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Enhancing Tomato Fruits Post-Harvest Resistance by Salicylic Acid and Hydrogen Peroxide Elicitors Against Rot Caused by *Alternaria solani*

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ABSTRACT

Postharvest diseases cause a great reduction in the quantity and quality of fruits yield. Treatment of tomato fruits with salicylic acid (SA) and Hydrogen peroxide (H_2O_2) elicitors enhanced the resistance to fruit rot caused by *Alternaria solani*. Moreover, the treatment decreased the development of post-harvesting fruit rot disease. The treatment with either SA or H_2O_2 individually or in combination on tomato shoots (in farm) decreased the diameter of fruit rotted area, decreased the PG activity, significantly increased PAL, PPO, POD enzyme activities and increased PR2 and PR3 genes expression after fruit harvesting. On the other hand, treatment by elicitors after harvesting decreased both diameter of rotten area and decreased PG activity but increased the activity of the PAL, PPO, POD enzymes. Also, the gene expression of PR2 and PR3 genes were high in compared to treatment by elicitors before fruits harvesting. The same observation was obtained by the combination of SA and H_2O_2, diameter rotten area was decreased and the PG as well. Significant incensement in the activity of enzymes PAL, PPO, POD, this combined with high expression of both PR2 and PR3 genes.

Keywords: Postharvest diseases**,** elicitors**,** gene expression**,** tomato fruit rot, *Alternaria solani*

INTRODUCTION

Tomato plants are one of the most important vegetable crops overall the world. The tomato belongs to the Solanaceae family along with other economically important crops such as potato, eggplant and pepper. Great loss post postharvest of vegetables and fruit causes by fungal plant pathogens (Tripathi and Dubey, 2004). Tomato (*Solanum lycopersicum*) fruit usually have a very short postharvest life. Decay is an important factor, which limits the storage of tomato, and results in appreciable losses at wholesale, retail, and consumer levels. Several fungal pathogens cause fruit tomatoes decay such as Rhizopus rot caused by *Rhizopus stolonifer,* black mold rot caused by *Alternaria arborescens*, Fusarium rots caused by *Fusarium* spp., buckeye rot caused by *Phytophthora* spp. and sour rot caused by *Geotrichum candidum* (Mahovic *et al.,* 2004). Tomato fruit rot disease caused by *A. solani* is the most severe disease of tomato fruits, causing in fields and during storage, marketing and transportation in Tikamgarh district of Madhya Pradesh use (Chaurasia *et al,* 2013) . Due to this disease, the tomato fruits not only lost their nutritional values but also quick and severe rotting makes them unfit for domestic. The control of post-harvest diseases by using synthetic fungicides (Eckert and Ogawa, 1988). But these fungicides has been restricted due to their carcinogenicity, teratogenicity, high and acute residual toxicity, long degradation period, environmental pollution and side-effects on human health (Tripathi and Dubey, 2004). For that , there are new alternatives have been explored to reduce use of synthetic fungicides.

The natural resistance of fruits and vegetables to diseases usually, leading to infection by pathogens decline after harvesting. Elicitors, as a part of integrated pest management (IPM) approach, are usually used to induce resistance against postharvest diseases (Terry and Joyce, 2004). The postharvest enhancing resistance to disease of postharvest horticultural crops have been studied today, including SA, chitosan, oxalic acid, $Cacl_2$ and the antagonistic yeast (Molloy *et al.,* 2004 and Liu *et al.,* 2007). It has been proven that induced resistance as an alternative for the control fruit postharvest diseases in fruits is effective in both the laboratory and in field (Tian and Chan, 2004). Different elicitors affect induction of many defense-related enzymes and production of phenolic compounds in plants (Thakur and Sohal, 2013).

The role of the Jasmonic acid , Salicylic acid and ethylene defense-signaling pathways in regulating of the resistance traits expression was determined in plants *Bemisia tabaci*. Studies in squash show that plants can discriminate the elicitors/effectors (chemical signals) introduced by two different *Bemisia tabaci* biotypes (van de Ven *et al.,* 2000). The functions of H_2O_2 as a stress signal in plants, mediating adaptive responses to various stresses. the of H_2O_2 accumulation results with exposure to various abiotic and biotic stresses (Desikan *et al.,* 2001). H_2O_2 can induce the expression of gene involved in antioxidant defense (Morita *et al*. 1999 and Jaiti *et al.* 2004).

The systemic acquired resistance (SAR) is considered to be activated more commonly by pathogens causing cell death reactions. SAR is mediated by SA dependent process (Gaffney *et al*. 1993 and Chaman *et al*., 2003). SAR to further infection by a broad range of pathogens induces of expression defense genes by SA (Verberne *et al*., 2000; Conrath *et al*., 2001 and Zhang *et al*., 2010).

The interaction between host and pathogen induces changes in cell metabolism; primarily activity of enzymes particularly phenylalanine ammonia lyase , Polyphenol oxidase, , lipoxygenase, superoxide dismutase , and β-1,3 glucanase (Cavalcanti *et al*., 2006; Ibrahim, 2012 and Nisha *et al*., 2012). The resistance to infection by were increased because of antioxidant defensive enzymes activity, PAL, POD, PPO, SOD, β-

1,3 glucanase and catalase (CAT) were increased (Mustafa and Alawami, 2012 and Ngadze *et al*., 2012).

Pathogenesis-related proteins (PRs) are plant species-specific proteins produced in response to infection with bacteria, fungi and viruses. Several plants produce PRs through a ubiquitous reaction during pathogen attack (Lee *et al.,* 2011). They have been associated with SAR and incipient anti-pathogen effects. These pathogenesis related responses and inhibition of fungal growth because of these proteins proved their defensive functions in the plant (Ebrahim *et al*., 2011).They are produced in large quantities in hypersensitive and resistant reactions. PRs prevent various pathogen invasions (Bowles, 1990). The aim of this study is; to examine the effect of both SA and H_2O_2 on the plant defense system and how to strength the plant immune response against the tomato fruit rot caused by *A.solani* and decreased the development of post-harvesting fruit rot disease.

MATERIALS AND METHODS

1.Plant Material, Growth Condition and Application of Elicitors

The present study was conducted in Kamal Shaheen farm on Nubaria area and the study was replicated in Saad el abd farm on Kafr Eldawar during 2015 and 2016. The farms were planted with tomato cultivar 1077, this economic cultivar produces high production. Two elicitors were used to induce resistance defense in tomato fruits post- harvesting against *A. solani*.

Chemical inducers: SA (2 µM) and H_2O_2 (5 mM) and combination of SA and H_2O_2 were sprayed each 10 days on shoots plant in field before 45 days from fruits harvesting (5 times). After harvest of tomato fruits were taken to the laboratory in polythene bags to complete the study. The tomato fruits treatment by each elicitor were divided into two groups: Group 1 included tomato fruits inoculated by *A .solani* and continue with spray by elicitor each 3 days after inoculation (five times), Group 2 included tomato fruits inoculated by *A .solani* and do not continue with treatment by any elicitor after inoculation. In addition to tomato fruits inoculated with *A. solani* this fruits untreated by any elicitors before (in field) or after the inoculation. Healthy tomato fruits, which uninoculated with *A. solani* and untreated by any elicitors.

2. Inoculation with *Alternaria solani*

The isolate of *A. solani* used in this study was isolated, purified and identified in previous study (Adss *et al.,* 2017). The *A. solani* isolate was cultured and maintained on PDA medium at 20 ^oC, cultures were sub-cultured on the PDA medium at 24 ± 1 ^oC for 6 days.

3. Determination of tomato resistance against the development of fruit rotting area after treatment by elicitors:

Tomato fruits semi ripe treated by each elicitor were divided into two groups (as mentioned before) to study the role of each elicitor to induce the resistance against the development of fruit rotting area caused by *A. solani.* Healthy fruits were selected and brought to the laboratory. sterilized by immersing in 3% sodium hypochlorite solution for 3 min, and washed thoroughly with sterilized distilled water. After surface sterilization the fruits were inoculated with 8.0 mm diameter inoculum discs by cavity method (Granger and Hornes, 1924, Chaurasia 2010). All the inoculated fruits were kept in moist chamber having 80-100 % relative humidity and then incubated at 28^oC for 3, 6, 9, 12 and 15 days. At the end of each incubation period, the diameter of rotten area was measured in mm. The diameter of rotten area as mm day was also calculated in each case with the help of the following formula:

Rotten area(mm/day)= Rotten area in mm/Total time period in days

4. Determination of Polygalacturonase (PG) activity *in vivo*

The tomato fruits treatment by elicitors were used to study PG enzyme production *in vivo* on intervals by method of Chaurasia *et al.(*2014) with some modifications. The samples were taken after 3, 6, 9, and 15 days of incubation. 20 g of diseased tissue was taken and mixed with 20 *ml* distilled water and the mixture was homogenized in blender for 10 min. the homogenate was centrifuged at 10.000 *rpm* / 20 min. The supernatant was used as enzyme extract. Enzyme extract for healthy tomato fruits (un inoculated and untreated by any elicitors) and tomato fruits inoculated with *A. solani* (untreated by any elicitors) were also prepared in a similar manner. PG activity was determined using the thiobarbituric acid (TBA) method (Lei *et al.,* 1985a; Lei *et al*., 1985b). The methods was described as (Adss *et al.,* 2017)

5. Defense-Related Enzymes

The activity of defense-related enzymes: POD, PAL, and PPO were estimated in tomato fruits after 0, 1, 3, 6, 9, 15 days of inoculation were described as (Nassar and Adss, 2016). Also, the same samples were used for the quantification of gene expression levels of PR1 and PR2 using real-time PCR (RT-PCR) technique after 1, 6, 9 days of inoculation.

6. Quantification of PR2, and PR3 Gene Expression

Total RNA was extracted from tomato fruit tissue using the GStract™ RNA Isolation kit II (Guanidium Thiocynate Method) (Maxim Biotech, Inc., USA) according to the manufacture procedure. The kit was provided by local chemical supply company.

1.Reverse Transcription-Polymerase Chain Reaction (RT-PCR) of mRNA

Reverse transcription (RT) to convert the mRNA to cDNA in the presence of dNTPS and reverse transcriptase. Reverse transcription reaction was performed using oligo (dT) primer . Each 25 µl reaction mixture contained 2.5 µl (5x) buffer with $MgCl_2$, 2.5 µl (2.5 mM) dNTPs, 1 µl (10 pmol) primer, 2.5 µl RNA (2 mg/ml) and 0.5 unit reverse transcriptase enzyme. PCR amplification was performed in a thermal cycler programmed at 42 °C for 1 h, 72 °C for 10 min (enzyme inactivating) and the product was stored at 4 °C until use.

2. PR2 and PR3 Gene Expression using RT-qPCR

Samples were analyzed using the Fermentase kit (Sigma Egypt, Cairo) (Peng *et al.,* 2004). Each reaction mixture had 12.5 µl of 2x Quantitech SYBR® Green RT Mix, 1µl of 25 pm/µl forward primer (Table 1), 1 µl of 25 pm/µl *reverse* primer,1 µl of the cDNA (50 ng), and 9.5 µl of RNase free water for a total of 25 µl. Samples were mixed by spinning before loading in the Rotor's wells. The real time PCR program was as the following: initial denaturation at 95 °C for 10 min, 40 cycles of 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. Data acquisition performed

during the extension step. This reaction was performed using Rotor-Gene- 6000-system (QIAGEN, USA).

3.Gene Expression Data Analysis

Comparative quantification analysis was done using Rotor-Gene-6000 Series instrument software according to Rasmussen (2001). The ratio of the target gene was expressed in the sample versus control in comparison to the reference gene. Relative expression of genes were estimated and analyzed using bioinformatics and statistical software. Results were normalized to 18S rRNA (reference gene). The data were statistically evaluated, interpreted and analyzed using Rotor-Gene-6000 software version 1.7.

7.Statistical Analysis

Enzymes assays were carried out as repeated measures over time with three replicates per treatment. Data were statistically analyzed as repeated measures over time using the MIXED procedure of the statistical analysis software (SAS) version 9.4 Cary, NC, SAS Institute Inc. (SAS, 2014). Least significant means were compared using Dunnett's *post-hoc* Test ($P < 0.05$).

Table1. Sequence of primers used in the real-time PCR

Primers		Primer sequence 5 →3̀	Annealing (°C)
PR3 chitinase	F	AACTATGGGCCATGTGGAAGA	60
	R	GGCTTTGGGGATTGAGGAG	
PR2glucanase	F	GGACACCCTTCCGCTACTCTT	60
	R	TGTTCCTGCCCCTCCTTTC	
18S Rrna	F	GGGCATTCGTATTTCATAGTCAGAG	60
	R	CGGTTCTTGATTAATGAAAACATCC	

RESULTS

Effect of SA and H_2O_2 elicitors on Development of of fruit rotting area on tomato caused by *A. solani*

Two elicitors were tested for their ability to enhance resistance of tomato cultivar 1077 to decrease the development of tomato fruit rot disease post-harvesting . The data in Table (2) showed that the treatment with SA or H_2O_2 or combination of both on tomato shoot in field and continue of treatment by elicitors after fruits harvesting enhanced resistance and decreased the diameter of rotted area of tomato fruits after harvesting compared with infection by *A.solan* (untreated by any elicitors). Also the treatment by elicitors after tomato fruits harvesting after infection by *A. solani* increased the resistance of tomato fruits by decreasing the diameter of rotten area compared with treatment by elicitors before fruits harvesting. The treatment by combination of SA and H_2O_2 increased resistance of tomato fruits by decreasing the diameter of rotten area followed by SA compared with H_2O_2 treatment

Fig. 1. Effect of two elicitors (SA and H_2O_2) on the rotting area of tomato fruits infected by *A. solani*

Table 2. Effect of two elicitors (SA and H_2O_2) on the rotting area of tomato fruits infected by *A. solani*.

Treatment		Diameter of Rotten area (in mm) Days after inoculation					Rotten area (mm/day)
		3	6	9	12	15	
SA	Before	11.00±1.00	17.00±2.00	30.00±1.00	40.00±2.00	51.66±1.52	9.977±2.00
	Before and after	10.00±2.00	14.00±1.00	23.00±3.00	38.00±2.00	47.00±1.00	8.80±1.00
H_2O_2	Before	11.0000±2.00	15.00±1.00	24.00±1.00	40.00±4.00	48.00±1.00	9.20±2.000
	Before and after	9.00±1.000	15.00±1.00	20.00±2.00	36.00±1.00	42.00±1.00	8.133±1.000
Combination	Before	8.666±0.577	12.00±2.00	17.66±1.52	26.00±1.00	34.00±2.00	6.554±0.577
	Before and after	8.333±0.577	11.33±0.57	15.00±1.00	21.00±2.00	28.00±2.00	5.577±0.57
Infected alone		17.00±1.000	28.00±1.00	42.00±2.00	59.33±0.57	75.66±0.57	14.799±1.00

***Data were average of three replicates** $LSD_{0.05} = 0.93$

Data in Table (3) indicate the PG enzyme activity in the healthy tomato fruits, diseased by *A.solani* and treatment by elicitors compared to tomato fruits inoculated by *A.solani* (untreated with any elicitors). The results showed that the PG enzyme activity present in healthy tomato fruits showing its constitutive nature. The maximum activity of PG enzyme was recorded. After 9 days of incubation the PG activity decreased gradually in healthy tissues to reach the maximum decrease activity at 15 days. The activity of PG enzyme was high in tomato fruits that infected by *A.solan* (untreated by elicitors) compared with tomato fruits treated with SA , H_2O_2 or combination of SA and H_2O_2 as shoots (in field) and treatment by elicitors after

fruits harvesting and inoculation by *A.solani*. Also the PG activity was low in tomato fruits that were treated by elicitors after tomato fruit harvesting after infection by *A. solani* compared with treatment by elicitors before fruits harvesting alone. The PG activity was low in tomato fruits treatment by combination of SA and H_2O_2 followed by SA and H_2O_2 treatments.

Table 3. Effect of two elicitors (SA and H_2O_2) on PG enzyme activity of tomato fruits infected by *A. solani*.

Treatment		Days after inoculation 3	6	9	15	Mean
SA	Before	0.424±0.032	0.554±0.083	0.623±0.056	0.411±0.008	0.503±0.103
	Before and after	0.397±0.021	0.512±0.068	0.586±0.060	0.378±0.042	0.468±0.099
Mean		0.411±0.029	0.534±0.071	0.605±0.056	0.395±0.032	0.486±0.101
H_2O_2	Before	0.503±0.060	0.577±0.035	0.702±0.008	0.508±0.049	0.572±0.091
	Before and after	0.462±0.084	0.586±0.077	0.697±0.083	0.466±0.042	0.553±0.119
Mean		0.482±0.069	0.582±0.054	0.699±0.053	0.487±0.047	0.563±0.104
Combination	Before	0.341±0.047	0.457±0.076	0.489±0.077	0.351±0.050	0.410±0.087
	Before and after	0.300±0.035	0.439±0.035	0.453±0.08	0.318±0.040	0.377±0.084
Mean		0.321±0.044	0.448±0.054	0.471±0.07314	0.335±0.04418	0.393±0.085
Infected		0.614±0.084	0.753±0.042	0.896±0.056	0.522±0.065	0.696±0.158
Healthy tomato fruits		0.332±0.008	0.300±0.035	0.281±0.024	0.018±0.014	0.233±0.132

*** Data were average of three replicates.**

$LSD_{0.05}$ (Treat.) = 0.05 **$LSD_{0.05}$ (Time) = 0.011**

In another experiment, tomato1077 cultivar was used to determine defense related enzyme activities of PAL, PPO and POD. Defense reactions were evaluated in tomato fruits 0, 1, 3, 6, 9 and 15 d post inoculations by *A. solani*. Tomato cultivar was treated with SA or H_2O_2 or combination of SA and H_2O_2 for 45 days before harvesting. Data in Table (4) showed that PAL enzyme activity significantly increased in tomato treatment with SA or H_2O_2 or combination of SA and H_2O_2 in field before fruits harvesting and treatment by elicitors after fruits harvesting compared with infection by *A.solani* (untreated by elicitors). Also PAL enzyme activity significantly increased in tomato fruits treated by elicitors after harvesting after infection by *A. solani* compared with treatment by elicitors before fruits harvesting alone. The treatment with combination of SA and H_2O_2 increased PAL enzyme activity followed by treatment by SA and treatment by H_2O_2. Data also revealed that, the high increase of PAL activity was observed from 3 to 6 days, then, declined.

Data in Table (5) showed that PPO enzyme activity significantly increased in tomato treated with SA or H_2O_2 or combination of them on tomato shoot in field and complete the treatment by elicitors after fruits harvesting compared with infection by *A.solan* (untreated by elicitors). Also PPO enzyme activity significantly increased in tomato fruits treated by elicitors after harvesting after infection by *A. solani* compared with treatment by elicitors before fruits harvesting alone. The treatment with combination of SA and H_2O_2 increased PPO enzyme activity, followed by treatment by SA compared with treatment by H_2O_2. Data also revealed that, the high increase of PPO activity was observed at 3 to 6 days, then, declined.

Data in Table (6) showed that POD enzyme activity significantly increased in tomato treated with SA or H_2O_2 or combination of SA and H_2O_2 on shoots in field and completed the treatment by elicitors after fruits harvesting compared with infection by *A. solani* (untreated by elicitors). POD enzyme activity significantly increased in tomato fruits treated by elicitors after tomato fruit harvesting and infection by *A. solani* compared with treatment by elicitors before fruits harvesting alone. The treatment with combination of SA and H_2O_2 increased POD enzyme activity, followed by treatment by SA compared with treatment by H_2O_2. Data also revealed that, the high increase of POD activity was observed at 3 to 6 days, then, declined.

Elicitors induces the expression of pathogenesis related protein defense (PR2 and PR3)

SA and H_2O_2 treatments enhanced the expression of PR-3 (chitinase) genes (Fig 3). Transcription of the gene reached its maximum level at 6 days in SA or H_2O_2 or combination of SA and H_2O_2 treated fruits compared with infection by *A. solani* (untreated by elicitors). Data also indicated that the highest increase in expression of PR3 gene reached the maximum level at 6 days in tomato fruits when the treatment was completed by elicitors after harvesting (in lab) and after infection by *A. solani* compared with fruits treated by elicitors in field only. The combination of SA and H_2O_2 was the most effective to increase the PR3 gene expression followed by SA .The control tomato fruit showed a continuous lower mRNA level of *PR3* throughout the experiment.

Table 4. Effect of two elicitors (SA and H_2O_2) on PAL enzyme activity of tomato fruits infected by *A. solani*.

Treatment		Days after inoculation 0	1	3	6	9	15	Mean
SA	Before	0.470±0.070	0.570±0.061	0.680±0.044	0.780±0.044	0.690±0.036	0.597±0.087	0.631±0.114
	Before and after	0.507±0.011	0.616±0.015	0.713±0.023	0.837±0.015	0.743±0.051	0.650±0.050	0.678±0.110
Mean		0.488±0.049	0.593±0.047	0.697±0.036	0.808±0.042	0.717±0.049	0.623±0.070	0.654±0.1123
H_2O_2	Before	0.363±0.040	0.5767±0.068	0.623±0.0165	0.700±0.010	0.653±0.047	0.597±0.087	0.586±0.1185
	Before and after	0.373±0.0642	0.540±0.0692	0.677±0.0589	0.753±0.045	0.697±0.045	0.607±0.1001	0.608±0.140
Mean		0.368±0.048	0.558±0.065	0.600±0.048	0.727±0.041	0.675±0.047	0.6027±0.085	0.597±0.128
Interaction	Before	0.423±0.006	0.670±0.010	0.760±0.030	0.887±0.0056	0.863±0.021	0.703±0.006	0.718±0.158
	Before and after	0.370±0.020	0.710±0.010	0.803±0.006	0.920±0.020	0.850±0.020	0.647±0.0056	0.717±0.184
Mean		.397±.032	.690±.024	.782±.031	.903±.023	.857±.019	.675±.0314	.712±.169
Infected		0.420±0.	0.407±0.025	0.620±0.020	0.650±0.010	0.670±0.010	0.630±0.020	0.561±0.113
Healthy tomato fruits		0.300±0.020	0.320±0.020	0.310±0.010	0.300±0.030	0.220±0.020	0.227±0.038	0.279±0.046

Data were average of three replicates

$LSD_{0.05}$ (Treat.) = 0.005 **$LSD_{0.05}$ (Time) = 0.0037**

Table 5. Effect of two elicitors (SA and H_2O_2) on PPO enzyme activity of tomato fruits infected by *A. solani*.

Treatment		Days after inoculation 0	1	3	6	9	15	Mean
SA	Before	0.697±0.100	0.733±0.029	0.757±0.032	0.893±0.025	0.827±0.015	0.733±0.076	0.773±0.083
	Before and after	0.690±0.056	0.733±0.049	0.854±0.052	1.017±0.161	0.963±0.040	0.700±0.052	0.843±0.136
Mean		0.693±0.073	0.748±0.039	0.805±0.066	0.955±0.123	0.895±0.079	0.752±0.061	0.808±0.117
H_2O_2	Before	0.606±0.101	0.633±0.032	0.737±0.006	0.790±0.026	0.770±0.061	0.657±0.051	0.699±0.086
	Before and after	0.600±0.089	0.743±0.049	0.840±0.053	0.837±0.025	0.790±0.010	0.673±0.038	0.747±0.099
Mean		0.603±0.085	0.683±0.071	0.783±0.066	0.833±0.034	0.780±0.041	0.665±0.041	0.721±0.095
Combination	Before	0.720±0.020	0.747±0.045	0.820±0.020	0.950±0.020	0.963±0.021	0.777±0.015	0.829±0.100
	Before and after	0.730±0.020	0.810±0.010	0.920±0.020	1.017±0.029	1.133±0.042	0.800±0.010	0.906±0.144
Mean		0.725±0.019	0.778±0.045	0.870±0.057	0.983±0.043	1.048±0.097	0.788±0.017	0.865±0.128
Infected		.520±.0100	.620±.0200	.690±.010	.720±.020	.760±.010	.410±.010	.667±.081
Healthy tomato fruits		0.300±0.020	0.307±0.025	0.320±0.020	0.200±0.050	0.200±0.046	0.200±0.020	0.254±0.063

*** Data were average of three replicates.**

$LSD_{0.05}$ (Treat.) = 0. .006 **$LSD_{0.05}$ (Time) = 0.001**

Table 6. Effect of two elicitors (SA and H_2O_2) on POD enzyme activity of tomato fruits infected by *A. solani*.

Treatment		Days after inoculation 0	1	3	6	9	15	Mean
SA	Before	0.170±0.061	0.240±0.044	0.463±0.071	0.460±0.046	0.333±0.059	0.190±0.017	0.317±0.142
	Before and after	0.200±0.026	0.273±0.055	0.510±0.113	0.403±0.025	0.333±0.049	0.293±0.021	0.327±0.100
Mean		0.185±0.045	0.257±0.048	0.487±0.089	0.432±0.045	0.333±0.048	0.241±0.059	0.323±0.121
H_2O_2	Before	0.163±0.050	0.240±0.030	0.390±0.053	0.253±0.042	0.240±0.061	0.170±0.053	0.243±0.087
	Before and after	0.153±0.015	0.273±0.064	0.397±0.045	0.257±0.050	0.150±0.020	0.140±0.010	0.222±0.089
Mean		0.158±0.034	0.257±0.048	0.373±0.0478	0.255±0.041	0.195±0.064	0.155±0.0378	0.232±0.087
Combination	Before	0.260±0.0200	0.303±0.006	0.590±0.010	0.650±0.020	0.620±0.020	0.303±0.006	0.454±0.172
	Before and after	0.210±0.010	0.350±0.020	0.603±0.015	0.680±0.020	0.640±0.020	0.303±0.006	0.464±0.188
Mean		0.235±0.031	0.327±0.029	0.597±0.014	0.665±0.024	0.630±0.021	0.303±0.0051	0.459±0.178
Infected		0.120±0.020	0.140±0.010	0.230±0.030	0.240±0.040	0.200±0.020	0.190±0.010	0.187±0.049
Healthy tomato fruits		0.100±0.020	0.120±0.020	0.100±0.020	0.100±0.020	0.090±0.010	0.070±0.010	0.097±0.021

*** Data were average of three replicates.**

$LSD_{0.05}$ (Treat.) = 0.0089 **$LSD_{0.05}$ (Time) = 0.0013**

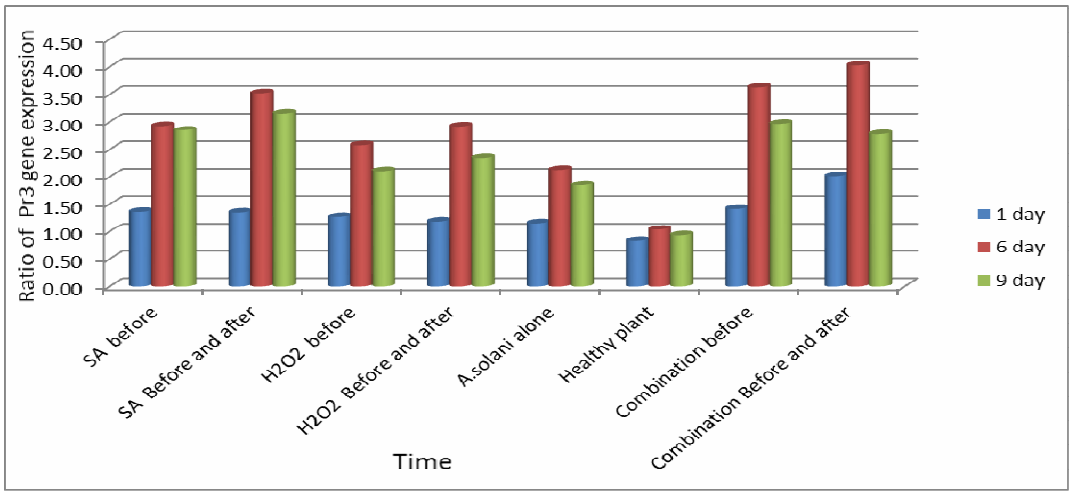

Fig. 2. Effect of two elicitors (SA and H_2O_2) on PR3 gene expression of tomato fruits infected by *A. solani*.

The level of PR2 gene expression reached its maximum level at 6 days in SA, H_2O_2 or combination of SA and H_2O_2 treated fruits compared with infection by *A.solani* (untreated by elicitors). Data indicated that the highest increase in expression of PR2 gene reached the maximum level at 6 days in tomato fruits when the treatment was completed by elicitors after harvesting and after infection by *A. solani* compared with treatment by elicitors before fruits harvesting. The combination of SA and H_2O_2 was the most effective to increase the PR2 gene expression, followed by H_2O_2. The treatment with H_2O_2 after harvesting increased PR2 gene expression in tomato fruits, followed by the treatment of SA after fruit harvesting compared with treatment by SA and H_2O_2 in field. The control tomato fruit showed a continuous lower mRNA level of *PR-2* throughout the experiment.

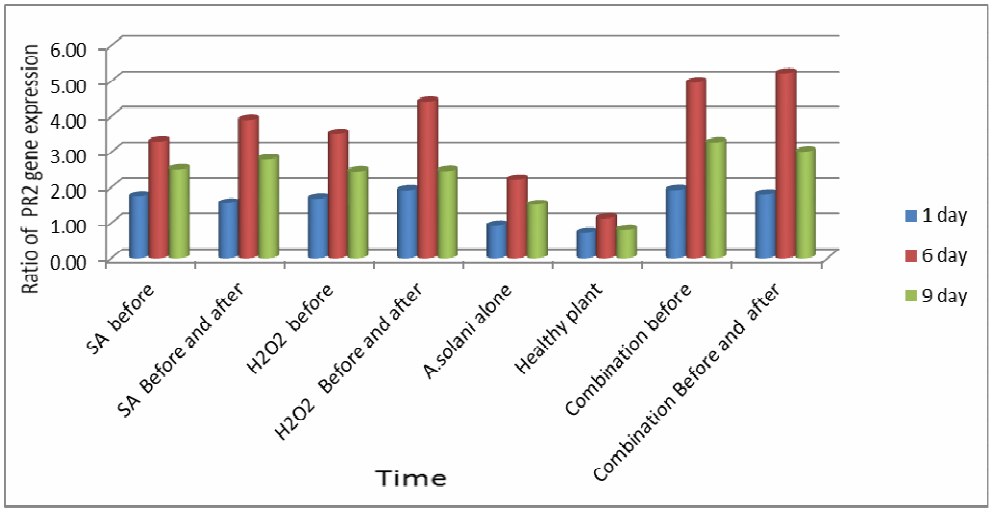

Fig. 3. Effect of two elicitors (SA and H_2O_2) on PR2 gene expression of tomato fruits infected by *A. solani*.

DISSCUSSION

Vegetable fruits are highly perishable crops. Thus, post-harvest handling, storage, transportation and marketing are seriously affecting their quality in the market. Improperly handling, packaging, storage and transportation could cause decay and increase the production of micro-organisms due to changing physiological state of the fruits and vegetables (Wilson *et al.,* 1991).

Elicitors can induce resistance against many plants diseases (Lou and Zhang, 2005 and Wang *et al.,* 2007). Fruit maturity influences the natural resistance against fungal pathogens (Prusky, 1996). Therefore, for protection of fruits from pathogen infection, it is necessary to investigate the mechanisms of fruit resistance at different maturity stages. Many hypotheses have been proposed to explain why ripened fruits are more susceptible to pathogens than non ripened ones. These include nutrient availability to trigger pathogen activation (Prusky, 1996), the ripening-related cell wall dissemble, facilitates pathogen expansion (Cantu *et al.,* 2008) and the weakness of antioxidant defense response to accelerate cell death of host tissue (Chan *et al.,* 2008). However, little attentions have been focused on the correlation of accumulation of PRs with the acquisition of resistance in fruits at different maturity stages. PRs are defined as proteins which are induced in plant tissues in response to pathogenic attack or related stimuli. In this study the development of fruits rot disease has been studied on semi ripped fruits of tomato, it is evident that all the taken fruits were found to be susceptible for the development of fruit rot. The symptoms disease appeared on third day of inoculation and the development of disease has been increased with increasing the incubation period up to fifteen days in each treatment.

Chaurasia *et al.,* (2013) three different tomato fruit types of various age (Green, Semi ripe and Ripe tomato) diseased by *A.solani* and the susceptibility was different between the three types and the rotted area increased with increasing the incubation period. In this study the treatment with SA or H_2O_2 elicitors enhanced resistance of tomato fruits rot caused by *A. solani* , development decreased and keep the fruits fresh long time. Treat with SA was more effective in controlling decay caused by *B. cienrea* and decrease of ethylene production and the decline of pH and lycopene content (Wang *et al.,* 2007). Induction of systemic resistance can lead to direct activation of defense-related proteins (Conrath *et al.,* 2001). Among these defense-related proteins are POD, PAL, and PPO that catalyze the formation of lignin, wound responses, pathogen attack, growth regulation, and synthesis of phytoalexins and phenolics (Ramamoorthy *et al.* 2002), which subsequently enhance plant's resistance to pathogens. The resistance to plant disease is associated with defense activation. The mechanisms of defense include preexisting physical and chemical barriers that interfere with pathogen establishment. Other methods of protection rely on inducible defense responses in the form of enzymes that are activated upon infection (Vanitha *et al.,* 2009). In this study the treatment with SA or H_2O_2 or combination of SA and H_2O_2 elicitors enhanced resistance of tomato fruit and increased the defense-related proteins POD, PAL, and PPO. The defense was more increased in fruits treated by elicitors in field and continue after fruits harvesting compared to treatment in field only, so that this fruits have low disease symptoms. Cota *et al.* (2007) showed that the red ripe fruits stage was more susceptible to *Alternaria alternate* than mature green stage in three different varieties of tomato , partly because of the changes in enzymatic activities of 1,3-glucanase and chitinase in response to fungal infection. Wang *et al.,* (2007) found that treatment with SA suppressed the decay of tomato fruits and induced the expression of PR1, PR2 and PR3. These results indicated that different PRs could be associated with different elicitor-induced fruit resistance. PR1 was regulated by SA pathway, whereas PR2 and PR3 were corresponded to JA/ethylene pathway (Van Loon *et al.,* 2006). Our results showed that the treatment with SA and H_2O_2 enhanced the expression of PR-2 and PR-3 gene.

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استحثاث المقاومة فى ثمار الطماطم بعد الحصاد بواسطة حمض السالسيلك وفوق اكسيد الهيدروجين ضد عفن ثمار الطماطم الذى يسببه فطر *Alternaria solani*

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تعتبر الطماطم واحدة من اهم محاصيل الخضر والذى يصاب بالعديد من المسببات المرضية وخاصة امراض ما بعد الحصاد والتى تحدث خسائر كبيرة فى كمية وجودة المحصول وتقلل من فترة تخزين الثمار وخاصة اعفان الثمار المتسببة عن فطر *Alternaria solani*. معاملة ثمار الطماطم بحمض السالسيلك اسيد SA وفوق اكسيد الهيدروجينH_2O_2 لاستحثاث المقاومة ضد عفن ثمار الطماطم المتسبب عن فطر *Alternaria solani* ادى الى تقليل تطور العفن فى الثمار بعد الحصاد. بينما معاملة ثمار الطماطم بSA و H_2O_2 او الاثنين معا على المجموع الخضرى فى المزرعة قبل الحصاد فقط او المعاملة بهم قبل وبعد الحصاد قلل تطور مساحة عرض العفن فى الثمار وقلل نشاط انزيم البولى جالاكتيورنيز PG وكانت هناك زيادة معنوية فى نشاط الانزيمات البيروكسيديز والبولى فينول اوكسيديز و الفنيل الانين ليز وزيادة التعبير الجينى للجينات المرتبطة بالمرضية PR2 و PR3 فى الثمارمقارنة بالعدوة بالفطر فقط دون اى معاملة بالمستحثات. المعاملة ب SA و H_2O_2 قبل وبعد الحصاد قلل تطور مساحة عرض العفن فى الثمار وقلل نشاط انزيم البولى جالاكتيورنيز PG وكانت هناك زيادة معنوية فى نشاط الانزيمات البيروكسيديز والبولى فينول اوكسيديز و الفنيل الانين ليز وزيادة التعبير الجينى للجينات PR2 و PR3 فى الثمارمقارنة بالمعاملة فقط بعد الحصاد. المعاملة ب SA و H_2O_2 معا قلل تطور مساحة عرض العفن فى الثمار وقلل نشاط انزيم البولى جالاكتيورنيز PG وكانت هناك زيادة معنوية فى نشاط الانزيمات البيروكسيديز والبولى فينول اوكسيديز و الفنيل الانين ليز وزيادة التعبير الجينى للجينات PR2 و PR3 فى الثمارمقارنة بالمعاملة بكل مادة لوحدها.