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Effect of Infection with Tylenchulus semipenetranson Enzymatic Activities in Citrus

Abd-Elgawad, M.M.M., M.H. Abou-Deif, M.M.A. Hammam, H. Abd-El-Khair, Faika F. H. Koura, A. E. Abd El-Wahab and S. A. Montasser

Abstract*- Six inocula 0, 5000, 10000, 15000, 20000 and 25000 second stage juveniles (J_2) and males/pot of *Tylenchulus semipenetrans* were tested on 1.5-year-old nematode-free seedlings of Citrus volkameriana. The inoculated seedlings were grown in plastic pots containing about 5 Kg of sterilized sandy clay soil (2:1) under greenhouse conditions for a year. The relationship between levels of Tylenchulus semipenetrans infection to sour orange seedling roots and activities of the isozymes superoxide dismutase, peroxidase and catalase were determined by electrophoresis. After three and six months of nematode inoculation, the enzyme activity of superoxide dismutase increased dramatically with increasing the nematode inoculation levels. After 12 months of nematode inoculation, the enzyme activity decreased with increasing the nematode inoculation levels. The enzyme activity of peroxidase after three and six months of nematode inoculation increased to 91.9% at nematode inoculum level of 5000 J_2/pot compared to 90.3% in the non-infested check. Similar results were obtained after 12 months of nematode inoculation. After 3 months of nematode inoculation, the enzyme activity of catalase increased to 88.5% at 5000 J_2/pot but to 85.7% in the control. After 6 months, catalase activity was 85.5, 85.1 and 85.5% with inoculum levels of 5000, 10000 and 25000 J_2/pot, respectively but 83.4% in the control. Similar results were obtained after 12 months of nematode inoculation. New isozymes of the tested enzymes appeared in the electrophoretic patterns when T. Semipenetrans inoculum levels increased.

***Index Terms*-Citrus, Nematode, Tylenchulus semipenetrans, Enzyme activity, Electrophoresis.**

I. INTRODUCTION

In plants, environmental conditions and some pathogens can cause oxidative stress damage by overproduction of toxic oxygen species [1,2] which can damage cellular components such as lipids, proteins, carbohydrates and nucleic acids [3]. Superoxide dismutase (SOD) is the first enzyme in the detoxifying process, converts O_2 radicals to H_2O_2. The enzymes catalase (CAT) and SOD are principle enzymes which scavenge active oxygen species and avoid lipid per-oxidation, cell membranes damage and chlorophyll degradation [4,5]. CAT controls H_2O_2 level in plant cells, regulates the germination rate of seeds [6] and participates to photosynthetic process [7]. It was reported that CAT activity increases in seedlings probably in order to neutralize H_2O_2 and thus avoid cellular damage caused by accumulation of the substrate [8].

Although numerous nematode pests are associated with the citrus rhizosphere, the citrus nematode, *Tylenchulus semipenetrans* Cobb, is the most important plant-parasitic nematode species of citrus in Egypt [9] and worldwide [10].

T. semipenetrans causes the disease 'slow decline' of citrus. The primary effect of this nematode in newly infested sites is a gradual reduction in tree quality so that over a period of years infested trees are smaller, less vigorous and less productive than normal. Symptom development depends on overall orchard conditions [11]. Infested trees growing under otherwise optimum conditions may yield somewhat less fruit while appearing quite healthy. As conditions become less suitable for tree growth, the effects of citrus nematode parasitism are more apparent. In new citrus plantings, symptom development progresses slowly as nematode populations develop to high levels. Symptoms are those associated with poor root development. Leaves are smaller and may become chlorotic. Heavily infected feeder roots are slightly thicker than healthy roots and have a dirty appearance due to soil particles that adhere to nematode-gelatinous egg masses on the root surface. This pest infects some other fruit trees like grape and olive all over the world. 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 [12] to be available in host tissues.

There are many reports of enhanced peroxidases (PX), polyphenol oxidize and ascorbate peroxides 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 the host plant [13,14,15]. Infected plants exhibit both enzymatic and non-enzymatic antioxidant defense systems to frustrate reactive oxygen species upon nematode infection. The accumulation of such materials in root tissues enhanced resistance in plants against invasion with new nematode larvae of these antioxidants; e.g. glutathione, SOD, CAT and ascorbate peroxides [15].Therefore, specific molecular markers of plant resistance to nematodes should be determined for unique pathogen/host systems to rate resistance/susceptibility to the most economically important nematode families, which would save effort, time and money [16]. Such markers may also be represented by enzymes with promise for use as genetically-based biochemical markers for screening breeding lines with potential for nematode resistance. More sensitive, rapid and accurate electrophoretic methods, such as those that are possible with miniaturized and automated equipment, should further facilitate identification of desirable markers.

Greater oxidase and PX activities were detected in the vascular bundles and cortical region of 45 infected roots

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than in un-inoculated check of tomato 'Pusa Ruby' seedlings after 60 days of inoculation with 1000 second stage juveniles (J_2) of *M. incognita* [17]. Axenized*M. incognita*-J_2 wasinoculated into 9-day-old seedlings of soybean variety Clark-63 and assayed PX activity by polyacrylamide gel electrophoresis [18]. The electro-phoretic analyses revealed presence of additional bands of PX isozymes that were separated 7, 14 and 21 days after inoculation. It was suggested that the elevated level of PX activity was due to de novo synthesis of PX isozymes. Also, aubergine var. Pusa Purple Long seedlings were inoculated with 1000 *M. incognita*- J_2 in a pot experiment [19]. Infested roots had higher contents of cell soluble proteins, PX and polyphenol oxidase. *M. incognita* resistant varieties of tobacco and tomato had significantly higher PX enzyme specific activity than susceptible varieties [20]. When seedlings of tomato cv. VFN8 (resistant to *M. incognita*) and cv. Roma VF (susceptible) were inoculated each with 60 *M. incognita* J_2 in pot experiment, the PX activity increased in all fractions but less so for the susceptible cultivar [21]. In resistant plants, the increased PX activity seems to be related to a lignification process around the necrosis and in the susceptible plants, to the production of secondary wall in the syncytium. The flexibility and swiftness of using such enzyme activity in rating plant genotypes for nematode resistance may enhance their potential utility as biochemical markers especially to expedite breeding programs. CAT activity was of lower magnitude and increased in cv. strain "B" but decreased in cvs "Dual Large" and "Castle Rock" as compared to their healthy controls.

The changes in oxidants and antioxidants such as enzyme activities (involved in defense mechanisms in plants against pathogens) in sour orange in response to infection with *T. semipenetrans* were studied [22]. They found that antioxidant substances, glutathione and ascorbic acid as well as, SOD, CAT and ascorbate peroxidase were increased; yet, the rates of increase differed according to nematode initial population.

The objectives of the present study were to estimate the activities of three systems of enzymes SOD, PX and CAT in sour citrus roots at different days after inoculation with different numbers of *T. semipenetrans*-J_2 and males to demonstrate the effect of infection on enzyme activities.

II. MATERIALS AND METHODS

Six inocula of *T. semipenetrans*-J_2 and males in aqueous suspension were obtained by incubation [23] of YousefiBaladi [*Citrus sinensis*(L.) Osbeck] roots from a mature citrus orchard [24]. The inocula were tested on 1.5-year-old nematode-free seedlings of *C. volkamariana* grown in plastic pots containing about 5 Kg of sterilized sandy clay soil (2:1). A very careful standardization of different variables was taken into consideration: the seedlings should not differ in appearance, size and health to avoid problems from hidden factors that affect trees and nematodes independently. Because the citrus nematode is a mild pathogen that likely requires a huge range in population density to measure significant effects, different nematode inocula were tested. The seedlings were established for 6 months before initiating the inocula 0, 5000, 10000, 15000, 20000 and 25000 nematodes (J_2+ males)/seedling as initial nematode population (Pi) in the spring season. The nematode suspension of each inoculum level was pipetted into five cores around the root system of a seedling and then immediately covered with wetted sandy soil. Eight replicates (seedlings) were used for each inoculum level treatment as well as the check. Pots were arranged in a randomized block design and seedlings were maintained on a greenhouse bench at 17 to 37°C. All pots received similar treatments of nutrition during the experimental period [25] and the citrus plants were watered as needed.

The enzyme activities of SOD, PX and CAT were determined in seedling roots of Volkameriana seedlings at different levels of nematode inoculation using polyacrylamide gel electrophoresis (PAGE) technique according to [26]. SOD, PX and CAT were determined at 3, 6 and twelve months after inoculation with nematode compared to the non-inoculated check.

Isozyme fractions were performed on vertical slab (20 cm x 20 cm x 0.2 cm) using the gel electrophoresis apparatus Shelton. A volume of 50 µl from the extraction of enzymes was mixed with 10 µlbromophenol blue and applied to each well. The gel was completely covered with electrode buffer. The electrodes were connected to power supply and adjusted at 150 V for four hours. After electrophoresis, the gels were stained according to the system of each enzyme and incubated at 37°C in dark for complete staining after adding the appropriate substrate for each enzyme and staining solution. Isozyme staining were made according to [27] for SOD, [28] for PX, [29] for CAT. After the appearance of the isozyme bands, the reaction was stopped by washing the gel two times with tap water, and then was photographed. Data were subjected to analysis using Computer Statistical Package (Total Lab from phoretix version 1.10).

III. RESULTS AND DISCUSSION

The SOD, PX and CAT isozyme patterns of PAGE showed a considerable variation in isozyme activities after inoculation of Volkamariana seedlings with different levels of nematode *T.semipenetrans*, measured as relative flow (RF) and density area of isozyme bands.

Superoxide dismutase activity

After 3 months of nematode inoculation, the enzyme activity of SOD, as density area of isozyme bands, increased with increasing the nematode inoculation levels which was 66.5% at 5000, 68.7% at 10000, 70.6% at 15000, 72.6% at 20000 and 72.3% at 25000 J2/pot, compared to 65.4% in the control. Five isozyme bands were presented in the pattern of SOD; this may indicate that these isozymes of SOD were activated in Volkameriana with nematode inoculation.

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After 6 months of nematode inoculation, the enzyme activity of SOD increased with increasing the nematode inoculation levels until 15000, which was 71.7% at 5000, 73.5 % at 10000 and 72.3% at 15000, compared to the control (71.4%). Also, different isozyme bands appeared and showed activity as band densities after treatments. After 12 months of nematode inoculation, the enzymatic activity of SOD decreased with increasing nematode inoculation levels which was 53.9% at 5000, 52.6% at 10000, 47.9% at 15000, 42.1% at 20000 and 38.1% at 25000, compared to the control (59.2%); as shown in Fig. 1 and 2 and Table 1.

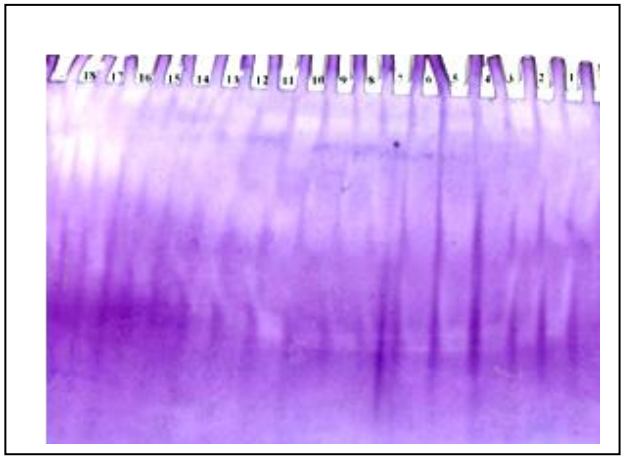

Fig. 1: Electrophoretic patterns of SOD isozymes from Volkameriana seedling roots at different levels of *T. semipenetrans* inoculations. Lanes from 1 to 6 after 3 months, 7-12 after 6 months and 13-18 after 12 months of nematode inoculation. Lanes of 1, 7 and 13are non-inoculated controls, Lanes of 2, 8 and 14 (5000 J_2), Lanes of 3, 9 and 15 (10000 J_2), Lanes of 4, 10 and 16 (15000 J_2), Lanes of 5, 11 and 17 (20000 J_2) and Lanes of 6, 12 and 18 (25000 J_2).

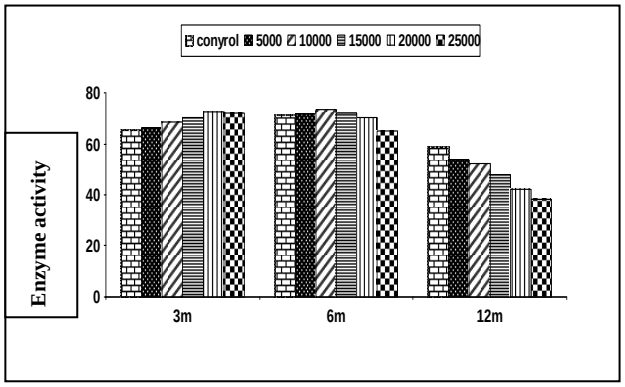

Fig 2: Superoxide dismutase enzyme activity from electrophoretic patterns of Volkameriana seedling roots after 3, 6 and 12 months of T. semipenetrans inoculation with different levels.

These results agreed with that obtained elsewhere[22].They found that SOD enzyme increased in sour orange in response to infection with *T. semipenetrans* according to nematode initial population level.

2- Peroxidase activity

After 3 and 6 months of nematode inoculation, the enzyme activity of PX increased to 91.9% and 90.7% as density area of isozyme bands at nematode inoculum level of 5000 J_2/pot compared to the control (90.3 and 86.4%), respectively. The enzymatic activities were generally low after nematode inoculation with 10000-25000J_2/pot levels. Similar results were observed after 12 months after inoculation with different levels of nematode (5000-25000J_2/pot), as shown in Fig. 3 and 4 and Table 1.

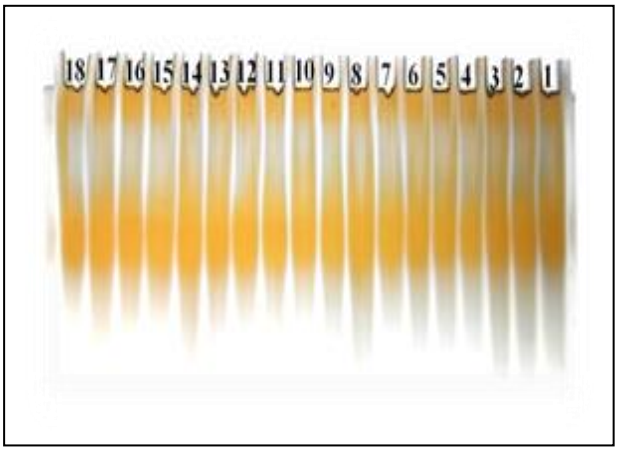

Fig. 3: Electrophoretic patterns of PX isozymes from Volkameriana seedling roots of citrus at different levels of *T. semipenetrans* inoculation. Lanes 1-6 after 3 months, 7-12 after 6 months and 13-18 after 12 months of nematode inoculation. Lanes 1, 7 and 13 are non-inoculated checks; Lanes 2, 8 and 14 (5000 J_2); Lanes 3, 9 and 15 (10000 J_2); Lanes 4, 10 and 16 (15000 J_2); Lanes 5, 11 and 17 (20000 J_2); Lanes 6, 12 and 18 (25000 J_2).

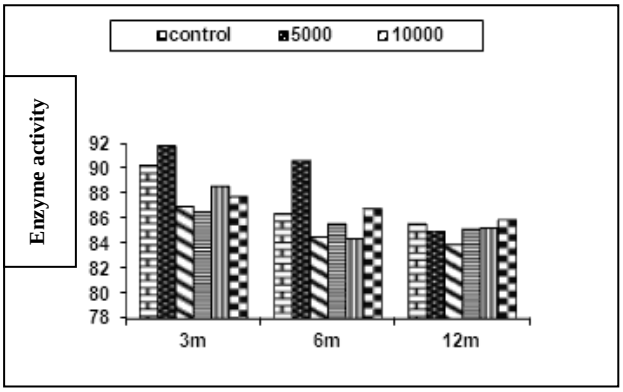

Fig. 4: Peroxidase enzyme activity from electrophoretic patterns of Volkameriana seedling roots after 3, 6 and 12 months of inoculation with different levels of *T. semipenetrans*.

These results agreed with others[17] who reported that greater oxidize and PX activities were detected in the vascular bundles and cortical region of 45 infected roots than in non-inoculated check of tomato 'PusaRuby' seedlings after 60 days of inoculation with 1000 *M. incognita*-J2. Presence of additional bands of PX isozymes in the electrophoretic analyses that were separated 7, 14 and 21 days after inoculation was also reported [18]. They suggested that the elevated level of PX activity was due to de novo synthesis of PX isozymes. In resistant plants, there were increases in PX activity following inoculation compared with susceptible plants [30]. This was associated with a pronounced defense reaction in resistant plants.

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Table 1: Superoxide dismutase, peroxidase and catalase activities from electrophoretic patterns of Volkameriana seedling roots after 3, 6 and 12 months of *T. semipenetrans* inoculation with different levels

Treatment	Superoxide dismutase activity %			Peroxidase activity %			Catalase activity %		
	3 months	6 months	12 months	3 months	6 months	12 months	3 months	6 months	12 months
Control	65.4	71.4	59.2	90.3	86.4	85.6	85.7	83.4	86.5
5000	66.5	71.7	53.9	91.9	90.7	85.0	88.5	85.5	88.7
10000	68.7	73.5	52.6	87.0	84.6	83.9	83.3	85.1	88.5
15000	70.6	72.3	47.9	86.6	85.6	85.2	79.7	82.9	84.0
20000	72.6	70.4	42.1	88.6	84.4	85.3	82.9	83.2	88.3
25000	72.3	65.3	38.1	87.8	86.8	85.9	83.7	85.5	88.5

The activity of the enzymes PX and polyphenol oxidase increased within infected roots, over their controls, of three tomato cultivars by *M. incognita* under greenhouse conditions [31]. After nematode infection in three cultivars of cucumber the PX activity reached as high as 103.8% at Alzaeem cultivar which might confirm the classical rating of its tolerance to nematode infection [32]. The enzyme activity increased after infection but to a less degree in two other susceptible cultivars of cucumber compared to the uninfected plants.

3. Catalase activity

The enzymatic activity of CAT increased, after three months of nematode inoculation, to 88.5% at 5000 J2/pot, while it decreased to 83.3, 79.7, 82.9, and 83.7% with inoculum levels of 10000, 15000, 20000 and25000 J2/pot, respectively compared to the control (85.7%), as shown in Fig. 5 and 6 and Table 1. After six months of nematode inoculation, the enzyme activity of CAT was increased to 85.5, 85.1 and 85.5% at inoculum levels of 5000, 10000 and 25000 J2/pot while it slightly decreased to 82.9 and 83.2 % at inoculums level of 15000 and 20000 J2/pot, respectively compared to the control (83.4%). After 12 months of nematode inoculation, the enzyme activity of CAT increased more than the control at all levels of inoculation, except 15000 J2/pot; it was 88.7% at 5000, 88.5% at 10000 and 25000 and 88.3% at 20000J2/pot compared to the control (86.5%). Such an increase in CAT was also reported by Mohamed et al. (1999) in susceptible tomato over its control after inoculation with nematode M. incognita [31].

Generally, the data indicated that the activity of antioxidant enzymes, SOD, PX and CAT in healthy plants were at low levels. Notable increase was observed as a result of nematode infection with *T. semipenetrans* at5000 J_2on sour orange after three and six months of inoculation, besides 12 months in CAT enzyme at other levels. The increment rates varied according to inoculums levels.

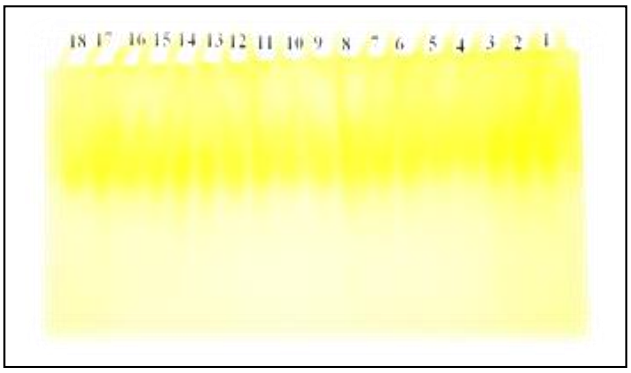

Fig. 5: Electrophoretic patterns of CAT isozymes from Volkameriana seedling roots at different levels of *T .semipenetrans* inoculation; lanes from 1 to 6 after 3 months, 7-12 after 6 months and 13-18 after 12 months of inoculation. Lanes of 1, 7 and 13 are controls, Lanes of 2, 8 and 14 (5000 J_2), Lanes of 3, 9 and 15 (10000 J_2), Lanes of 4, 10 and 16 (15000 J_2), Lanes of 5, 11 and 17 (20000 J_2), Lanes of 6, 12 and 18 (25000 J_2).

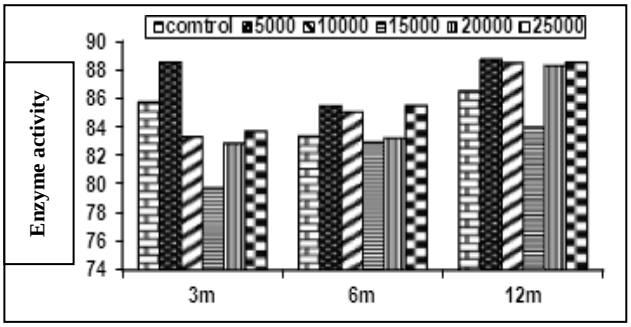

Fig. 6: Catalase enzyme activity from electrophoretic patterns of Volkameriana seedling roots after 3, 6 and 12 months of inoculation with different levels of *T. semipenetrans*.

Increasing of SOD,PX and CAT activities seem to be a result of an adaptive response which provides the plant with protection against biotic stress as reported by **[33]**. This increase in SOD is probably to prevent the deleterious effect of O_2 radicals in root cells and to transform it to H_2O_2 which is then transformed by catalase to harmless $O_2$and H_2O, as reported others**[1]**. Yet, the protocol applied herein is not intended to discriminate susceptible and resistant accessions/genotypes of plants to nematodes. For such discriminations, we should use very young seedlings, as specific enzyme activities decrease with aging of plants. Also, we don't need to use roots, young leaves may be enough to have a sound value of CAT activity per the intendedgenotypes. Moreover, previously determined susceptible and resistant genotypesshould be available to use as standards, and preferably use as many accessions as possible to biochemically design their host suitability. Finally, for working on hydrogen peroxide, the root/leaf extracts must be ultra filtered with a cutting set at 10,000 MW to get ridof phenols. The presence of phenols in the extracts markedly inhibit catalase, so we should get rid of them [34]. On the other hand, symptoms may not be apparent on lightly infected root systems so that *T. semipenetrans*-infected nursery stock may easily go undetected [10]. So,

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the biochemical markers recorded herein may help in characterizing such infection. This is especially important since feeder roots decay faster due to loss of integrity at the epidermis and at feeding sites in the cortex, resulting in invasion by secondary organisms. This may be expressed as lesions on lightly infected roots. On the contrary, heavy infections result in cortical sloughing and root death. Hence, the name 'slow decline' is less appropriate when young trees are replanted into heavily infested soil where effects on tree growth may be noted soon after planting [10].

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REFERENCES

[1] Bowler, C., M. Van Montagu and D. Inze (1992). Superoxide disniutase and stress tolerance. Plant Mo1. Biol. 43: 173-180.

[2] Bowler, C. and Fluhr, R. (2000). The role of calcium and active oxygen as signals for controlling cross tolerance. Trends Plants Sci. Rev., 5:241-246.

[3] Monk, L.S.,K.V. Fagerstedt and R.M.M. Crawford (1989).Oxygen toxicity and superoxide dismutase as an antioxidant in physiological stress. Physiol. Plant, 76: 456-459.

[4] Smirnoff, N. (1993). The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol., 125: 27-58.

[5] Foyer, C.H., P. Descourvieres and K.J. Kunert (1994).Photoxidative stress in plants. Physiol. Plant, 92: 696-717.

[6] Wadsworth, G.L. and J.G. Scandalios (1990). Molecular characterization of a catalase null allele at cat3 locus in maize. Genetics, 125: 867-872.

[7] Willekens, H., S. Chamnogpol, M. Davey, M. Schravdner, C. Langebartels, M.C. Van, D.Inze and C.W. Van (1997). Catalase is a sink for H_2O_2 and is indispensable for stress in C3 plants. EMBO J., 16: 4806-4816.

[8] Willekens, H., C. Langebartels, C. Tire, M.M. Van, D.Inze and C.W. Van (1994).Differential expression of catalase genes in Nicotianaplumbaginifolia. Proc. Natl. Acad. Sci. U.S.A., 91: 10450-10454.

[9] Abd-Elgawad, M. M. M., F. A. Al-Yahya and Z. A. Stephan (2010). Nematodes of citrus (In Arabic), pp.553-602. In: "Plant Nematodes in Arab Countries" W. A. Abu-Gharbieh, A. S. Al-Hazmi, Z. A. Stephan and A. A. Dawabah (Eds). Darwael for publishing, Arab Society of Plant Protection, Amman, Jordan.

[10] Duncan, L.W. 2005. Nematode parasites of citrus. In: Luc, M., Sikora, R.A., and Bridge, J. (Eds). Plant Parasitic Nematodes in Subtropical and Tropical Agriculture, 2nd edition: Wallingford, U. K. CABI Publishing, pp. 437-466.

[11] Duncan, L.W. and E. Cohn 1990. Nematode parasites of citrus. In: Luc, M., Sikora, R.A & Bridge, J. (Eds). Plant parasitic nematodes in subtropical and tropical agriculture. Wallingford, UK, C.A.B International: pp. 321-346.

[12] Baldacci C.F., C. Chang, M. Macourt, C. Deborde, J. Hopkins, P. Lecomte, S. Bernillon, R. Brouquisse, A. Moing, P. Abad, D. Herouart, A. Puppo, B. Favery andP. Frendo (2012). Homo glutathione deficiency impairs root-knot nematode development in Medicagotruncatula. Plos Pathogen / www.Plospathogen. Org. 8(1):e1002471.

[13] Siahpoush S, N. Sahebani and H. Aminian (2011). Change in some defense compounds of cucumber treated with Bacillus cereus and salicylic acid against Meloidogynejavanica. Afr. J. Plant Sci. (14):829-834.

[14] Aryal S.K., R.F. Davis, K.L. Stevenson, P. TimperandJ. Pingsheng (2011). Meloidogyne incognita enzymes involved in systemic acquired resistance. J. Nematol. 43(3-4):152-159.

[15] El-Beltagi H.S., A.A. Farahat, A.A. Alsayed and N.A. Mahfoud (2012). Response of antioxidant substances and enzymes activities as a defense mechanism against root-knot nematode infection. Not. Bot. Hort. Agrobot. Cluj. 40(1):132-142.

[16] Abd-Elgawad M. M. and Molinari, S. (2008). Markers of plant resistance to nematodes: classical and molecular strategies. Nematologiamediterranea, 36: 3-11.

[17] Haseeb, A.,A.M. Khan and S.K. Saxena (1986). The histochemical localization of oxidase, peroxidase and lignin in the roots of tomato infected by the root-knot nematode, Meloidogyne incognita. Indian Journal of Nematology, 16(2): 263.

[18] Smite H.C. and D.R. Dasgupta (1987). De novo synthesis of peroxides isozymes of soybean var. Clark-63 infected with the root-knot nematode, Meloidogyne incognita. Indian J. Nematol., 17(2): 247-253.

[19] Ahuja, S., M.C.Mukhopadhyaya, A.Singh and S.P. Ahuja (1987). Effects of infestation of egg plant (Solanummelongena) with root knot nematode (Meloidogyne incognita) on the oxidative enzymes and cell wall constituents of their roots. Capsicum-Newsletter, 6: 98-99.

[20] Shukla, Y.M and M.K. Chakraborty (1988). Biochemical studies on response of tobacco and tomato plants to root knot nematode infection. Tobacco-Research. 14(1): 43-50.

[21] Zacheo, G., S. Molinari and D. Pacoda (1988).Hydroxyproline-rich proteins and peroxides in tomato roots infested by root-knot nematode. Nematol. Medit., 16(2): 235-237.

[22] Afify A. E. M. M. R., A.A. Farahat, Al-Sayed A. A. and Mahfoud N. A. M. (4014). Antioxidant enzymes as well as oxidant activities involved in defense mechanisms against the root-knot, reniform and citrus nematodes in their host plants. International Journal of Biotechnology and Food Science, 2 (6): 102-111.

[23] Young, R.W. 1954. An incubation method for collecting migratory end parasitic nematodes. Plant Disease Reporter 38:794-795.

[24] Abd-Elgawad, M. M. M., Duncan, L. W., Koura, F. H. F., Abd El-Wahab, A. E., Montasser, S. A., and Hammam, M.

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M. A. 2011. Management revision and observations on Tylenchulus semipenetrans on citrus yield in Egypt. Egyptian Journal of Agronematology 10(1):64-77.

[25] Ibrahim, A.M. and Khaleef, M.N.H. 1997.The citrus: cultivation, care, and production (In Arabic) p. 718. MonshaatAlmaarf, Alexandria, Egypt.

[26] Stegemann, H.,A.E.T. Shehata and M. Hamza (1980). Broad bean Protein (Viciafaba. L.). Electrophoretic studied on seeds of some German and Egyptian cultivars. J. Agronomy and crops sci., 149: 447-453.

[27] Larsen, A.L. and W.C. Benson (1970). Variety-specific variants of oxidative enzymes from soybean seed. Crop Science 10 (5): 493-495.

[28] Graham, R.C., U. Lundholm and M.J. Karnovsku (1965). Cytochemical demonstration of peroxidase activity with 3-amino 9-ethylcardazole. J. histochrm. Cytochem., 13: 150-156.

[29] Jonathan, F.W. and N.F. Wendel (1990). Visualization and interpretation of plant isozymes. In: D.E. Soltis and P.S. Soltis eds. Isozymes in plant Biology, London Chamran and Holl, 5-45.

[30] Sharma, O.N. (1993). Enhancement of biological defense mechanisms in resistance and its activation in susceptible tomato cultivars infested with root-knot nematode, Meloidogyne incognita. Plant Disease Research, 8(1): 47-53.

[31] Mohamed, M.A.,M.M.A. Youssef and M.M. Abd-Elgawad (1999). Measuring reaction of tomato cultivars to Meloidogyne incognita through plant appearance and enzyme activity. Int. J. Nematol., 9(2):174-180.

[32] Abd-Elgawad, M.M.M., M.H. Abou-Deif, F.H.F. Koura, A.E. Abdel-Wahab, M.M.A. Hammam (2008). Enzymatic activity as biochemical markers for detecting cucumber and squash tolerance to nematode infection. Egyptian Journal of Plant Breeding, 12 (1): 303-313.

[33] Guida G, G. Zacheo and T.B. Zacheo (1992). Activation of detoxifying enzymes in tomato roots following paraquat treatment and nematode infection. Nematol. medit. (20):203-209.

[34] Molinari, S. and M. M. Abd-Elgawad 2007. Catalase inhibition as a biochemical marker of resistance to root-knot nematodes in tomato. Nematologiamediterranea, 35: 237-22.

AUTHOR BIOGRAPHY

First Author: Department of Plant Pathology, National Research Centre – Egypt, Email:mahfouzian2000@yahoo.com.

Second Author: Genetic and Cytology Department, National Research Centre, Dokki 12622, Giza, Egypt/ Biology Department, Faculty of Science and Arts, University of Geddah, Khulais, KSA. Email: aboudeif@yahoo.com

Third Author: Department of Plant Pathology, National Research Centre – Egypt, Email:mmahammam@yahoo.com

Fourth Author: Department of Plant Pathology, National Research Centre – Egypt, Email:khairhfz@yhoo.com.