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Research Paper

Antioxidant enzymes as well as oxidant activities involved in defense mechanisms against the root-knot, reniform and citrus nematodes in their host plants

Afify A. E. M. M. R.[1]* • Farahat A. A.[2] • Al-Sayed A. A.[2] • Mahfoud N. A. M.[2]

[1]Deparment of Biochemistry, Faculty of Agriculture, Cairo University, Egypt.
[2]Department of Zoology and Agricultural Nematology, Faculty of Agriculture, Cairo University, Egypt.

*Corresponding author: E-mail: abdelmoneimafify@yahoo.com. Tel: 01004352965.

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Abstract. The changes in oxidants and antioxidants such as enzyme activities (involved in defense mechanisms in plants against pathogens)in cowpea, eggplant, jasmine, papaya and sour orange in response to infection with *Meloidogyne incognita*, *Rotylenchulus reniformis* and *Tylenchulus semipenetrans* were held. Different hosts react the same way to nematode infection, whereas oxidants, lipid peroxidase (MDA) and hydrogen peroxide (H_2O_2) were increased after nematode infection. Also antioxidant substances, Glutathione (GSH) and ascorbic acid (AA) as well as, superoxide dismutase (SOD), Catalase (CAT) and ascorbate peroxidase (APX) were increased. The rates of increase differed according to nematode species, host plant and nematode initial population.

Keywords: Oxidants, antioxidant enzymes, *Meloidogyne incognita, Rotylenculus reniformis*, *Tylenculus semipenetrans*.

INTRODUCTION

The root-knot nematodes *Meloidogyne* spp. are the most important nematode pests worldwide due to their great damage resulted on the very wide host range which include more than 2000 plant species (Gugino et al., 2008).The reniform nematode *Rotylenchulus reniformis* (Linford et al., 1940) come after the root-knot nematode especially in the warmer parts of the world causing great losses in different field crops, vegetables, ornamentals and some orchards of fruit trees. The citrus nematode, *Tylenchulus semipenetrans*, the nematode pest is responsible for the slow decline in citrus orchards and some other fruit trees like grape and olive all over the world. All the three nematode genera depend, upon their development on their hosts, on the formation of feeding sites (Giant cells, syncytia, hypertrophied cells and nurse cells). The success of nematode reproduction on their compatible hosts depends on the successful formation of such feeding sites which rely on the availability of certain concentrations of some chemicals and enzymes (Baldacci-Cresp et al., 2012) to be available in host tissues.

In plants attacked by nematodes, selective changes occur in the metabolism either as consequence of the establishment of a susceptible host-pathogen interaction or as a result of resistance between host and parasite. Several models for resistance/susceptibility have been developed based on biochemical changes (Kaplan et al., 1980; Zacheo, 1987). There are many reports of enhanced peroxidases, polyphenoloxidase, and ascorbate peroxidase following the interaction of nematodes with their hosts especially the resistant ones and this has led to the hypothesis that these enzymes may be important in the defense mechanism of host (Siahpoush et al., 2011; Aryal et al., 2011; El-Beltagi et al., 2012).

Kaplan et al. (1980) stated that, generally, incompatibility to nematodes is expressed after infection and active mechanisms involve compounds produced postinfectionally rather than performed constitutive plant products. Accordingly, plants develop defense mechanisms right away after nematode invasion. Most of these defense mechanisms are incompatible resistant interactions between plants and pathogens of which the formation of reactive oxygen species (ROS) are common (Bakker et al., 2006). Such reactive oxygen species induced lipid peroxidation accounting for cell death after pathogen invasion. H_2O_2 acts as signaling molecule that triggers gene activation, or as cofactor in a process that requires new gene expression for both localized cell death and induction of defense genes in adjacent cells as well as its direct effect on nematode development (Siahpoush et al., 2011; Karajeh, 2008). Infected plants exhibit both enzymatic and non enzymatic antioxidant defense systems to frustrate ROS upon nematode infection. The accumulation of such materials in root tissues enhanced resistance in plants against invasion with new nematode larvae (El-Beltagi et al., 2012), of these antioxidants, GSH, SOD, Catalase and ascorbate peroxidase. Biochemical changes in antioxidants enzymes were recorded in *Tetranychus urticae* Koch as a result of treatments with natural extracts (Afify et al., 2011).

The present study was carried out to study enzymatic and non enzymatic antioxidants involved in defense mechanisms of cowpea, eggplant, jasmine, papaya and sour orange against the root-knot, the reniform and the citrus nematodes.

MATERIALS AND METHODS

Stock cultures

Pure cultures of the root-knot nematode, *Meloidogyne incognita* the reniform nematode, *Rotylenchulus reniformis* (Linford, 1940) were obtained from the stock cultures belong to Nematology Research Center, Zoology and Agriculture Nematology Department, Faculty of Agriculture, Cairo University. These cultures are maintained on eggplant and pigeon pea, respectively. Pure culture of the citrus nematode, *Tylenchulus semipenetrans* (Cobb, 1913) was obtained from a citrus orchard at the Farm of the Fac. of Agric. Cairo Univ., Giza, Egypt.

Test plants

Cowpea (*Vigna sinensis*) cv. Kareem 7, eggplant (*Solanum melongena*) hyb. Oneta F1, Papaya (*Carica papaya*) cv. Solo, Jasmine (*Jasminum grandiflora*) cv. Balady and sour orange (*Citrus aurantium*) were used in the present study.

One single seedling of each host crop grown in 15 cm diameter clay pots filled with sandy loam soil (1:1 v/v) was inoculated with 2000 or 4000 infective stage of one of the tested nematode species. Treatments were replicated 8 times as well as another untreated 8 to serve as a check. Pots of all treatments were arranged on a clean bench in a greenhouse at 30 ± 5°C for 6 weeks and horticultural treatments carried out the same.

Nematode extraction

At the end of the experimental time, 4 pots of each treatment were soaked lonely in a plastic bucket half full of tab water until the root system could be separated. Each root system was stored in 5% formaldehyde in plastic jars. Soil suspension was quite stirred and nematodes were extracted using sieves and Baermann technique. Soil population was calculated using Hawkesly counting slide. Root population was determined under stereo binocular after staining the root with acid fuchsine (Goody, 1957). Fresh and dry samples of the inoculated and non inoculated plants of the other 4 replicates were sent to chemistry Laboratory, Department of Biochemistry, Fac. of Agric. Cairo Univ. for determination of oxidant and antioxidant substances and enzymes.

Chemical analysis

Determination of oxidative burst

Lipid peroxidation (MDA contents): Thiobarbituric acid reaction (TBA) was measured and calculated from the absorbance according to (Hodges et al., 1999).

Assay of hydrogen peroxide concentration: Hydrogen peroxide was measured by the method described by Capaldi and Taylor (1983). The ground samples in 5% TCA (2.5 mlper 0.5 g fresh shoots or roots) with 50 mg active charcool at 0°C, and centrifugated for 10 min. at 15,000 x g. Supernatant was collected neutralized with 4 NKOH to pH 3.6 and used for H_2O_2assay.The reaction mixture contained 200 µl of leaf extract, 100 µl of 3.4 mM 3-methylbenzothiazolinehydrazone (MBTH). The reaction was inhibited by adding 500 µl of horse radish peroxidase solution (90 µ per 100 ml) in 0:2 M sodium acetate (pH 3.6). Two minutes later 1400 µl of 1N HCl was added. Absorbance was read at 630 nm after 15 min.

Determination of antioxidant substances

Determination of total glutathione: The level of total acid-soluble SH compound (Glutathione GSH) was determined with Ellman's reagent according to De Vos et al. (1992).

Ascorbic acid determination: Levels of AA were determined following the procedure described by Singh et al. (2006). Fresh shoots or roots sample of a known weight (1 g) was extracted with 3 ml of 5% (w/v) trichloroacetic acid (TCA) and centrifuged at 18,000 x g for 15 min. AsA was determined in a reaction mixture consisting of 0.2 ml of supernatant, 0.5 ml of 150 mM phosphate buffer (pH 7.4, containing 5 mM EDTA) and 0.2 ml of deionized water. Color was developed in reaction mixtures with the addition of 0.4 ml of 10% (w/v) TCA, 0.4 ml of 44% (v/v) phosphoric acid, 0.4 ml of α- α-dipyridyl in 70 % (v/v) ethanol and 0.2 ml of 3% (w/v) $FeCl_3$. The reaction mixtures were incubated at 40°C for 40 min and the absorbance was read at 532 nm.

Determination of antioxidant enzymes

Assay of superoxide dismutase (SOD) activity (SOD; EC.1.15.1.1): The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of NBT using the method of Beauchamp and Fridovich (1971).

Assay of ascorbic peroxidase APX activity (APOX; EC.1.11.1.11): Ascorbate peroxidase activity was estimated according to the method of Nakano and Asada (1981).

Assay of catalase activity (CAT; EC 1.11.1.6): Catalase activity was determined by consumption of H_2O_2 using the method of Dhindsa et al. (1981).

Assay of Glutathione-S-transferase (GST; EC. 2.5.1.18): Glutathione-S-transferase activity was measured according to the method of Mannervik and Guthenberg (1981).

RESULTS

The reproduction pattern and the rate of buildup of the root-knot, *M. incognita*; the reniform, *R. reniformis* and the citrus nematode, *T. semipenetrans* on eggplant, papaya, cowpea, jasmine and sour orange as initially inoculated with 2000 or 4000 infective stage/plant are illustrated in Table 1. Data showed significant variations in the number of galls, egg-masses and final populations between the two initial inoculum levels of nematodes. The rate of nematode build up decreased in all cases by increasing the initial inoculums from 2000 to 4000 infective stage/pot. The highest rate of build up and the significant highest final populations of *M. incognita* were achieved on eggplant and papaya, while those of *R. reniformis* were achieved on jasmine and cowpea. The citrus nematode reproduced normally on sour orange.

Concerning the changes in plant enzymes involved in defense mechanisms against plant pathogens, data in Table 2 indicated that lipid peroxidation (MDA) and hydrogen peroxide (H_2O_2) in shoots and roots of healthy cowpea, eggplant, jasmine, papaya and sour orange were at the lowest levels and varied significantly in the majority of cases with those infected with *M. incognita*, *R. reniformis* or *T. semipenetrans*. Nematode infection increased levels of MDA and H_2O_2 in all plants. In all cases, higher inoculum levels (4000/ plant) resulted in significant higher increases in MDA and H_2O_2. The highest significant increase was observed in shoots and roots of eggplant infected with 4000 J_2 of *M. incognita,* followed by those in shoots and roots of jasmine infected with *R. reniformis* then in shoots and roots of papaya infected with *M. incognita* at the same inoculum level. Sour orange behaved the same in response to infection with *T. semipenetrans*. MDA and H_2O_2 increased significantly especially at the high inoculum level.

Non enzymatic antioxidants, glutathione (GSH) and ascorbic acid significantly increased in response to nematode infection. The highest significant increase was observed in shoots and roots of eggplant infected with 4000 infective stages of *R. reniformis* followed by those in shoots and roots of cowpea infected with 4000 J_2 of *M. incognita* then those in papaya infected with 4000 infective stage of *R. reniformis* (Table 3). Similarly, the citrus nematode resulted in increasing GSH and ascorbic acid significantly as compared to healthy plants.

With the activity of antioxidant enzymes, superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT), data in Table 4 indicated that the activities of such enzymes in healthy plants were at low levels without significant differences between different hosts. Significant increase was observed as a result of nematode infection, *R. reniformis* on eggplant at 4000 infective stage achieved the highest activity of the three enzymes which varied significantly with other nematodes on other hosts. *Meloidogyne incognita* at the same inoculum level on cowpea came after, followed by *R. reniformis* on papaya, *M. incognita* on jasmine then *T. semipenetrans* on sour orange (Tables 4 and 5).

Glutathione-S-transferase (GST) activity in shoots and roots of different hosts was likely increased significantly by nematode infection. *M. incognita* at the high inoculum level recorded the highest significant activity in shoots and roots of eggplant, followed by *R. reniformis* in shoots and roots of jasmine and then shoots and roots of cowpea. GST activity was also increased significantly in shoots and roots of sour orange as a result of infection with *T. semipenetrans* (Table 5).

Infection of eggplant and jasmine with concomitant population of R+M resulted in higher rates of oxidants and antioxidants comparing to infection of eggplant with single population of *R. reniformis* or jasmine with single population of *M. incognita* at the same inoculum level.

Comparing the percentage increase in oxidants, antioxidant substances and enzymes in perennial hosts in response to infection with 4000 infective stage of the three nematode species, data in Figure 1 illustrate that the highest percentages of increase in lipid peroxidation (MDA) were resulted in jasmine shoots and roots in response *R. reniformis* followed by those accomplished

Table 1. Infectivity and build-up of the root-knot, the reniform nematode and the citrus nematode on different host plants.

Host	Inoculum level	Counts of *Meloidogyne incognita*		
		Galls	**Final population**	**Pf/Pi**
Eggplant	2000	1146 ± 4.32[b]	7284 ± 14.17[c]	3.6
	4000	1244 ± 5.22[a]	1048 ± 8.54[a]	2.6
Papaya	2000	896 ± 3.63[d]	5648 ± 7.54[d]	2.8
	4000	1022 ± 3.67[c]	7793 ± 9.00[b]	1.9
Cowpea	2000	311 ± 7.95[f]	4250 ± 16.82[f]	2.1
	4000	451 ± 5.24[e]	5500 ± 19.07[e]	1.4
Jasmine	2000	469 ± 2.63[e]	2926 ± 6.92[h]	1.5
	4000	522 ± 7.01[e]	3763 ± 12.49[g]	0.9
		Counts of *Rotylenchulus reniformis*		
		Egg-masses	**Final population**	**Pf/Pi**
Eggplant	2000	207 ± 5.29[h]	4380 ± 13.22[h]	2.2
	4000	243 ± 3.60[g]	6398 ± 9.16[f]	1.6
Papaya	2000	274 ± 3.60[f]	6321 ± 13.22[g]	3.2
	4000	402 ± 3.60[e]	8164 ± 11.7[e]	2.0
Cowpea	2000	1013 ± 3.60[d]	9843 ± 13.45[d]	4.9
	4000	1689 ± 3.60[b]	13066 ± 15.13[c]	3.3
Jasmine	2000	1547 ± 4.58[c]	16044 ± 13.74[b]	8.0
	4000	1969 ± 3.60[a]	20659 ± 17.05[a]	5.1
		Counts of *Tylenchulus semipenetrans*		
		Egg-masses	**Final population**	**Pf/Pi**
Sour orange	2000	255 ± 5.29	3205 ± 15.13	1.6
	4000	292 ± 3.60**	3594 ± 10.00**	0.9

*Values followed by the same letter are not significantly different ($p \leq 0.5$) according to Duncan's Multiple Range Test. P_f = Final population. P_i = Initial population.

by *M. incognita* in papaya shoots and roots; however, the lowest rates of increase in such oxidants were recorded in sour orange shoots and roots infected with *T. semipenetrans*. Regarding the oxidant substances Glutathione (GSH) and ascorbic acid (AA)and enzymes SOD, APX and CAT, the highest percentages of increase were observed in sour orange shoots and roots, followed by papaya and then jasmine in response to infection with *T. semipenetrans, R. reniformis* and *M. incognita*, respectively. In case of antioxidant enzymes, percentage increase in GST was the highest comparing to other antioxidant enzymes with superiority of *R. reniformis*, then *M. incognita* and *T. semipenetrans*.

It could be generally concluded that host plants respond to nematode infection, to great extent, by the same way. The oxidant and antioxidant enzymatic and non enzymatic substances are involved in the defense mechanisms exerted by plants against different nematode pests. Yet, the amounts produced differed from one host to another, nematode species to another, host compatibility as well as the size of nematode population.

DISCUSSION

It is generally known that incompatibility to nematodes expressed after infection and active mechanisms involved compounds produced postinfectionally rather than performed constitutive plant products (Zacheo et al., 1987). From this point of view, plants develop defense mechanisms right away after nematode invasion. Most of

Table 2. Comparison between the contents of shoots and roots of different host plants of oxidants, lipid peroxidation (MDA) and hydrogen peroxide (H_2O_2) in response to infection with different nematode species and inoculum levels.

Host	Nema.	Inoc. level	MDA (µmol/g fw)				H_2O_2 (µmol/g fw)			
			Shoot	% inc.	Root	% inc.	Shoot	% inc.	Root	% inc.
Cowpea	Control		2.50 ± 0.36^{or}		3.08 ± 0.07^{no}		4.06 ± 0.08^{w}		5.86 ± 0.07^{m}	
	M. incog.	2000	2.56 ± 0.14^{or}	2.4	3.32 ± 0.13^{mn}	7.8	5.75 ± 0.10^{r}	41.6	7.48 ± 0.13^{lm}	26.6
	M. incog.	4000	6.05 ± 0.03^{i}	142.0	7.55 ± 0.27^{f}	145.1	13.55 ± 0.17^{i}	233.7	19.41 ± 0.14^{f}	231.2
	R. renifo.	2000	3.29 ± 0.07^{m}	31.6	3.95 ± 0.02^{jk}	28.3	8.50 ± 0.17^{o}	109.4	9.63 ± 0.17^{jk}	64.3
	R. renifo.	4000	7.92 ± 0.08^{d}	216.8	8.50 ± 0.36^{d}	176.0	17.99 ± 0.08^{f}	343.1	24.40 ± 0.12^{d}	316.4
Eggplant	Control		2.44 ± 0.04^{r}		2.92 ± 0.05^{o}		4.54 ± 0.10^{u}		6.09 ± 0.03^{m}	
	M. incog.	2000	6.41 ± 0.14^{h}	162.7	7.01 ± 0.08^{g}	140.1	16.54 ± 0.18^{g}	264.3	18.24 ± 0.10^{f}	199.5
	M. incog.	4000	12.65 ± 0.03^{a}	418.4	13.98 ± 0.06^{a}	378.8	38.89 ± 0.06^{a}	756.6	45.89 ±0.07^{a}	653.5
	R. renifo.	2000	2.83 ± 0.09^{op}	16.0	2.96 ± 0.04^{o}	1.4	4.69 ± 0.08^{u}	3.3	6.11 ± 0.10^{m}	0.3
	R. renifo.	4000	5.59 ± 0.02^{j}	129.1	6.04 ± 0.08^{h}	106.9	9.80 ± 0.07^{m}	115.9	10.55 ± 0.07^{ij}	73.2
	R+M	2000	2.87 ± 0.07^{op}	17.6	3.03 ± 0.08^{o}	3.8	7.68 ± 0.08^{p}	69.2	9.94 ± 0.02^{jk}	63.2
	R+M	4000	6.99 ± 0.03^{f}	186.5	8.06 ± 0.06^{e}	176.0	13.56 ± 0.07^{i}	198.7	16.40 ± 5.26^{j}	169.3
Jasmine	Control		2.66 ± 0.05^{po}		3.23 ± 0.04^{mn}		4.86 ± 0.06^{t}		6.13 ± 0.10^{m}	
	M. incog.	2000	2.98 ± 0.02^{no}	12.0	3.88 ± 0.08^{k}	20.1	8.46 ± 0.09^{o}	74.1	9.63 ± 0.10^{jk}	51.1
	M. incog.	4000	6.63 ± 0.08^{g}	149.3	7.82 ± 0.08^{ef}	142.1	18.40 ± 0.09^{e}	278.6	21.60 ± 0.12^{e}	252.9
	R. renifo.	2000	4.89 ± 0.07^{k}	83.8	5.59 ± 0.06^{i}	73.1	11.08 ± 0.04^{j}	128.0	13.60 ± 0.07^{h}	121.9
	R. renifo.	4000	11.00 ± 0.26^{b}	313.5	12.05 ± 0.35^{b}	273.3	30.11 ± 0.01^{b}	519.6	35.56 ± 0.07^{b}	480.1
	R+M	2000	2.98 ± 0.03^{no}	12.0	3.96 ± 0.06^{jk}	22.6	9.61 ± 0.11^{n}	97.7	9.94 ± 0.03^{jk}	62.2
	R+M	4000	7.22 ± 0.07^{e}	171.4	8.53 ± 0.25^{d}	164.1	19.83 ± 0.13^{d}	308.0	23.10 ± 0.48^{d}	276.8
Papaya	Control		2.80 ± 0.07^{op}		3.03 ±0.08^{o}		4.36 ± 0.10^{v}		5.96 ± 0.12^{m}	
	M. incog.	2000	3.95 ± 0.26^{l}	41.1	4.19 ± 0.23^{j}	38.3	10.91 ± 0.10^{k}	150.2	12.11 ± 0.20^{hi}	103.2
	M. incog.	4000	8.13 ± 0.05^{c}	190.4	9.82 ± 0.02^{c}	224.1	23.20 ± 0.06^{c}	432.1	29.13±0.01^{c}	388.8
	R. renifo.	2000	3.09 ± 0.03^{mn}	10.4	3.62 ±0.16^{l}	19.5	4.96 ± 0.11st	13.8	6.33 ± 0.10^{m}	6.2
	R. renifo.	4000	6.69 ± 0.05^{g}	138.9	7.56 ±0.13^{f}	149.5	10.13 ± 0.02^{l}	132.3	13.46 ± 0.08^{h}	125.8
Citrus	Control		3.01 ^{no}±0.17		3.90 ^{k}±0.06		5.11 ^{s}±0.09		6.36 ± 0.09^{m}	
	T. semi.	2000	3.11 ^{mn}±0.16	3.32	3.56 ^{lm}**±0.11**	0.0	7.12 ^{q}±0.11	39.3	8.28 ±0.07^{kl}	30.2
	T. semi.	4000	6.90 ^{f}±0.07	129.2	7.59 ^{f}±0.15	94.6	15.56 ^{h}±0.08	204.4	21.60 ±0.11^{e}	239.6

*Values followed by the same letter are not significantly different ($p \le 0.5$) according to Duncan's Multiple Range Test. Inc. = Increase; Inoc. = Inoculum.

Table 3. Comparison between the contents of shoots and roots of different host plants of antioxidant substances, glutathione (GSH) and ascorbic acid (AA) in response to infection with different nematode species and inoculum levels.

Host	Nematode	Inoc. level	GSH (µmol/g fw)				Ascorbic acid (mg /g fw)			
			Shoot	% inc.	Root	% inc.	Shoot	% Inc.	Root	% Inc.
Cowpea	Control		15.10 ± 0.17^{o}		17.9 ± 0.10^{p}		8.55 ± 0.26^{q}		11.71 ± 0.17^{q}	
	M. incog.	2000	24.9 ± 0.20^{i}	64.9	29.7 ± 0.20^{j}	65.9	21.40 ± 0.36^{g}	150.3	25.30 ± 0.36^{g}	116.1
	M. incog.	4000	45.4 ± 0.62^{b}	200.7	55.6 ± 0.26^{b}	210.6	28.93 ± 0.20^{b}	238.4	35.77 ± 0.26^{b}	205.5
	R. renifo.	2000	21.1 ± 0.55^{j}	39.7	23.2 ± 0.45^{l}	29.6	14.50 ± 0.26^{l}	69.6	17.21 ± 0.26^{i}	47.0
	R. renifo.	4000	30.6 ± 0.26^{g}	102.7	35.96 ± 0.10^{h}	100.9	19.55 ± 0.26^{h}	128.7	25.14 ±0.26^{g}	114.7
Eggplant	Control		14.4 ± 0.36^{p}		16.8 ± 0.26^{q}		8.95 ± 0.26^{q}		10.88 ± 0.26^{r}	
	M. incog.	2000	13.4 ±0.26^{p}	0.00	16.1 ± 0.65^{r}	0.00	8.60 ± 0.17^{q}	0.0	10.20 ± 0.36^{s}	0.0
	M. incog.	4000	16.7 ± 0.20^{m}	16.0	19.8 ± 0.26^{n}	17.9	11.68 ± 0.26^{n}	30.5	13.57 ± 0.20^{o}	24.7
	R. renifo.	2000	31.9 ± 0.26^{f}	121.5	36.5 ± 0.20^{g}	117.3	23.50 ± 0.17^{e}	162.6	28.30 ± 0.36^{f}	160.1
	R. renifo.	4000	56.7 ± 0.26^{a}	293.8	68.8 ± 0.26^{a}	309.5	32.92 ± 0.36^{a}	267.8	37.73 ± 0.20^{a}	246.8
	R+M	2000	19.7 ± 0.20^{k}	36.8	22.3 ± 0.45^{m}	32.7	11.10 ± 0.75^{o}	24.02	16.40 ± 0.36^{m}	50.7
	R+M	4000	38.7 ± 0.26^{d}	168.8	42.9 ± 0.20^{e}	155.4	16.62 ± 0.20^{j}	85.7	20.93 ± 0.26^{j}	92.4
Jasmine	Control		14.1 ± 0.17^{p}		15.9 ± 0.10^{r}		9.23 ± 0.36^{p}		11.51 ± 0.20^{q}	
	M. incog.	2000	19.7 ± 0.20^{k}	39.7	21.9 ± 0.26^{m}	37.7	18.60 ± 0.17^{i}	101.5	21.60 ± 0.20^{i}	87.7
	M. incog.	4000	32.1 ± 0.36^{f}	127.7	43.6 ± 0.20^{d}	174.2	22.93 ± 0.26^{f}	148.4	30.77 ± 0.26^{d}	167.3
	R. renifo.	2000	14.0 ± 0.52^{p}	0.0	16.9 ± 0.26^{d}	6.3	11.30 ± 0.26^{no}	22.4	12.40 ± 0.36^{p}	7.7
	R. renifo.	4000	18.2 ± 0.17^{i}	29.1	22.4 ± 0.45^{q}	40.9	13.90 ± 0.20^{m}	50.6	15.80 ± 0.26^{n}	37.3
	R+M	2000	14.5 ± 0.26^{p}	2.8	18.1 ± 0.45^{m}	13.8	13.60 ± 0.17^{m}	47.4	17.21 ± 0.65^{l}	49.5
	R+M	4000	30.4 ± 0.26^{g}	115.6	36.8 ± 0.26^{p}	131.5	19.53 ± 0.20^{h}	111.6	21.49 ± 0.26^{i}	86.7
Papaya	Control		13.9 ± 0.17^{p}		16.2 ± 0.36^{r}		8.37 ± 0.36^{q}		10.92 ± 0.26^{r}	
	M. incog.	2000	16.1 ± 0.65^{n}	15.8	18.2 ± 0.36^{p}	12.3	11.60 ± 0.17^{n}	38.6	16.70 ± 0.20^{lm}	52.9
	M. incog.	4000	21.1 ± 0.36^{j}	51.8	24.4 ± 0.26^{k}	50.6	19.33 ± 0.36^{h}	130.9	23.24 ± 0.55^{h}	112.8
	R. renifo.	2000	26.3 ± 0.55^{h}	89.2	32.5 ± 0.26^{i}	100.6	21.50 ± 0.17^{g}	156.9	25.20 ± 0.36^{g}	130.8
	R. renifo.	4000	41.4 ± 0.26^{c}	197.8	47.3 ± 0.43^{c}	192.0	27.51 ± 0.26^{c}	228.7	33.27 ± 0.40^{c}	204.7
Citrus	Control	16.22	16.22 ± 0.10^{mn}		18.64 ± 0.11^{o}		9.32 ± 0.13^{p}		11.73 ± 0.09^{q}	
	T. semi.	2000	21.3 ± 0.55^{j}	31.3	24.3 ± 0.55^{k}	30.4	15.20 ± 0.36^{k}	63.09	19.80 ± 0.26^{k}	68.8
	T. semi..	4000	35.9 ± 0.06^{e}	121.3	38.73 ± 0.09^{f}	`54.1	24.28 ± 0.07^{d}	160.5	29.59 ± 0.02^{e}	155.3

*Values followed by the same letter are not significantly different (p ≤ 0.5) according to Duncan's Multiple Range Test. Inc. = Increase; Inoc. = Inoculum.

Table 4. Comparison between the activities of antioxidant enzymes, Super oxide dismutase (SOD) and ascorbate peroxidase (APX) in shoots and roots of different host plants in response to infection with different nematode species and inoculum levels.

Host	Nematode	Inoc. level	SOD (Unit/g fw)				APX (Unit/mg fw)			
			Shoot	% Inc.	Root	% Inc.	Shoot	% Inc.	Root	% Inc.
	Control		89.1 ± 1.05^{lm}		73.3 ± 0.45^{s}		14.9 ± 0.26^{r}		11.3 ± 0.36^{q}	
	M. incog.	2000	136.9 ± 0.26^{fgh}	53.7	119.9 ± 0.26^{j}	63.6	24.6 ± 0.26^{h}	65.1	19.4±0.36^{h}	71.7
Cowpea	*M. incog.*	4000	186.8 ± 0.26^{ab}	109.7	153.4 ± 0.26^{d}	109.3	35.4 ± 0.36^{b}	137.6	31.7 ± 0.20^{b}	180.5
	R. renifo.	2000	112.6 ± 0.26^{ijk}	26.4	98.6 ± 0.26^{n}	34.5	19.8 ± 0.26^{lm}	32.9	14.4 ± 0.26^{m}	27.4
	R. renifo.	4000	159.3 ± 0.36^{de}	78.8	143.1 ± 0.36^{f}	95.2	26.9 ± 0.26^{g}	80.5	22.5 ± 0.20^{f}	99.1
	Control		93.1 ± 0.36^{klm}		84.3 ±0.36^{q}		16.1 ± 0.65^{q}		12.9 ± 0.26^{op}	
	M. incog.	2000	103.8 ± 0.41^{jkl}	11.50	86.3 ± 0.36^{q}	14.8	16.7 ± 0.26^{p}	3.7	13.2 ± 0.60^{nop}	2.3
	M. incog.	4000	111.8±1.99^{ijk}	20.0	100.3 ± 0.26^{m}	19.0	17.9 ± 0.20^{n}	11.2	13.8 ± 0.26^{mn}	7.0
Eggplant	*R. renifo.*	2000	143.2 ± 0.45^{efg}	53.8	129.1 ± 0.65^{i}	53.1	28.3 ± 0.26^{f}	75.8	25.1 ± 0.65^{e}	94.6
	R. renifo.	4000	202.5 ± 0.43^{a}	117.5	196.7 ± 0.35^{a}	133.2	41.0 ± 0.26^{a}	154.7	38.5 ± 0.20^{a}	198.5
	R+M	2000	117.9 ± 0.20^{hij}	26.6	108.6 ± 0.26^{l}	28.8	20.5 ± 0.36^{k}	27.3	17.0 ± 0.75^{j}	31.8
	R+M	4000	174.5 ± 0.20^{bcd}	87.4	139.6 ± 0.45^{h}	65.6	28.5 ± 0.36^{f}	77.0	21.3 ± 0.55^{g}	65.1
	Control		88.3 ± 0.26^{m}		71.5 ± 0.20^{t}		15.7 ± 0.26^{q}		12.6 ± 0.20^{p}	
	M. incog.	2000	129.9 ± 0.26^{ghi}	46.6	110.3 ± 0.41^{k}	54.3	20.0 ± 0.45^{kl}	27.4	16.1 ± 0.55^{k}	27.8
	M. incog.	4000	173.3 ± 0.55^{bcd}	95.6	159.0 ± 2.64^{c}	122.4	29.5 ± 0.26^{e}	87.9	25.7 ± 0.36^{de}	104.0
Jasmine	*R. renifo.*	2000	93.6 ± 0.20^{klm}	5.6	84.3 ± 0.36^{q}	17.9	15.3 ± 0.36^{r}	0.0	11.5 ± 0.36^{q}	0.0
	R. renifo.	4000	116.1 ± 0.65^{ij}	31.0	108.8 ± 0.10^{l}	52.2	19.4 ± 0.26^{m}	23.6	15.5 ± 0.36^{kl}	23.0
	R+M	2000	112.8 ± 0.26^{ijk}	27.3	96.1 ± 0.65^{o}	34.4	17.6 ± 0.26^{no}	12.1	13.8 ± 0.36^{mn}	9.5
	R+M	4000	156.1 ± 0.52^{def}	76.2	141.6 ±0.26^{g}	98.0	22.5 ± 0.26^{i}	43.3	18.3 ± 0.36^{i}	45.2
	Control		90.7 ± 0.20^{lm}		82.1 ± 0.36^{r}		15.4 ± 0.36^{r}		12.8 ± 0.26^{p}	
	M. incog	2000	103.6 ± 0.20^{jkl}	14.2	94.3 ± 0.36^{p}	14.9	17.3 ± 0.36^{o}	12.3	13.1 ± 0.55^{nop}	2.3
Papaya	*M. incog.*	4000	156.5 ± 0.26^{def}	72.6	139.9 ± 0.26^{h}	70.4	21.5 ± 0.20^{j}	39.6	15.2 ± 0.55^{l}	18.8
	R. renifo.	2000	132.6±0.26^{ghi}	46.2	119.7 ± 0.20^{j}	45.8	22.7 ±0.20^{i}	47.4	18.9 ± 0.26^{ghi}	47.7
	R. renifo.	4000	179.3 ^{bc}±0.26	97.7	163.1 ± 0.36^{b}	98.7	33.8 ±0.36^{c}	119.5	27.4 ± 0.26^{c}	114.1
	Control		92.9 ± 0.17^{klm}		70.5 ± 0.26^{t}		15.4 ± 0.36^{r}		13.6 ± 0.26^{no}	
Citrus	*T. semi..*	2000	122.3±0.26^{hij}	31.6	109.2 ± 0.55^{l}	54.9	22.4 ± 0.36^{i}	45.5	16.8 ± 0.17^{j}	23.5
	T. semi.	4000	165.1 ± 0.75^{cd}	77.7	150.7 ± 0.17^{e}	113.8	30.1 ± 0.55^{d}	95.5	26.3 ± 0.26^{e}	93.4

Values followed by the same letter are not significantly different ($p \leq 0.5$) according to Duncan's Multiple Range Test. Inc. = Increase. Inoc. = Inoculum.

Table 5. Comparison between the activities of antioxidant enzymes, catalase (CAT) and glutathione-S-transferase (GST) in shoots and roots of different host plants in response to infection with different nematode species and inoculum levels.

Host	Nematode	Inoc. level	CAT (unit/g fw)				GST (unit/g fw)			
			Shoot	% Inc.	Root	% Inc.	Shoot	% Inc.	Root	% Inc.
Cowpea	Control		34.4 ± 0.45^{w}		24.6 ± 0.26^{v}		5.56 ± 0.08^{v}		6.11 ± 0.14^{y}	
	M. incog.	2000	61.2 ± 0.40^{j}	77.9	48.0 ± 0.26^{l}	93.1	14.40 ± 0.11^{s}	159.0	17.13 ± 0.09^{t}	180.5
	M. incog.	4000	103.4 ± 0.36^{b}	200.6	94.1 ± 0.65^{b}	282.5	29.84 ± 0.09^{h}	436.7	36.86 ± 0.07^{h}	503.3
	R. renifo.	2000	47.1 ± 0.36^{o}	36.9	38.9 ± 0.36^{o}	58.1	18.33 ± 0.10^{o}	229.7	20.68 ± 0.07^{o}	238.5
	R. renifo.	4000	78.5 ± 0.20^{f}	128.2	66.4 ± 0.45^{f}	169.9	37.76 ± 0.10^{c}	579.1	40.58 ± 0.12^{d}	564.2
Eggplant	Control		39.8 ± 0.26^{t}		29.3 ± 0.26^{t}		5.16 ± 0.08^{w}		6.36 ± 0.07^{x}	
	M. incog.	2000	45.9 ± 0.26^{p}	15.3	33.4 ± 0.26^{r}	14.0	24.68 ± 0.08^{k}	378.3	27.38 ± 0.09^{l}	330.5
	M. incog.	4000	48.9 ± 0.20^{n}	22.6	38.8 ± 0.26^{o}	32.4	51.31 ± 0.10^{a}	894.4	56.67 ± 0.08^{a}	791.0
	R. renifo.	2000	68.2 ± 0.36^{i}	71.6	54.8 ± 0.26^{j}	87.0	12.66 ± 0.10^{t}	145.4	15.84 ± 0.07^{u}	149.1
	R. renifo.	4000	117.8 ± 0.26^{a}	196.0	98.8 ± 026^{a}	237.2	25.39 ± 0.06^{j}	392.1	31.58 ± 0.07^{k}	396.5
	R+M	2000	44.6 ± 0.26^{q}	12.1	36.4 ± 0.36^{pq}	24.2	16.25 ± 0.09^{r}	214.9	19.13 ± 014^{s}	200.8
	R+M	4000	78.6 ± 0.26^{f}	97.5	64.7 ± 0.20^{g}	120.8	28.40 ± 0.19^{i}	450.4	36.32 ± 012^{i}	471.1
Jasmine	Control		36.9 ± 0.26^{v}		26.1 ± 0.65^{u}		4.58 ± 0.14^{x}		6.39 ± 0.08^{x}	
	M. incog.	2000	53.9 ± 0.26^{l}	46.1	44.4 ± 0.36^{m}	70.1	17.31 ± 0.07^{p}	278.0	19.40 ± 0.19^{r}	203.6
	M. incog.	4000	90.3 ± 0.36^{d}	144.7	78.5 ± 0.20^{d}	200.8	31.51 ± 0.16^{e}	588.0	38.65 ± 0.08^{f}	504.9
	R. renifo.	2000	43.6 ± 0.26^{r}	18.2	30.8 ± 0.26^{s}	18.0	22.27 ± 0.07^{l}	386.2	24.58 ± 0.09^{m}	284.7
	R. renifo.	4000	56.09 ± 0.36^{k}	52.0	43.6 ± 0.26^{n}	67.1	43.51 ± 0.23^{b}	850.0	49.57 ± 0.08^{b}	675.7
	R+M	2000	45.0 ± 0.55^{q}	22.0	36.1 ± 0.36^{q}	38.3	18.76 ± 0.08^{n}	309.6	20.35 ± 0.09^{p}	218.5
	R+M	4000	70.5 ± 0.26^{h}	91.1	61.1 ± 0.36^{i}	134.1	33.37 ± 0.07^{d}	628.6	39.48 ± 0.14^{e}	517.9
Papaya	Control		42.1 ± 0.26^{s}		30.7 ± 0.65^{s}		4.50 ± 0.22^{x}		6.95 ± 0.03^{w}	
	M. incog.	2000	45.7 ± 0.20^{p}	8.6	36.9 ± 0.20^{p}	20.2	21.31 ± 0.12^{m}	373.6	23.35 ± 0.12^{n}	236.0
	M. incog.	4000	74.1 ± 0.45^{g}	76.0	62.6 ± 0.36^{h}	103.9	43.65 ± 0.16^{b}	870.0	48.37 ± 0.09^{c}	596.0
	R. renifo.	2000	61.0 ± 0.62^{j}	44.9	48.9 ± 0.20^{k}	59.3	16.82 ± 0.13^{q}	273.8	19.11 ± 0.10^{s}	175.0
	R. renifo.	4000	92.8 ± 0.36^{c}	120.4	84.0 ± 0.26	173.9	30.68 ± 0.08^{f}	581.8	36.07 ± 0.18^{j}	419.0
Citrus	Control		38.6 ± 0.26^{u}		29. ± 0.36^{t}		7.60 ± 0.07^{u}		8.36 ± 0.02^{v}	
	T. semi.	2000	50.9 ± 0.26^{m}	31.8	43.6 ±0.17^{n}	47.8	16.26 ± 0.04^{r}	113.9	19.84 ± 0.08^{q}	137.3
	T. semi.	4000	84.5 ± 0.17^{e}	118.9	73.3 ± 0.26^{e}	148.5	30.43 ± 0.05^{g}	300.4	38.25 ± 0.1^{g}	357.5

*Values followed by the same letter are not significantly different ($p \leq 0.5$) according to Duncan's Multiple Range Test. Inc. = Increase; Inoc. = Inoculum.

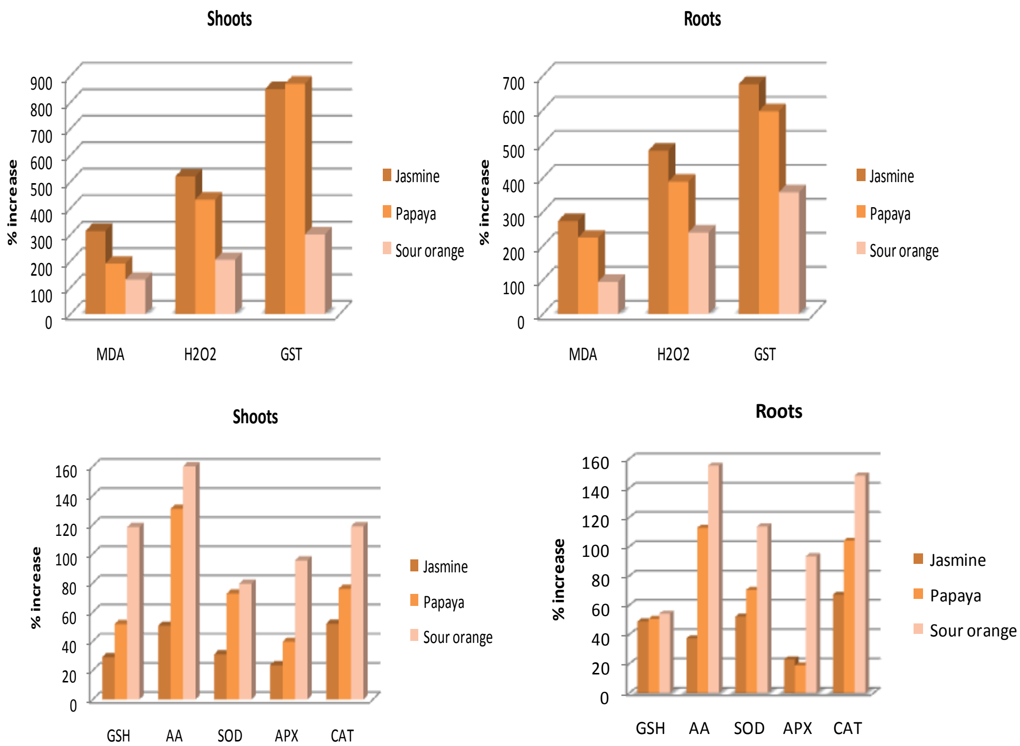

Figure 1. % increase in oxidants (A) and antioxidants (B) in shoots and roots of jasmine, papaya and sour orange in response to infection with *R. reniformis*, *M. incognita* and *T. semipenetrans*, respectively.

these defense mechanisms are incompatible resistant interactions between plants and pathogens of which the formation of reactive oxygen species (ROS) are common (Bakker et al., 2006; Montes et al., 2004). Such reactive oxygen species induced lipid peroxidation accounting for cell death after pathogen invasion. Hence, increasing the rates of MDA and H_2O_2 in different hosts in response to infection with *M. incognita*, *R. reniformis* and *T. semipenetrans* in the present study as compared to healthy plants accounted for the defense mechanism against nematode invasion. The rates of increase in both oxidants depend on nematode species and inoculum levels as well as host plant. Our results agreed with those of Davis et al. (2004) and Huang et al. (2004) who state that the initial reaction of the susceptible cultivars is similar to that of resistant host and may be result from nematode secretions into plant tissues. These results evinced that eggplant and papaya were preferable hosts to *M. incognita* than jasmine and cowpea which were more preferable to *R. reniformis*. It could be generally observed that the increase in such antioxidants depends on nematode species; inoculum level and host plant.

Infected plants exhibited both enzymatic and non-enzymatic antioxidant defense systems to frustrate ROS upon nematode infection. The significant increase of non-enzymatic antioxidants such as glutathione (GSH) and total ascorbic acid may be resulted from the enhancement of MDA and H_2O_2 production after nematode invasion. In the present study, infection with the three nematode species resulted in significant increase in GSH and ascorbic acid in the different host plants. The highest significant increase was observed in shoots and roots of eggplant infected with 4000 infective stages of *R. reniformis* followed by those in shoots and roots of cowpea infected with 4000 J_2 of *M. incognita*. The significant lower levels of both antioxidants in preferable hosts to both nematodes may be attributed to its consumption in scavenging higher levels of peroxidases produced in such hosts. The involvement of GSH in cellular metabolism and its potential agro-biotechnological application has been realized to resist biotic and abiotic stresses and pests. Regulation of GSH was considered to enhance pathogen resistance and maintain antioxidant capacity of these substances in scavenging free radicals (Afify et., 2011; Hari et al., 2011; Afify et al., 2012).

The role of ascorbic acid in plant defense mechanisms against nematodes was illustrated by Arrigoni et al. (1979). They reported that ascorbic acid depletion in plants attenuated resistance in tissues to nematode infections. They hypothesized that plants utilized ascorbic acid for synthesis of mitochondrial hydroxyproline proteins which control the development of cyanide resistant respiration. They also stated that ascorbic acids synthesis was stimulated in resistant plants. Cyanide-resistant respiration (CCR), commonly associated with wounds is requisite to activations of biological defense mechanism. Increase in superoxide dismutase (SOD) and peroxidase activity seems to be result of an adaptive response which provides the plant with protection against biotic and abiotic stress (Guida et al., 1992).The protective activity of SOD, and catalase (CAT) was enhanced in susceptible but decreased in resistant plants (Zacheo et al., 1990). Superoxide dismutase prevents the deleterious effect of O^{-}_2 radicals in root cells and transforms it to H_2O_2 which is then transformed by catalase to harmless O_2 + H_2O. Accordingly, in susceptible tomato roots infested with *M. incognita*, SOD activity considerably increased in comparison to uninfected controls and decreased in resistant cultivars (Zacheo et al., 1987). These findings are in accordance with our results whereas superoxide dismutase (SOD), ascorbate oxidase (APX), Glutathione-S-transferase (GST) and catalase (CAT) increased after nematode infection. The increment rates varied according to host plant, nematode species and inoculum level.

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