas fluorescens</i>	50 $\mu\text{g/ml}$ + $10^8$ cfu/ml
16.	Water	-

ml of 80% methanol and agitated for 15 min at 70°C. One ml of methanolic extract was added to 5 ml of distilled water and 250 µl of Folin-ciocalteu reagent, after this the solution was kept at 25°C. After 3 min, 1 ml of a saturated solution of Na<sub>2</sub>CO<sub>3</sub> and 1 ml of distilled water were added, and the reaction mixture was incubated for 1 hr at 25°C. The absorption of the developed color was measured using spectrophotometer at 725 nm. The total soluble phenolic content was calculated by comparison with a standard curve obtained from Folin-Ciocalteu reaction with catechol. Results were expressed as phenol equivalent in µg/mg g<sup>-1</sup> of fresh weight.

The data was analyzed statistically by Complete Randomized Block design (CRD) [26] using STPR (GBPUA&T statistical software and MS Excel. Data recorded were first transformed (angularly transformed) to make them homogenous before analysis and the treatment were compared by means of critical differences at one per cent level of significance.

## Results and Discussion

Induction of different defense related enzymes in pea against *U. viciae-fabae* (Pers.) J. Schrot through abiotic and biotic elicitors were estimated as follows:

### Total phenols (mg/gm of fresh leaf)

Data pertaining to effect of different elicitors on activity of total phenols in pea revealed that induction of total phenols was found significantly at 72 hrs after spray of elicitors as compare to control (Table 2).

Between all abiotic elicitors, induction was found maximum in salicylic acid (21.70) followed by oxalic acid (21.66) and isonicotinic acid (21.66). Whereas chitosan showed comparatively less induction

(18.47). In biotic elicitors, *Pseudomonas fluorescens* (27.31) was found most effective in induction followed by *Trichoderma harzianum* + *Pseudomonas fluorescens* (27.07) and *Trichoderma harzianum* (20.96). Among all combinations, salicylic acid + *Pseudomonas fluorescens* (24.29) was most effective followed by salicylic acid + *Trichoderma harzianum* (23.22), isonicotinic acid + *Pseudomonas fluorescens* (22.96), chitosan + *Trichoderma harzianum* (22.40), chitosan + *Pseudomonas fluorescens* (21.72), oxalic acid + *Pseudomonas fluorescens* (21.60), oxalic acid + *Trichoderma harzianum* (21.07), isonicotinic acid + *Trichoderma harzianum* (20.27). Lowest induction was observed in control (12.96).

Salicylic acid, *Pseudomonas fluorescens* and salicylic acid + *Pseudomonas fluorescens* were found most effective among all the treatments.

### Peroxidase (µmol/min/mg/protein)

Induction of peroxidase in pea by different elicitors was found significantly at 72 hrs after spray of elicitors as compare to control.

Among all abiotic elicitors, induction was found maximum in salicylic acid (52.43) followed by chitosan and isonicotinic acid (49.12) whereas oxalic acid (28.54) showed very less induction. In biotic elicitors, *Pseudomonas fluorescens* (58.41) showed high induction followed by *Trichoderma harzianum* + *Pseudomonas fluorescens* (47.12) and *Trichoderma harzianum* (35.84). Between all combinations, salicylic acid + *Pseudomonas fluorescens* (52.43) was found most effective followed by isonicotinic acid + *Pseudomonas fluorescens* (51.77), oxalic acid + *Pseudomonas fluorescens* (49.78), chitosan + *Pseudomonas fluorescens* (48.45), isonicotinic acid + *Trichoderma harzianum* (42.48), salicylic acid + *Trichoderma harzianum* (39.82), chitosan + *Trichoderma harzianum* (37.17), oxalic acid + *Trichoderma harzianum* (28.54). Lowest induction was observed in control (26.55) (Table 3).

**Table 2:** Effect of elicitors on total phenols accumulation in pea under controlled condition.

TOTAL PHENOLS (mg/gm of fresh leaf)				
Elicitors	Time intervals			
	24hr	48hr	72hr	96hr
<b>Abiotic</b>				
Salicylic acid	9.74 ± 0.24	19.04 ± 0.84	21.70 ± 0.26	14.10 ± 1.13
Chitosan	12.82 ± 0.06	17.10 ± 0.38	18.47 ± 0.49	14.11 ± 0.33
Oxalic acid	17.26 ± 0.63	16.82 ± 0.36	21.66 ± 0.76	11.35 ± 0.90
Isonicotinic acid	9.40 ± 0.18	17.48 ± 1.41	21.66 ± 0.76	12.50 ± 1.00
<b>Biotic</b>				
<i>Pseudomonas fluorescens</i>	13.52 ± 0.01	25.15 ± 1.05	27.31 ± 1.21	18.38 ± 0.49
<i>Trichoderma harzianum</i>	4.96 ± 0.03	16.48 ± 0.33	20.96 ± 0.70	16.85 ± 0.36
<i>Trichoderma harzianum</i> + <i>Pseudomonas fluorescens</i>	9.17 ± 0.16	22.00 ± 0.79	27.07 ± 1.23	16.98 ± 0.37
<b>Combinations</b>				
Salicylic acid + <i>Trichoderma harzianum</i>	13.99 ± 0.56	16.61 ± 0.34	23.22 ± 0.98	21.38 ± 0.74
Salicylic acid + <i>Pseudomonas fluorescens</i>	5.28 ± 0.03	16.82 ± 0.36	24.29 ± 0.89	17.95 ± 0.45
Chitosan + <i>Trichoderma harzianum</i>	9.02 ± 0.05	15.53 ± 0.25	22.40 ± 0.82	18.94 ± 0.53
Chitosan + <i>Pseudomonas fluorescens</i>	8.17 ± 0.13	15.24 ± 0.22	21.72 ± 0.76	18.30 ± 0.48
Oxalic acid + <i>Trichoderma harzianum</i>	11.08 ± 0.29	12.77 ± 0.02	21.07 ± 0.71	13.46 ± 1.08
Oxalic acid + <i>Pseudomonas fluorescens</i>	8.58 ± 0.09	14.64 ± 0.17	21.60 ± 0.75	16.49 ± 0.33
Isonicotinic acid + <i>Trichoderma harzianum</i>	10.57 ± 0.34	17.15 ± 0.38	20.27 ± 0.64	14.65 ± 1.18
Isonicotinic acid + <i>Pseudomonas fluorescens</i>	7.38 ± 0.22	19.43 ± 0.57	22.96 ± 0.87	18.11 ± 0.46
Water (Control)	8.21 ± 0.05	15.82 ± 0.27	12.96 ± 1.03	12.53 ± 1.00
<b>CD at 1%</b>	<b>a= 0.42**</b>	<b>b= 0.21**</b>	<b>a*b= 0.84**</b>	
<b>SEM</b>	<b>a= 0.11</b>	<b>b= 0.57</b>	<b>a*b= 0.22</b>	
<b>CV</b>	<b>2.42</b>			

± = Standard error, a= Time interval, b= Elicitors, \*\* Significant level at the 0.01.

Salicylic acid, *Pseudomonas fluorescens* and salicylic acid + *Pseudomonas fluorescens* were found most effective between all the treatments.

oxidase activity in pea revealed that polyphenol oxidase induction was found at 72 hrs after spray of elicitors as compare to control (Table 4).

### Polyphenol oxidase (μmol/min/mg/protein)

Between all abiotic elicitors, induction was found highest in oxalic acid (17.67) followed by isonicotinic acid (16.02), chitosan

Data pertaining to effect of different elicitors on polyphenol

**Table 3:** Effect of elicitors on Peroxidase (PO) activity in pea under controlled condition.

PEROXIDASE (μmol/min/mg/protein)				
Elicitors	Time interval			
	24hr	48hr	72hr	96hr
<b>Abiotic</b>				
Salicylic acid	30.65 ± 0.97	35.84 ± 0.22	52.43 ± 0.55	22.57 ± 0.20
Chitosan	8.63 ± 0.08	39.82 ± 0.75	49.12 ± 0.73	23.23 ± 0.34
Oxalic acid	21.24 ± 0.71	13.94 ± 0.15	28.54 ± 0.23	21.24 ± 0.11
Isonicotinic acid	35.18 ± 0.55	45.80 ± 0.32	49.12 ± 1.02	24.56 ± 0.97
<b>Biotic</b>				
<i>Pseudomonas fluorescens</i>	22.57 ± 0.04	15.93 ± 0.46	58.41 ± 0.53	17.92 ± 0.24
<i>Trichoderma harzianum</i>	25.22 ± 0.52	24.56 ± 0.44	35.84 ± 0.61	18.58 ± 0.27
<i>Trichoderma harzianum</i> + <i>Pseudomonas fluorescens</i>	22.57 ± 0.69	40.49 ± 0.47	47.12 ± 1.27	35.84 ± 1.13
<b>Combinations</b>				
Salicylic acid + <i>Trichoderma harzianum</i>	16.59 ± 0.09	15.93 ± 0.67	39.82 ± 1.26	27.88 ± 0.78
Salicylic acid + <i>Pseudomonas fluorescens</i>	23.23 ± 0.44	27.88 ± 1.08	52.43 ± 0.65	29.87 ± 0.86
Chitosan + <i>Trichoderma harzianum</i>	12.61 ± 0.24	23.89 ± 0.95	37.17 ± 1.17	26.55 ± 0.50
Chitosan + <i>Pseudomonas fluorescens</i>	23.23 ± 0.48	35.84 ± 0.94	48.45 ± 0.39	40.49 ± 1.46
Oxalic acid + <i>Trichoderma harzianum</i>	43.81 ± 0.71	18.58 ± 0.37	28.54 ± 0.08	24.56 ± 0.11
Oxalic acid + <i>Pseudomonas fluorescens</i>	40.49 ± 0.11	30.53 ± 0.25	49.78 ± 0.57	41.81 ± 0.21
Isonicotinic acid + <i>Trichoderma harzianum</i>	29.87 ± 0.05	34.51 ± 0.93	42.48 ± 1.03	33.85 ± 0.85
Isonicotinic acid + <i>Pseudomonas fluorescens</i>	23.89 ± 1.00	45.13 ± 0.57	51.77 ± 0.21	44.47 ± 0.39
Water (Control)	7.96 ± 0.07	19.25 ± 0.85	26.55 ± 0.02	18.58 ± 0.75
<b>CD at 1%</b>	<b>a= 0.93**</b>	<b>b= 0.46**</b>	<b>a*b= 1.86**</b>	
<b>SEM</b>	<b>a= 0.25</b>	<b>b= 0.12</b>	<b>a*b= 0.50</b>	
<b>CV</b>	<b>2.78</b>			

± = Standard error, a= Time interval, b= Elicitors, \*\* Significant level at the 0.01.

**Table 4:** Effect of elicitors on Polyphenol oxidase (PPO) activity in pea under controlled condition.

POLYPHENOL OXIDASE (μmol/min/mg/protein)				
Elicitors	Time interval			
	24hr	48hr	72hr	96hr
<b>Abiotic</b>				
Salicylic acid	5.73 ± 0.08	5.81 ± 0.25	10.29 ± 0.20	7.77 ± 0.16
Chitosan	6.05 ± 0.24	6.52 ± 0.12	11.93 ± 0.48	3.53 ± 0.03
Oxalic acid	4.71 ± 0.04	12.72 ± 0.37	17.67 ± 0.10	8.17 ± 0.13
Isonicotinic acid	4.71 ± 0.07	10.99 ± 0.15	16.02 ± 0.32	9.81 ± 0.12
<b>Biotic</b>				
<i>Pseudomonas fluorescens</i>	5.65 ± 0.23	5.89 ± 0.18	7.77 ± 0.14	2.36 ± 0.07
<i>Trichoderma harzianum</i>	5.57 ± 0.10	6.83 ± 0.01	10.36 ± 0.02	2.04 ± 0.03
<i>Trichoderma harzianum</i> + <i>Pseudomonas fluorescens</i>	6.44 ± 0.08	7.77 ± 0.21	13.82 ± 0.56	8.95 ± 0.24
<b>Combinations</b>				
Salicylic acid + <i>Trichoderma harzianum</i>	6.05 ± 0.01	7.62 ± 0.26	14.84 ± 0.44	8.32 ± 0.37
Salicylic acid + <i>Pseudomonas fluorescens</i>	6.36 ± 0.24	5.10 ± 0.18	9.34 ± 0.19	8.24 ± 0.19
Chitosan + <i>Trichoderma harzianum</i>	2.43 ± 0.07	7.14 ± 0.09	8.17 ± 0.31	6.28 ± 0.04
Chitosan + <i>Pseudomonas fluorescens</i>	5.73 ± 0.17	4.95 ± 0.17	16.57 ± 0.40	8.87 ± 0.02
Oxalic acid + <i>Trichoderma harzianum</i>	3.61 ± 0.08	7.38 ± 0.22	15.47 ± 0.61	1.65 ± 0.01
Oxalic acid + <i>Pseudomonas fluorescens</i>	4.63 ± 0.09	10.68 ± 0.02	15.15 ± 0.52	14.52 ± 0.48
Isonicotinic acid + <i>Trichoderma harzianum</i>	4.32 ± 0.05	6.28 ± 0.20	15.00 ± 0.09	12.33 ± 0.27
Isonicotinic acid + <i>Pseudomonas fluorescens</i>	5.57 ± 0.17	9.50 ± 0.24	14.21 ± 0.28	8.32 ± 0.25
Water (Control)	2.90 ± 0.05	4.63 ± 0.13	6.60 ± 0.08	3.22 ± 0.03
<b>CD at 1%</b>	<b>a= 0.24**</b>	<b>b= 0.12**</b>	<b>a*b= 0.49**</b>	
<b>SEM</b>	<b>a= 0.67</b>	<b>b= 0.33</b>	<b>a*b= 0.13</b>	
<b>CV</b>	<b>2.88</b>			

± = Standard error, a= Time interval, b= Elicitors, \*\* Significant level at the 0.01.

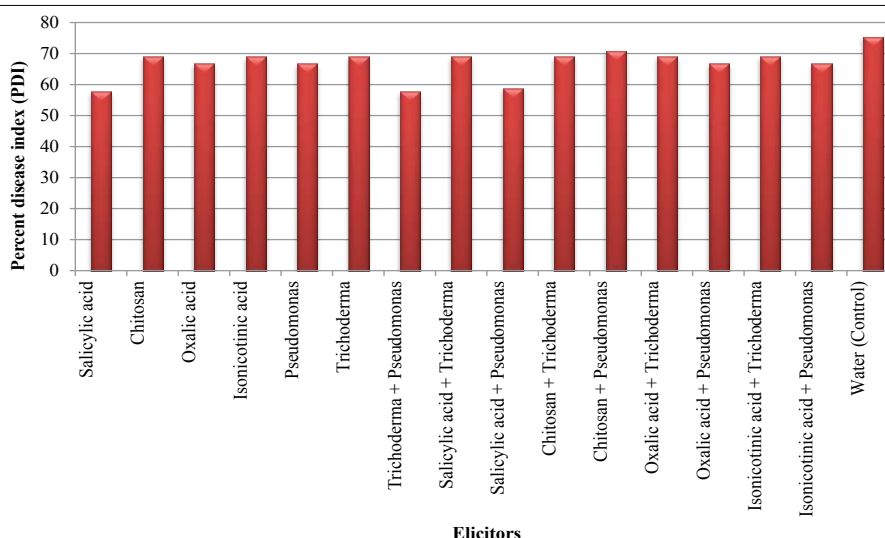
(11.93) and salicylic acid (10.29). In biotic elicitors, *Trichoderma harzianum* + *Pseudomonas fluorescens* (13.82) showed maximum induction followed by *Trichoderma harzianum* (10.36) and *Pseudomonas fluorescens* (7.77). Among all combinations, chitosan + *Pseudomonas fluorescens* (16.57) was most effective followed by oxalic acid + *Trichoderma harzianum* (15.47), oxalic acid + *Pseudomonas fluorescens* (15.15), isonicotinic acid + *Trichoderma harzianum* (15.00), salicylic acid + *Trichoderma harzianum* (14.84), isonicotinic acid + *Pseudomonas fluorescens* (14.21), salicylic acid + *Pseudomonas fluorescens* (9.34) and chitosan + *Trichoderma harzianum* (8.17). Lowest induction was observed in water (6.60) (Figure 1).

Oxalic acid, *Trichoderma harzianum* + *Pseudomonas fluorescens* and chitosan + *Pseudomonas fluorescens* were found most effective between all the treatments.

### Phenylalanine ammonia lyase (PAL) (mg/gm of fresh leaf)

Effect of different elicitors on PAL activity in pea revealed that induction of PAL was found at 48 hrs after spray of elicitors as compare to control (Table 5).

Among all abiotic elicitors, induction was found maximum in oxalic acid (48.54) followed by salicylic acid (47.44), chitosan (38.68) and



**Figure 1:** Effect of elicitors on percent disease index (PDI) of rust in pea under controlled condition.

**Table 5:** Effect of elicitors on Phenylalanine ammonia lyase (PAL) activity in pea under controlled condition.

PHENYLALANINE AMMONIA LYASE (mg/gm of fresh leaf)				
Elicitors	Time interval			
	24hr	48hr	72hr	96hr
<b>Abiotic</b>				
Salicylic acid	20.62 ± 0.89	47.44 ± 0.42	17.33 ± 0.26	15.69 ± 0.51
Chitosan	20.25 ± 0.73	38.68 ± 1.01	14.78 ± 0.37	9.85 ± 0.15
Oxalic acid	28.46 ± 0.41	48.54 ± 0.13	37.59 ± 0.43	15.87 ± 0.29
Isonicotinic acid	16.79 ± 0.68	13.50 ± 0.37	17.88 ± 0.47	8.21 ± 0.25
<b>Biotic</b>				
<i>Pseudomonas fluorescens</i>	33.76 ± 0.88	51.45 ± 0.42	33.94 ± 0.18	29.38 ± 0.77
<i>Trichoderma harzianum</i>	10.58 ± 0.30	27.92 ± 1.03	19.89 ± 0.22	9.85 ± 0.43
<i>Trichoderma harzianum</i> + <i>Pseudomonas fluorescens</i>	30.11 ± 1.11	52.18 ± 1.21	35.58 ± 0.55	30.11 ± 0.30
<b>Combinations</b>				
Salicylic acid + <i>Trichoderma harzianum</i>	6.20 ± 0.06	31.57 ± 0.63	21.53 ± 0.58	15.33 ± 0.29
Salicylic acid + <i>Pseudomonas fluorescens</i>	13.50 ± 0.09	25.00 ± 0.23	35.95 ± 1.23	17.70 ± 0.64
Chitosan + <i>Trichoderma harzianum</i>	19.52 ± 0.25	36.49 ± 0.48	33.21 ± 0.33	32.48 ± 0.43
Chitosan + <i>Pseudomonas fluorescens</i>	19.16 ± 0.19	28.65 ± 0.39	20.80 ± 0.82	10.40 ± 0.12
Oxalic acid + <i>Trichoderma harzianum</i>	6.57 ± 0.04	26.27 ± 0.12	19.16 ± 0.74	8.21 ± 0.22
Oxalic acid + <i>Pseudomonas fluorescens</i>	14.23 ± 0.40	19.89 ± 0.48	17.70 ± 0.51	9.31 ± 0.25
Isonicotinic acid + <i>Trichoderma harzianum</i>	21.17 ± 0.11	44.70 ± 0.37	29.56 ± 0.85	23.54 ± 0.30
Isonicotinic acid + <i>Pseudomonas fluorescens</i>	17.15 ± 0.01	31.75 ± 0.18	27.92 ± 1.21	21.90 ± 0.51
Water (Control)	11.86 ± 0.27	15.87 ± 0.29	8.21 ± 0.28	7.91 ± 0.59
<b>CD at 1%</b>	<b>a= 0.71**</b>	<b>b= 0.35**</b>	<b>a*b= 1.43**</b>	
<b>SEM</b>	<b>a= 0.19</b>	<b>b= 0.97</b>	<b>a*b= 0.38</b>	
<b>CV</b>	<b>2.89</b>			

± = Standard error, a= Time interval, b= Elicitors, \*\* Significant level at the 0.01.

isonicotinic acid (13.50). In biotic elicitors, *Trichoderma harzianum* + *Pseudomonas fluorescens* (52.18) was found most effective followed by *Pseudomonas fluorescens* (51.45) and *Trichoderma harzianum* (27.92). Between all combinations, Isonicotinic acid + *Trichoderma harzianum* (44.70) was most effective followed by chitosan + *Trichoderma harzianum* (36.49), Isonicotinic acid + *Pseudomonas fluorescens* (31.75), salicylic acid + *Trichoderma harzianum* (31.57), chitosan + *Pseudomonas fluorescens* (28.65), oxalic acid + *Trichoderma harzianum* (26.27), salicylic acid + *Pseudomonas fluorescens* (25.00), oxalic acid + *Pseudomonas fluorescens* (19.89). Lowest induction was observed in water (15.87).

Oxalic acid, *Trichoderma harzianum* + *Pseudomonas fluorescens* and Isonicotinic acid + *Trichoderma harzianum* were found most effective among all the treatments.

### Effect of different elicitors on per cent disease index (PDI) for rust in pea

The data on effect of different elicitors on percent disease index (PDI) for rust in pea showed less PDI in all the elicitors treated plants as compare to control.

Among all abiotic elicitors, PDI was found maximum in chitosan and isonicotinic acid (68.88) followed by oxalic acid (66.66) and salicylic acid (57.77). In biotic elicitors, *Trichoderma harzianum* (68.88) showed highest PDI followed by *Pseudomonas fluorescens* (66.66) and *Trichoderma harzianum* + *Pseudomonas fluorescens* (57.77). Between all combinations, salicylic acid + *Trichoderma harzianum*, chitosan + *Trichoderma harzianum*, oxalic acid + *Trichoderma harzianum*, Isonicotinic acid + *Trichoderma harzianum* showed similar PDI of 68.88 followed by oxalic acid + *Pseudomonas fluorescens* and isonicotinic acid + *Pseudomonas fluorescens* (66.66), salicylic acid + *Pseudomonas fluorescens* (58.88) and chitosan + *Pseudomonas fluorescens* (57.77) (Figure 1).

Salicylic acid, *Trichoderma harzianum* + *Pseudomonas fluorescens* and Chitosan + *Pseudomonas fluorescens* recorded least plant disease index as compare to all the treatments.

Outcome of present investigations revealed that all fifteen elicitors tested alone/or in combination for induction of defense related enzymes in pea against *U. viciae-fabae* (Pers.) J. Schrot results in significant induction of total phenols, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in all the treatment as compare to control. Salicylic acid, *Pseudomonas fluorescens*, salicylic acid + *Pseudomonas fluorescens* were found most effective in induction of total phenols and peroxidase at 72 hrs after spray of elicitors. Polyphenol oxidase induction was found significantly high in oxalic acid, *Pseudomonas fluorescens* + *Trichoderma harzianum* and chitosan + *Pseudomonas fluorescens* at 72 hrs after spray of elicitors. Among all the treatments, maximum induction of Phenylalanine ammonia lyase activity was found in oxalic acid, *Trichoderma harzianum* + *Pseudomonas fluorescens* and isonicotinic acid + *Trichoderma harzianum* after 48hrs of spray of elicitors. Effect of different elicitors on percent disease index (PDI) 20 days after inoculation with uredospores of *U. viciae-fabae* showed least PDI in salicylic acid, *Trichoderma harzianum* + *Pseudomonas fluorescens* and chitosan + *Pseudomonas fluorescens* treated plants.

Several reviews have highlighted the potential of chemical treatments to activate and enhance natural plant disease resistance [17,18]. Dann and Deverall [27,28] stated that inoculation of unifoliate leaves of nine days old green bean (*Phaseolus vulgaris*) with

spore suspension of *Colletotrichum lindemuthianum* (10<sup>4</sup> conidia/ml), causing local lesions, or spraying with 2-6-dichloroisonicotinic acid (20µg/ml) induces development of resistance in the upper leaves against challenge inoculation of *U. appendiculatus* afterwards. Rauscher et al. [29] reported the treatment of broad bean leaves with salicylic acid or 2, 6; dichloroisonicotinic acid induces resistance against the rust fungus *Uromyces viciae-fabae* resulting in reduced rust pustules density.

Pea (*Pisum sativum* L.) plants treated with different concentrations of salicylic acid and 4-aminobutyric acid increased activities of phenol metabolizing enzymes implicated in the defense of plants. The enzymes peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase and superoxide dismutase responded to treatment with variation in their activities. Phenolic content also varied following treatment with the inducers [30]. SA was the first synthetic compound shown to induce enhanced activation of a variety of defence responses against major pathogens on various crops [16,17]. Peroxidase activity in cucumber (*Cucumis sativa* L.) and tobacco (*Nicotina tabaccum* L.) after treatment with SA have been reported [31]. Exogenous applications of salicylic acid (SA) and benzothiadiazole (BTH) solutions have been used in faba bean to induce systemic acquired resistance (SAR) to rust (*Uromyces viciae-fabae*), ascochyta blight (*Ascochyta fabae*) and broomrape (*Orobanche crenata*) by Sillero et al. [32]. Surekha et al. [33] conducted experiment to understand the role of *Trichoderma viride* in inducing defense enzymes (Peroxidase, Polyphenol Oxidase and Phenyl Alanine ammonia Lyase) and total phenolic content in black gram exposed to pathogens *Fusarium oxysporum* and *Alternaria alternata*. He found that the biocontrol agent, *T. viride* induce higher levels of defense enzymes in black gram during pathogenesis by *F. oxysporum* and *A. alternata*. Nikoo et al. [34] also mentioned that plant-mediated systemic resistance against the *M. javanica* in tomato cv. CALJN3 was triggered using salicylic acid (SA) and *Pseudomonas fluorescens* CHAO as elicitors. Biochemical changes in *T. harzianum* treated plants, *M. phaseolina* inoculated plants and healthy plants were assayed at different stages of infection by Sreedevi et al. [35]. She found that treatment with *T. harzianum* and challenge inoculation of *M. phaseolina* enhanced induction of defense enzymes such as peroxidase (PO) and polyphenol oxidase (PPO) and defense compounds like total phenol and ortho-dihydric phenol. Numerous findings in other plant pathogen system such as *Puccinia helianthi*/sunflower [36,37], *Uromyces appendiculatus*/common bean [38], or *Uromyces pisi*/pea [39] based on reduction of infection frequency has also been reported. Systemic acquired resistance seems to be a mechanism different from the pre-existing resistance and is associated to the induction of pathogenesis related (PR) proteins [40]. Activation of SAR by exogenous application of elicitors can protect from a broad pathogens spectrum [29,38].

Therefore the above mentioned treatments might be an alternative for the conventional pesticides in pea crop protection, with the advantage of a low environmental impact.

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