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Research Article
Fusarium oxysporum Induced Defense Response in Resistant and Susceptible Cultivars of *Eruca sativa* (Miller)

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Abstract
Background and Objective: Plants provide defense mechanism against biotic stress through various strategies. There is involvement of secondary metabolites of plants in defense against pathogens. The objective of the study was to determine the polyphenol content and phenylalanine ammonia lyase (PAL), peroxidase and polyphenol oxidase (PPOx) enzyme activities in the pathogen inoculated and control plants of *Eruca sativa* (*E. sativa*) cultivars of Rajasthan. **Materials and Methods:** The present study described polyphenol, PAL, peroxidase and polyphenol oxidase activity in three cultivars of *E. sativa* viz. RTM-2002 (susceptible), RTM-314 (susceptible) and RTM-1212 (resistant) against *Fusarium oxysporum* and the polyphenols were analyzed by TLC and HPLC. Comparison of mean method was used for statistical analysis of the data, which was analyzed using SPSS-16 statistical software (SPSS Inc.) at Bioinformatics Centre in Banasthali University, Rajasthan. Statistical comparison of mean values was performed using paired t-test. **Results:** TLC and HPLC analysis of polyphenol content showed that it was maximum in resistant cultivar RTM-1212 and minimum in susceptible cultivar RTM-314. As compared to control plants, the activities of enzymes (PAL, peroxidase and PPOx) were higher in pathogen inoculated plants with an increase till 168 h. Thus, based on phytochemical analysis in the pathogen infected plants, it was observed that RTM-1212 cultivar of *E. sativa* responded more actively to pathogen attack as compared to other cultivars. **Conclusion:** The results indicated a role of polyphenol, PAL, peroxidase and polyphenol oxidase in the defense response of *E. sativa*, which is an economically important oilseed crop of semi-arid regions.

Key words: *Eruca sativa*, *Fusarium oxysporum*, defense response, resistant cultivars, susceptible cultivars, biotic stress

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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

After pathogen attack, multiple defense reactions are invoked by plants, such as production of phytoalexin and antimicrobial proteins. In response to pathogen attacks, plants demonstrate constitutive and inducible defenses. Plants exhibit diverse responses to attack pathogens. In one of the most capable forms of plant defenses, known as hypersensitive response (HR), local changes at the site of pathogen infection are observed and this form of response is associated with induced mechanism[1].

Phenolic compounds are a significant group of secondary metabolites as these compounds are involved in resistance to pathogens due to their antimicrobial activity. It is well chronicled that inoculation of tobacco plants with tobacco virus coat proteins causes hypersensitive response and accumulation of phenolic compounds[2].

A series of morphological and biochemical changes triggers the systemic induction of plant resistance in the host plant. These changes are introduced by specific strains of fungi leading to colonization and penetration of plant root tissues and result in the synthesis of defense chemicals against pathogens[3].

Phenylalanine ammonia lyase (PAL) (E.C.4.1.3.5), the non-oxidative deamination enzyme, catalyzes the conversion of L-phenylalanine to trans-cinnamic acid and ammonia[4]. This is an important enzyme responsible for the activation of plant defense mechanism.

Peroxidase (PO) (E.C. 1.11.1.7) belongs to Class-9 PR proteins. It plays a significant role in the defense mechanism of plants against pathogens. This enzyme oxidizes phenolic components into quinines, which are toxic to pathogen[5].

Polyphenol oxidase (E.C. 1.10.3.2, PPOx) is an oxidative enzyme. Based on the browning reactions, PPOx have been suggested to play a defensive role in plants[6].

Eruca sativa (*E. sativa*) is originated in Mediterranean region. It is also known as taramira, rocket salad, roquette and white pepper. *E. sativa* belongs to rapeseed-mustard group and Brassicaceae family[7]. It contains a high amount of vitamin C and displays various medicinal properties viz., laxative, stimulant, astringent, antiphlogistic, diuretic, digestion, stimulant and anti-inflammatory for colitis[8]. One of the dreaded disease of taramira, fungal pathogen *Fusarium oxysporum* is a soil borne pathogen that infects a number of plants, including cotton, tomato, banana, *Arabidopsis* and *E. sativa*[9]. It is the main cause of "root rots" disease and it leads to 5-60% reduction of crop production under the field conditions[10]. The main goal of this study was to estimate defense response of *E. sativa* when attacked by fungal pathogen (*Fusarium oxysporum*).

MATERIALS AND METHODS

Description of the study site: This study was conducted at Department of Biosciences and Biotechnology, Banasthali University, Rajasthan, India from September, 2015 to April 2016, located at 26°24'05.1"N and 75°52'19.9"E.

Plant material and growth conditions: Swami Keshwan and (S.K.N) Rajasthan Agricultural University, Jobner, Rajasthan provided the seeds of three taramira cultivars viz. var. RTM-314 (susceptible), RTM-1212 (resistant) and RTM-2002 (susceptible). The 0.1% $HgCl_2$[11] was used for surface sterilization of seeds. These seeds were then grown in plastic pots in sterilized soils. Controlled conditions in growth chamber viz., photoperiod of 14 h light and 10 h dark with relative humidity of 60%, temperature (26 ± 2°C), were used for growing these plants. Also, these plants were watered once daily. For investigation under *in vivo* condition, 25 days old plants were selected and plant parts of same age were harvested for *in vivo* at long time interval (24, 48, 72, 96, 120, 144 up to 168 h) and short time interval (0, 2, 4, 6, 8 up to 10 h). Then, 8-10 plants were planted in each of the 10 pots taken for each cultivar of taramira. IMTECH, Chandigarh provided the fungal strain of *Fusarium oxysporum* (MTCC NO. 2480).

Activation and maintenance of fungal culture: Activation of the lyophilized fungal strain was performed on potato dextrose broth under aseptic conditions in the laminar flow. Temperature of 28 ± 29°C was maintained for incubation of flasks in incubator shaker for 120 h at shaking speed of 120 rpm. The fungal strain was grown and maintained on potato dextrose agar (PDA) slants by sub culturing at an interval of 30 days.

Preparation of spore suspension and mode of infection: Fungal suspension was prepared under aseptic conditions in autoclaved distilled water. In order to uniformly suspend fungal spores, the flask was kept in incubator shaker, shaking at a speed of 120 rpm for 2 h. The fungal spore concentration was tuned to 10^5 spores mL^{-1}. Pathogen infection in both *in vivo* experiments was introduced through spore suspension. Sprayer was used to spray these spore suspensions on the leaves.

Determination of polyphenol: Phenolic content of taramira leaves was done using modified Bray and Thorpe[12] method.

Analysis of polyphenol by thin layer chromatography: The polyphenol extract from both control and inoculated plants were separated by applying them on TLC silica gel (20×20 cm) aluminum sheets from MERCK (Darmstadt, Germany). In Thin Layer Chromatography (TLC), different solvents were used for the identification of phenolic compounds with different spraying solutions. Polyphenol samples from control and inoculated plants were applied 2 cm above the lower edge of TLC plate. Then, TLC plates were dipped in different solvent mixtures for 2-3 h. TLC plates were allowed to dry for 1 h and sprayed with different spraying solutions according to the solvent used.

The spots were marked with pencil and their R_f values were calculated using the below Eq.[13]:

$$R_f = \frac{\text{Distance (cm) travelled by the spot from the origin}}{\text{Distance (cm) travelled by the solvent from the origin}}$$

Analysis of polyphenol by High Performance Liquid Chromatography (HPLC)

Reagent used: Solvent A-2% glacial acetic acid in HPLC grade water; solvent B-2% glacial acetic acid+30% acetonitrile and 68% HPLC grade water.

These solvents were mixed in a ratio of A:B (60:40). Filtration of polyphenol extracts of the samples was performed using 0.22 µm Millipore filter. Twenty microliters of the samples were injected into a loop injection valve of HPLC (Azilant) in which a SCL-10AVP analog pump and SPD 10AV detector were connected to the system controller. Maximum and minimum pressures maintained were 400 and 0 kgf cm^{-2}, respectively. The HPLC of the standard and samples was performed at 320 nm using a reverse phase C-18 column with a run time of 20 min. In the course of HPLC run time, a flow rate of 1 mL min^{-1} was maintained with the help of a binary mode of the gradient system. In order to quantitatively determine various peaks, a comparison of the integration area values of different standards with known concentration was performed with the sample peaks and the polyphenol content was calculated accordingly.

Determination of PAL activity: Camm and Towers[14] method was used for detecting the PAL activity. Initially, 1 g plant tissue was homogenized in 0.3 mL of 0.05 mM borate buffer (pH 8.8). Filtration and centrifugation of homogenate was conducted at 10,000 rpm for 15 min at 4°C. Then, a mixture of 0.1 mL enzyme extract and 0.3 mL of 50 mM L-phenylalanine was prepared. The total volume was adjusted to 3 mL with 0.05 M borate buffer, (pH 8.8). Incubation of the reaction mixture was performed at 30°C for 15 min and absorbance was taken at 290 nm. The amount of the product formed was calculated using the standard curve of cinnamic acid and represented as µmole of cinnamic acid mg^{-1} protein min^{-1} of all three cultivars (RTM-314, RTM-1212 and RTM-2002) of *E. sativa*.

Determination of polyphenol oxidase activity: Determination of polyphenol oxidase (PPOx) activity was done using the procedure given by Mayer *et al.*[15]. The reaction mixture consisted of 2 mL of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µL of the enzyme extract. In order to initiate the reaction, 200 µL of 0.01 M catechol was added and enzyme activity (expressed as change in absorbance) was recorded at 30 sec intervals up to 30 min at 430 nm.

Determination of peroxidase activity: Peroxidase (PO_X) activity was carried out according to the procedure described by Putter[16] method. The reaction mixture consisted of 3 mL of a mixture containing 0.5 mL guaiacol solution in 0.1 M sodium phosphate buffer of pH 7.0, 0.03 mL hydrogen peroxide and 0.1 mL of sample extract. Crude enzyme preparations were diluted to give changes in absorbance at 436 nm, after every 30 sec, up to 3 min. The enzyme activity was calculated using the extinction coefficient of guaiacol dehydrogenation product which was 6.39 μmol^{-1} at 436 nm.

Statistical analysis: Comparison of mean method was used for statistical analysis of the data, which was analyzed using SPSS-16 statistical software (SPSS Inc.) at Bioinformatics Center in Banasthali University. Statistical comparison of mean values was performed by paired t-test[17]. It was found to be significant at $\leq$0.05% level. The data reported in graphs were mean values of two experiments each containing three replications per treatment (n-6).

RESULTS

Determination of polyphenol: The result of defense response in taramira plant was the induction of biosynthesis of polyphenol by the progression of *Fusarium oxysporum* invasion. In inoculated 15 days old cultivars viz., RTM-1212, RTM-2002 and RTM-314, values were in ranges in increase in polyphenol content at 6 h, was approximately 43.86±0.12, 41.93±0.09 and 39.74±0.05 while in 25 days old cultivars, values were in ranges increase was 53.26±0.11, 51.83±0.09 and 49.97±0.14, respectively (Fig. 1). The values of polyphenol contents in 15 days old (long term) inoculated three investigational cultivars i.e., RTM-1212, RTM-2002 and RTM-314 to be 53.28±0.11, 48.34±0.17 and 46.33±0.08 mg g^{-1} fw which were higher as compared to

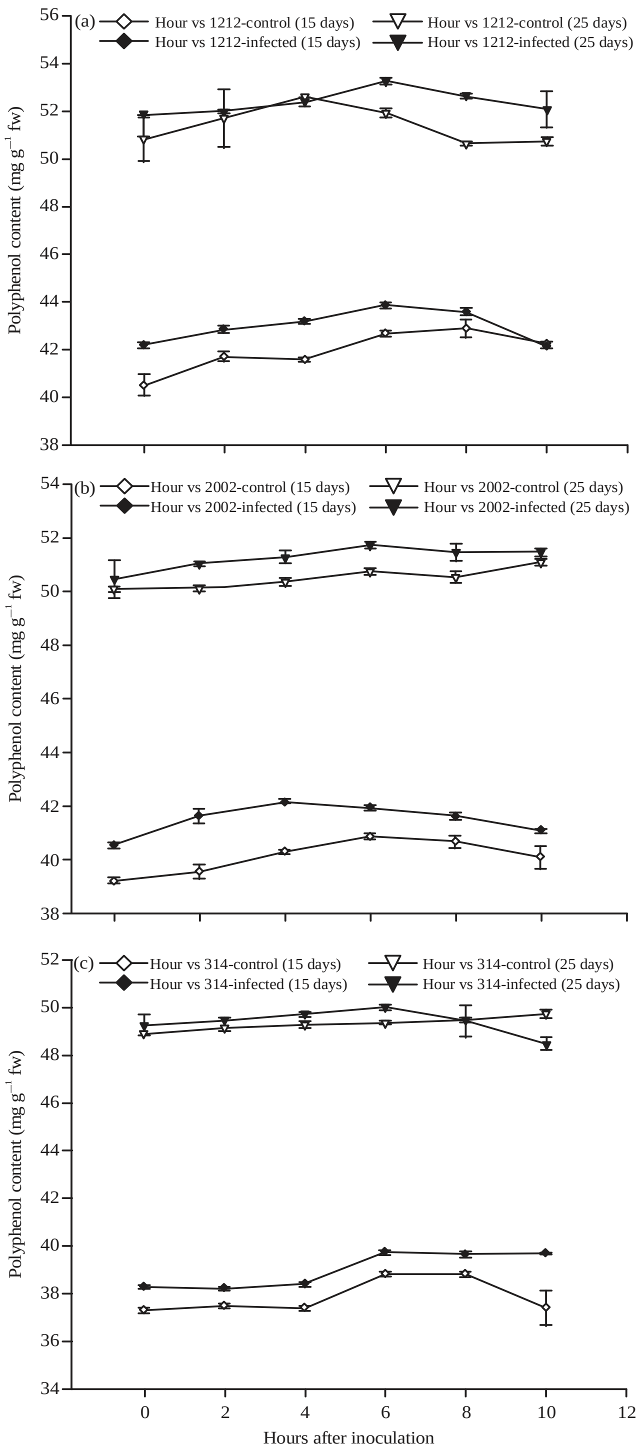

Fig. 1(a-c): Polyphenol content (mg/g^{-1} fw) of control and inoculated of (a) RTM-1212 (b) RTM-2002 and (c) RTM-314 cultivar at short time interval after inoculation with *Fusarium oxysporum*
Values shown are Mean±SD, (n = 5)

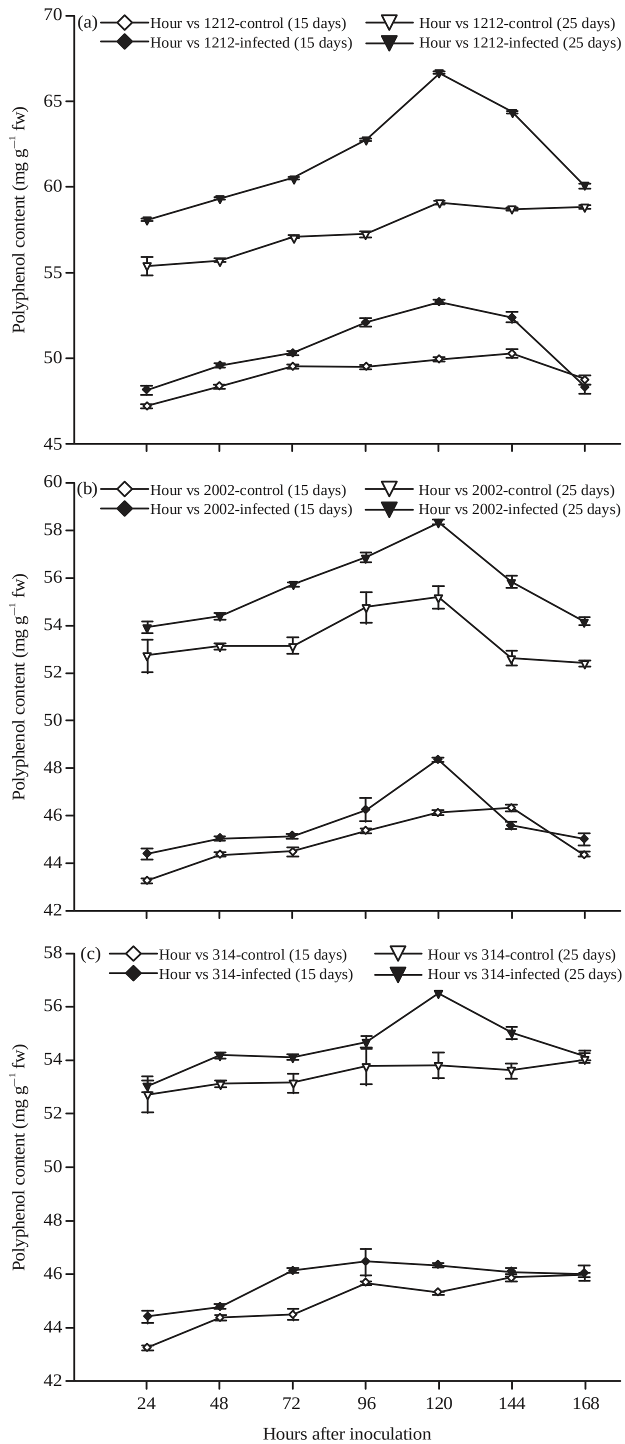

Fig. 2(a-c): Total polyphenol content (mg/g^{-1} fw) of control and inoculated of (a) RTM-1212 (b) RTM-2002 and (c) RTM-314 cultivar at long time interval after inoculation with *Fusarium oxysporum*
Values shown are Mean±SD, (n = 5)

control ones. Whereas, in 25 days old investigational cultivars the polyphenol values were found as 66.68±0.08, 58.31±0.05 and 56.51±0.08 mg g^{-1} fw found in inoculated ones than the corresponding control ones (Fig. 2).

Table 1: Thin layer chromatography of polyphenols extracted from *Eruca sativa*

		Plant samples R_f values					
Standards R_f values		314-C	314-I	1212-C	1212-I	2002-C	2002-I
CINA	**FA**						
0.93	0.95	0.400	0.480	0.470	0.520	0.470	0.490
		0.560	0.720	0.700	0.760	0.780	0.800
		0.740	0.740	0.800	0.830	0.900	0.920
		0.880	0.910	0.850	0.850	0.960	0.900
CA	**p-COU-A**						
0.89	0.91	0.700	0.710	0.820	0.880	0.910	0.920
		0.790	0.840	0.900	0.920	0.930	0.930
		0.810	0.880	0.680	0.700	0.950	0.960
SA	**o-COU-A**						
0.78	0.82	0.750	0.756	0.754	0.807	0.783	0.784
		0.762	0.766	0.771	0.832	0.752	0.756
		0.800	0.816	0.821	0.833	0.793	0.796

CIN A: Cinnamic acid, FA: Ferulic acid, CA: Caffeic acid, p-Cou A: p-coumaric acid, SA: Salicylic acid, o-cou A: o-coumaric acid, C: Control, I: Infected

Table 2: Concentration of phenolic compounds (%) in control and infected taramira cultivars of 25 days old plants of taramira at 120 h of infection

		RTM-1212				RTM-2002				RTM-314			
		C		I		C		I		C		I	
Phenolic compounds	Std. area	Sample area	Conc. (%)	Sample area	Conc. (%)	Sample area	Conc. (%)	Sample area	Conc. (%)	Sample area	Conc. (%)	Sample area	Conc. (%)
Cinnamic acid	477095	312326	0.07	162285	0.34	243218	0.01	152326	0.320	101176	0.02	125726	0.05
Sinapic acid	558281	20872.9	0.04	752284	0.13	*	*	104721	0.002	71278	1.26	*	*
Ferulic acid	587863	3892.63	0.07	138068	0.23	135279	0.002	151746	0.260	*	*	6849	0.002
Gallic acid	501040	26892.8	0.31	91367	0.33	*	*	64101	0.130	*	*	*	*
o-coumaric acid	173125	121885	0.07	453649	0.26	767725	0.04	421319	0.250	286464	0.16	186280	0.04

C: Control, I: Infected, Conc.: Concentration. *Compound absent, Std.: Standard

Qualitative determination of polyphenol content by using Thin Layer Chromatography (TLC): As observed from the data, the chromatogram showed the presence of yellow, orange and brown colored spots which confirmed the presence of phenolic compounds (Table 1). The R_f value of brown and yellow spots in the sample almost matched the R_f value of caffeic acid and coumaric acid, respectively.

Analysis of polyphenol content by using High Performance Liquid Chromatography (HPLC): Analysis of peaks was performed by comparing the Retention Time (RT) of the standard phenolic compounds with the methanolic extracts of all the three taramira cultivars (Table 2). Maximum concentration for polyphenol was observed at 120 h of infection. Five peaks were found in infected RTM-1212 cultivar. Similarly, infected RTM-2002 also showed 5 peaks and infected RTM-314 showed total of 3 peaks (Fig. 3a-c). For instance, the concentration of cinnamic acid and sinapic acid in inoculated RTM-1212, RTM-2002 and RTM-314 was found to be elevated to 0.34 and 0.13%, 0.32 and 0.00%, 0.05% which is found higher than that of 0.07 and 0.04%, 0.01, 0.02 and 1.26% in control cultivars of taramira, respectively. Comparable results were also obtained in remaining three phenolic compounds.

PAL activity: In 15 days old plants, the amount of PAL was recorded as 2.59±0.02, 2.46±0.02 and 2.35±0.02 μmol of cinnamic acid mg^{-1} protein min^{-1} in inoculated RTM-1212, RTM-2002 and RTM-314 cultivars, respectively at 6 h of inoculation in comparison to control ones. Similar results were also found in 25 days old plants, the amount of PAL in inoculated RTM-1212, RTM-2002 and RTM-314 cultivars was also recorded as 3.10±0.03, 3.08±0.03 and 2.98±0.02 at 6 h after inoculation (Fig. 4). Fifteen days old pathogen inoculated plants of RTM-1212 showed PAL activity of2.88±0.02 at 48 h in contrast to control plants. Similarly, in 25 days old plants the PAL activity was observed as 3.45±0.03 at 48 h of infection in inoculated plants of cultivars RTM-1212 (Fig. 5).

Peroxidase activity: The specific activity of peroxidase in 15 days old three investigational cultivars i.e., RTM-1212, RTM-2002 and RTM-314 were observed as 1.45±0.06, 1.43±0.03 and 2.25±0.04 μmol mg^{-1} protein min^{-1} at 4 h of inoculation. Similarly, the corresponding values were noticed as 2.38±0.04, 1.44±0.03 and 2.45±0.04 μmol mg^{-1} protein min^{-1} in 25 days old infected cultivars (Fig. 6). The values were noticed as 2.78±0.02, 2.56±0.04 and 2.25±0.05 μmol mg^{-1}protein min^{-1}in 15 days old cultivars of

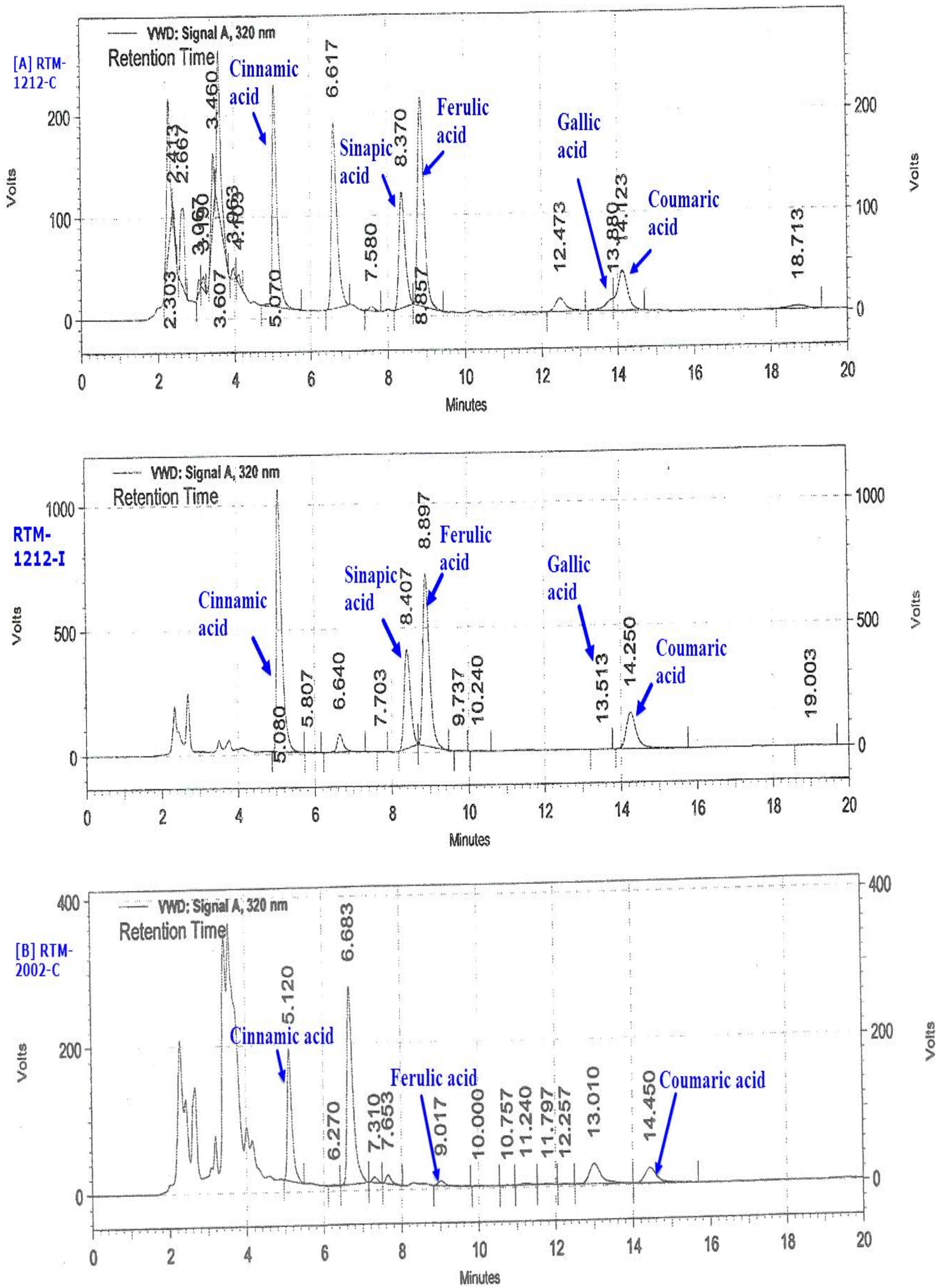

Fig. 3(a-c): Continued

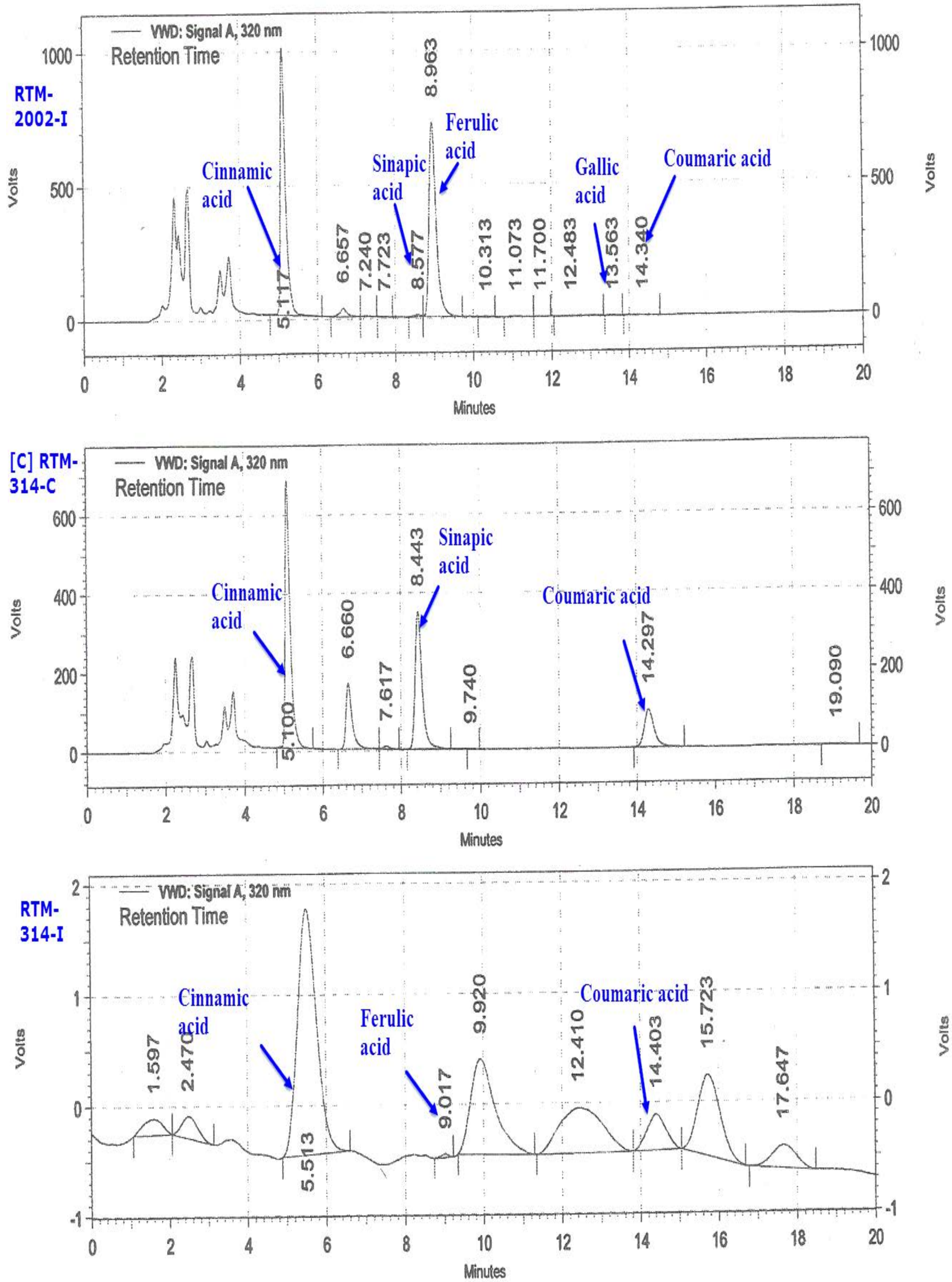

Fig. 3(a-c): HPLC chromatogram of control and infected plants of (a) RTM-1212, (b) RTM-2002 and (c) RTM-314 after 120 h of infection

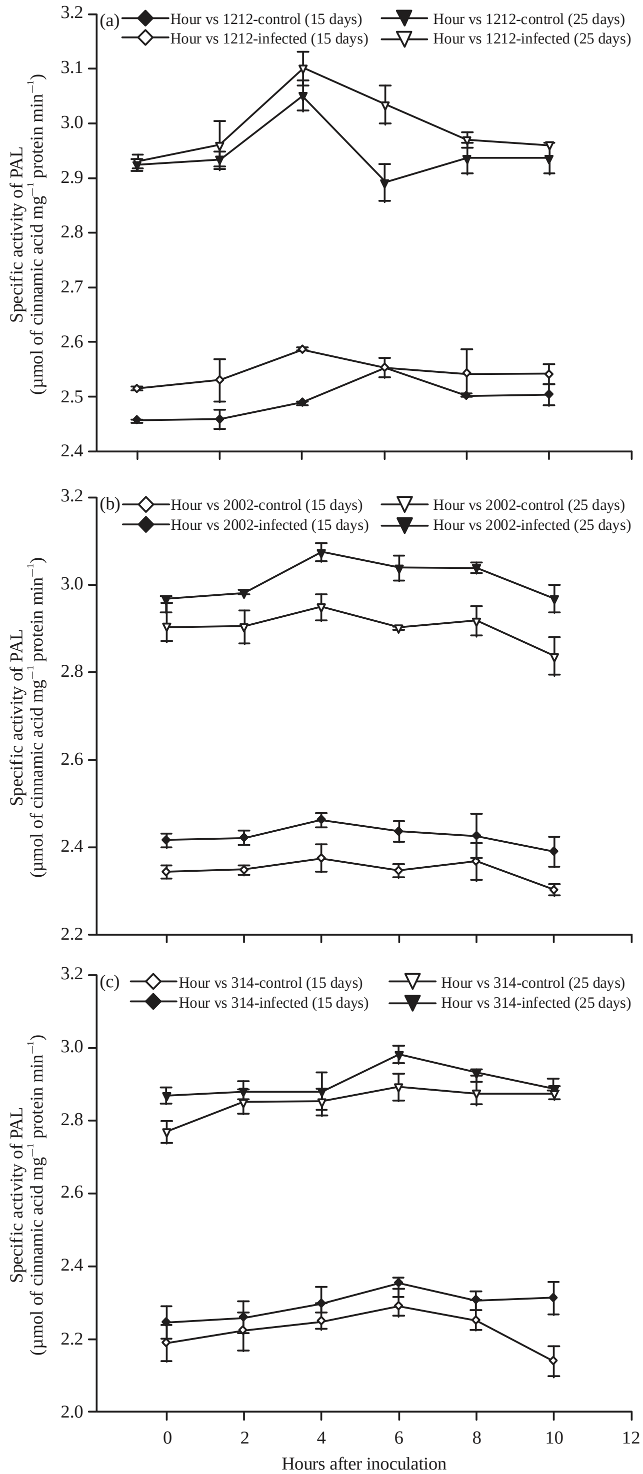

Fig. 4(a-c): Specific activity of PAL (μmol of cinnamic acid mg^{-1} protein min^{-1}) of control and inoculated (a) RTM-314, (b) RTM-1212 and (c) RTM-2002 cultivar at short time interval after inoculation with *Fusarium oxysporum*

Values shown are Mean±SD, (n = 5)

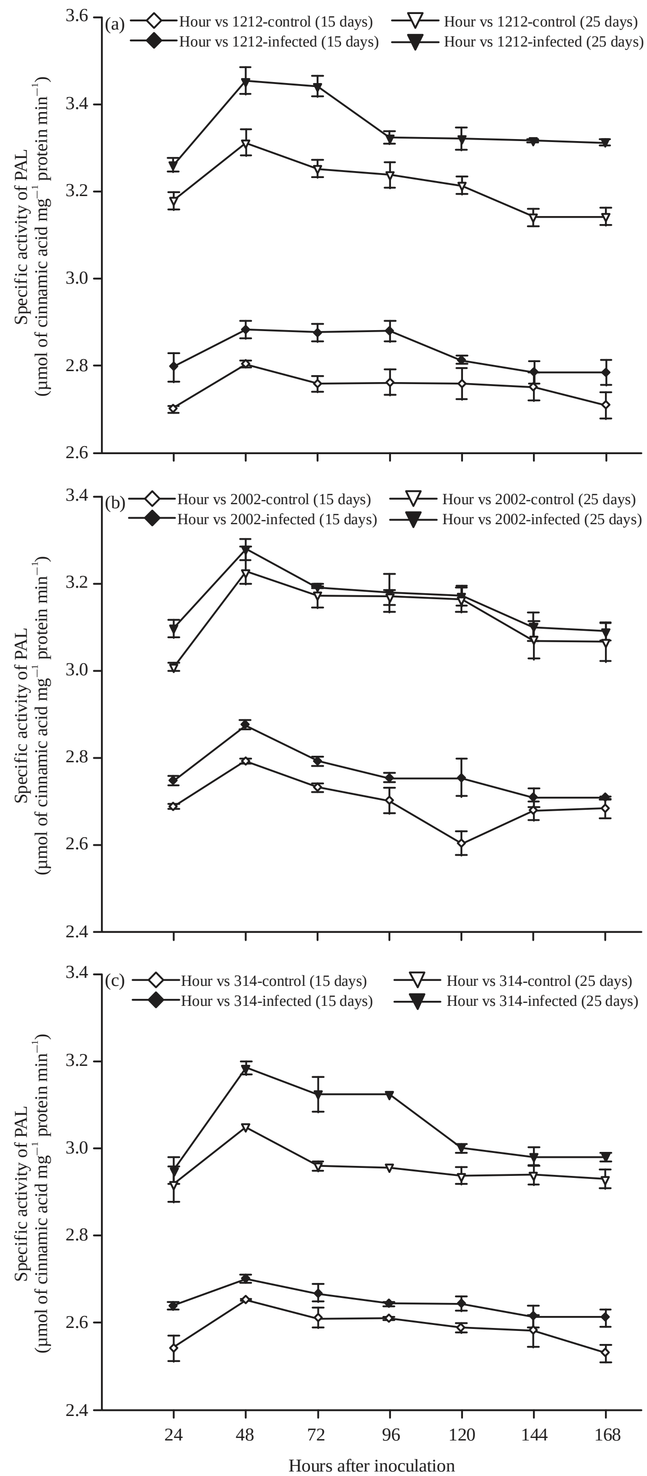

Fig. 5(a-c): Specific activity of PAL (μmol of cinnamic acid mg^{-1} protein min^{-1}) of control and inoculated (a) RTM-314, (b) RTM-1212 and (c) RTM-2002 cultivar at long time interval after inoculation with *Fusarium oxysporum*

Values shown are Mean±SD, (n = 5)

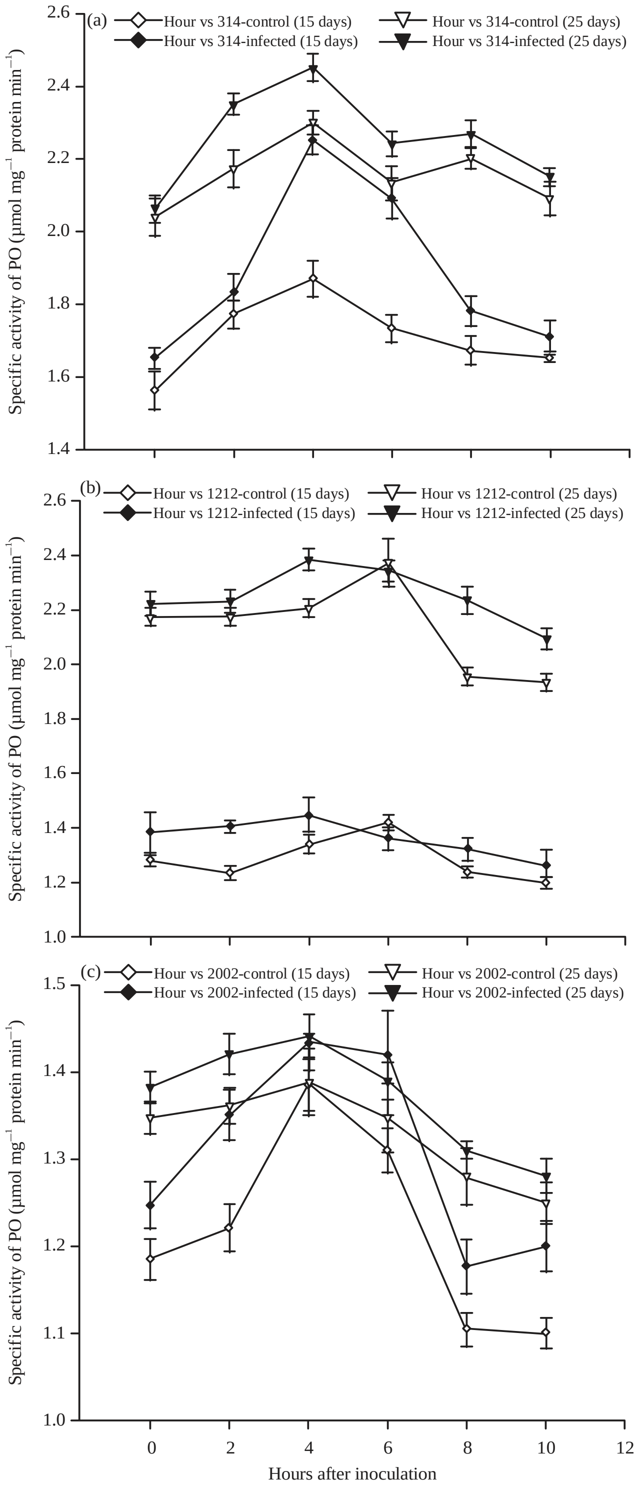

Fig. 6(a-c): Specific activity of peroxidase (μmol of mg^{-1} protein min^{-1}) of control and inoculated of (a) RTM-314, (b) RTM-1212 and (c) RTM-2002 cultivar at short time interval after inoculation with *Fusarium oxysporum*

Values shown are Mean±SD, (n = 5)

RTM-1212, RTM-2002 and RTM-314, respectively at 96 h of inoculation. Similarly, in 25 days old plants the peroxidase activity was observed as 4.84±0.03, 4.05±0.00 and 3.59±0.00 μmol mg^{-1} protein min^{-1} at 96 h of inoculation (Fig. 7).

Polyphenol oxidase (PPOx) activity: It was noted that maximum production of polyphenol oxidase occurs in 15 days old three investigational cultivars i.e., RTM-1212, RTM-2002 and RTM-314 were observed as 1.89±0.01, 1.74±0.04 and 1.71±0.05 μmol mg^{-1} protein min^{-1} at 4 h. Similarly, the corresponding values were noticed as 2.64±0.04, 2.55±0.02 and 2.30±0.05 μmol mg^{-1} protein min^{-1} in 25 days old infected cultivars (Fig. 8). The values were noticed as 2.59±0.01, 2.63±0.11 and 2.40±0.02 μmol mg^{-1} protein min^{-1} in 15 days old cultivars of RTM- 1212, RTM-2002 and RTM-314 respectively at 96 h of inoculation. Similarly, in 25 days old plants the peroxidase activity was observed as 2.77±0.00, 2.54±0.00 and 2.71±0.05 μmol mg^{-1} protein min^{-1} at 96 h of inoculation (Fig. 9).

DISCUSSION

As per the study, the phenolic compounds, PAL, peroxidase and polyphenol oxidases enzymes activities in inoculated plants increased greatly ($p \leq 0.05$) as compared to control plants. Also, it was observed that the healthy taramira plants contained significantly less phenols as compared to the inoculated ones. Both age groups across all time intervals conform to this observation. Polyphenol contents were evaluated every 2 h (0, 2, 4, 6, 8 and 10 h) after the infection for identification of the activation of biosynthesis of phenolic compounds. In both 15 and 25 days old plants, increased value was observed at 6 h after inoculation. The results mentioned here are in conformation with the ones achieved by investigation of Martin *et al.*[18]. As per the results, the infection of young grapevine plants with *Phaeomoniella chlamydospora* lead to an up regulation of plant phenolic content. Present study are also in coordination with Gadzovska *et al.*[19], who observed that phenolic compounds in *Hypericum perforatum* cells suspension increases by 60 times after JA (Jasmonic acid) elicitation. Later, it was noted that there was a minimal decline in the polyphenol levels when investigated at 144 and 168 h of pathogen inoculation in all the three cultivars.

As per this comparison, it can be confirmed that both caffeic acid and coumaric acid were present in the sample. Mobility of spot in both control and pathogen inoculated plant extract was almost identical as per the comparison of

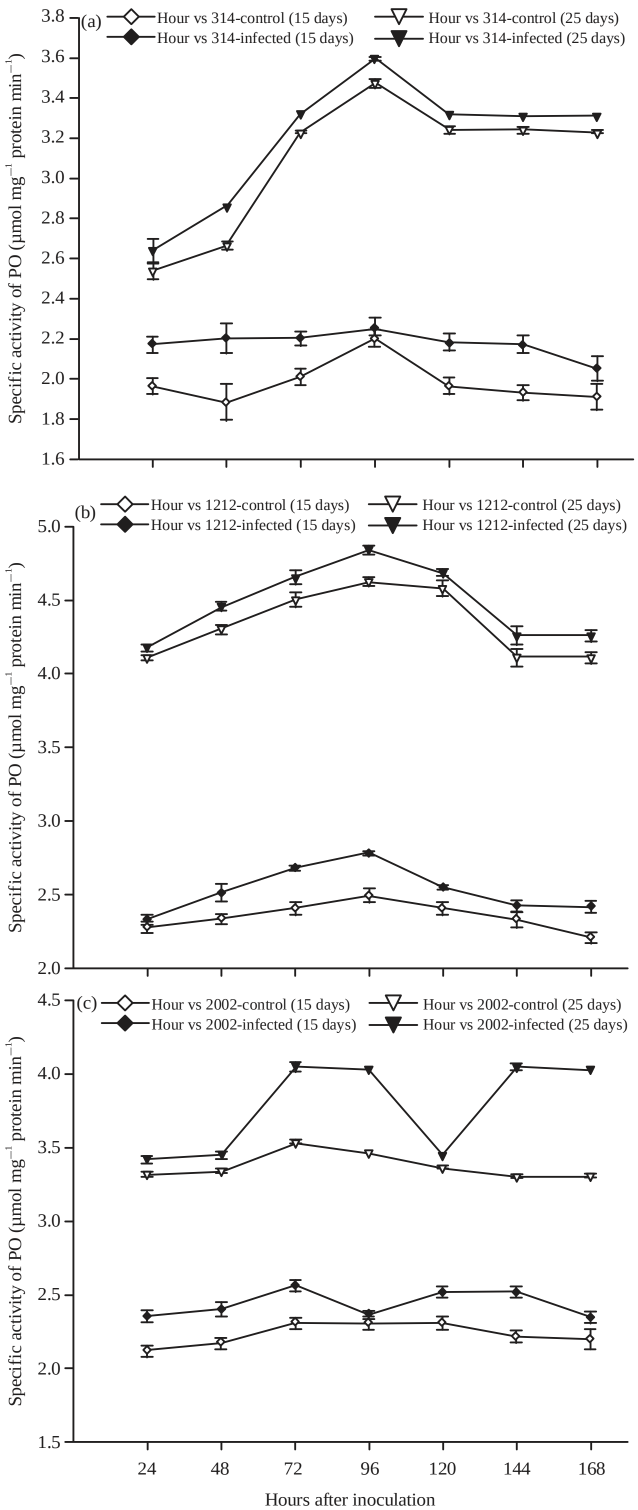

Fig. 7(a-c): Specific activity of peroxidase (µmol of mg^{-1} protein min^{-1}) of control and inoculated (a) RTM-314, (b) RTM-1212 and (c) RTM-2002 cultivar at long time interval after inoculation with *Fusarium oxysporum*

Values shown are Mean±SD, (n = 5)

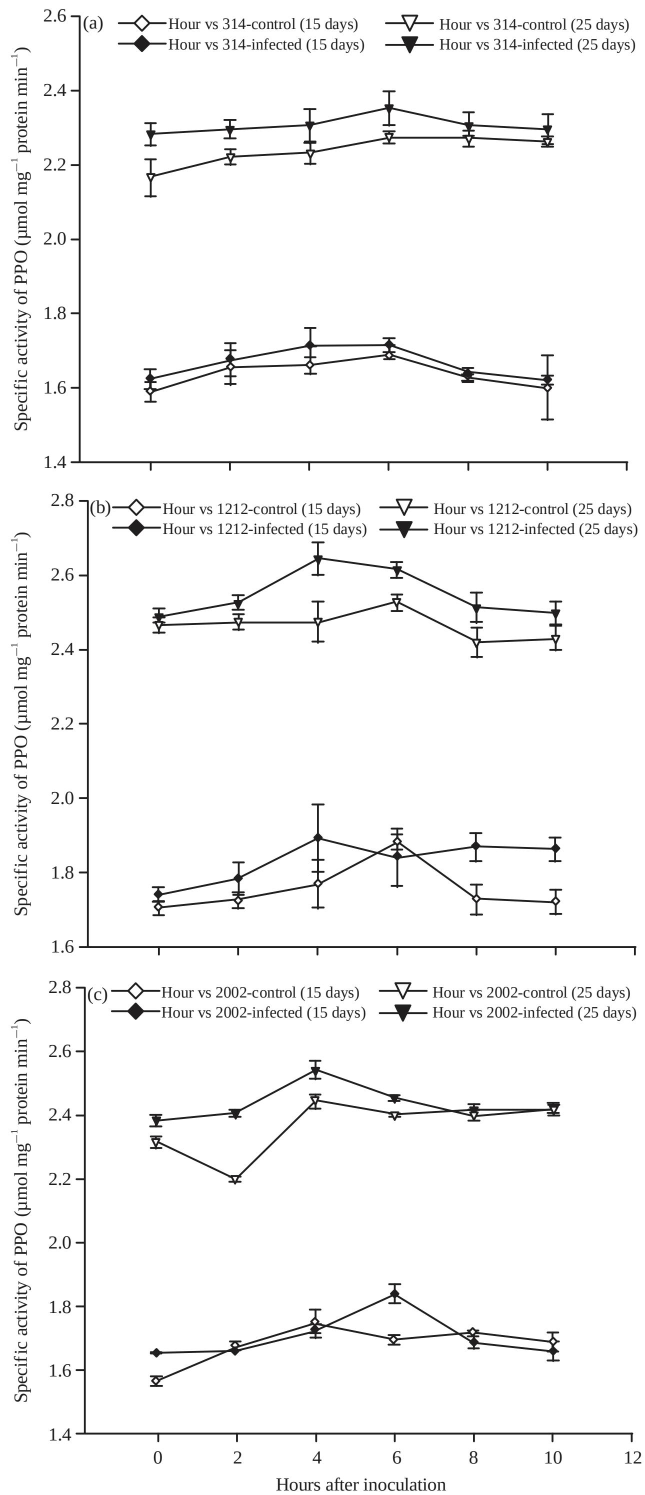

Fig. 8(a-c): Specific activity of PPO (µmol mg^{-1} protein min^{-1}) of control and inoculated (a) RTM-314, (b) RTM-1212 and (c) RTM-2002 cultivar at short time interval after inoculation with *Fusarium oxysporum*

Values shown are Mean±SD, (n = 5)

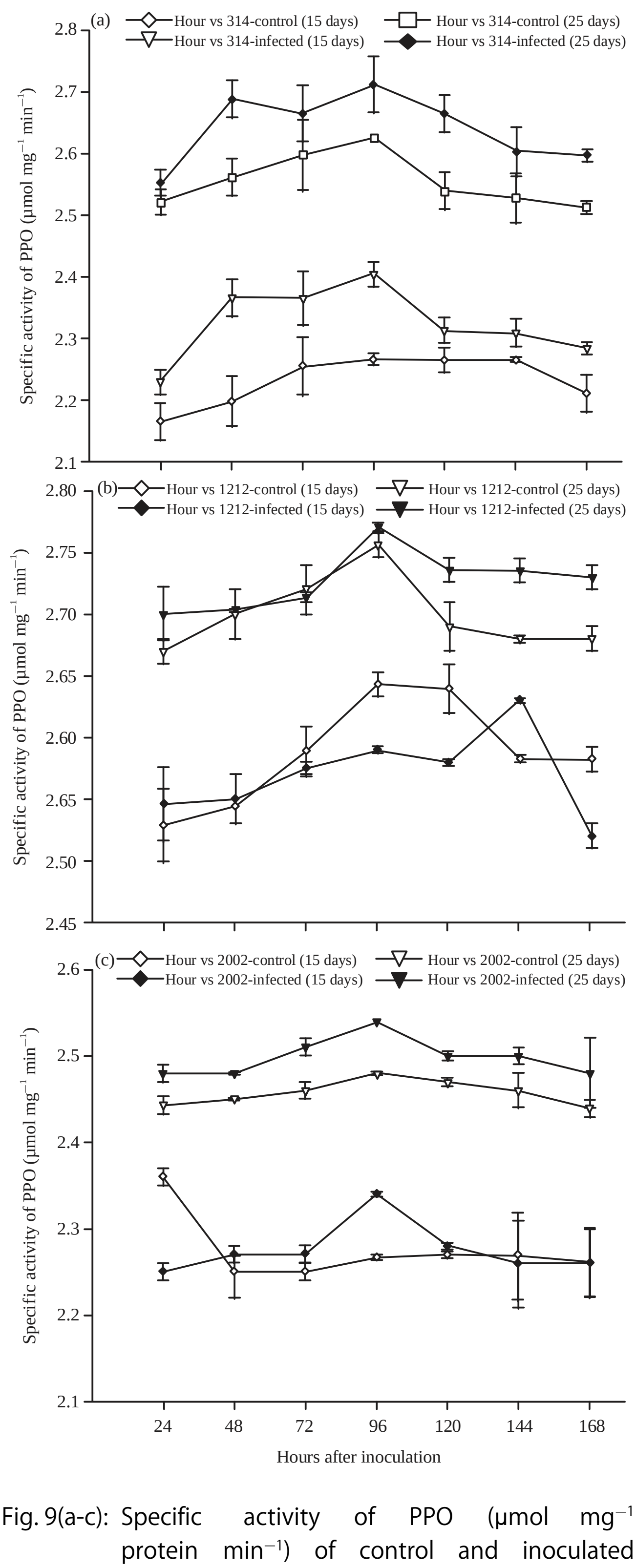

Fig. 9(a-c): Specific activity of PPO (μmol mg^{-1} protein min^{-1}) of control and inoculated (a) RTM-314, (b) RTM-1212 and (c) RTM-2002 cultivar at long time interval after inoculation with *Fusarium oxysporum*

Values shown are Mean±SD, (n = 5)

TLC plates of control and inoculated sample. But the R_f value in infected sample was found to be more than that of control samples. These results were compatible with reports of Balogun and Teraoka[20], which showed the presence of some phenolic spots in potato virus X and tobacco mosaic virus in infected tomato plants, which were not present in non-infected ones, as revealed by thin layer chromatographic analysis.

From the above-mentioned data, it was clear that RTM-314 showed poor results since it produced polyphenols in low concentration because of late recognition of pathogen. In investigational cultivars, the concentration of phenolic compounds was found to be increased after infection, as compared to healthy cultivars. Similar results were observed in apple plants after they were infected with *Venturia inaequalis* (apple scab), which implies that there was more p-coumaric acid in resistant varieties of apple as compared to susceptible varieties[21]. It was observed that resistant cultivars i.e. RTM-1212 showed maximum increase in polyphenols while susceptible cultivar i.e. RTM-314 showed minimum production. In addition to maximum concentration of phenolic compound, RTM-1212 also showed the presence of maximum number of phenolic compounds.

The PAL activity was as certained by exposure of 15 and 25 days old plants to the pathogen under *in vivo* conditions after every 2 h up to 10 h. In 15 days old plants, maximum activity of PAL was recorded at 6 h and it was noticed that amount of PAL was always higher in inoculated plants in comparison to control plants at every stage of infection. PAL activity in pathogen treated tomato plant increased significantly[22]. Thus, it can be concluded that both 15 and 25 days old inoculated plants showed an increase in specific activity of PAL even at shorter time intervals i.e. 4 h after inoculation of fungal pathogen. The maximum enzyme activity was observed at 48 h of infection in all the three cultivars (RTM-314, RTM-1212 and RTM-2002) in both 15 and 25 days plants. The increase in PAL activity initiates defense reaction of plants to pathogen attack. It shows significant increase after infection by pathogen or wounding in forms of resistance to *Fusarium oxysporum* fungi[23].

In plant protection, peroxidase is one of the crucial enzyme. After assaying the peroxidase (PO) activity in the leaf samples of all the three cultivars, PO activity was observed to be significantly higher in the resistant cultivars as compared to the susceptible ones. Infection of systemic acquired resistance (SAR) seedlings of pearl millet (*Pennisetum glaucum*) by downy mildew (*Sclerospora adminicle*) leads to 1.5-2.6-fold increase in peroxidase activity[24].

To recognize the role of peroxidase in plant defense system, long term experiments were also conducted at the difference of 24 h post inoculation up to 168 h. Among all the three-investigational cultivar, RTM-1212 has the highest content which was observed at 96 h of inoculation. The specific activity of peroxidases was observed to increase after 24 h of infection, reaching maximum at 96 h in RTM-1212, RTM-2002 and RTM-314 cultivars in both 15 days as well 25 days old varieties and later on declined drastically up to 168 h of inoculation. Infection by pathogen like *Fusarium oxysporum* in plants lead to stimulation of peroxidases, which is one of the major defense system enzymes.

The current study was conducted to ascertain the maximum activity of PPO enzyme in pathogen inoculated and control plants after short time interval (0-10 h) in 15 and 25 days old cultivars. These results are in support with development of resistance in plants against *Pseudomonas syringae* (*P. syringae*), which is majorly caused due to increased amount of PPO in tomato after infection with *P. syringae*[25].

This study also helps to develop the thematic, multidisciplinary center that enhance and broadens humans ability to attain more insights of plant-specific events that are crucial for evading pathogen by plant defenses. Some of the applications of this study include development of new research tools to study plant-pathogen interactions, identification of new correlates, diagnostics and therapeutics for infectious and inflammatory diseases. Results of this study can be applied for improving the growth of this crop in future by making it resistant to pathogens.

CONCLUSION

It can be concluded from the above results that secondary metabolites are candidate for the study of induced defense responses and the resistance/susceptibility of taramira plants when attacked by a particular pathogen. Hence, it can be inferred that this is a best study for the improvement of this major crop in future. This study can also be used in breeding programs for crop improvement of taramira plant, thereby leading to development of new knowledge.

SIGNIFICANCE STATEMENT

The present study discovers *Fusarium oxysporum* induced defense response in resistant and susceptible cultivars of *E. sativa* (Miller). This defense response helps in inhibiting fungal growth in *E. sativa* plant. This study of fungus-plant interaction can help in breeding of new resistant varieties of taramira plant cultivars, which are resistant to stresses such as biotic stress (fungal diseases). Also, the results of this study can be referenced in further research by the researchers for developing new resistant varieties of *E. sativa* by genetic modification of its susceptible varieties.

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