academicJournals

Vol. 8(4), pp. 167-177, April 2014
DOI: 10.5897/AJPS2012.0720
Article Number: 8FA9C0444190
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Camel urine, a potent tool for plant protection

E. Nafie

Department of Botany, College for Women (Arts, Science and Education), Ain Shams University, Cairo, Egypt.

Received 20 November, 2012; Accepted 27 March, 2014

Cross-tolerance is the phenomenon by which a plant resistance to stress results in resistance to another form of stress. Such intriguing links has made the author to suggest that foliar camel urine (CU) application depending on its amazing constituents may mimic elicitor actions and prime tissues to pathogens in normally susceptible host plants. This study was conducted to assess anatomical and biochemical pathogenesis alterations in tomato water T1, *Fusarium oxysporum* infected T2 plants and also to evaluate the defense-related responses resulting from CU application in either nonT3 and/or T4 infected seedlings at anatomical and molecular levels. Fast and consistent increment in detoxifying enzymes peroxidase, phenylalanine ammonia-lyase, polyphenol oxidase and soluble proteins were recorded in T4 roots. In contrast, tomato roots, T2, T4 showed retard response in catalase activity. T4 accumulate highly significant level (80%) of total free and bound phenolics within its root. Root of T4 SA concomitant with coumarins had significant increment in both free and conjugated phenolic part. Anatomical microscopy shows alterations in T2, T3 as compared to T1 tomato root vascular bundle diameter. Results showed earlier reduction explained as adaptation mechanism in resisting the imposed stress regardless of its type. In contrast, T4 seedlings showed great increase in vascular bundle diameter concomitant with xylem lignin deposition and on epidermis cell walls. Tomato shoot administered CU implicate phenylpropanoid pathway in prime root to *F. oxysporum* invasion by enhancing innate immunity mechanisms, fast accumulation of H_2O_2 concomitant with cell wall modifications as physical barrier.

Key words: Cross-resistance, signaling, defense mechanism, detoxification enzymes, free and glycoside-phenolics, phenylpropanoid pathway.

INTRODUCTION

Plants are frequently exposed to different and simultaneous environmental stresses, which can be both biotic and/or abiotic. Exposure to abiotic stress, in some cases, enhances resistance to pathogens indicative of crosstalk between biotic and abiotic stress signaling (Bowler and Fluhr, 2000). Bowler and Fluhr (2000) and Abuqamar et al. (2009) stated that biotic and abiotic stress have similarities in plant responses to pathogens and abiotic stresses and partially share induced signaling cascades, among which are production of reactive

E-mail: oeetezaz@yahoo.com. Tel: +202-22736361.

oxygen species (ROS), accumulation of hormones such as salicylic acid, ethylene, jasmonic acid and abscisic acid. These signals ultimately induce expression of nuclear defense genes that lead to the assembly of the overall defense reaction.

Mittler et al. (2004) suggested that during plants evolution they were able to regulate ROS toxicity, through a highly balanced and tightly coordinated network of at least 152 genes which encode both ROS-producing and ROS-scavenging enzymes. For this reason, ROS molecules have been used as signaling molecules and accordingly, the interplay between the ROS-producing and ROS-scavenging pathways will determine the intensity, duration and localization of the ROS signals (Mittler et al., 2004).

Usually, cellular signaling cascade via ROS generated by plant-pathogen interactions, can also be activated by the use of elicitors, stable molecules that induce an immune defense response in plants (Spoel and Dong, 2012). Elicitor-induced plant signaling, serves as a guide to a series of intracellular events that end in the activation of transduction cascades and hormonal pathways, which trigger induced resistance and consequently activate plant immunity to environmental stresses (Spoel and Dong, 2012).

The exploitation of natural resources origin in conferring resistance to plants against abiotic and biotic stresses is gaining much attention. Urine and milk of camels (*Camelus dromedarius*) have been considered to have medicinal significance due to the active substances contained in desert plants (AL-awadi and AL-Jedabi, 2000). An inhibitory and antibiotic activity of camel urine against the growth of *Candida albicans* (yeast), *Aspergillus nige*r, *Fusarium oxysporum* were proven even after it's boiling to 100°C (AL-awadi and AL-Jedabi, 2000).

The urine of camel, was very much in use as remedies for the treatment of dropsy, abdominal enlargements, anaemia, abdominal tumor, tuberculosis, haemorrhoids, leucoderma, leprosy and in mental diseases (Thakur, 2004). Al-Attas (2008), using neutron activation analysis, estimated some essential and rare elements in milk and urine of camels.

In fact, it is known that treatment of plants with elicitors, or attack with pathogens, causes a set of defense reactions such as the accumulation of defensive secondary metabolites in edible and inedible parts of plants, specific gene expression and enzymatic induction (García-Mier et al., 2013).

Plants undergo also anatomical changes in response to biotic and abiotic factors. De Micco et al. (2013) conducted a study to reveal histology alterations in *Phaseolus vulgaris* L. plants when subjected to increasing dose of X-rays (0.3, 10, 50 and 100 Gy), results showed that even at high levels of radiation, general anatomical structure was not severely perturbed. There seems to be no report in the literature on the effect of CU on physiology or anatomy of higher plants. The present study suggested that CU could encourage tomato defensive mechanisms which will prime the plant against further invasion depending on its amazing constituents and its potency to mimic elicitor's role. This work aim to first, assess tomato root responses to *F. oxysporum* challenge and CU foliar application, second to test if pretreatment aerial shoot evoke root defense mechanisms against next *Fusarium* challenge. To do this, histological concomitant with biochemical changes in treated tomato seedlings different parts were assessed.

MATERIALS AND METHODS

Tomato seeds (*Lycopersicon esculentum* vs. *castlerock*) susceptible to *F. oxysporum* f. sp. *radicis- lycopersici* were kindly provided by Field Crops Research Institute at the National Research Centre, Giza, Egypt. Seeds were surface disinfected in 1% hydrochloric acid for 30 min and rinsed repeatedly in sterile double-distilled water prior to sowing (Fuchs et al., 1997), planted and grown in vermiculite in an environmentally controlled greenhouse at 24 ±2°C with 16/8 h of light - dark condition till desired age. Plants were fertilized twice a week, young plants, approximately with five or six fully expanded leaves were then transplanted into pots.

Camel urine was received from Maryout Research Station of Desert Research Center after collection from male camel of about seven years old in the early morning.

Fungal inoculums' preparation

Authentic fungal culture was received from Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt. Fungus *F. oxysporum* f. sp. *Radicis lycopersici* were sub cultured in 9-cm Petri dishes on potato dextrose agar (PDA) at 25°C in the dark. Conidia were removed from 10-15-day-old cultures into distilled water and their concentration was adjusted to 10^6 - 10^7 conidia per ml with a cytometer before inoculation.

Experimental design and treatments application

Upon the appearance of the fifth leaf, pots were randomly assigned to four groups of twenty replicates each, sprayed equally with either water (T1), and challenged with *F. oxysporum* (T2), sprayed with camel urine (T3), and challenged with *F. oxysporum* (T4). Challenging of T2 and T4 were carried out with pathogen two days later after foliar applications. The experiment was left till harvesting time: two, four, seven and fourteen days after inoculation.

Preparation of tomato tissues to study enzymatic activity

Seedlings representing all treatments at the harvesting time were separated, immediately frozen in liquid N_2 and stored at - 80°C.

Plant biochemical analysis

Phenylalanine ammonia-lyase enzyme extraction and assay (PAL) (EC 4.3.1.5)

The ratio of frozen tissue (g) and extraction buffer volume (ml) was 1:5. The extraction was carried out following the method proposed

by Lister et al. (1996). Frozen plant material was ground at 4°C in buffer (50 mM Na_2HPO_4/KH_2PO_4, pH 7, polyvinylpyrrolidone (PVP) 0, 5% (Mr, 44 000), 50 mM sodium ascorbate, 18 mM mercaptoethanol, 0.1% (v/v) Triton X-100). The homogenate was filtered through four layers of cheesecloth and centrifuged at 20 000 g for 10 min. $(NH_4)_2SO_4$ was added to the supernatant (to 35% saturation), which was then centrifuged for 20 min at 20 000 g to remove the PVP. More $(NH_4)_2SO_4$ was added to this supernatant to reach a final saturation of 80%. This fraction was centrifuged at 20 000 g for 20 min, and the pellet was re suspended in extraction buffer (without PVP and Triton). This solution was used for PAL assays.

PAL activity was assayed after adapting (McCallum and Walker, 1990) method. The assay mixture consisted of 0.06 M sodium borate buffer, pH 8.8, and partially purified enzyme. The reaction was started by the addition of 11 mM L-phenylalanine. The reaction mixture was incubated at 30°C for 15 min, and the reaction was terminated by the addition of 6 N HCl and then measured at 290 nm (Lee et al., 2007). Enzyme activity was expressed as µM cinnamic acid h^{-1} g^{-1} FW.

Peroxidase enzyme extraction and assay (POD) (EC 1.11.1.7)

Frozen plant parts were homogenized (1:5) in appropriate ice cold extraction buffer: 0.1 M potassium phosphate buffer (pH 6.8) containing 1% Triton X-100 and 0.15 g of Polyclar AT (insoluble polyvinylpolypyrrolidone, Sigma Chemical Co., St. Louis). The supernatants were used directly in the enzyme assays.

Peroxidase activity was determined following Hammerschmidt and Kuc (1982) method. The reaction mixture consisted of 0.5 ml plant extract, 1.5 ml of 0.05 M pyrogallol and 0.5 ml of 1% hydrogen peroxide. The reaction was incubated in a water bath, and then terminated after incubation for 30 min; absorbance was recorded at 420 nm. The enzyme activity was expressed as change in the absorbance of the reaction mixture, min^{-1} g^{-1} FW.

Catalase enzyme extraction and assay (CAT) (EC 1.11.1.6)

Frozen plant parts stored at - 80°C, were extracted in 0.4 ml of buffer (potassium phosphate, 50 mM at pH 7.4, containing 10 mM DTT). Catalase activity was assayed at 25°C following the decrease in absorption at 240 nm in 10 mM sodium phosphate buffer, pH 7(Aebi, 1984). The enzyme activity was expressed as mM H_2O_2, min^{-1} g^{-1} FW.

Polyphenol oxidase enzyme extraction and assay (PPO) (EC 1.14.18.1)

The extraction method proposed by Thypyapong et al. (1995) after some modifications were used. Frozen tissue was grounded in extraction buffer (100 mM Tris-HCl, pH 7.0, 100 mM KCl, 1 mM phenylmethanesulfonyl fluoride (PMSF) and 3% [w/v] PVP). The homogenates were centrifuged at 12 000g for 15 min, and the supernatant was used to measure the PPO enzyme activity. The PPO activity was determined according to Mayer (1995) with some modifications. The reaction mixture consisted of 100 mM buffer (Na_2HPO_4/ KH_2PO_4), pH 7.0 and 1.5 ml of 0.01 M catechol and reaction was initiated by the addition of 200 µl enzyme extract, the activity was expressed as changes in absorbance at 495 nm.

Protein determination

From each treatment, 0.5 g samples were grinded in mortar with 5 ml of phosphate buffer pH 7.6 and centrifuged at 8000 g for 20 min. The supernatant was then made equal by adding phosphate buffer solution. Coomassie Brillaint Blue G-250 solution was then added and mixed thoroughly (Bradford, 1976). The total volume was 3 ml. All treatments were incubated for 5 min at room temperature and absorbance at 595 nm was recorded against the reagent blank. A standard sample (100 µg in 100 µl) was used to estimate soluble protein concentrations in the unknown treated samples as mg g^{-1}.

Phenolics extraction

Tomato seedlings harvested from two, four, seven and fourteen days old plants, representing each of the four treatments were used to extract a free-phenolic fraction part, and a conjugated fraction consisting of the aglycones released after hydrolysis. The extraction method of Fawe et al. (1998) was used. Fine powder samples preserved in liquid N_2 were extracted in 80% acidified methanol (10 g/100 ml). The extract was then filtered with glass fiber filters (GF/C; Schlei and Schuell), and the filtrate was concentrated. The aqueous residue was adjusted to pH 2.0 and partitioned against hexane. The aqueous phase containing the phenolic constituents was further partitioned against ethyl acetate and then subjected to acid hydrolysis, 4 N HCl in an autoclave for 15 min. The hydrolysate was cooled and partitioned against ethyl acetate. The two ethyl acetate fractions obtained were dried, and the residues designated as free-phenolic fraction and conjugated-phenolic fraction, respectively, were re suspended in absolute methanol (2.5 g/ml).

Total phenolic concentration determination

Total phenolic (TP) concentration was measured in both free and glycosidic-bound phenolic extract by using Folin Ciocalteus reagent (Sigma, St. Louis, Mo.) according to Singleton and Rossi (1965) method. Determination of total phenols concentration was done using a Jenway (UV/Vis 6405) spectrophotometer monitoring 750 nm. TP content was expressed as mg FW.

Phytoalexins separation using high-performance liquid chromatography (HPLC)

Tomato leaves and roots from all treated plants after 48 h of infection were harvested, pooled and kept at -80°C until use (approximately 20 g). The phenolic compounds extraction was performed as described previously. The resultant free and conjugated-phenolic fractions after separation as previously described were used to determine their profiles.

The HPLC system (Thermo Separation Products, Riviera Beach, Fla.) consisted of an auto sampler (AS3000), injector (100 µl), column oven (30), pump (P3000) and diode array detector (UV6000). A reverse-phase C18 column (250 by 4.6 mm; Luna 2; Phenomenex, Torrance, Calif.), with a pre column of similar resin, was employed. Elution was performed using phosphate buffer (50 mM, pH 2.5) and methanol at a flow rate of 1 ml min^{-1}. A linear-gradient program was developed as follows: C18 column (time [in minutes]/methanol [percent]) = 0/0, 6/0, 31/95, 32/0. In all instances, the software was programmed to show peaks at their maximum absorbance. For each treatment, HPLC analyses were repeated twice, showing similar results.

Tissue processing for ultra structural investigation

For optical light microscopy (OLM) investigation, roots (covering 7 and 14 day harvesting time) sections from chosen tomato plant parts were fixed in FAA (ethanol 50% + formaldehyde 5% + glacial acetic acid 10%, in water) for 48 h. After fixation, segments were

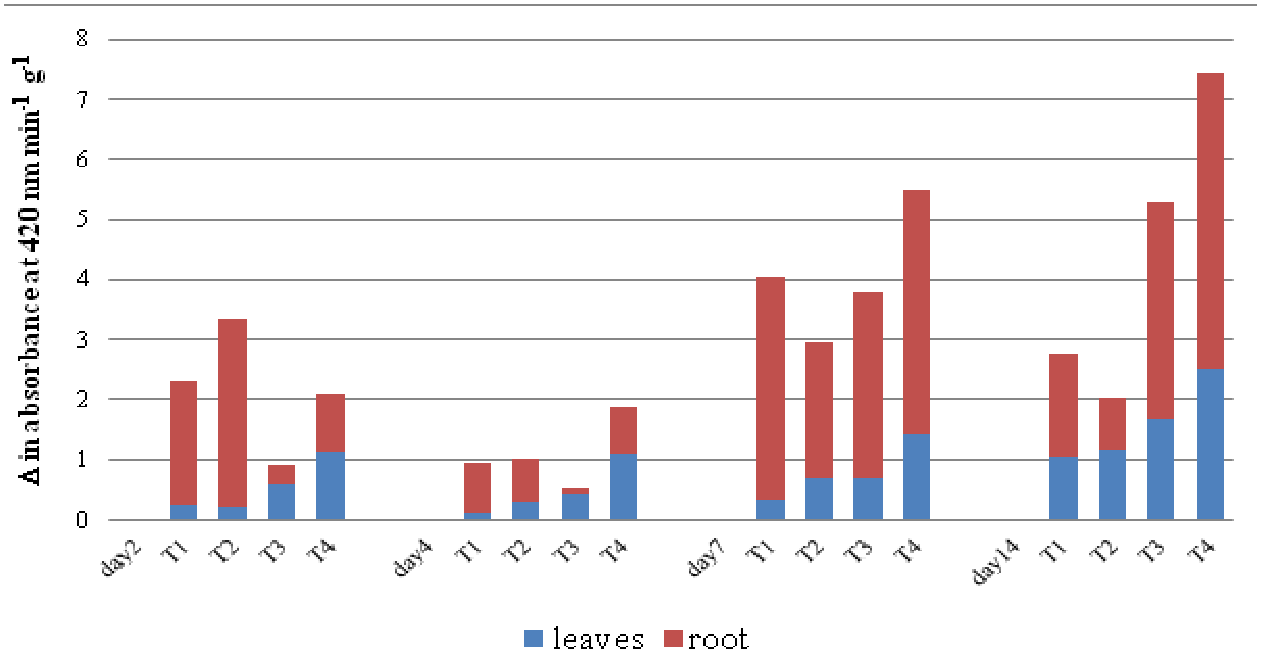

Figure 1. Peroxidase enzyme activity estimated in water (T1), and challenged with *F. oxysporum* (T2), sprayed with camel urine (T3), and challenged with *F. oxysporum* (T4), harvested at day two, four, seven and 14 post infection.

dehydrated through a gradient series of ethanol, cleared with xylene, and finally embedded in paraffin. Fifteen-micrometer-thick sections were cut with a microtome and transferred onto glass slides coated with egg albumin. Sections were de-paraffinized with xylene and rehydrated through a gradient series of ethanol. Sections were stained with safranin O (at ethanol 50% step) to show lignin following Balatinecz and Kennedy (1967) with slight modifications, washed well, then light green was used (at ethanol 100% step) for visualizing the plant sections anatomy. Photographs were taken using Olympus microscope BH2 with camera attachment (model U-ACAD-2). Calibrated linear ocular was used for tissue measurements.

Statistical analysis

The test of least significant difference (L.S.D) at the level of 0.05% significance was used to examine differences among treatment means and interactions. Data were statistically analyzed using MSTAT-C software package according to the described method by Freed et al. (1989).

RESULTS

Biochemical changes

Camel urine application enhanced tomato resistance responses

To assess pathogenesis and if pre CU application may prime and condition plant response to successive pathogen attack, changes in detoxification enzymes were elucidated in samples harvested at the required time.

Peroxidase and catalase activities

The fungus inoculation resulted in rapid increment in T2 root enzyme activity after 48 h thereafter fluctuated, while leaves exhibited progressive levels. Peroxidase activity in T4 treated plants showed progressive local and systemic enhancement *in vivo* plant leaf and root. Root displayed higher activity than leaves as shown in Figure 1. Leaves of infected tomatoes exhibited CAT increment two days later while their roots negatively responded. T4 leaves during same time showed similarity with the control while roots enzyme sharply decreased. At the 14th day, enzyme activity in roots of all treated plants were increased, on the other hand leaves responded in a different manner as shown in Figure 2.

Alterations in phenolic compounds metabolism

Phenylalanine ammonia-lyase and polyphenol oxidase activities

Phenylalanine ammonia-lyase and polyphenol oxidase activities in T4 roots progressively increased till 14 day post challenge. Roots of T2 treated plants had highest level after 24 h in PAL and PPO activity thereafter

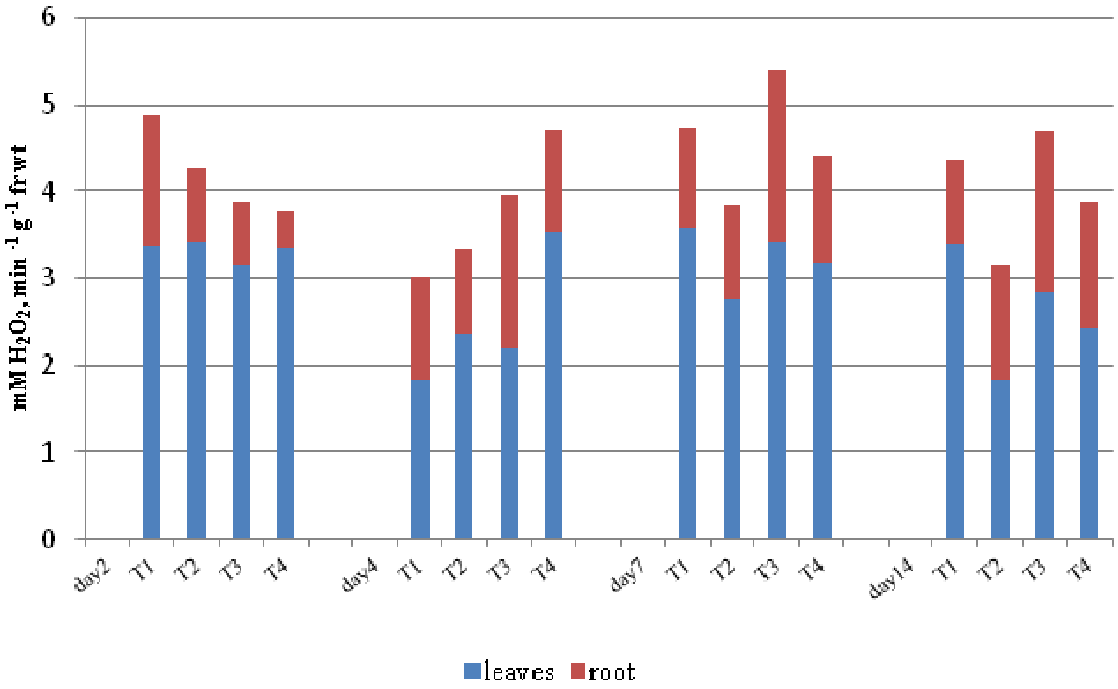

Figure 2. Catalase enzyme activity estimated in water (T1), and challenged with *F. oxysporum* (T2), sprayed with camel urine (T3), and challenged with *F. oxysporum* (T4)l, harvested at at day two ,four, seven and 14 post infection.

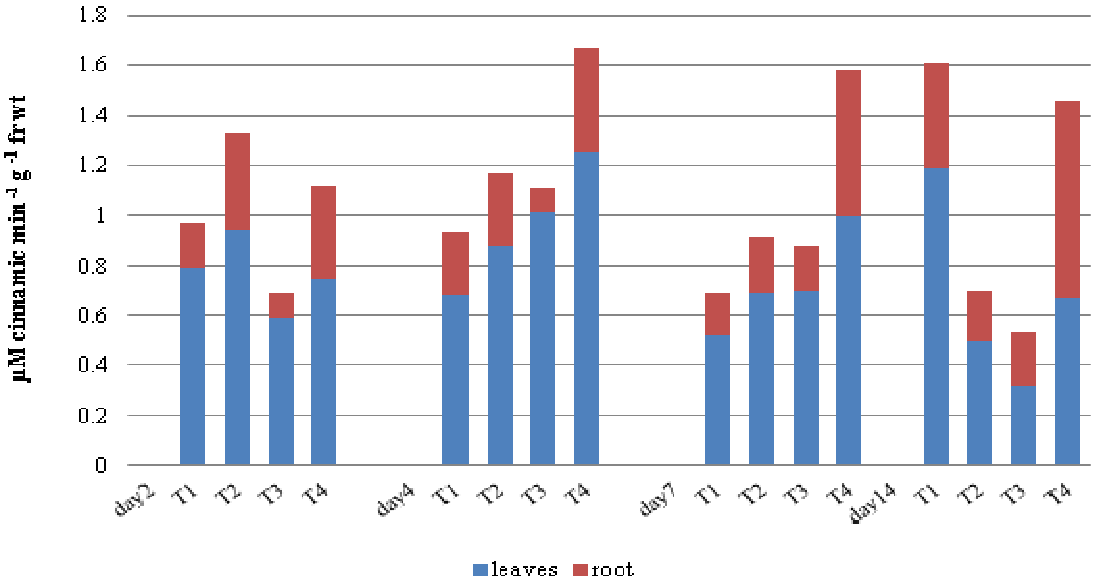

Figure 3. Phenylalanine ammonia-lyase enzyme activity estimated in water (T1), and challenged with *F. oxysporum* (T2), sprayed with camel urine (T3), and challenged with *F. oxysporum* (T4), harvested at day two, four, seven and 14 post infection.

generally decreased as compared to the control. Fungus inoculation caused rapid increment in leaves PAL activities then gradually ceased while PPO showed fluctuations as shown in Figures 3 and 4.

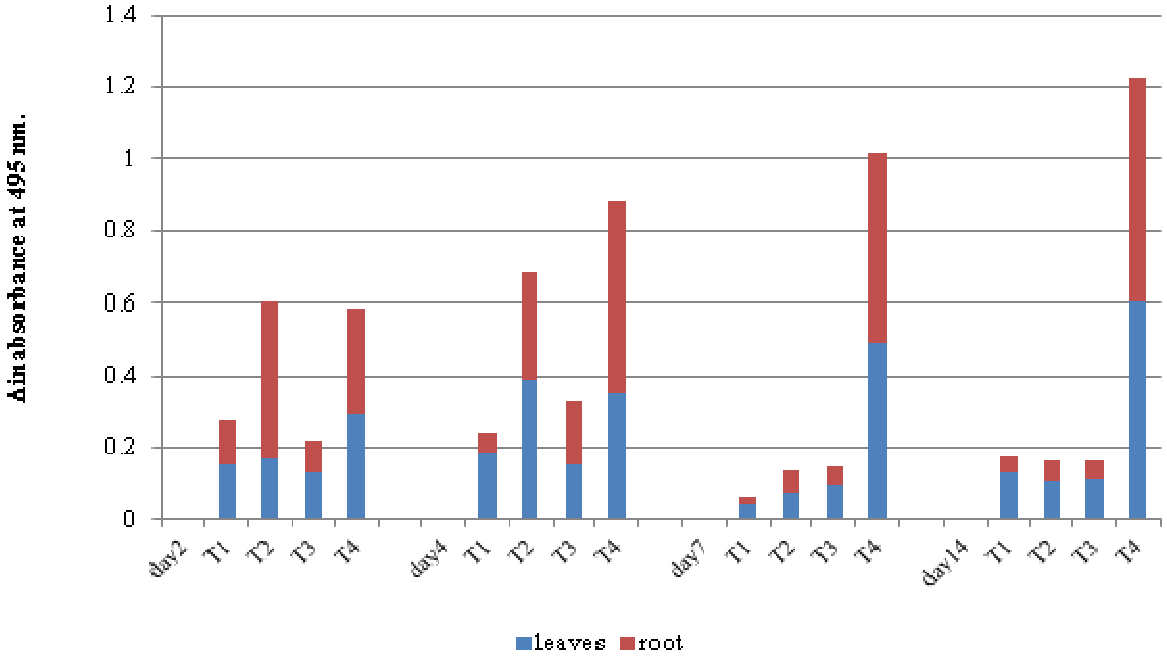

Figure 4. Polyphenol oxidase enzyme activity estimated in water (T1), and challenged with *F. oxysporum (*T2), sprayed with camel urine (T3), and challenged with *F. oxysporum* (T4), harvested at two day two, four, seven and 14 post infection.

Quantification of total phenolics in free and conjugated phenolic extracts

Increment in total phenols throughout the study was recorded in both free and conjugated phenols in different tomato parts as shown in Table 1. Alterations in phenolic compounds recorded throughout the experiment were plant part dependent, in which T4 root recorded the highest level. T2 and T4 TP in tomato leaves showed increment reaching 43.8 and 29% respectively, and in root, 40 and 81.7% at the 14 day post infection with respect to each control.

HPLC separation of phytoalexins

A comparative study for 48 h post harvesting of plants leaves and roots represent all treatments profile of certain fractions analyzed in conjugated and free phenolics, using reverse-phase HPLC. Four phenolic compounds were observed, and changes in their relative amount distribution between conjugate and free state were recorded as shown in Table 2.

Leaves and roots of challenged tomato plant with *F. oxysporum* T2 showed general retarded levels in free and conjugated coumarins, pyrogallol, catechol and salicylic acid amounts relative to the control T1 that was not challenged. In T4 tomato roots, coumarins, and salicylic acid were enhanced significantly in free and glycosidic-bound phenolic fraction while leaves salicylic acid enhanced bound phenolic fraction.

Soluble proteins

Soluble proteins in T2 or T4 roots showed continuous increment over the control. In T2 and T4 seedlings leaves rapid and negative response was recorded and continued to increase in T4 only, results are illustrated in Figure 5.

Histological changes

A part of root transverse sections from all treated tomato seedlings seven and fourteen days post-harvest are in Table 3 and Figures 6 and 7 to explore histological changes.

Tomato root section showed abundance in xylem ray with large prominent metaxylem vessels in early root as shown in Figure 6a. The structural changes associated with pathogen infection in T2 tomato seedlings root cortex and vascular bundle was reduction in both diameters, as compared to the control, T1 (Figure 6). In seedling root T3, cortex diameter was larger while vascular bundle was reduced as compared to the control T1 (Figure 6). Infection after foliar pretreatment of seedlings T4 enhanced lateral root initiation, xylem cell wall lignifications in the form of dark lesions encircling the cells as shown in Figure 6 and increment is not only in

Table 1. Phenolic compounds (free and conjugate) estimated in tomato leaves and roots in water (T1), challenged with *F. oxysporum* (T2), sprayed with camel urine (T3) and challenged with *F. oxysporum* (T4) harvested at two, four, 7 and 14 day post infection.

Harvesting days		2 day			4 day			7 day			14 day			
Phenolic (mg/g FW) treatments		Free	Glycosidic-bound	Total	Free	Glycosidic-bound	Total	Free	Glycosidic-bound	Total	Free	Glycosidic-bound	Total	Total (%)
Leaves	T1	0.4c	0.52b	0.92d	0.73b	0.85b	1.58c	0.8c	0.90d	1.7d	0.92c	1.20c	2.12d	
	T2	0.75b	0.60b	1.35c	1.32a	1.11b	2.43b	1.46a	1.22c	2.68c	1.66ab	1.39c	3.05c	43.80
	T3	0.68b	1.43a	2.11b	1.2a	2.20a	3.40a	1.27b	2.51b	3.78b	1.50b	2.65b	4.15b	
	T4	1.07a	1.46a	2.53a	1.4a	2.44a	3.84a	1.45a	2.90a	4.35a	1.85a	3.52a	5.37a	29.30
	LSD at 5%	0.21	0.45	0.32	0.27	0.48	0.63	0.30	0.26	0.64	0.28	0.65	0.73	
Roots	T1	0.69c	0.94b	1.63bc	0.95c	1.14c	2.09c	0.98 c	1.40b	2.38 c	1.00 c	1.50c	2.50c	
	T2	1.00b	0.92b	1.92b	1.48b	1.34c	2.82b	1.61 b	1.53b	3.14 b	1.80 b	1.70c	3.50b	40
	T3	0.55c	0.99b	1.54c	0.57d	1.60b	2.17c	0.75 c	1.78b	2.53 c	1.86 b	2.35b	4.21 b	
	T4	1.56a	2.45a	4.01a	1.87a	3.67a	5.54a	2.60 a	4.41a	7.01 a	2.74 a	4.91a	7.65 a	81.70
	LSD at 5%	0.26	0.35	0.23	0.31	0.22	0.54	0.48	1.44	0.50	0.68	0.57	0.85	

Table 2. Certain phenolic compounds estimated using HPLC (free and glycoside) in leaves and roots extracted after forty-eight hours (after infection) in either water (T1), and challenged with *F. oxysporum* (T2), sprayed with camel urine (T3), and challenged with *F. oxysporum* (T4). Phenolics estimated as µg/g FW.

Parameter		Free phenolics				Glycosidic –bound				Total			
		Coumarins	Pyrogallol	catechol	salicylic acid	coumarins	pyrogallol	catechol	salicylic acid	coumarins	pyrogallol	catechol	Salicylic acid
Leaves	T1	101.63a	50.10b	65.25a	13.9b	217.36a	101.6a	114.21a	7.95c	318.99a	151.70a	179.46a	21.85a
	T2	58.62b	59.00a	60.85a	6.4bb	68.50b	27.5c	36.60b	2.10d	127.12b	86.50b	97.45b	8.50d
	T3	32.12c	21.00c	26.36b	1.9c	66.74b	72.52b	14.35c	13.22a	98.86c	93.52b	40.71c	15.12b
	T4	33.74c	17.13c	22.00b	2.1c	32.50c	14.31d	12.64c	9.62b	66.24d	31.44c	34.64c	11.72c
	LSD at 5%	5.32	4.85	8.67	4.62	10.38	9.88	11.05	1.26	13.22	10.58	15.35	2.87
Roots	T1	40.76b	35.54b	32.50a	4.27c	4.50c	10.20c	29.18b	1.75c	45.26c	45.74b	61.68b	6.02c
	T2	-	30.90b	14.63c	0.30d	6.31c	9.70c	24.44c	4.23c	6.31d	40.60bc	39.07d	4.53c
	T3	38.62b	50.00a	36.00a	8.44b	59.68b	39.63a	40.00a	11.00b	98.3b	89.63a	76.00a	19.44b
	T4	78.00a	10.60c	27.52b	10.66a	65.33a	23.45b	23.62c	17.10a	143.33a	34.05c	51.14c	27.76a
	LSD at 5%	10.24	8.65	4.32	2.08	4.65	5.34	3.98	4.21	9.25	8.62	7.36	5.14

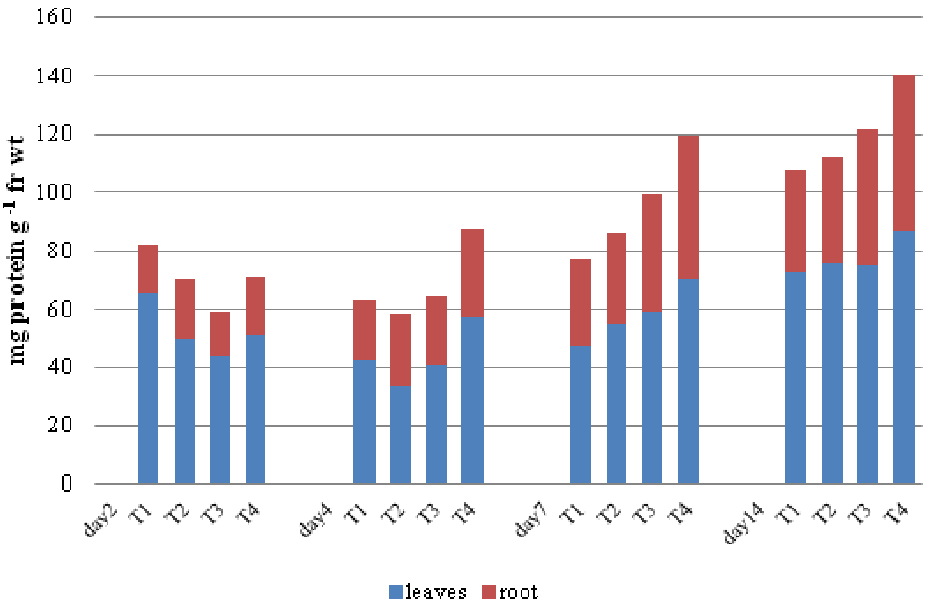

Figure 5. Soluble proteins estimated in water (T1), and challenged with *F. oxysporum* (T2), sprayed with camel urine (T3), and challenged with *F. oxysporum* (T4), harvested at day two, four, seven and 14 post infection.

Table 3. Tomato seedlings root, cortex and stele cross section diameter (µm) in either water (T1), and challenged with *F. oxysporum* (T2), sprayed with camel urine (T3), and challenged with *F. oxysporum* (T4), harvested at 7 and 14 days post infection.

Treatments	Root		Cortex		Stele	
	7 day	14 day	7 day	14day	7 day	14 day
T1	21.6	26.0ab	4.0b	5.0b	12.0b	13.0b
T2	20.0	24.0b	3.4b	4.8b	11.4b	12.6b
T3	21.0	24.60.b	5.0a	6.0a	11.0b	12.4b
T4	23.6	27.8a	3.4b	5.0b	14.4a	16.4a
LSD at 5%	NS	2.44	0.85	0.79	0.2.10	0.57

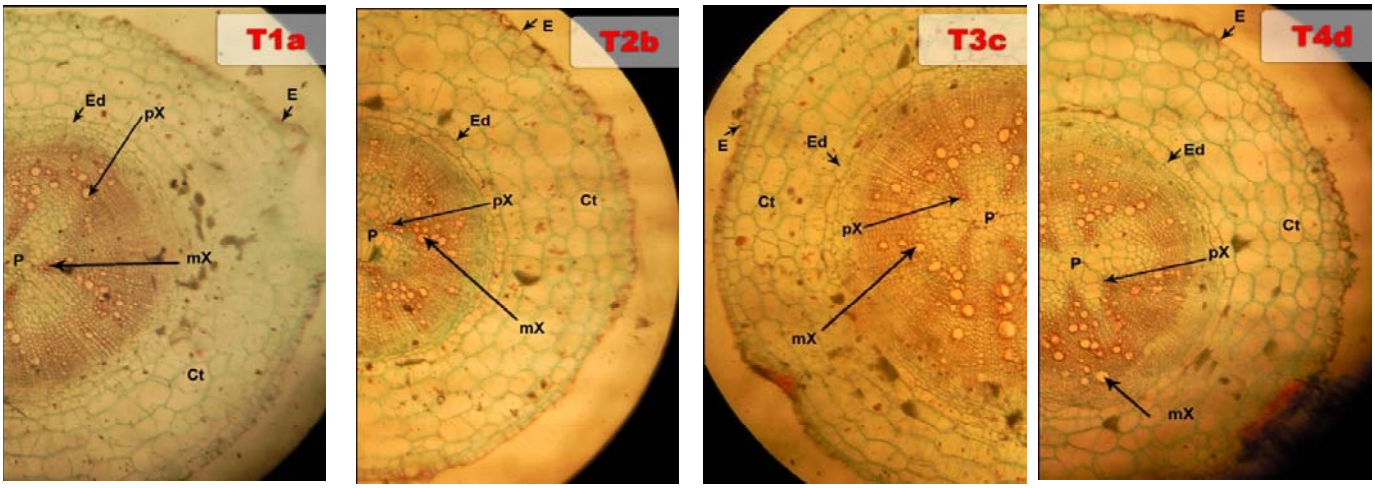

Figure 6. Light Microscopic images showing cross sections of root from 7-day post infection tomato seedlings. Different treatments were: T1a, control water non infected; T2b, *F. oxysporum* infected; T3c, urine camel foliar spraying; T4d, urine camel sprayed and infected. Ct, tomato seedlings cortex; E, epidermis; Ed, endodermis; mX, metaxylem; P, pith; Ph, phloem; pX, protoxylem.

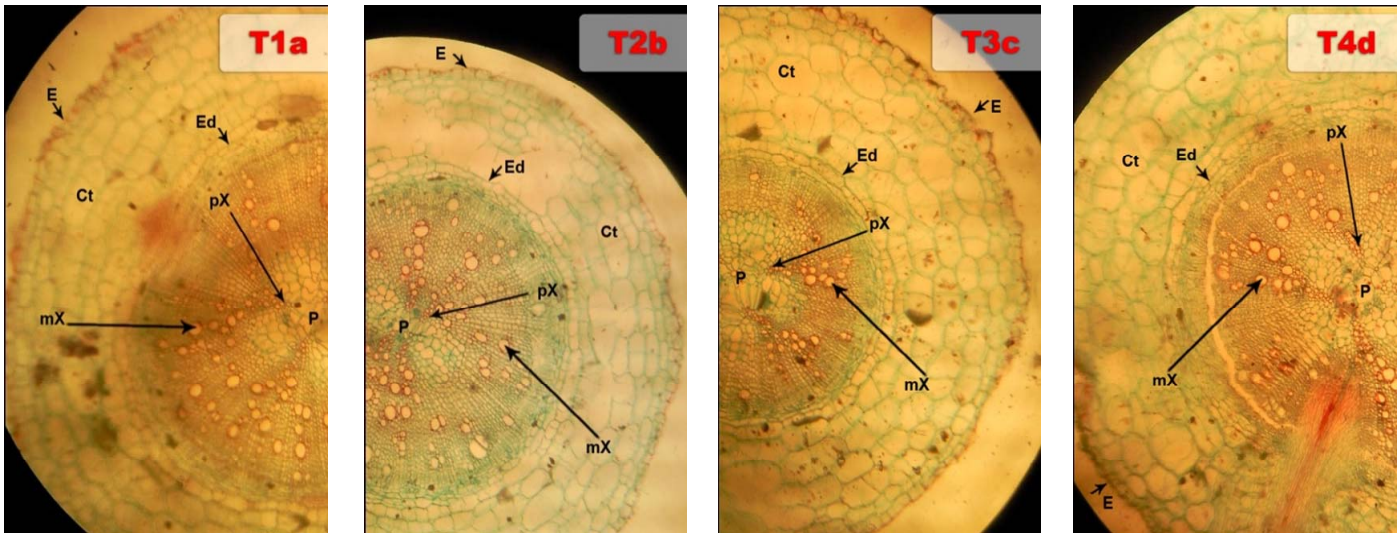

Figure 7. Light Microscopic images showing cross sections of root from 14-days post infection tomato seedlings. Different treatments were: T1a, control water non infected; T2b, *F. oxysporum* infected; T3c, urine camel foliar spraying; T4d, urine camel sprayed and infected. Ct, tomato seedlings cortex; E, epidermis; Ed, endodermis; mX, metaxylem; P, pith; Ph, phloem; pX, protoxylem.

xylem cell width but also in number. Root age follow the same trends (Figure 7).

DISCUSSION

In nature, plants often deal with simultaneous or subsequent abiotic and biotic stresses which can influence the primary induced defense responses (Abuqamar, 2009). Activation of plant defense mechanisms is associated with ecological fitness costs.

Hence, plants need regulatory mechanisms to effectively and efficiently adapt to changes in their complex environment. Share between different signaling pathways provides the plant with such a powerful regulatory potential (Abuqamar, 2009).

Presented results show that tomato plants responded to exogenous, foliar applications of the CU solution. The amazing solution was found to sensitize susceptible tomato plants to react more rapidly and more efficiently to *F. oxysporum* f. sp. *Radicis lycopersici* attack.

Peroxidase has often been used as an enzymatic marker in studies of defense-related processes (Karthikeyan et al., 2006). The enhanced peroxidase activity shown by the *pschit*, transgenic tobacco lines, was linked to their increased pathogen resistance (Dana et al., 2006). Tomato tissues responded to CU application and this point to CU recognition. Tomato leaves tissues point to fast and consistent POD increments thereafter CU systemic transporting, preferentially enhanced the roots peroxidase. In seedlings roots, peroxidase activity increments inT4 implicate wall-bound phenolics accumulation in lignifications with pathogen restriction in systemic parts as a result of pre CU foliar application (Karthikeyan et al., 2006). Root CAT depletion (in the present work) may be explained by salicylic acid binding affinity to iron-containing enzymes (Klessig et al., 2000) resulting in H_2O_2 accumulation to directly award off pathogen spreading.

PAL is a key enzyme in the biosynthesis of phenyl propane unit. Tomato plant pretreated by camel urine as compared to water spray before challenging both implicated total phenolics and PAL activity increment in roots within the plant strategy to lignin deposition which had been confirmed by the histological part resulted in physical protection enhancement. Increased PAL activity level in response to pathogen or elicitor like raw cow's milk spray has been reported (Arun et al., 2010).

Karthikeyan et al. (2006) stated that infected chickpea and palm seedlings treated with biocontrol agents resulted in increased accumulation of phenolics and PAL. Tomato T2 roots suggested that immediate transient activation in PAL after infection could re-establish the notion that plant recognize its enemy and respond to invasion by PAL expression.

PPOs are a group of copper containing enzymes that catalyze oxidation of hydroxy phenols to their quinone derivatives, which have antimicrobial activity (Thipyapong and Stiffens, 1997). Recently, study on elicitation of resistance and defense related enzymes by amino acids and raw cow's milk (RCM) in Pearl millet against downy mildew disease (Arun et al., 2010) recorded increased PPO in both RCM and amino acids treated plants.

The prominent increase in PPO roots of T4 treatments exhibited the highest enzyme levels, involved camel urine spray in conditioning root defense responses against

pathogen toxic metabolites which may activate phenol-oxidizing enzymes where *F. oxysporum* was found to produce such a toxic metabolite.

Emerging evidence suggests that hormones signaling pathways, as well as ROS, play key roles in the crosstalk between biotic and abiotic stress signaling (Abuqamar, 2009). They act synergistically or antagonistically with each others to regulate plant responses to pathogens and abiotic stress factors (Abuqamar, 2009). SA quantity in roots of T4 treated seedlings, showed that SA could be a signaling molecule forming a feedback amplification cycle in concert with ROS (Abuqamar, 2009). In this way, SA induction is not required but the endogenous SA present amplifies the effects of ROS initial levels. This study contributes to defining the role of SA during the camel urine application. Asselbergh et al. (2007) found that in tomato, increase in SA-regulated defense gene expression, faster accumulation of H_2O_2 and the associated cell wall modifications explain the increase in resistance to *Botrytis cinerea*. In the present work, root of the pretreated seedlings with camel urine and pathogen challenged record enhancement conjugated SA concomitant with catalase inhibition and peroxidase increment so, shoot perception to camel urine and the cascade signaling pathways cross-talk *via* specific systematic signals like SA to prime root to other stress by enhanced defense gene expression, fast accumulation of H_2O_2 concomitant with cell wall modifications as physical barrier after lignin deposition. Cvikrová et al. (2006) conducted a study to identify the defense-related alterations in phenylpropanoid pathway, they explain the rise of phenolic contents in the reaction zones by scoring predominately derivatives of cinnamic acid, in particular *p*-coumaric acid, content of which increased by about 2.3- and 3.0-fold.

Many of the pathogen-induced phenylpropanoids like coumarins are considered phytoalexins because they exhibit antimicrobial properties (Cvikrová et al., 2006). Immediate excellent increment after two days post infection in total coumarins in T4 seedlings roots share increments in total antioxidant defenses. Further physiological and molecular research has to be done in order to clearly define the signaling pathways involved in camel urine induced resistance. Current accumulation of proteins in CU treated tomato may provide a storage form of nitrogen that is re-utilized when stress is over, while increasing soluble proteins in infected and pretreated may be due to synthesis structural proteins in particular synthesis of those proteins which are involved in modification of cell wall especially in roots to collaborate defense against pathogen infection.

In the present work, root of seedlings received both foliar application and pathogen challenged showed no tissue injury or disorganization in vascular tissues. Enhanced lignifications on epidermis cell wall and enhanced thickness inside xylem cell wall which were correlated with peroxidase activity and related to camel urine unique constituents especially Ca and Se ions as compared to water treated seedling. Selenium ions as one of CU constituents (Al-Atta, 2008) has been shown to protect plants from both herbivore and pathogen infection (Hanson et al., 2004).

In the light of the above recorded results, the present study may suggest that tomato aerial parts can recognize and respond to CU, evoke cascade signaling pathways, systematically crosstalk with roots using SA and sensitize tomato underground parts to respond faster and to a greater extent to *F. oxysporum* attack. A better understanding of these mechanisms will make it possible to genetically engineer crops disease resistance, leading to greater food production.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The author wish first to acknowledge the Botany Department, Ain Shams University for permitting this work to be done in the Botany Garden, second to thank Faculty of Agriculture, Ain Shams University, for providing *F. oxysporum* cultures, finally, I wish to express my thanks to Dr. Azza Elshafey, Professor of Plant Physiology, Botany Department, Faculty of Science, Ain Shams University, for her scientific support, encouragement and revising the manuscript.

REFERENCES

Abuqamar S, Luo, H, Laluk K, Mickelbart M, Mengiste T (2009). Crosstalk between biotic and abiotic stress responses in tomato is mediated by the *AIM1* transcription factor. Plant J. 58(2):347-60. Epub 2008 December 25. Erratum in: Plant J. 2009 December 60(5):929.

Aebi H (1984). Catalase *in vitro.* Meth. Enzy.105:121-126.

Al-Atta (2008). Determination of essential elements in milk and urine of camel and in *Nigella sativa* seeds. Arabian J. Chem. 1(2):123-129.

AL-awadi A, AL-Jedabi A (2000). Antimicrobial agents in camel's urine. Microbiol. Viruses (9B) 8-11 November, pp. 265-281.

Arun K, Mali P, Manga V (2010). Changes of some phenolic compounds and enzyme activities on infected pearl millet caused by *Sclerospora graminicola*. Int. J. Plant Physiol. Biochem. 2(1):6-10.

Asselbergh B, Curvers K, Franca S, Audenaert K, Vuylsteke M, Van Breusegem F, Hofte M (2007). Resistance to *Botrytis cinerea* in *sitiens*, an abscisic acid-deficient tomato mutant, involve timely production of hydrogen peroxide and cell wall modifications in the epidermis. Plant Physiol. 144:1863-1877.

Balatinecz J, Kennedy R (1967). Maturation of ray parenchyma cells in pine. For. Prod. J. 17:57-64.

Bowler C, Fluhr R (2000). The role of calcium and activated oxygens as signals for controlling cross-tolerance. Trends Plant Sci. 5: 241–246.

Bradford M (1976). A rapid and sensitive method for quantization of microgram quantities of protein utilizing the principle of protein-dye binding .Anal. Biochem. 72:248-254.

Cvikrová M, Malá J, Hrubcová M, Eder J (2006). Soluble and cell wall-bound phenolics and lignin in *Ascocalyx abietina* infected *Norway pruces.* Plant Sci. 170:563-570.

Dana M, Pintor-Tor J, Beatriz Cubero B (2006). Transgenic Tobacco plants over expressing chitinases of fungal origin show enhanced resistance to biotic and abiotic stress agents. Plant Physiol. 142:722-730.

De Micco V, Arena C, Aronne G (2013). Anatomical alterations of *Phaseolus vulgaris* L. mature leaves irradiated with X-rays.

Fawe A, Abou-Zaid J, Menzies G, Bélanger R (1998). Silicon-mediated accumulation of flavonoid phytoalexins in cucumber. Phytopathol. 88:396-401.

Freed R, Einmensmith SP, Guetz S, Reicosky D, Smail VW, Wolberg P (1989). User's guide to MSTAT-C, an analysis of agronomic research experiments. Michigan State University, USA.

Fuchs J, Moenne-Loccoz Y, Defago G (1997). Nonpathogenic *Fusarium oxysporum* strain *Fo47* induces resistance to Fusarium Wilt in tomato. Plant Dis. 81:492-496.

García-Mier L, Guevara-González RG, Mondragón-Olguín VM, del Verduzco-Cuellar BR, Torres-Pacheco I (2013). Agriculture and bioactives: achieving both crop yield and phytochemicals. Int. J. Mol. Sci.14:4203-4222.

Hammerschmidt R, Kuc J (1982). Lignification as a mechanism for induced systemic resistance in cucumber. Physiol.Pl. Pathol. 20:61-71.

Hanson B, Lindblom S, Loeffler M, Pilon-Smits E (2004). Selenium protects plants from phloem-feeding aphids due to both deterrence and toxicity. New Phytol. 162:655-662.

Karthikeyan M, Radhika K, Mathiyazhagan S, Bhaskaran R, Samiyappan R, Velazhahan R (2006). Induction of phenolics and defense-related enzymes in coconut (*Cocos nucifera* L.) roots treated with biocontrol agents. Braz. J. Plant Physiol. 18 (3).

Klessig FD, Durner J, Nod R, Navarre AD, Wendehenne D, Kumar D, Ma Zhou J, Shah J, Zhang S, Kachroo P, Trifa Y, Pontier D, Lam E, Silva H (2000). Nitric oxide and salicylic acid signaling in plant defense. Proceedings of the National Academy of Sciences of the United States of America. 97(16):8849-8855.

Lee R, Kim, Y, Jung J, Avice C, Ourry A, Kim H (2007). Peroxidases and lignification in relation to the intensity of water-deficit stress in white clover (*Trifolium repens* L.). J. Exp. Bot. 58(6):1271-1279.

Lister C, Lancaster J, Walker J (1996). Phenylalanine Ammonia-Lyase activity and its relationship to anthocyanin and flavonoid levels in New Zealand-grown apple cultivars. Am. Soc. Hortic. Sci.121:281-285.

Mayer AM, Harel E, Shaul RB (1965). Assay of catechol oxidase a critical comparison of methods. Phytochem. 5:783-789.

McCallum J, Walker J (1990). Phenolic biosynthesis during grain development in wheat: changes in phenylalanine ammonia-lyase activity and soluble phenolic content. J. Cereal Sci. 11:35-49.

Mittler R, Vanderauwera S, Gollery M, van Breusegem F (2004). Reactive oxygen gene network of plants Trends. Plant Sci.9:490-498.

Singleton V, Rossi J (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16:144-158.

Spoel SH, Dong X (2012). How do plants achieve immunity? Defense without specialized immune cells. Nat. Rev. Immunol.12:89–100.

Thakur A (2004).Therapeutic use of urine in early Indian medicine, Indian J. History Sci. *39*(4):415-427.

Thipyapong P, Stiffens J (1997). Tomato Polyphenol oxidase differential response of the PPO F Promoter to injuries and wound signals. Plant Physiol. 115:409-418.

Thypyapong P, Hunt M, Steffens J (1995). Systemic wound induction of potato (*Solanum tuberosum*) polyphenol oxidase. Phytochem 40:673-676.