DOI : 10.5958/0974-0112.2017.00048.2

Indian J. Hort. 74(2), June 2017: 233-239

# Induced systemic resistance (ISR) in hot pepper against *Phytophthora capsici* infection triggered by cell wall oligosaccharide elicitors from *Trichoderma* species

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## ABSTRACT

**Induced systemic resistance, one of the mechanisms of biological control is elicited either by oligosaccharides or glycoproteins released from cell wall of fungal bioagents like *Trichoderma* species. Oligosaccharide elicitors from 10 *Trichoderma* isolates having biocontrol potential were tested for their ability to elicit ISR in hot pepper against *Phytophthora capsici* infection. Treatment with elicitors from isolates Th10, Th9, Th33 and Th28 reduced *P. capsici* infection in hot pepper by 70-80% compared to 100% infection in pathogen inoculated control. Assays of peroxiase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and glucanase revealed that elicitor treatment significantly induced higher enzyme activity in elicitor treated plants compared to control. Induction of more number of isoforms of PO and PPO was also observed. Elicitors from Th10 and Th33 induced two fold increase in PPO and PAL and three-fold increase in PO, while Th28 and Th9 increased PPO and glucanase by two folds. Different enzymes (involved in phenyl propanoid metabolism or hydryolytic enzymes) contributed to ISR in different isolates.**

**Key words:** Elicitors, hot pepper, induced systemic resistance (ISR), *Phytophthora capsici*, *Trichoderma*.

## INTRODUCTION

*Trichoderma* species are the most common commercially available biological control agents used to manage soil borne pathogens. Earlier the mechanisms of action, *viz*. mycoparasitism, antibiotics production and competition for space and nutrition were explored for identifying the potential isolates of *Trichoderma*. In the recent years, another mechanism, *i.e.* induced systemic resistance is being explored. Treatment with bioagents to the seeds or seedlings induces an array of defense mechanism systemically in plants that includes hydrolytic enzymes like chitinase or glucanase or enzymes involved in phenyl propanoid metabolism like phenylalaline ammonia lyase, peroxidase or polyphenol oxidase. Fungal bioagents like *Trichoderma* releases elicitors that induce the defense mechanism in plants. Elicitors may be glucans, chitin oligomers, chitosan, glycoproteins, proteins or fatty acids (Brotman *et al*., 1). In the present study, hot pepper - *P. capsici* pathosystem was taken up to understand the induction of defense mechanism cell wall glucan elicitors from *Trichoderma* isolates that had been earlier identified as potential biocontrol agents. The main objectives were to determine whether treatment of roots with cell wall elicitors from *Trichoderma* isolates could induce systemically defense enzymes in leaves against foliar infection by *P. capsici*, quantify the defense responses and correlate them to ISR against the pathogen.

## MATERIALS AND METHODS

From the *Trichoderma* isolates maintained at ICAR-NBAII, Bengaluru ten isolates were selected based on their ability to induce systemic resistance in our earlier study (unpublished data). *Trichoderma* isolates used in the study and the disease incidence due to challenge inoculation with *P. capsici* in plants treated with their talc formulations were: *T. harzianum* isolates Th9 (45%), Th10 (35%), Ta101 (65%), Th19 (50%), Th28 (45%) and Th33 (50%); T. *asperellum* isolates Ta30 (48%) and Ta102 (52%) and *T. virens* isolates Tvs5 (55%) and Tvs8 (50%). In *P. capsici* alone inoculated plants there was 100% infection. *Trichoderma* cultures were maintained on potato dextrose agar (HiMedia) slants at 4ºC. A highly virulent isolate of *P. capsici* (PC 06-16) capable of causing serious foliar infection as blight was used. Zoospores at $2 \times 10^6$ per ml were used for inoculation.

Cell wall elicitors were extracted from *Trichoderma* isolates and carbohydrate content was measured as described by Sriram *et al*. (13). Hot pepper cv. Byadagi Kaddi (susceptible to *P. capsici*) was used. Before sowing, seeds were thoroughly surface sterilized with 1% sodium hypochlorite for 2 min. and washed in sterile water and air-dried. Biopeat SG compost was used as substrate for raising the

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seedlings, and 30-day-old seedlings were used in the experiment. Hot pepper seedlings were treated with elicitor preparation by dipping seedlings for 10-15 min., in elicitor preparation and transplanted. Untreated plants served as control. Two sets each of five replications were maintained and in each replication 10 plants were maintained. One day after elicitor treatment one set of plants were spray-inoculated with *P. capsici* (2 × $10^6$ zoospore/ ml) and plants treated with pathogen alone served as pathogen inoculated control. Disease incidence was recorded 30 days after pathogen inoculation.

Leaf samples were collected at weekly intervals (1, 7, 14, 21, and 28 days). Biochemical studies included colorimetric assays of peroxiase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), total proteins, total phenols and glucanase activity. Activity gel electrophoresis with native gels was carried out to study the isoforms of PO and PPO induced by elicitor treatment. One gram leaf sample was homogenized in two 5 ml portions of 80% ethanol and total phenols were estimated using Folin-Ciocalteau reagent (Sriram *et al*., 11). Leaf samples were homogenized with liquid nitrogen for enzyme assays. For total protein, PO and PPO assays, one gram powdered sample was extracted with 0.5 ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4°C and centrifuged for 20 min. at 10,000 rpm. Total protein content in the samples was estimated by Lowry's method. Activities of PAL, PO, PPO and glucanase were assayed as described earlier (Dickerson *et al*., 4; Sriram *et al*., 11; Karthikeyan *et al*., 6; Pan *et al.* 9, respectively). Activity gel electrophoresis with native gels was carried out to study the induction of PO and PPO isoforms due to elicitor treatment as described by Marri *et al.* (8). All gels were analyzed for presence or absence of isoforms after staining. The data related to different assays were analyzed by ANOVA using Statistical Product and Service Solution (SPSS) version16.0 software.

## RESULTS AND DISCUSSION

Carbohydrate estimation of cell wall elicitor confirmed that extracted cell wall elicitor contained carbohydrate moiety. No significant difference (P<0.280, F = 1.46) was found in the concentration of carbohydrate in terms of glucose units per g of fresh weight cell wall elicitor preparation from different *Trichoderma* isolates (20-60 µg/ml). Treatment with different *Trichoderma* cell wall elicitors significantly (P<0.01, F = 20.08) reduced infection by *P. capsici* in plants compared to pathogen alone inoculated plants where 100% disease incidence was observed. Disease incidence was reduced by 70-80% in plants treated with elicitors from Th10 (80%), Th9 (75%), Th33 (70%) and Th28 (70%). Plants treated with elicitors from Tvs5, Ta102, Tvs8 and Th19 did not show significant reduction in leaf blight incidence by *P. capsici* (Fig. 1).

In plants treated with elicitors from Th9, Th10, Th33 and Th28, there was enhanced high protein content (2.7, 2.8, 2.7 and 2.7 mg/g, respectively) till 28$^{th}$ day (Fig. 2A) compared to 1.5 mg/g in control. Challenge inoculation with *P. capsici* enhanced the protein content in plants treated with these elicitors ((2.8, 3.4, 3.0 and 2.9 mg/g respectively).

Peroxidase activity significantly increased in all plants treated with cell wall elicitor preparation from Th33 and Ta102 (11.17 and 11.23 change in abs/min./g of plant tissue). Challenge inoculation also enhanced PO activity (12.35 change in abs/min./g) (Fig. 3B). Native-PAGE analysis revealed the presence of

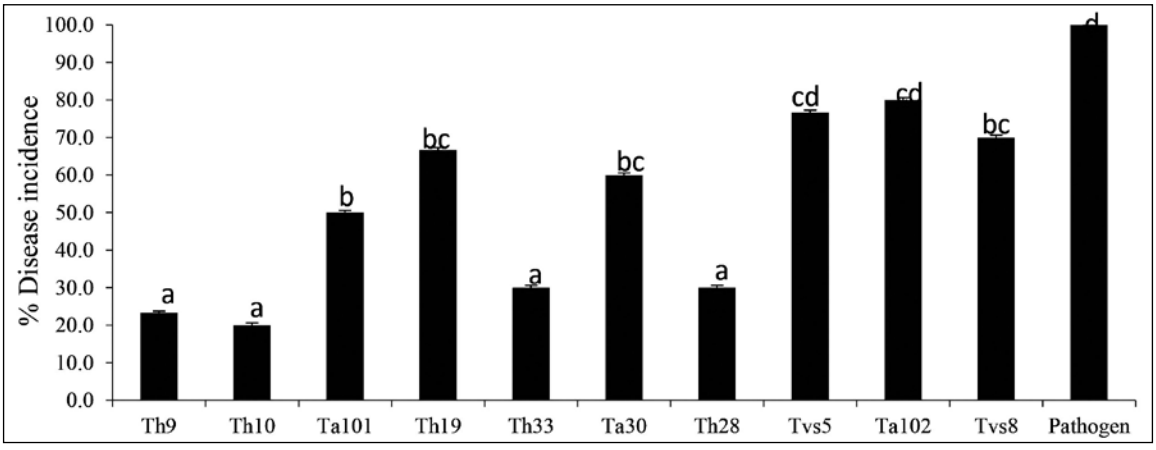


**Fig. 1.** Per cent disease incidence in hot pepper plants treated with different *Trichoderma* elicitor (glucan) and challenge inoculated with pathogen (*P. capsici*). Values represented are back transformed. Different letters indicate significant difference according to Duncan's test (P<0.05).

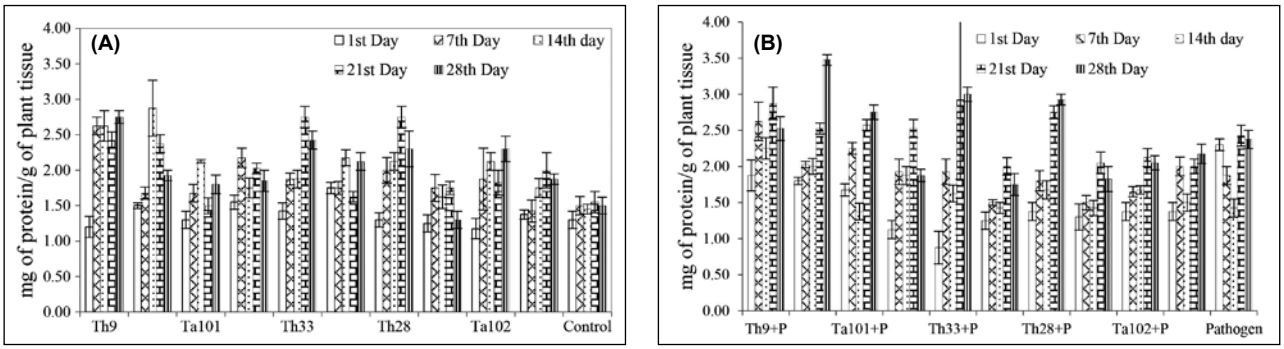


**Fig. 2.** Total proteins content in hot pepper plants. (A) Plants treated with elicitors only, (B) Plants treated with elicitor and challenge inoculated with *P. capsici* and bars represent ± SE.

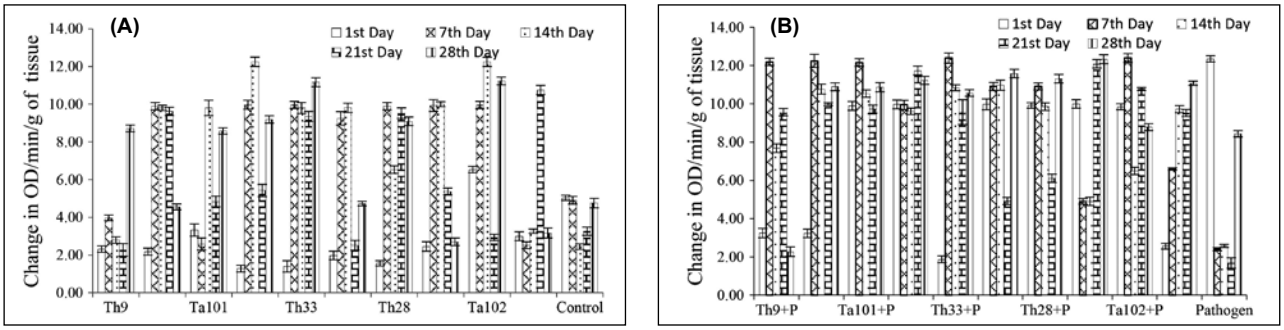


**Fig. 3.** Peroxidase activity in hot pepper plants. (A) Plants treated with elicitors only, (B) Plants treated with elicitor and challenge inoculated with *P. capsici* and bars represent ± SE

maximum of six PO isoforms designated as PO1-PO6 with greater band intensity in plants treated with elicitors alone and challenged with *P. capsici*, whereas in control plants only three PO isoforms were seen that too were faint or less intense bands indicating the role of cell wall elicitor in inducing high PO activity in red pepper and in turn ISR (Table 1). Treatment with elicitors from isolates Th9 and Th28 enhanced PPO activity (0.18 and 0.17 change in abs/min./g of plant tissue, Fig. 4A). There was no significant difference in PPO activity in plants treated with elicitors of other isolates compared to control. Challenge inoculation with pathogen enhanced PPO activity in plants treated with elicitors from Th9, Th28 and TVS8 (Fig. 4B). Plants treated with elicitor alone and challenged with *P. capsici* expressed 4 or 5 PPO isoforms (PPO1, PPO2, PPO3, PPO4 and PPO5) compared to 3 isoforms in control and 4 isoforms in *P. capsici* alone inoculated plants (Table 2). PPO5 was present only in elicitor treated plants while PPO4 was induced either by pathogen or elicitor treatment. Significant increase in phenol content was observed in plants

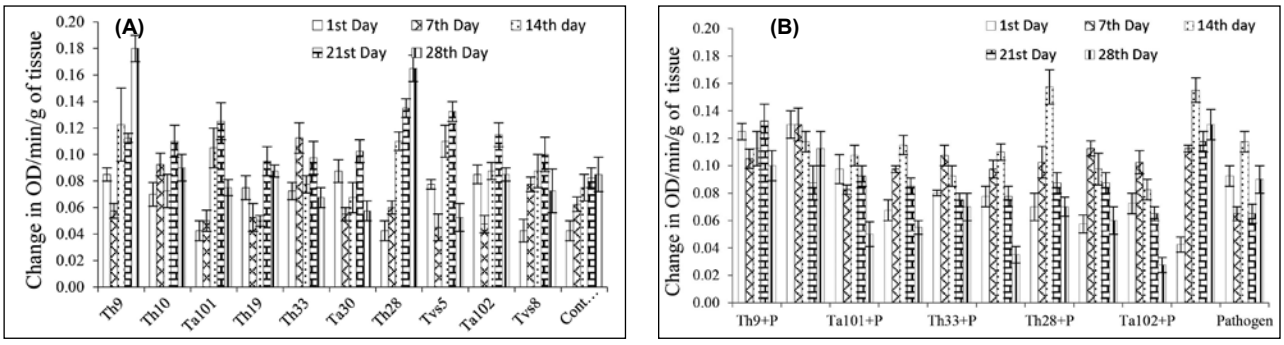


**Fig. 4.** Polyphenol oxidase activity in hot pepper plants. (A) Plants treated with elicitors only, (B) Plants treated with elicitor and challenge inoculated with *P. capsici* and bars represent ± SE.

**Table 1.** Number of peroxidase isoforms observed under activity gel electrophoresis in hot pepper in response to treatment with elicitors from *Trichoderma* species.

| Treatment | 1 day | | 7 day | | 14 day | | 21 day | | 28 day | |
|---|---|---|---|---|---|---|---|---|---|---|
| | E-P | E+P | E-P | E+P | E-P | E+P | E-P | E+P | E-P | E+P |
| Th9 | 4 | 4 | 4 | 5 | 5 | 3 | 6 | 6 | 5 | 6 |
| Th10 | 5 | 5 | 6 | 6 | 5 | 5* | 6* | 3# | 5 | 3 |
| Ta101 | 5 | 5 | 5 | 5 | 5 | 5* | 6 | 5 | 5* | 5* |
| Th19 | 5 | 5 | 5 | 5 | 5 | 5 | 6 | 5# | 5* | 6* |
| Th33 | 5* | 5 | 4 | 5 | 5 | 5 | 5 | 3# | 5* | 5* |
| Ta30 | 5* | 5* | 5 | 5 | 4 | 5 | 6 | 5 | 5 | 5 |
| Th28 | 5 | 5* | 5 | 5 | 4# | 6 | 5 | 5 | 3# | 4 |
| Tvs5 | 6 | 5 | 5 | 5 | 4 | 6 | 5 | 6 | 5 | 5* |
| Ta102 | 5 | 5 | 5 | 5 | 6 | 4 | 5 | 4 | 4 | 4 |
| Tvs8 | 6 | 5 | 5 | 5 | 4# | 6 | 4 | 6 | 4 | 4 |
| Control | 3 | - | 4 | - | 3 | - | 4# | - | 3# | - |
| Pathogen | - | 5 | - | 5 | - | 6* | - | 6* | - | 6 |

E-P = Elicitor without pathogen; E+P = Elicitor with pathogen, # light band (thin); * thick band

**Table 2.** Number of polyphenol oxidase isoforms observed under activity gel electrophoresis in hot pepper in response to treatment with elicitors from *Trichoderma* species.

| Treatment | 7 day | | 28 day | |
|---|---|---|---|---|
| | E-P | E+P | E-P | E+P |
| Th9 | 4 | 4 | 4 | 4 |
| Th10 | 5 | 5 | 4 | 4 |
| Ta101 | 5 | 5 | 5 | 5 |
| Th19 | 4 | 4 | 5 | 5 |
| Th33 | 5 | 5 | 5 | 5 |
| Ta30 | 5 | 5 | 4 | 4 |
| Th28 | 4 | 3 | 3 | 3 |
| Tvs5 | 4 | 4 | 4 | 4 |
| Ta102 | 5 | 5 | 4 | 4 |
| Tvs8 | 5 | 5 | 4 | 4 |
| Control | 3 | - | 3 | - |
| Pathogen | - | 4 | - | 4 |

E-P = Elicitor without pathogen; E+P = Elicitor with pathogen

treated with elicitors from Th9 (840 µg/g), Ta101 (720 µg/g), Ta102 (800 µg/g), Th10 (940 µg/g) and Th28 (780 µg/g) compared to 440 µg/g in control (Fig. 5A). Pathogen alone inoculated plants the phenol content was 460 µg/g and it did not significantly change the phenol content in elicitor treated plants. (Fig. 5B).

A two-fold significant increase in β-1,3-glucanase activity was observed in plants treated with elicitors of Th9 and Th28 (11.6 and 14.1 mg of glucose released/min/g of plant tissue) compared to control and reached maximum on 14$^{th}$ day (Fig. 6A). Challenge inoculation with pathogen followed by treatment with elicitors from Th10, Ta30, Th28 and Th33 enhanced glucanase activity (16.9, 12, 10.7 and 13.2 mg of glucose released/min./g of plant tissue, respectively) (Fig. 6B). Glucanase as well as PPO induction was observed as a result of both systemic acquired resistance and induced systemic resistance. Enhanced PAL activity was observed in plants treated with elicitor from Th10 and Ta101 (190 and 110 µM of trans-cinnamic acid/h/g of plant tissue) that declined after 7 days, while in control it was 30 µM of trans-cinnamic acid/h/g of plant tissue (Fig. 7A). Challenge inoculation enhanced PAL activity in plants treated with elicitors from Th9 and Th10 (180 and 130 µM, respectively) (Fig. 7B).

Differential expression of defense enzymes, *viz*., PR-proteins and enzymes of phenyl propanoid pathway was observed in response to elicitor treatment. Among the isolates studied, elicitors of Th9, Th10, Th28 and Th33 showed promising results in activating defense mechanism in plants. Treatment with elicitors of the isolates Th9, Th10, Th33 and Th28 resulted in enhanced activities of defense enzymes (PO, PPO, PAL and β-1,3-glucanase) (Fig. 8). Treatment with elicitors of Th9, Th28 and Th33 showed early increased PAL activity as PAL catalyses the first step in phenyl propanoid pathway causing increased production of phenolic phytoalexins, which resulted in reduced pathogen invasion. Isolates Th9, Th28 and Th33 showed more induction of β-1,

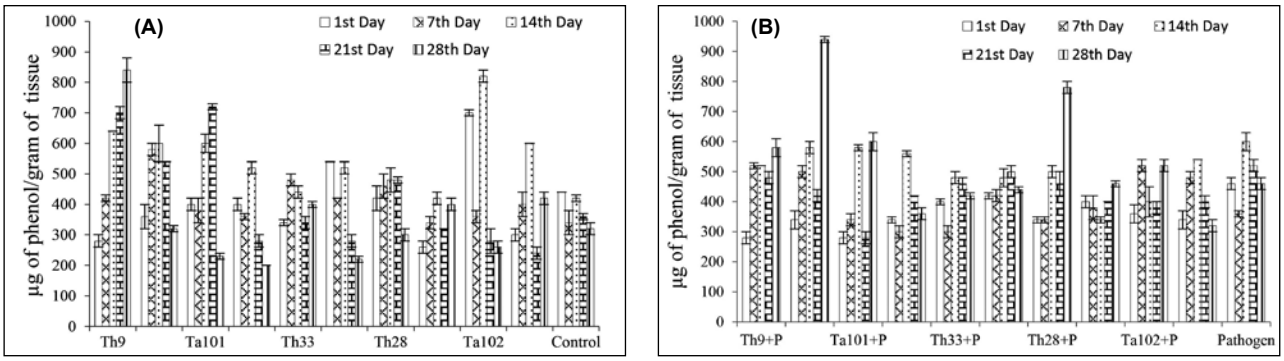


**Fig. 5.** Total phenols content in hot pepper plants (A) Plants treated with elicitors only, (B) Plants treated with elicitor and challenge inoculated with *P. capsici* and bars represent ± SE.

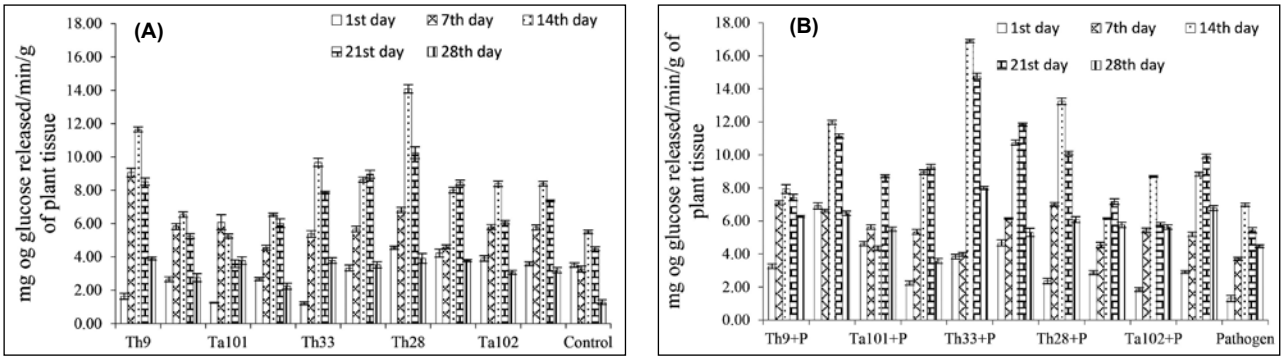


**Fig. 6.** β-1,3-glucanase activity in hot pepper plants (A) Plants treated with elicitors only, (B) Plants treated with elicitor and challenge inoculated with *P. capsici* and bars represent ± SE.

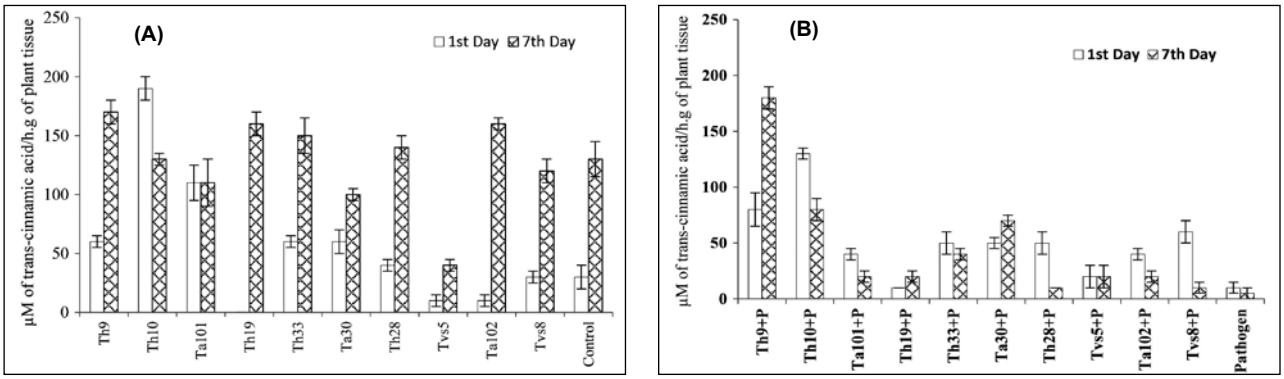


**Fig. 7.** Phenylalanine ammonia lyase activity in hot pepper plants (A) Plants treated with elicitors only, (B) Plants treated with elicitor and challenge inoculated with *P. capsici* and bars represent ± SE.

3-glucanase activity which is triggered by cell wall elicitor treatment which diffuse toward and affect the glucan support structure of *P. capsici*. Since, *P. capsici* cell wall contains glucan, the enhanced glucanase activity might be one of the reasons for increased disease reduction. Elicitor from Th10 enhanced PO activity that catalyses the last steps in the biosynthesis of lignin and hydrogen peroxide that are deposited in cell walls and papillae and interferes with further growth and development of pathogen.

Reports on the induction of defense enzymes due to treatment with bioagents or their elicitors have been reported earlier. Buensanteai *et al*. (2) observed up-regulation of PAL gene in maize plants treated with extracellular protein elicitor from *T. virens*. Karthikeyan *et al.* (6) reported the induction of

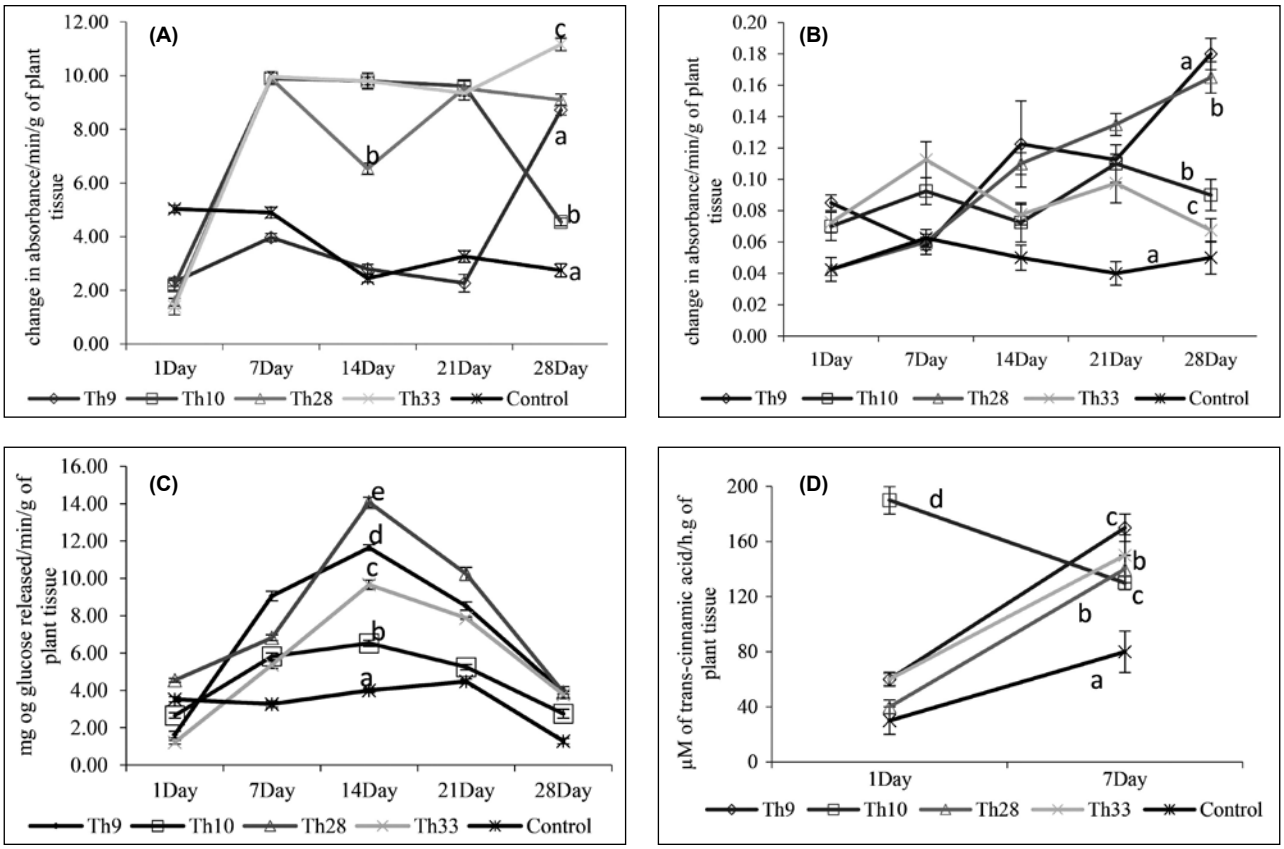


**Fig. 8.** Enzymatic activities of selected isolates. a. Peroxidase, b. Polyphenol oxidase, c. Glucanase, d. PAL. Different letters indicate significant difference according to Duncan's test (P = 0.05).

PPO in coconut plants in response to treatment with *Pseudomonas fluorescens* and *T. viride*. Increased hydrogen peroxide production in rice plants and cotton cotyledons treated with protein elicitor from *Trichoderma virens* was observed by Djonović *et al*. (5). Patil *et al.* (10) reported the induction of phenol content in tomato plants treated with elicitors from non-pathogenic *Fusarium* isolates that provided protection against *F. oxysporum* f. sp. *lycopersici*. Sriram *et al.* (12) observed higher induction of phenol content in hot pepper plants treated with cell wall elicitors from *T. harzianum* and protection against infection by *P. capsici*. The induction of defense mechanism using cell wall fragments of *T. viride* and *T. longibrachiatum* has been documented earlier (Koch *et al*., 7; Chang *et al*., 3). In our earlier study, cell wall glucan elicitor from *T. harzianum* induced systemic resistance by significant increase in glucanase and phenol content in hot pepper plants against *P. capsici* (Sriram *et al*., 12). The treatment with elicitors from isolates Th9, Th10, Th28 and Th33 triggered different defense mechanism (ISR) in hot pepper plants, which resulted in reduced disease incidence by ISR mediated protection either through induction of glucanase or enzymes involved in phenyl propanoid pathway or shikimic acid pathway.

## ACKNOWLEDGEMENTS

This work was supported by the PhytoFuRa project funded by ICAR. We are thankful to the Director, NBAII, Bengaluru for providing lab facilities.

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Received : October, 2015; Revised : January, 2017; Accepted : March, 2017