INTERNATIONAL JOURNAL OF ENVIRONMENT
Volume-4, Issue-4, Sep-Nov 2015 ISSN 2091-2854
Received:26 Nov 2014 *Revised:13 April 2014* *Accepted:2 Nov 2015*

PHYTOALEXINS STIMULATION IN THE INFECTED *Sesamum indicum* L. WITH FUNGUS *Macrophomina phaseolina* (Tassi) Goid (Rot Disease)

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Abstract

Plants respond to a wide variety of pathogen attack. They show the local response in originally attacked plant organ and systemic response in unaffected plant parts with the *de novo* production of phytochemical compounds. Phenolics (polyphenols) play an important role in the defense mechanism of the plants. So, this study was carried out to analyze the metabolic modifications in *Sesame* plant after the infection with the pathogen (*Macrophomina phaseolina*) by estimating the levels of polyphenol in 7 days and 14 days old *Sesame* plants. The polyphenol contents in infected plants are considerably exceeded in contrast to control plants. This *in vivo* study of *M. phaseolina* infection reveals the differences of resistance levels in *Sesame* against the pathogen. The obtained results give important information concerning the plant-pathogen interactions, in the defense response for *Sesame* improvement programs seeking the adaptation to the diverse range of fungal attack along with adverse environmental factors.

Key Words**:** defense response, *Macrophomina phaseolina*, Polyphenols, *Sesamum indicum*

Introduction

Plants respond to pathogen attack or elicitor treatments by activating a wide variety of protective mechanisms designed to prevent pathogen replication and spreading (Malolepsza and Rozalska, 2005). The defense mechanisms, including the rapid production of reactive oxygen species (De Gara *et al.,* 2003); alterations in the cell wall constitution, accumulation of antimicrobial secondary metabolites known as phytoalexins (Agrios, 2005). In nature, plants are generally resistant to most pathogens because they have an innate capability to recognize prospective invading pathogens and to accumulate successful defenses by the production of phytoalexins. These induced defense mechanisms are expressed at sites of attack which is known as hypersensitive response and as well as the surroundings of the attacked site or wound location of primary infection and protect the plant from the increase of infection. When constitutive defenses enlarge above by these pathogens in plant, then a complex signaling cascade of inducible defense responses is activated (Asselbergh *et al*., 2008). As a result of which secondary metabolites are produced to combat the plant against pathogen infection. Comparing metabolic outline of infected plants with the corresponding controls is a powerful tool to unravel the biochemical pathways involved in multi-factorial disorders. Phenolic acids are signaling molecule which regulates the induced resistance by signal transduction pathways (Feys and Parker, 2000).Sesame (*Sesamum indicum* L.) is an annual plant belongs to family *Pedaliaceae* and it is grown for oil having high antioxidant activity. Sesame is produced in the warm regions of the world, mainly in India, but it has great loss of its yield because it is mainly affected by the soil borne fungus *Macrophomina phaseolina* causes charcoal or root rot disease in this plant. *M. phaseolina* elaborates a number of phytotoxins, namely, asperlin, isoasperlin, phomalactone, phaseolinic acid, phomenon and phaseolinone (Bhattacharya *et al.*,1992a). Phaseolinone appears to be most important of these toxins and it induces disease symptoms in plants similar to those produced by the pathogen (Bhattacharya *et al.,* 1992b; Sharma *et al*., 2014). This phytopathogen plays a crucial role in losses of 500 plant species, but the plant has endowed a mechanism to defend themselves against the phytopathogen, they have the capability to resist themselves

by activating a signalling cascade and producing phytoalexins at the infected and uninfected site.

Thus, the principle of the present work is to compare the defense response of four different cultivars viz. RT-46, GT-2, T-12 and TMV-3 in 7 days and 14 days old *Sesame*plant at different time interval respectively, against the pathogenic fungus i.e. *M. phaseolina* in terms of polyphenols.

Material and Methods

Plant material and growth conditions

Seeds of different cultivars of *Sesamum indicum* L. were procured from Rajasthan, Tamil; Nadu and U.P. Seeds were sterilized with 0.1% $HgCl_2$ and grown under controlled conditions in the greenhouse at temperature 30±2 ℃ and 77% humidity. For investigation, 7 days and 14 days old plants were selected for *in vivo* systems at two time intervals, i.e. 0, 2, 4, 6, 8 up to 10 h and 24, 48, 72, 96, 120, 144 up to 168 h. Spore suspension of *Macrophomina phaseolina*(10^5 spores per ml) (MTCC 166) was sprayed on sesame plants.

Assay of Polyphenols

Phenolic content of sesame leaves was estimated by using modified Bray and Thorpe (1954) method. The polyphenol extract obtained was mixed with Folin-Ciocalteu's reagent. After 3 minutes of incubation (25℃±2), 1ml of 25% sodium carbonate was added. The absorbance was recorded at 725 nm. The polyphenol content was calculated as µg $gfw^{-1}$of plant tissue.

Analysis of Polyphenols by high performance liquid chromatography (HPLC)

Reagents used: Solvent A- 1.5% orthophosphoric acid; Solvent B- 20% glacial acetic acid + 25% acetonitrile + 1.5% orthophosphoric acid. Mixed in a ratio of A: B (60:40).

The polyphenol extracts of the samples were filtered using 0.22 µm Millipore filter, 20 µl of the samples were injected into a loop injection valve of HPLC (Shimadzu LC-10A) equipped with SCL-10AVP analog pump and SPD 10AV detector connected to the system controller. A maximum pressure of 400kgf/cm^2 and minimum of 0kgf/cm^2 was maintained. The HPLC

of samples was run at 320 nm using a reverse phase C-18 column. During the run, a flow rate of 1ml/min was maintained using a binary mode of the gradient system.

Various combinations of the percentage of the solvents viz. 30:70, 40:60, 50:50, 60:40, 70:30 of A and B respectively were used for achieving best resolution of peaks. Finally, the experiments were carried out using 60:40 ratio of A and B solvents for 10 min as best results were obtained in this concentration.

For the quantitative determination of various peaks, the integration area values of different standards with known concentration were compared with the sample peaks and the polyphenol content was calculated accordingly.

Statistical analysis

All data of healthy and diseased plants were subjected to one-way analysis of variance (ANOVA; Tables 1 and 2) (Petkovšek *et al.*, 2008). The data was statistically analyzed using the Statistical Package for Social Sciences (SPSS; version 17.0). One way analysis of variance with *Macrophomina phaseolina* using *in vivo* system.

(ANOVA) based on the general linear model was used to assess the mean differences among the variables (enzyme category and hours of exposure). The p-values of <0.05 and <0.01 were considered to be statistically significant.

Table 1: Descriptive Analysis of Polyphenol content (µg gfw^{-1}) at short time and long time interval in *Sesamum indicum* plants in control and after infection with *Macrophomina phaseolina* using *in vivo* system

Variety	Groups	N	Mean	Std. Deviation	Std. Error
RT- 46	Control	33	17.7915	5.73044	.99754
	Infected	33	20.5460	1.08387	.18868
	Total	66	19.1688	4.32096	.53187
GT-2	Control	33	13.4342	.49390	.08598
	Infected	33	14.5376	1.07018	.18629
	Total	66	13.9859	.99649	.12266
T-12	Control	33	8.4559	.57614	.10029
	Infected	33	9.6020	.97345	.16946
	Total	66	9.0290	.98149	.12081
TMV-3	Control	33	4.6758	.47069	.08194
	Infected	33	4.4872	2.36000	.41082
	Total	66	4.5815	1.69117	.20817

Table 2: One way analysis of variance (ANOVA) for polyphenol content at short time and long time interval in *Sesamum indicum* plants in control and after infection with *Macrophomina phaseolina* using *in vivo* system

Variety		Sum of Squares	df (degree of freedom)	Mean Square	F	Sig.
RT- 46	Between Groups Within Groups	125.189 1088.409 1213.598	1 64 65	125.189 17.006	7.361	.009
GT-2	Between Groups Within Groups	20.089 44.455 64.544	1 64 65	20.089 .695	28.922	.000
T-12	Between Groups Within Groups	21.670 40.945 62.616	1 64 65	21.670 .640	33.872	.000
TMV-3	Between Groups Within Groups	.587 185.317 185.904	1 64 65	.587 2.896	.203	.654

Results and Discussion

Plants defense system is under genetic control. When a plant come in contact to a pathogen a large number of defence related genes are activated to produce antimicrobial secondary defense related compounds (phenolics, PR proteins, ROS, defense related enzymes etc.) which are essential for plants to counter pathogen attack. For example, Martin et al. (2009) explained that the infection of young grapevine plants with *Phaeomoniella chlamydospora* induced an upregulation of plant phenolic content. In the present study, in order to determine the maximum activity of polyphenols in pathogen inoculated and control plants after short time interval (0, hour up to 10 hours) and long time interval (24 hours up to 168 hours), the polyphenol content was determined in 7 days old and 14 days old cultivars.

Quantitative determination of polyphenol content in *Sesamum indicum* L. plants at short time interval using *in vivo* system after foliar spray of spore suspension

In 7 days old RT- 46 cultivar values are ranges 70.36±0.24, 71.56±0.2, 70.97±0.2, 72.11±0.27, 72.66±0.24 and 71.48±0.14µg gfw^{-1} in control and in infected cultivars the values 71.52±0.24, 71.99±0.17, 73.4±0.03, 78.24±0.14, 76.3±0.07 and 75.24±0.12 µg gfw^{-1} are observed at 0, 2, 4, 6, 8 and 10 hpi (hours post inoculation). Therefore, these values in 14 days old control RT- 46 cultivar reaches 74.70±0.07, 75.75±0.30, 76.42±0.18, 76.38±0.20,

75.22±0.14 and 75.62±0.36 µg gfw^{-1} but in inoculated ones values 75.64±0.34, 76.50±0.20, 77.09±0.20, 79.81±0.17, 79.09±0.31 and 78.89±0.14 µg gfw^{-1} are observed at 0, 2, 4, 6, 8 and 10 hpi (Table 3). In both the cases i.e., 7 and 14 days old plants the value was raised at 6 hours after inoculation. The percentage increased in polyphenol content at 6 hours in inoculated 7 days old cultivars i.e., RT-46, GT-2, T-12 and TMV-3 is approx. 9%, 7%, 4% and 4% and in 14 days old cultivars is approx. 5%, 6%, 10% and 6% respectively in comparison of control plants. On the basis of above, data it could be concluded that the raised value at 6 hour using *in vivo* system shows the activation of biosynthesis of polyphenol content after inoculation of fungal pathogen. The results presented here, are in coordination with obtained by investigation of Martin et al. (2009). They explained that the infection of young grapevine plants with *Phaeomoniella chlamydospora* induced an up regulation of plant phenolic content. Our results also show the resemblance with Gadzouska *et al.,* 2007, he observed a 6-fold increases in phenolic compounds in *Hypericum perforatum* cells suspension after JA (Jasmonic acid) elicitation.

Table 3: Polyphenol content (µg gfw^{-1}) at short time intervals in *Sesamum indicum* plants after infection with *Macrophomina phaseolina* using *in vivo* system

7 days Old Plants								
Time (Hr)	RT 46		GT 2		T 12		TMV 3	
	C	I	C	I	C	I	C	I
0	70.36±0.24	71.52±0.24	42.34±0.1	43.75±0.00	25.24±0.35	25.26±0.14	21.23±0.1	22.23±0.1
2	71.56±0.2	71.99±0.17	42.65±0.37	44.49±0.24	26.42±0.09	26.56±0.32	21.76±0.18	22.3±0.21
4	70.97±0.2	73.4±0.03	43.45±0.51	44.92±0.31	26.66±0.09	27.15±0.09	21.7±0.1	22.38±0.24
6	72.11±0.27	**78.24±0.14**	43.1±0.2	**46.2±0.34**	26.95±0.09	**28.01±0.14**	21.81±0.2	**22.79±0.14**
8	72.66±0.24	76.30±0.07	42.77±0.59	45.94±0.24	26.46±0.1	27.69±0.12	21.81±0.2	22.72±0.07
10	71.48±0.14	75.24±0.12	43.16±0.51	45.49±0.14	26.7±0.1	27.44±0.09	21.7±0.1	22.76±0.12
14 Days Old Plants								
Time (Hr)	RT 46		GT 2		T 12		TMV 3	
	C	I	C	I	C	I	C	I
0	74.70±0.07	75.64±0.34	50.82±0.15	51.31±0.14	27.73±0.18	27.93±0.06	23.07±0.07	24.44±0.14

2	75.75±0.30	76.50±0.20	50.53±0.07	52.92±0.1	27.13±0.07	28.13±0.53	23.19±0.17	24.5±0.20
4	76.42±0.18	77.09±0.20	50.76±0.07	54.15±0.12	27.42±0.07	28.87±0.2	23.13±0.15	24.56±0.24
6	76.38±0.20	**79.81±0.17**	52.55±0.03	**55.66±0.15**	27.91±0.03	**30.97±0.09**	23.62±0.14	**25.07±0.17**
8	75.22±0.14	79.09±0.31	51.92±0.31	55.29±0.18	27.58±0.12	30.30±0.09	23.72±0.27	25.03±0.15
10	75.62±0.36	78.89±0.14	53.02±0.24	55.62±0.1	28.09±0.14	29.58±0.2	23.81±0.31	24.99±0.1

Quantitative determination of polyphenol content in *Sesamum indicum* L. plants at long time interval using *in vivo* system after foliar spray of spore suspension of *Macrophomina phaseolina*

The polyphenol value conditions is higher in infected plants at every stage of infection in comparison of control ones (Table 4). The value of polyphenol contents in 7 days old control four investigational cultivars i.e., RT-46, GT-2, T-12 and TMV-3 is observed 82.97±0.1, 70.89±0.14, 41.08±0.21 and 27.2±0.17 µg gfw^{-1} and in infected cultivars values are 96.08±0.06, 80.11±0.07, 55.25±0.14 and 39.32±0.27 µg gfw^{-1}. Whereas in 14 days old investigational cultivars the polyphenol value is found 87.49±0.16, 71.48±0.14, 45.43±0.12 and 33.56±0.15 µg gfw^{-1} in control cultivars and 99.55±0.18, 80.89±0.18, 58.58±0.15 and 40.16±0.20 µg gfw^{-1} found in inoculated ones. It is found from table 4 that the highest amount of polyphenol content is at 120 hours and then declined drastically in all the four experimental cultivars, the reason could be that successful multiplication and establishment of pathogen by overcoming structural barriers formed by plants (Thakker et al., 2013).The percentage is noticed in 7days old RT-46, GT-2, T-12 and TMV-3 cultivars 16%, 13% 34% and 45% in comparison of control ones. Similarly, this value is noticed 14%, 13%, 29% and 17% in 14 days old infected cultivars when compared to control ones correspondingly. These results are found in coordination with Thakker et al., 2013, they observed marked accumulation of phenolics on the 7^{th} day in dead fungus (*Fusarium oxysporum* f.sp. *cubense*) treated plants, while this increase in total phenolics content was observed on 3rd third day in live fungus (*Fusarium oxysporum* f.sp. *cubense*) treated plants as compared to control plants.

Table 4: Polyphenol content (µg gfw^{-1}) at long time interval in *Sesamum indicum* plants after infection with *Macrophomina phaseolina* using *in vivo* system

7 days Old Plant								
Hours	RT 46		GT 2		T 12		TMV 3	
	C	I	C	I	C	I	C	I
24	80.89±0.18	85.26±0.06	66.37±0.18	70.93±0.18	37.46±0.39	40.1±0.18	25.5±0.24	26.95±0.18
48	81.61±0.33	87.1±0.19	67.66±0.14	73.05±0.21	38.53±0.21	44.3±0.07	26.3±0.32	30.34±0.18
72	82.5±0.27	90.51±0.09	69.72±0.18	74.23±0.27	40.04±0.1	48±0.27	26.42±0.37	32.26±0.21
96	82.44±0.2	94.24±0.12	70.17±0.07	77.01±0.18	41.96±0.24	50.04±0.1	27.49±0.35	34.2±0.12
120	82.97±0.1	**96.08±0.06**	70.89±0.14	**80.11±0.07**	41.08±0.21	**55.25±0.14**	27.2±0.17	**39.32±0.27**
144	83.57±0.17	91.49±0.06	71.99±0.17	79.73±0.1	43.94±0.18	54.12±0.07	28.17±0.18	37.46±0.39
168	81.54±0.24	89.83±0.15	70.27±0.21	76.93±0.07	44.39±0.21	51.33±0.16	28.87±0.2	36.63±0.18
14 Days Old Plant								
Hours	RT 46		GT 2		T 12		TMV 3	
	C	I	C	I	C	I	C	I
24	85.44±0.06	89.32±0.06	68.99±0.32	71.52±0.24	39.32±0.27	42.34±0.1	30.4±0.2	33.28±0.21
48	85.67±0.16	90.26±0.1	69.64±0.07	73.42±0.03	41.02±0.14	46.14±0.3	32.1±0.16	34.14±0.03
72	86.18±0.12	94.18±0.03	71.6±0.21	76.34±0.07	41.14±0.24	49.18±0.21	32.61±0.18	36.2±0.14
96	86.48±0.09	96.06±0.03	72.58±0.17	78.32±0.24	44.94±0.17	58.00±0.18	33.14±0.15	39.24±0.7
120	87.49±0.16	**99.55±0.18**	71.48±0.14	**80.89±0.18**	45.43±0.12	**58.58±0.15**	33.56±0.15	**40.16±0.20**
144	88.26±0.06	97.14±0.12	72.97±0.1	80.22±0.24	45.37±0.03	56.35±0.18	34.06±0.12	39.4±0.31
168	88.53±0.18	91.47±0.03	72.62±0.21	79.77±0.15	46.08±0.24	53.65±0.17	34.55±0.21	38.04±0.1

Qualitative determination of polyphenol content by using high performance liquid chromatography

Quantitative analysis of polyphenol contents was also done by using HPLC. Two solvent systems were used at a concentration of 60:40 with a flow rate of 1 ml min^{-1}at 320 nm wavelengths. The peaks were analyzed by comparing the retention time (RT) of the standard phenolic compounds with the methanolic extracts of all the four sesame cultivars. The extracts were collected after 120 hour of infection according to the quantitative analysis (table 4) of polyphenols, maximum concentration was found at 120 hour of infection. For the

HPLC analysis, 14 days old healthy and inoculated all the four sesame cultivars (*in vivo*) were taken. The reason behind this is that 14 days old plants were showing the best results by producing polyphenols in more concentration when compared to 7 days old plants. It is clear from the data that total integration of area of peaks increases after infection in all the investigational cultivars. For example, infected RT-46 shows the peak area 2226 units which found more than that of control i.e. 2136 units. Similar criterion of peak area also has to be seen in rest three investigational cultivars i.e. 1693 units found in infected GT-2, 1451 units found in infected T-12 and 1351 units in infected TMV-3 while these area values in control plants were lower in comparison of infected plants as control GT-2 shows the peak area 1651 units, while control T-12 shows the peak area value 1418 units and control TMV-3 shows 1335 units which are seen to be lower in comparison of inoculated ones. On the other hand, number of peaks was also found more in infected RT-46 cultivar (7). Similarly, infected GT-2 showed 7 numbers of peaks while infected T-12 showed 6 numbers of peaks and infected TMV-3 showed 5 numbers of total peaks. From the above mentioned data, it is seen that TMV-3 showing the poor results by producing polyphenols in low concentration, it could be due to the late recognition of pathogen and slow process of polyphenol synthesis in the TMV-3 cultivar. Along with area, number of peaks are also found to be increased in RT-46 when compared to rest three investigational cultivars.

The concentration of phenolic compounds in investigational cultivars is found to be elevated after infection in comparison of healthy cultivars. For example, the concentration of caffeic acid and coumaric acid in inoculated RT-46 is found to be elevated to 0.885µg gfw^{-1} and 0.571µg gfw^{-1} which is found higher than that of 0.849µg gfw^{-1} and 0.548µg gfw^{-1} in control RT-46. Similar results are also obtained in rest three investigational cultivars. Analogous results were demonstrated by Picinelli et al. (1995) in apple plants after infection with *Venturia inaequalis* (Apple scab), they interpret that resistant varieties of apple contained more p-coumaric acid in comparison with susceptible varieties.

Chlorogenic acid, Gallic acid, Caffeic acid, Cinnapic acid, Coumaric acid, Ferrulic acid, Cinnamic acid and Kaemferol, these 8 standard samples were used to identify the corresponding components in sesame leaves polyphenols. To recognize the presence of

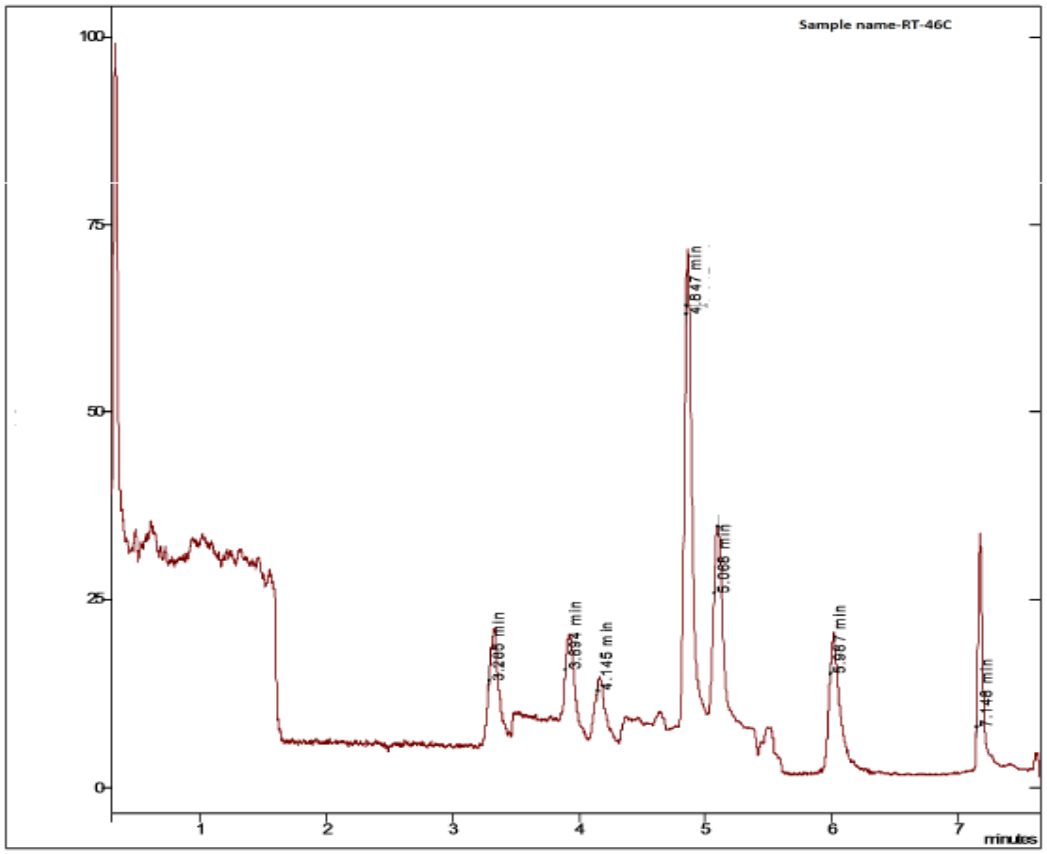
specific phenolic compounds, an alignment was made between the retention time of standards and samples with chromatogram. The concentration of an individual compound was calculated on the basis of peak area measurements. It is obvious from the chromatogram that 8 peaks of different standards have to be seen in control and inoculated plants, but few extra peaks were also noticed in inoculated ones which could not be identified. So, it could be presumed that other compounds in small concentration are also present in inoculated plants.

The elevated concentration of other phenolic compounds viz. caffeic acid, chlorogenic acid, gallic acid, sinapic acid, coumaric acid, ferulic acid and cinnamic acid were also found in increasing fashion in pathogen infested cultivars while concentration of these phenolics in comparison of inoculated ones were found to be inferior in healthy cultivars. These results are in support of Del Río et al., 2003 who found the presence of some main phenolic compounds i.e. oleuropein, catechin and tyrosol by using HPLC-MS in *Oleaeuropaea* plant extracts against *Phytophthora* sp.

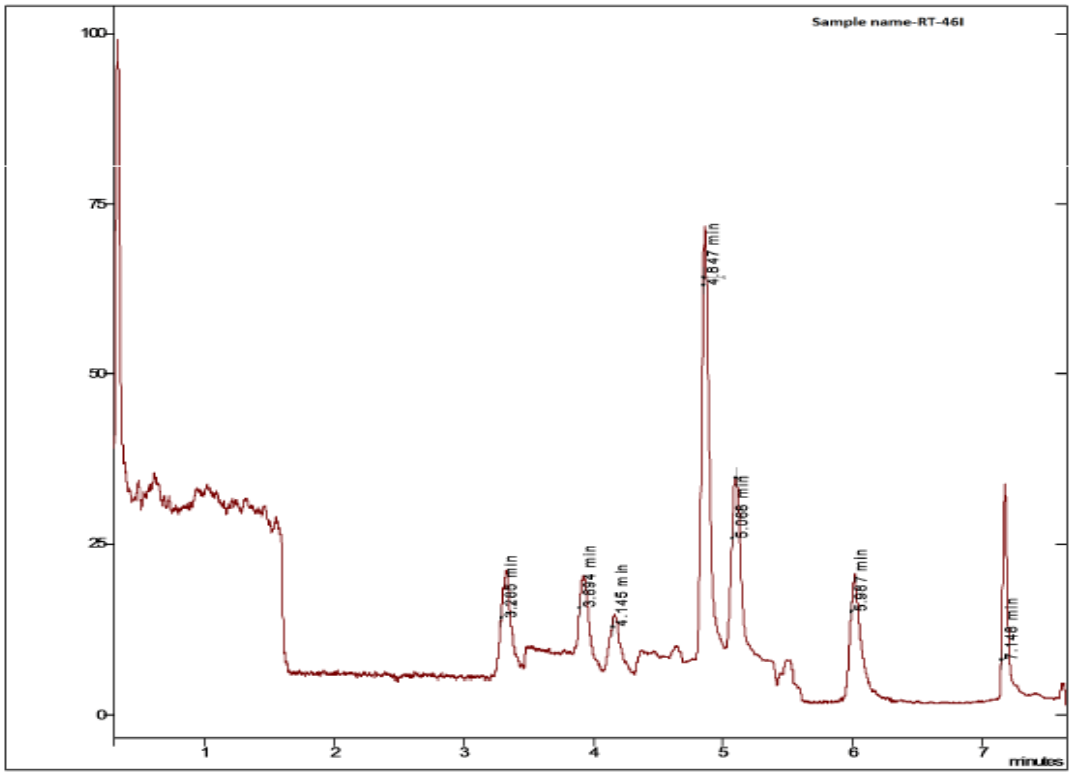
It has been observed through HPLC chromatogram that kaemferol is absent in all the four cultivars. On the other hand, few phenolic compounds were found to be absent in control plants but present in inoculated ones. For example, gallic acid and sinapic acid was not observed in GT-2 control but found in GT-2 infected cultivar. Similarly, chlorogenic acid was not found in control T-12 cultivar but present in inoculated one but this compound is totally absent in control and inoculated TMV-3 cultivar. In the same way, sinapic acid was not observed in control and inoculated T-12 and TMV-3 cultivar. Maximum increase in polyphenols was observed in resistant cultivar i.e. RT-46 and minimum production was in susceptible cultivar i.e. TMV-3. Along with a maximum concentration of phenolic compound maximum number of phenolic compounds was also found in RT-46 cultivar. So, according to the depicted data RT-46 is responding more actively to pathogen attack in comparison of rest three investigational cultivars. Depicted results are in support of Schovánková and Opatová (2011) who described that the elevation of all major phenolic compounds – chlorogenic acid, epicatechin and phloridzin was initiated in apple peel after the inoculation of the pathogen which was found more intensive in the part surrounding the rotten zone. Similarly, Wang et

al. 2008 found HPLC analyses of the soluble phenolics resulting from the activation of expression of *PAL* genes in infected potato leaves in response to different genotypes of *P. infestans* showed that the accumulation of these compounds started from 24 h.a.i. and reached maximum at 120 h.a.i (hours after infection). According to Hammerschmidt (2005), polyphenols are very important for plants to contribute resistance against microorganisms, herbivores and insects (Table 5).

A

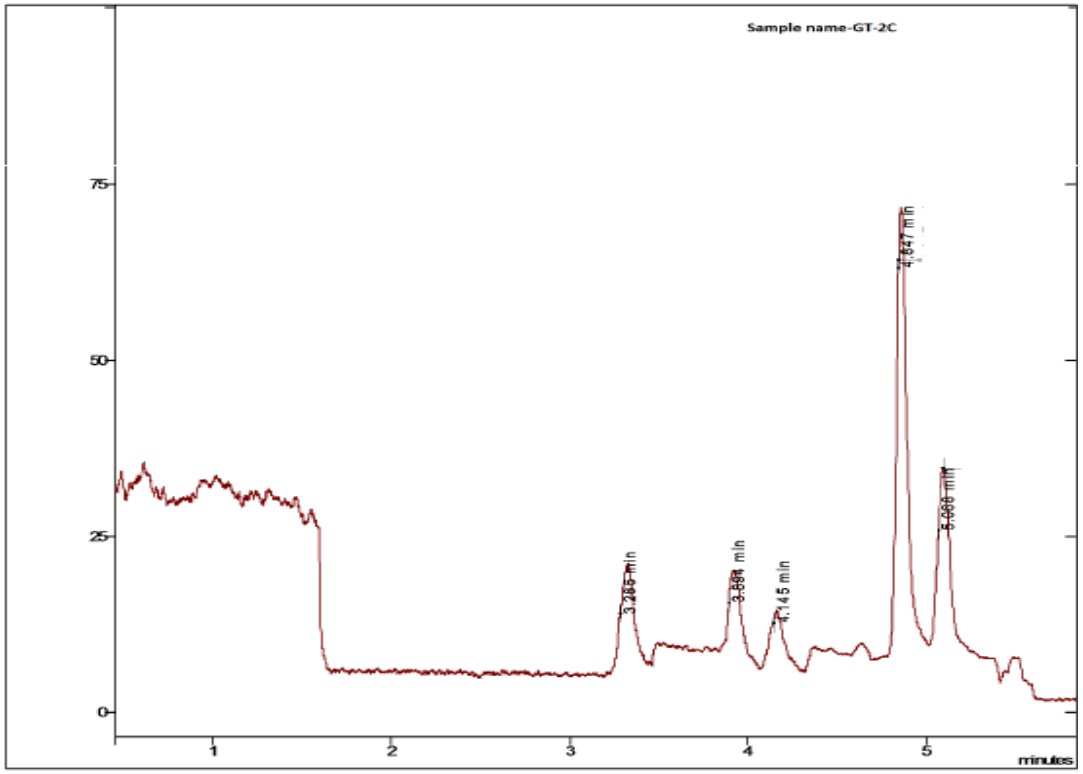


Control (RT46)

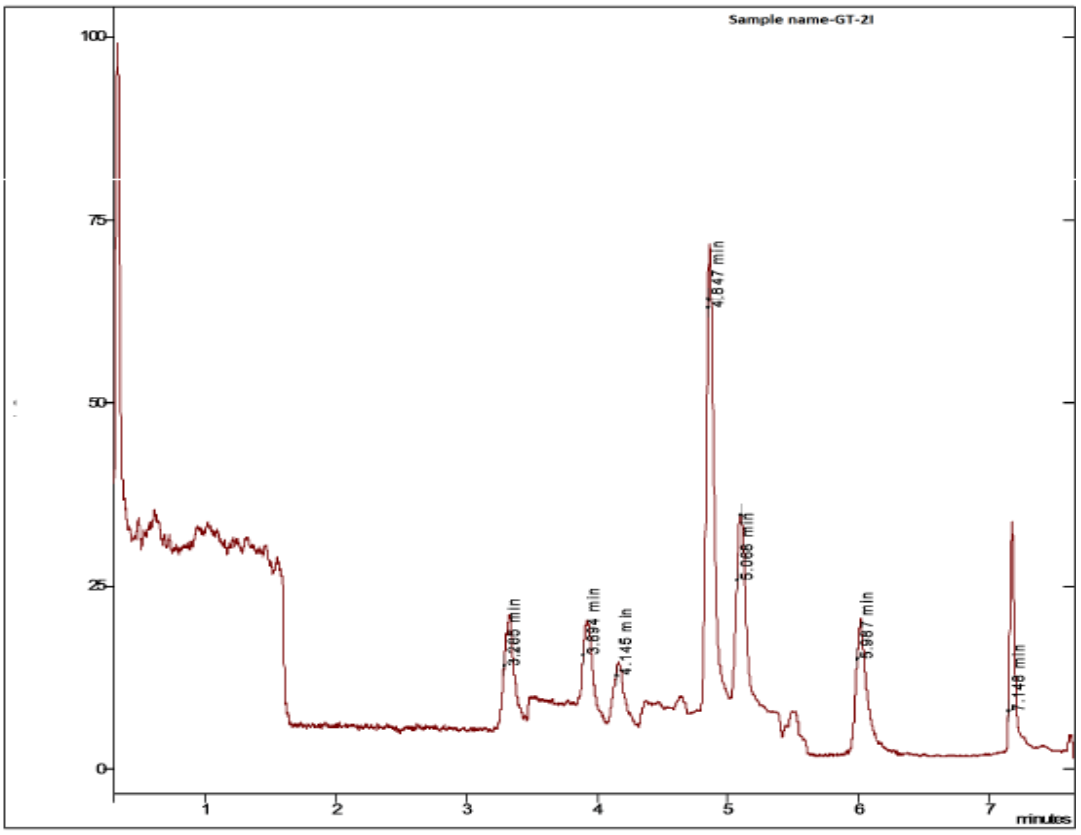


Infected (RT46)

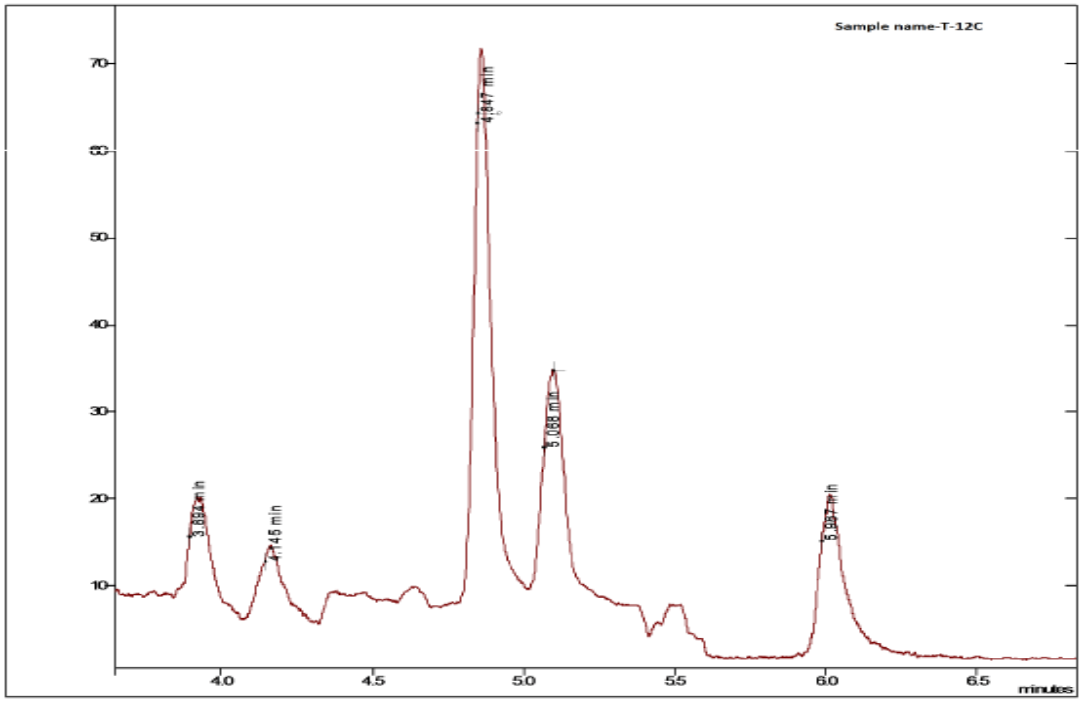
B



Control (GT2)

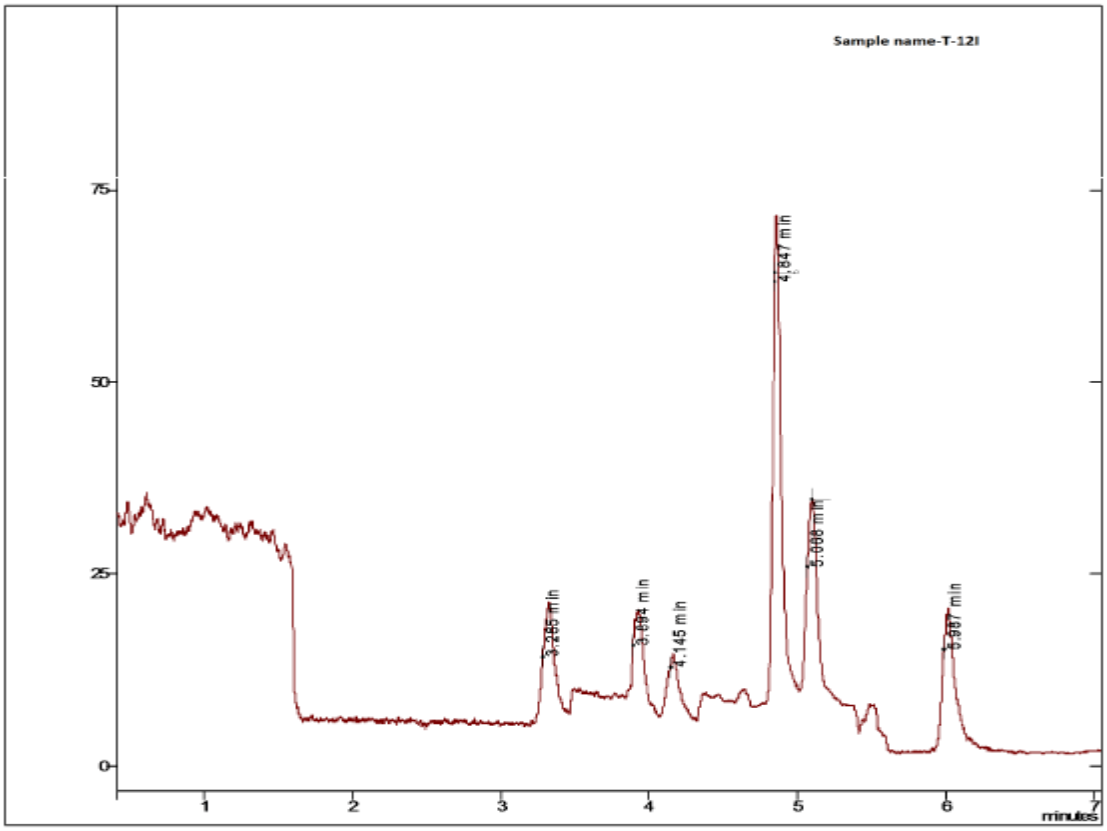


Infected (GT2)

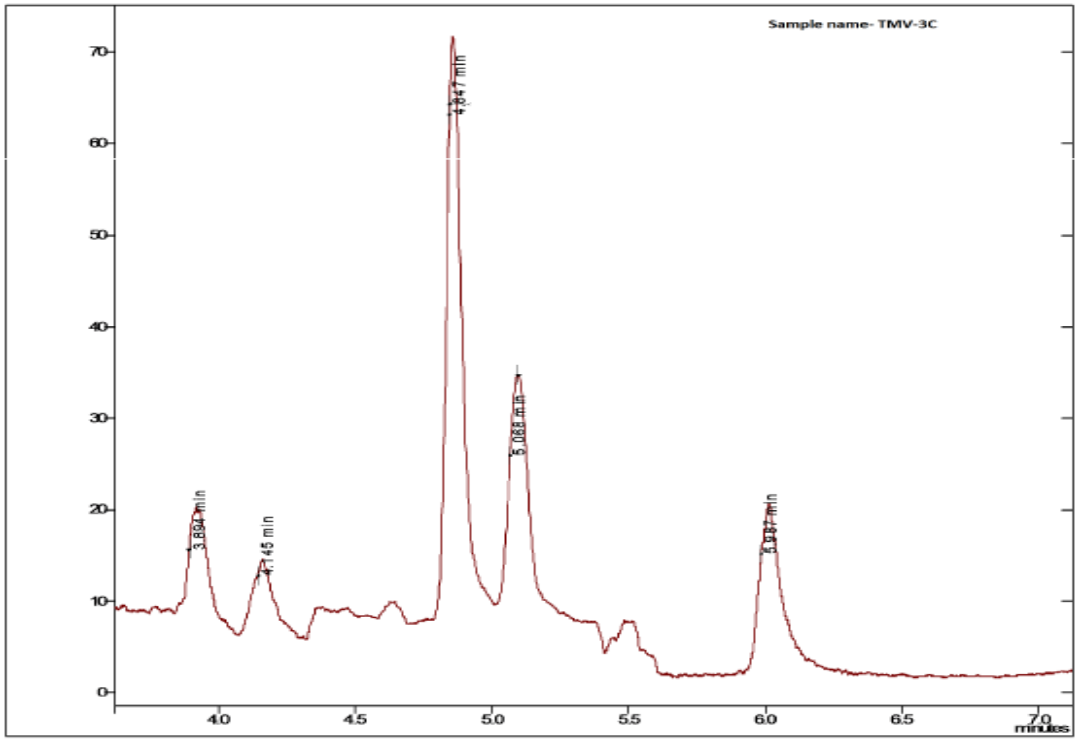


Control (T12)

C

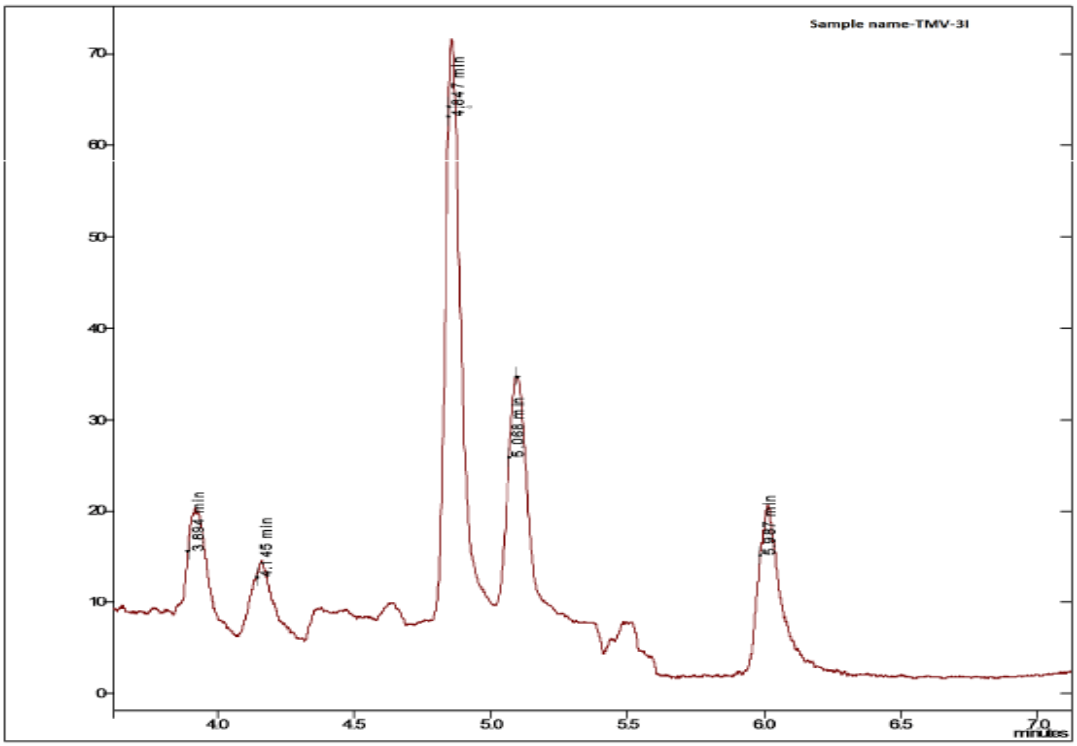


Infected (GT2)



Control (TMV3)

D



Infected (TMV3)

Figure 3: HPLC chromatogram of control and infected plants of all four cultivars [(A) RT-46, (B) GT-2, (C) T-12 and (D) TMV-3] after 120 hours of infection

Table 5: Concentration of phenolic compounds (µg gfw^{-1}) in control and infected sesame cultivars of 15 days old plants of Sesame at 120 hours of infection

Phenolic compounds	RT-46		GT-2		T-12		TMV-3	
	C	I	C	I	C	I	C	I
Caffeic acid	0.849	0.885	0.596	0.609	0.564	0.577	0.444	0.458
Chlorogenic acid	0. 239	0.264	0.103	0.110	*	0.101	*	*
Gallic acid	0.098	0.118	*	0.095	0.090	0.099	0.073	0.078
Sinapic acid	0.043	0.054	*	0.032	*	*	*	*

Coumaric acid	0.548	0.571	0.512	0.525	0.489	0.494	0.411	0.419
Kaemferol	*	*	*	*	*	*	*	*
Ferulic acid	0.155	0.171	0.154	0.166	0.121	0.132	0.055	0.065
Cinnamic acid	0.076	0.084	0.075	0.081	0.071	0.077	0.058	0.065

Abbreviations: C- Control, I- Infected, *-Compound absent

On the basis of the above results, it can be concluded that secondary metabolites can be used as tools to study the induced defense responses as well as the resistance / susceptibility against a particular pathogen of the Sesame plants. So, these results are the best study to improve this important crop in the future.

Acknowledgement

Authors are thankful to Prof. Aditya Shastri, Vice-Chancellor, Banasthali Vidyapith, Rajasthan (India) for providing basic facilities of research work.

References

Agrios, G. N., 2005. Plant Pathology.5th Edition. Academic Press, San Diego, USA. 922pp.

Asselbergh, B., David, De V. and Monica H., 2008. Global Switches and Fine-Tuning-ABA Modulates Plant Pathogen Defense. *The American Phytopathological Society*, 21 (6), 709–719.

Bhattacharya, D., Dhar, T. K. and Ali, E., 1992b. An enzyme immunoassay of phaseolinone and its application in estimation of the amount of toxin in *Macrophomina phaseolina*-infected seeds. *Applied and Environmental Microbiology*, 58, 1970–1974.

Bhattacharya, D., Siddiqui, K. A. I. and Ali, E., 1992a. Phytotoxic metabolites of Macrophominaphaseolina. *Indian Journal of Mycology and Plant Pathology*, 22, 54–57.

Bray, H. G. and Thorpe, W. V., 1954. Analysis of phenolic compounds of interest in metabolism. *Methods in Biochemical Analysis,* 1, 27-52.

De Gara, L., M. de, Pinto and Tommasi, F., 2003. The antioxidant systems via-á-via reactive oxygen species during plant-pathogen interaction. *Plant Physiology and Biochemistry,* 41, 863-870.

Del Río, J. A., Báidez, A. G., Botía, J. M., Ortuño, A., 2003. Enhancement of phenolic compounds in olive plants (*Oleaeuropaea* L.) and their influence on resistance against *Phytophthora* sp. *Food Chemistry,* 83, 75–78.

Feys, B. and Parker, J. E., 2000. Interplay of signaling pathways in plant disease resistance. *Trends in Genetics,* 16, 449-55.

Gadzouska, S., Delaunnay, A., Spasenoski, M., Maury, S., Joseph, C., and Haðege, D., 2007. Jasmonic acid elicitation of *Hypericum perforatum* L. cell suspensions and effects on the production of phenylpropanoids and naphtodianthrones. *Plant Cell Tissue and Organ Culture,* 89(1), 1-13.

Hammerschmidt, R., 2005. Phenols and plantepathogen interactions: the saga continues. *Physiological and Molecular Plant Pathology*. 66, 77-78.

Malolepsza, U. and Rózalaska, S., 2005. Nitric oxide and hydrogen peroxide in tomato resistance. Nitric oxide modulates hydrogen peroxide level in O.hydroxyethylorutin-induced resistance to *Botrytis cinerea* in tomato. *Plant Physiology and Biochemistry*, 43, 623-635.

Martin, N., Vesentini, D., Rego, C., Monteiro, S., Oliveira, H. and Ricardo Boavida, F., 2009. *Phaeomoniellachlamydospora* infection induces changes in phenolic compounds content in *Vitisvinifera*. *Phytopathologia Mediterranea,* 48, 101 – 116.

Petkovšek, MM.,Štampar, F. and Veberič, R.,2008. Increased phenolic content in apple leaves infected with the apple scab pathogen. *Journal of Plant Pathology*, 90, 49–55.

Picinelli, A., Dapena, E. andMangas, J.J., 1995. Polyphenolic pattern in apple tree leaves in relation to scab resistance. A preliminary study. *Journal of Agricultural and Food Chemistry*, 4, 2273-2278.

Schovánková, J. and Opatová H., 2011. Changes in phenols composition and activity of phenylalanine-ammonia lyase in apples after fungal infections. *Horticultural Science* (Prague), 38(1), 1–10.

Sharma, S., Sharma, V and Alam, A. (2014). Alteration in β-1, 3-glucanases enzyme in *Sesamum indicum* L. infected with *Macrophomina phaseolina*. *Mycopath*, 12(2), 67-75.

Thakker, J. N., Patel, S. and Dhandhukia Pinakin, C., 2013. Induction of Defense-Related Enzymes in Banana Plants: Effect of Live and Dead Pathogenic Strain of *Fusarium oxysporum* f. sp. *cubense*. ISRN Biotechnology, 2013, 1-6.

Wang, X., El Hadrami, A., Adam, L. R. and Daayf, F., 2008. Differential activation and suppression of potato defence responses by *Phytophthora infestans* isolates representing US-1 and US-8 genotypes. *Plant Pathology*, 57, 1026–1037.