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Research Article
Physiological and Biochemical Changes in the Wheat Plant (*Triticum aestivum* L.) Infected with Nematodes

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Abstract

Background and Objective: Cereal Cyst Nematodes (CCNs) *Heterodera avenae* are from the most critical nematode pests that restrict the production of cereals worldwide. These nematodes alone are savage pests that harm wheat and reduce their production by 10(%) globally. This study was done to study the changes in the physiological and biochemical activity of wheat plants infected with nematodes. **Materials and Methods:** The pot experiments were conducted in the greenhouse of the National Research Centre, Dokki, Cairo, Egypt. Wheat seeds variety Misr-1 was used. Treated pots were infested with nematode cysts to give a population density of 8000 j_2 and eggs 1 kg^{-1} soil. The roots and shoots of seedlings were processed for physiological and biochemical analysis. The mean comparisons among treatments were determined by Duncan's multiple range tests at a 5(%) level of probability. **Results:** Our results proved the promotion effects of CCN on Reactive Oxygen Species ROS as Malondialdehyde MDA production that concomitant with the inhibitory effects on photosynthetic pigment contents, altering endogenous phytohormones, Indole-3-Acetic Acid (IAA), Abscisic Acid (ABA), Gibberellic Acid (Gas), Zeatin and reconstructing the production of assimilates (Total Soluble Solids (TSS), total carbohydrates and protein). These variations stimulate the production of enzymatic antioxidants, Superoxide Dismutase (SOD), Catalase (CAT) and Peroxidase (POX) and non-enzymatic antioxidant, phenol. The infection also affects the absorption and translocation, production of minerals, P, K, Fe, Zn, Mn and Cu. All of these changes reflected on the yield production and the nutritional values of yielded grain grown under such conditions. **Conclusion:** Most nutritional values, physiological and biochemical aspects of wheat decreased in the infected plants as compared with non-infected plants.

Key words: Nematodes, endogenous hormones, zeatin, antioxidant enzymes, photosynthetic pigments, wheat, yield quality

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Wheat (*Triticum aestivum* L.), seeds are full of nutritional values as carbohydrates, minerals, proteins and vitamins. Cereals provide about 20 (%) of the consumed calories worldwide. So, they are considered the essential source of energy for humans worldwide. In developing countries, the expenditure rate surpassed the productivity[1]. The present production rates will not be adequate to satisfy the projected global request due to the huge increase in population density. For wheat, global production must be increased by 60 (%) to satisfy the population density in 2050[2].

The Cereal Cyst Nematodes (CCNs) *Heterodera avenae* woll. has been detected in many countries with different climate types throughout the world[3,4]. It causes considerable yield losses of several economic yielded grains[5,6]. So, it is considered a major limiting factor of wheat in many parts of the world[7,8]. *H. avenae* was 1st reported on wheat in northwest Egypt and northeast Egypt[9,10].

Invading host roots with cyst nematodes leads to the initiation of the formation of different creatures, multicellular cells called Syncytium which become the main radix of nutrients for nematodes during their lifecycle[11,12]. Formation of these Syncytia was found to be associated with profound physiological and biochemical changes involved in water and nutrients relations and the phytohormones originating in roots that concern not only the infection site but the whole plant[13]. The plant's uptake of nutrients and water is considerably lowered due to its impaired root system, which leads to fragility and low yield[14]. They added that nematode infection causes alterations in morphological and physiological processes in host plants, including inhibiting growth, promoting chlorosis and lowering photosynthetic rates.

So, this study aimed to evaluate the changes in the physiological and biochemical activity of wheat plants infected with *Heterodera avenae* to the quantity and quality of grain yield. Such information is important for more understanding of host nematode interactions and nematode sustainable management.

MATERIALS AND METHODS

Experimental design: The pot experiments were conducted in the greenhouse of the National Research Centre, Dokki, Cairo, Egypt, during the winter season of 2018/2019 (from 25th November-5th May,). The daytime temperature ranged from 14.5-30.2°C with an average of 23.2±3.8°C, whereas temperature at night was 12.4±1.8°C, with minimum and maximum of 8.0 and 17.6°C, respectively. Daily relative humidity averaged 57.7±9.6 (%) in a range from 38.1-78.7 (%). Seeds of *Triticum aestivum* variety Misr-1 were obtained from Agricultural Research Centre, Ministry of Agriculture and Land Reclamation, Egypt. The experiment was carried out in the twenty plastic pots 30 cm diameter containing sterilized loamy soil each filled with about 8 kg.

Methodology: The experimental soil was analysed according to the method described by De Rooij[15] (Table 1). Fertilization was done with the recommended dose i.e., (5 g phosphorous/pot as triple phosphate, 6 g nitrogen/pot as urea and 5 g potassium/pot as potassium sulphate) during the preparation of pots and after sowing. Watering was carried out according to the usual practice. The selected grains were washed with distilled water, sterilized with 1 (%) sodium hypochlorite solution for about 2 min and thoroughly washed again with distilled water. Five uniform air-dried wheat grains were sown in each pot. Ten pots were infested with nematode cysts of *H. avenae* to give a population density of 8000 j_2 and eggs 1 kg^{-1} soil, other 10 pots were left without nematodes (control). About 55 days after seed germination, wheat seedlings from 10 pots (5 infected+5 controls) were gently extracted from the soil and washed with tap water. The roots and shoots of seedlings were processed for physiological and biochemical analysis. The remaining (5 infected+5 controls) of pots were left for assaying grain yield and quality at harvest (in May, 2019).

Nematode population: The population of *Heterodera avenae* was collected from a wheat-growing area heavily infected with nematodes from Ismailia Province. Nematode cysts were extracted from soil according to the previously described method[16]. Nematode Cysts were dried at room temperature (20±2°C) and kept at 7°C until further use. Ten cysts were quashed according to the previous method[17] and the total number of eggs and second-stage juveniles (J_2) per cyst was counted. The average of eggs and J_2 was 80 per cyst.

Table 1: Chemical analysis of the experimental soil

pH	7.74
EC (ds m^{-1})	0.29
mL L^{-1}	
Ca^{++}	0.30
Mg^{++}	0.20
Na^{+}	1.10
K^{+}	0.40
CO_3^{-}	0.60
HCO_3^{-}	-
Cl^{-}	1.00
SO_4^{-}	0.40
ppm	
N	21.00
P	2.40

Physiological and biochemical studies

Photosynthetic pigments: Both chlorophyll a and b and carotenoids contents in fresh leaves were estimated using the previously described method[18], using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). The values of photosynthetic pigments were expressed in µg/100 g FW.

Endogenous phytohormones: Extraction, separation and determination of phytohormones: The method of hormone extraction was done according to the previous method adopted by Zhang *et al.*[19] and the Methylation process was carried out according to the previously described method[20]. Identification and determination of auxins, gibberellins and abscisic acid were carried out by Hewlett Packard gas-liquid chromatography (5890) with a flame ionization detector[21]. Cytokinin fractions (zeatin and benzyl adenine) were detected by HPLC isocratic UV analyzer according to the method described by Tarkowski *et al.*[22].

Total phenolic compounds: The total phenolic compound was determined using a spectrophotometer as previously described method[23].

Total soluble sugars, total carbohydrates and total protein: Total sugar and total carbohydrate concentrations were determined by using the phenol sulphuric acid method according to Mecozzi[24]. Total protein was estimated according to the previously described method[25].

Lipid peroxidation: Lipid peroxidation was determined by measuring the amount of produced Malondialdehyde (MDA) by the Thiobarbituric Acid (TBA) reaction as previously described method[26].

Assay of enzymes activity: Enzyme extracts were collected following the previously described method[27].

Peroxidase: (POX, EC 1.11.1.7) the activity was assayed spectrophotometrically by the previously described method[28].

Superoxide dismutase: (SOD, EC 1.12.1.1) the activity was spectrophotometrically assayed at 560 nm by Nitro-Blue-Tetrazolium (NBT) reduction as previously described method[27].

Catalase: (CAT, EC 1.11.1.6) the activity was determined spectrophotometrically following the decrease in absorbance at 240 nm[27]. The enzyme activities were calculated as previously described method[29].

Mineral contents: The oven-dried samples of wheat shoots, roots and yielded grains (at 70°C for 72 hrs (were powdered. N, P, K, Zn, Mn, Fe and Cu were determined. The nitrogen content of plant leaves was measured by Kjeldahl protocol according to Paul *et al.*[30]. The phosphorus percentage was estimated by the colorimetric method according to Paul *et al.*[30]. The potassium content of the plants was measured by using a flame photometer method according to Paul *et al.*[30]. Zinc, manganese, iron and copper were determined using atomic absorption spectrophotometer, D. P. 3300 Parken Elmer according to Paul *et al.*[30].

Grain yield and quality determination: The weight of grain (g/pot) was determined in infected and non-infected plants. Then grain total protein was calculated by multiplying Nitrogen (%) in 6.25 as the method previously described by Maehre *et al.*,[25]. Gluten content (%) was determined using a non-destructive grain analyser, Model Infratec TM 1241, 15W 5.00 valid from S/N 12414500, 1002 5017/Rev.1, Foss analytical AB, Hoganas, Sweden. Glutenin was prepared by the previously modified method[31].

Statistical analysis: The experiment was conducted in a completely randomized design with 5 replicates. Data on physiological and yield characters and seed quality were subjected to conventional methods of analysis of variance according to Snedecor and Cochran[32]. The mean comparisons among treatments were determined by Duncan's multiple range test at a 5% level of probability.

RESULTS

Photosynthetic pigments: Data presented in (Fig. 1a-e) presented that, infected plants with nematodes decreased photosynthetic pigment contents (chlorophyll a, chlorophyll b, carotenoids and total pigments) compared to control plants. The percent of reduction reached 38.60, 40.86, 29.50 and 36.6% in chlorophyll a, chlorophyll b, carotenoids and total pigment levels of the plant leaves, respectively.

Change in endogenous phytohormones: Results in (Fig. 2a-e) showed that endogenous phytohormones (IAA, ABA, GA and Zeatin) contents induced regarding Cereal Cyst Nematodes

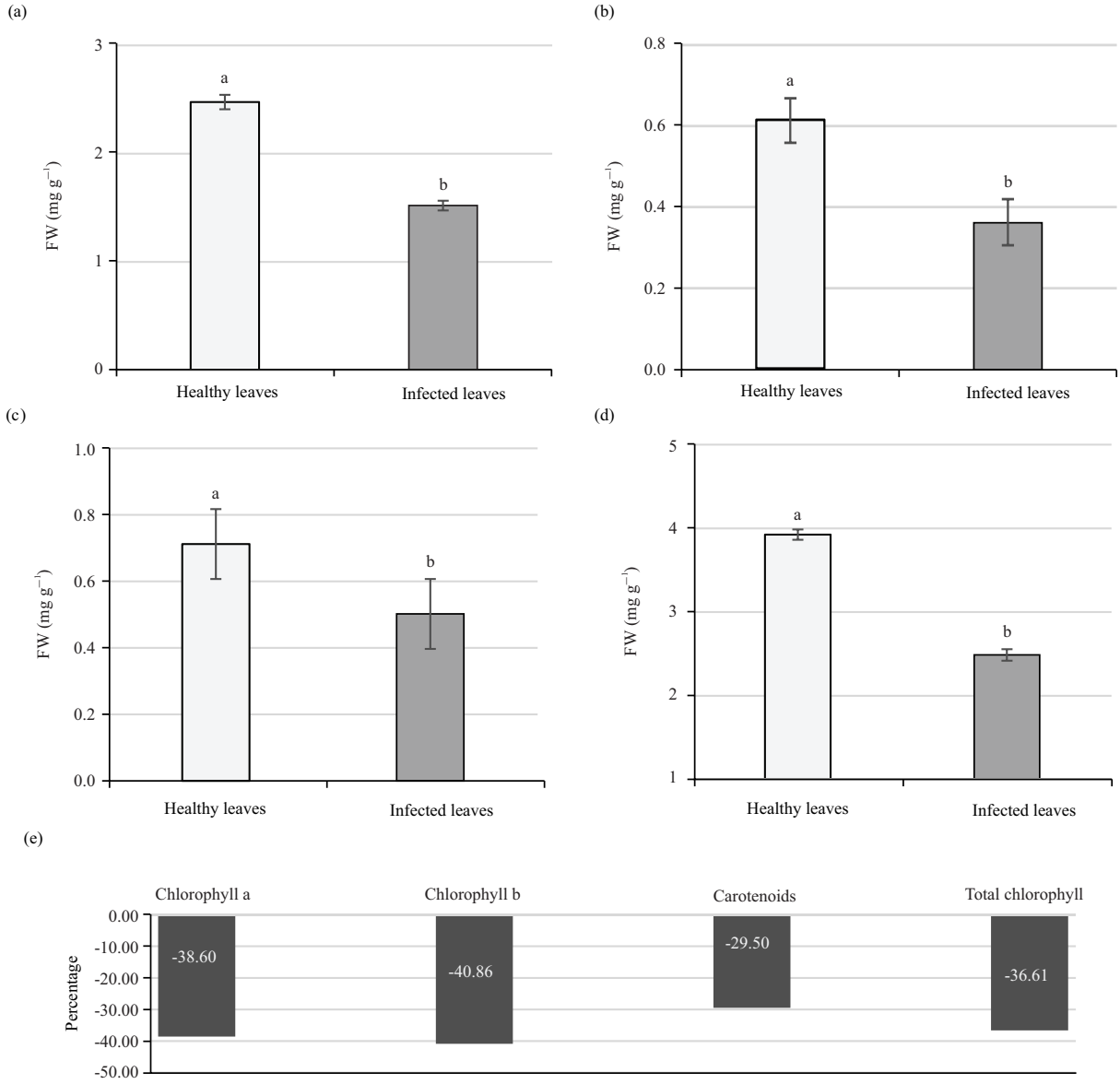

Fig. 1(a-e): Photosynthetic pigment contents (mg/g fresh weight), (a) Chlorophyll a, (b) Chlorophyll b, (c) Carotenoids, (d) Total pigment and (e) Increase or decrease percentages of wheat (Masr-1) leaves infected with the nematode

(CCN (infection onto the wheat plant. Generally, ABA surpassed other hormones in percent of increment. This percent increase reached 91.04 (%), 164.286 and 114.028 (%) of IAA, ABA and GA of infected roots, respectively. While, the percent of endogenous hormones increases in leaves reached 15.62 (%) in IAA, 177.89 (%) in ABA. GA contents in leaves of the wheat plant showed a significant decrease by about14.40 (%). Infection with nematodes induced a significant decrease in zeatin contents of both roots and leaves. The maximum reduction percent was 32.50 (%) in root followed by 16.66 (%) in leaves.

Total soluble sugars, total carbohydrates, total proteins and phenolic contents: The results in (Fig. 3a-e) showed the variation in total soluble sugar, total carbohydrates, total proteins and total phenolic contents in fresh leaves and roots of the wheat plant in response to the nematode. The results exhibited that, the infection with nematode promote a significant increase in all tested parameters of roots and leaves as compared with the corresponding non-infected plant. Wheat leaves have the percent of increases 139.79, 84.121, 29.310 and 9.446% in TSS, total phenolic compounds, total proteins and total carbohydrates, respectively as compared

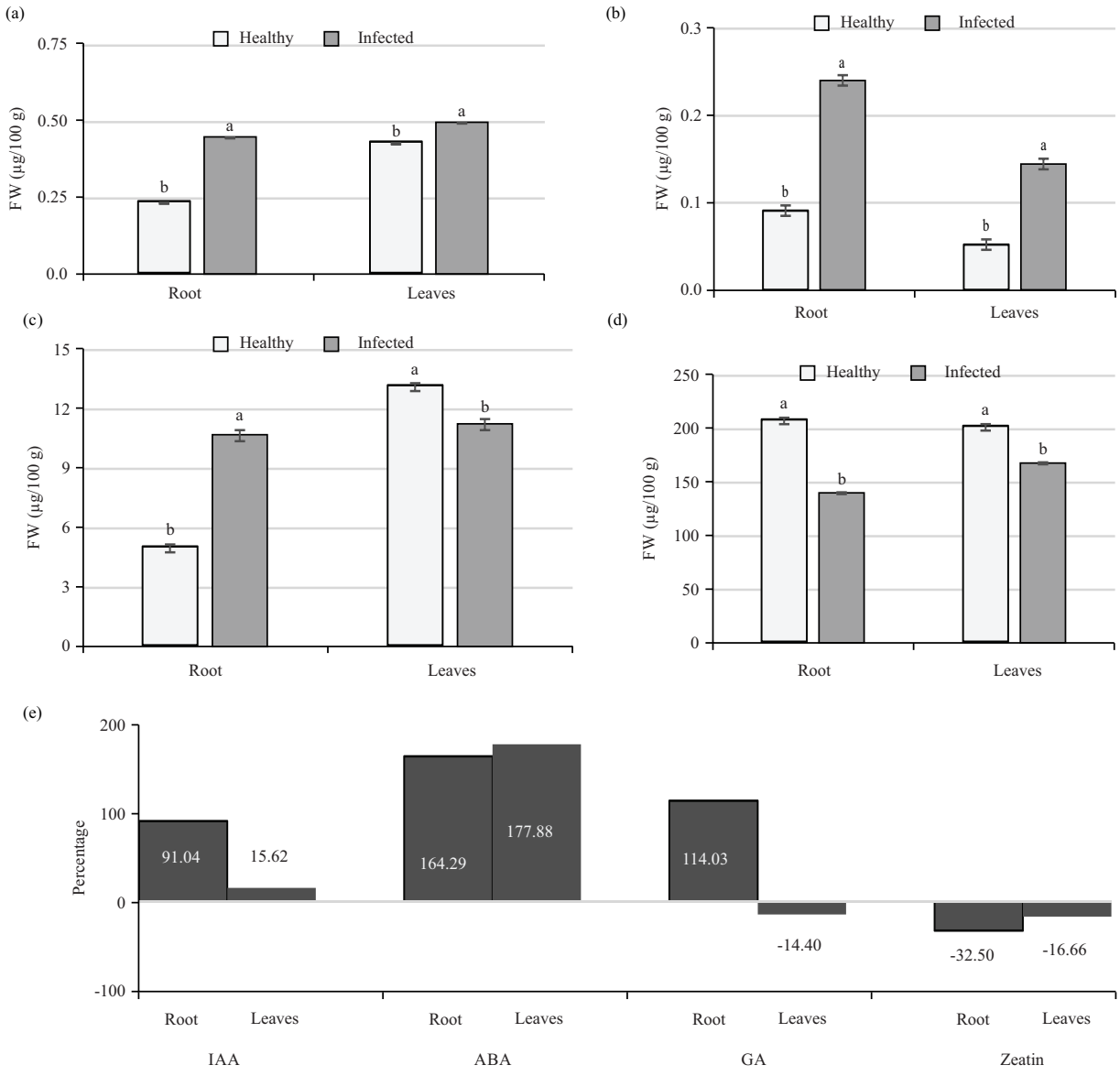

Fig. 2(a-e): Endogenous phytohormone contents (µg/100 g fresh weight), (a) IAA, (b) ABA, (c) GA, (d) Zeatin and (e) Increase or decrease percentages of wheat (Masr-1) roots and leaves infected with the nematode

with the corresponding non-infected wheat leaves. While, the percent of the increase in roots recorded 10.446 and 53.437 (%) in TSS and phenolic compounds respectively as compared with the corresponding non-infected wheat roots.

Lipid peroxidation: The results appeared that infection with nematodes induced significantly Malondialdehyde (MDA) in roots and leaves compared to control plants (Fig. 4a). The highest percentage of increase in the Malondialdehyde (MDA) was recorded in roots (61.217 (%)) followed by (18.237 (%)) in leaves.

Enzyme activities: According to the impact of infection with nematodes on some oxidative enzymes activities of wheat plants, (Fig. 4b-e) showed that, the infection-induced significant increases in SOD activity in both wheat roots and shoots as compared to the non-infected plant. However, the infection-induced increase in POX activity in roots and exhibited a little reduction in shoots.

The infection limited the CAT activity of roots while, the opposite effect was recorded on wheat leaves as compared to the control plant. The highest stimulation percent was recorded by POX activity (164.506 (%)) in the infected root. At the same time, the maximum percent of

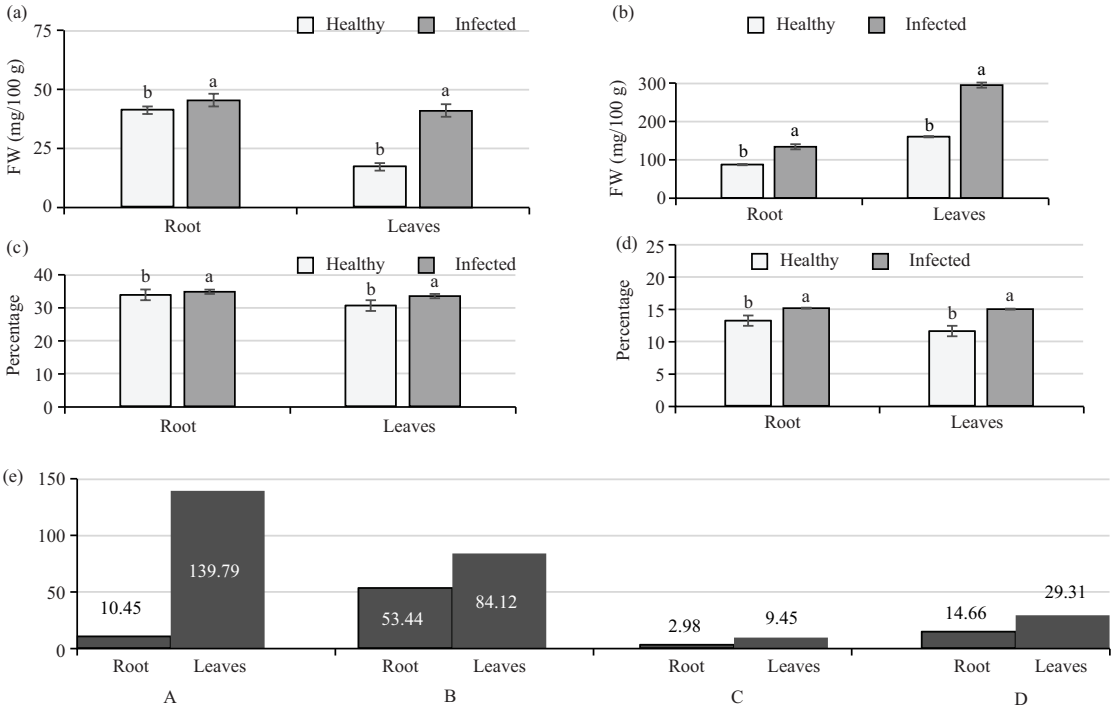

Fig. 3(a-e): Osmolytes of wheat (Masr-1) roots and leaves infected with the nematode, (a) Total soluble sugars, (b) Total phenolic compounds (mg/100 mg fresh weight), (c) Total carbohydrates, (d) Total protein (%) and (e) Increase and decrease percentage of wheat (Masr-1) roots and leaves infected with the nematode

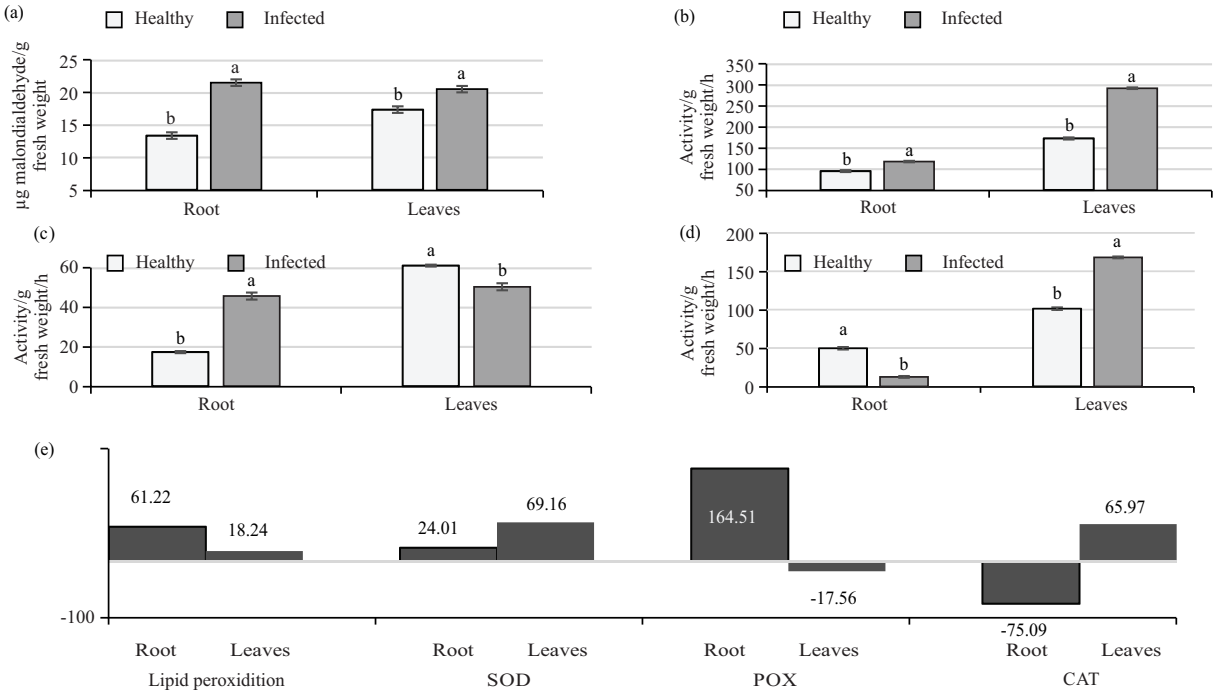

Fig. 4(a-e): Lipid peroxidation (µg malondialdehyde/g fresh weight) and enzyme activities (activity/g fresh weight/h), (a) Lipid peroxidation, (b) SOD, (c) POX, (d) CAT and (e) Increase and decrease percentage of wheat (Masr-1) roots and leaves infected with the nematode

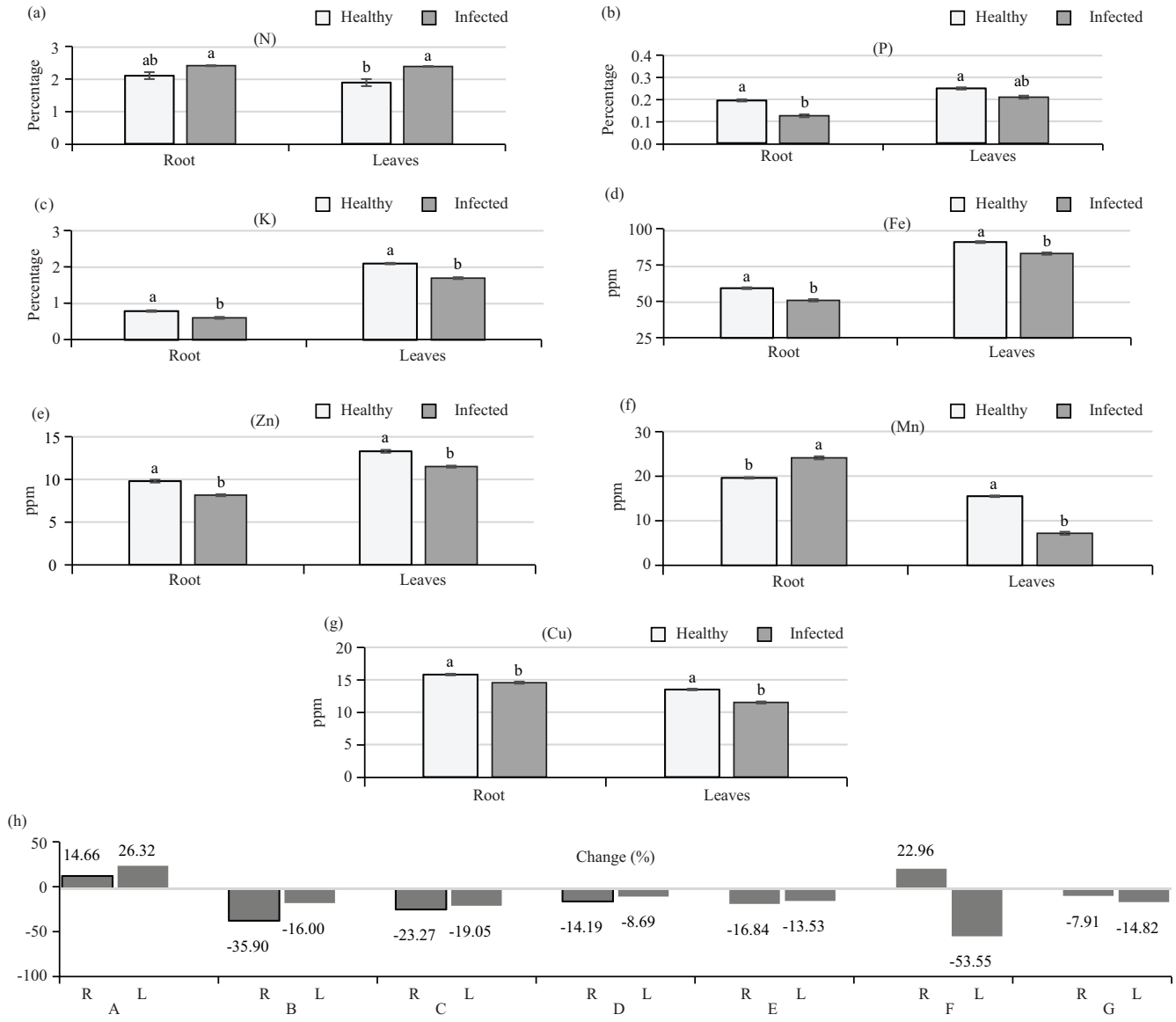

Fig. 5(a-h): Mineral contents of wheat (Masr-1) roots and leaves infected with the nematode, (a) Nitrogen, (b) Phosphorous, (c) Potassium, (d) Iron, (e) Zinc, (f) Manganese, (g) Cupper and (h) Their increase and decrease percentage

decrease was recorded by CAT activity (-75.086 (%)) in the infected root.

Mineral composition of plant: The results in (Fig. 5a-h) showed the mineral contents of the root and shoot of wheat plants infected with nematodes. The results cleared that the contents of all studied minerals (P, K, Fe, Mn, Zn and Cu) were decreased with infection as compared with non-infected plants. Nitrogen content exhibited the opposite trend in infected plants with an increase percent of 26.316 (%) in the wheat shoot as compared with the infected plant.

Yield and yielded grain quality: Results presented in (Fig. 6a-d) showed that infection with nematodes decreased significantly grain yield (g/pot), seed protein (%) and gluten (%) as compared with the non-infected plant. The percent of reduction reached 30.30, 9.30 and 16 (%) of tested parameters, respectively. The results also indicated that most of the studied minerals (N, P, Mn and Cu) were decreased in yielded grains with infection. On the other hand, the other minerals (K, Fe and Zn) were increased in response to infected plants as compared with non-infected plants. The percent of increments reached 10.60, 57.00 and 1.40 (%), respectively (Fig. 7a-h).

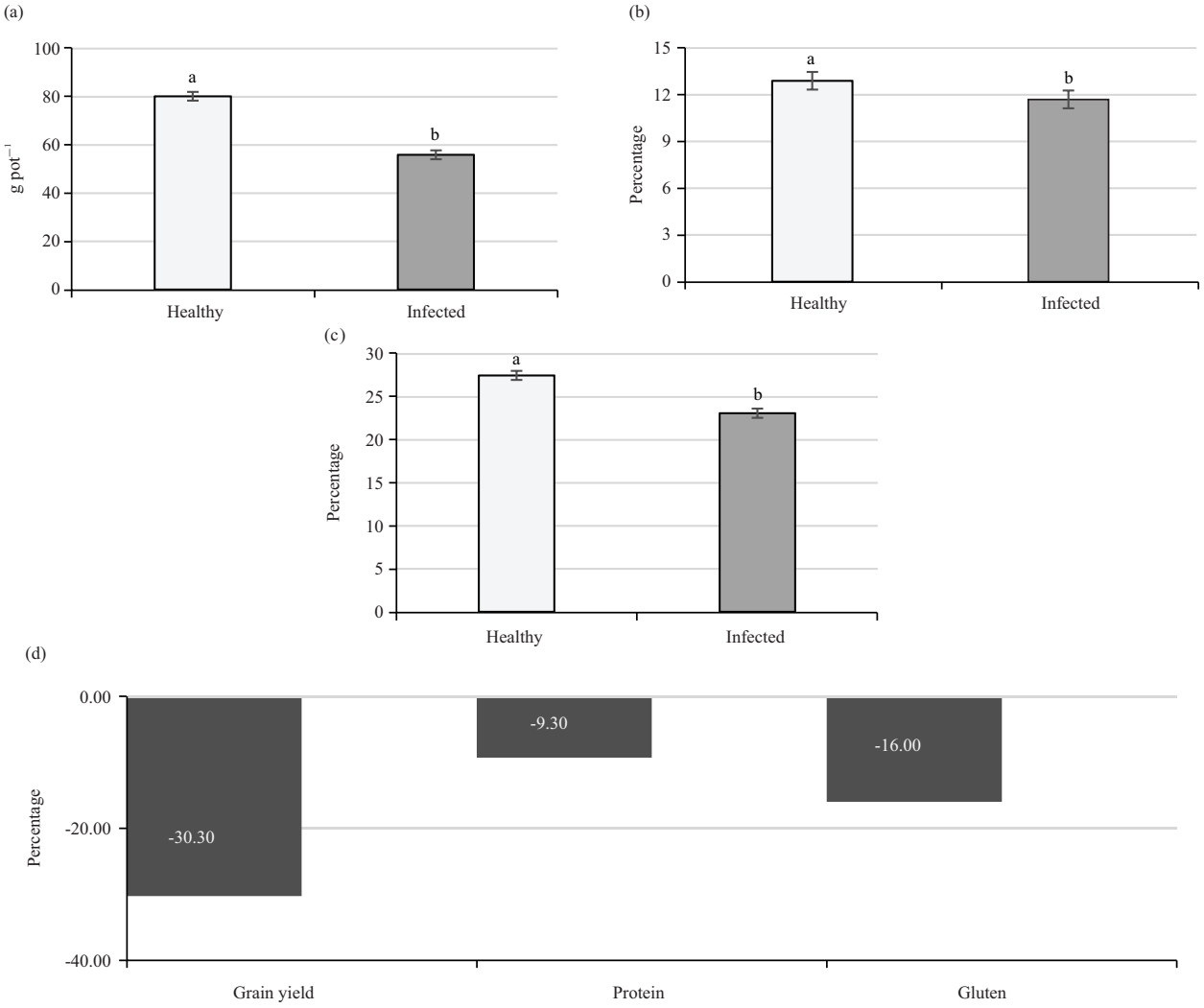

Fig. 6(a-d): Grain yield and nutritional values of yielded grains, (a) Grain yield, (b) Protein, (C) Gluten and (d) Their increase and decrease percentage of wheat (Masr-1) infected with the nematode

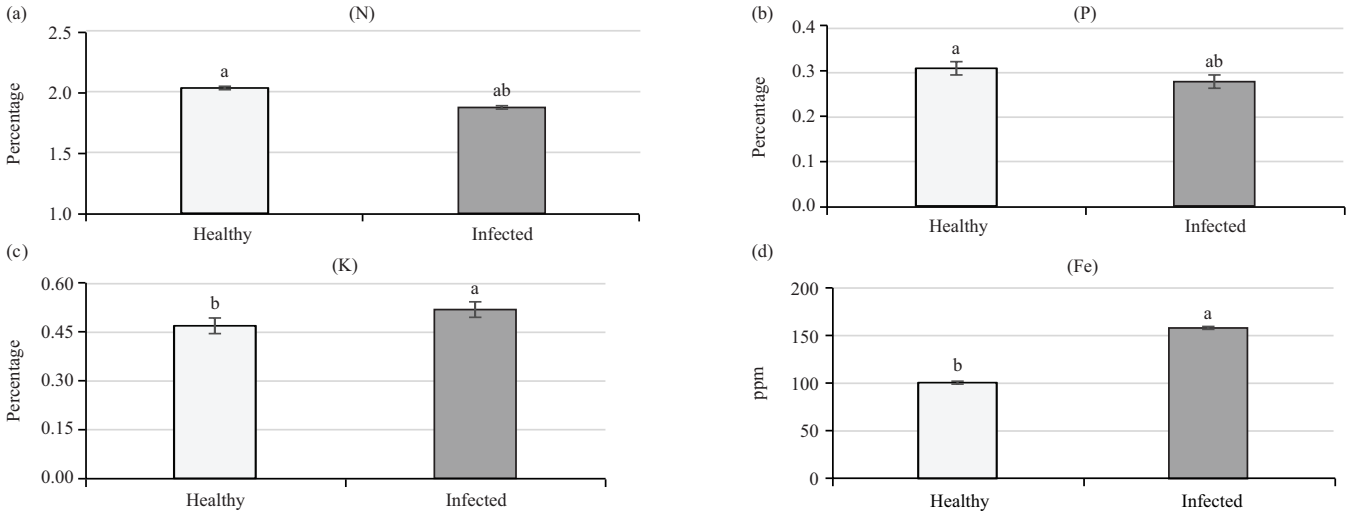

Fig. 7(a-h): Continue

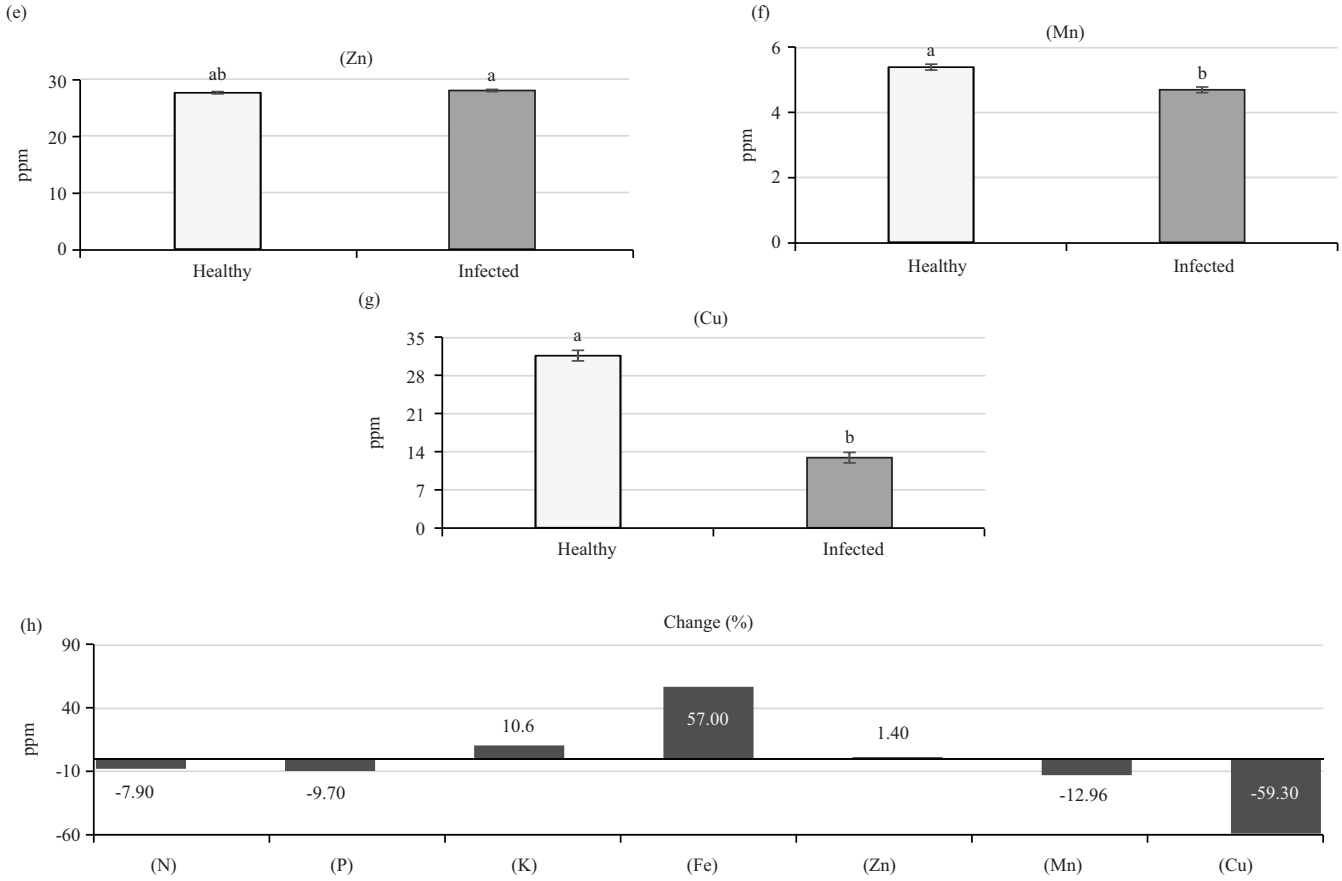

Fig. 7(a-h): Mineral contents of yielded grains, (a) Nitrogen, (b) Phosphorous, (c) Potassium, (d) Iron, (e) Zinc, (f) Manganese, (g) Cupper and (h) Increase and decrease percentage of wheat (Masr-1) roots and leaves infected with the nematode in wheat (Masr-1) infected with the nematodes

DISCUSSION

Our results revealed that physiological and biochemical operations were disturbed in wheat plants infected with the cyst nematodes *Heterodera avenae* compared to healthy plants (Fig 1-5). Cyst nematodes invade host roots and initiate the formation of syncytia, which become the only source of nutrients for nematodes throughout their entire lifecycle. The wheat cyst nematodes (CCN) *Heterodera avenae* like other sedentary endoparasites nematodes parasitize their hosts causing damage at the feeding sites, as they break down the cell structure of the roots inducing cells to form permanently modified structure: multicellular cells could Syncytium which become the main radix of nutrients for nematodes during their life cycle[11,12]. The formation of these Syncytia was found to be associated with profound physiological and biochemical changes that may concern not only the infection site but the whole plant also[13]. In this regard, photosynthetic pigment contents (chlorophyll a, chlorophyll b, carotenoids and total pigment) were significantly decreased in the infected plants with nematodes as compared with non-infected plants (Fig. 1). Similar results were also obtained by previous results[33-35] they reported that, infestation with RKN markedly decreased photosynthetic pigment contents and altered several photosynthetic traits (WUE and photosynthesis rate). They also revealed that the pictures of photosynthetic pigments, content and composition, are considered the poignant markers for nematode infection. Moreover, the decrease in photosynthetic values in plants infected with nematodes was also accompanied by proteolytic enzyme activities[36].

Plant endogenous hormones have a pivotal role in mediating plant defence against several environmental biotic and abiotic stresses. Our results showed that, IAA, ABA and GAS increased in both root and leaves of wheat plants infected with nematodes, while cytokinins as zeatin showed a marked decrease in both root and leaves of wheat infected plants as compared with healthy plants (Fig. 2). These findings may be a result of activating systemic defense versus

nematodes through altering antioxidant systems, hormone pathways (Fig. 4) and redox homeostasis (Fig. 3 and 5). In this regard Fraire-Velázquez[37] concluded that, the hormones act as part of a complicated network of synergistic and antagonistic interactions to overcome biotic stress conditions. Also, phytohormones such as IAA and ABA act as signalling molecules and alternate the protection response to biotic stress[38].

Accordingly Guo *et al.*[39] reported that, there is a close relationship between the hormone profiles, increase IAA content significantly in the leaf tissues, which trigger auxin synthesis and increase lower hypocotyl elongation in a plant grown under biotic stress conditions. Also, nematode infection increases root elongation as a result of auxin increments which are accompanied with enhances nutrient demand for cell division and differentiation at the early stage of nematode infection[40]. Moreover, Gheysen and Mitchum[41] proved that, infection with nematodes principally up-regulate auxin biosynthesis and auxin-responsive genes, encouraging the role of auxin at infected plants.

Regarding ABA, it interacts antagonistically with other hormones in plant defence against *Meloidogyne graminicola* and ruffles nematode-caused disease symptoms[42]. Also, inoculation with nematodes enhanced ABA content in leaves of watermelon and it has a little role in vindication of plants against RKN infection. GA is important for the susceptibility of the plant to *M. graminicola*[43]. On the other hand, GA treatment increases the resistance of plants against *nematodes*[44,45]. Cytokinins have a role in the amelioration of plant growth in response to biotic stresses and have a key role in plant-pathogen interactions[46]. In this regard, Kyndt *et al.*[47] reported that, alteration in cytokinin contents due to RKNs could be crucial for swelling of the root meristem and could be vital for the altering of galls into nutrient sinks. In this connection, Dowd *et al.*[48] reported that, both plant- and nematode produced cytokinins and added that, cytokinin signalling mutants and plants that reduced cytokinin levels become less susceptible to nematodes.

Invading of the plant by several pests leads to accumulation for primary metabolites, mainly carbohydrates and assumes the activation of a defense response necessitate promote the production of secondary metabolites[49]. Our results concluded that, TSS, total carbohydrate, total protein and total phenolic compounds were promoted in both root and leaves as a result of inoculation of wheat plants with CCN (Table 3). These findings may be results of these metabolites ameliorating the inhibitory effect of nematodes on the plant. In this connection, Singh *et al.*[50] reported that, plants under stress conditions produce sugars which act as essential signs for osmotic adjustment, carbon storage, osmoprotectant and scavenging of free radicals. Also, Korayem *et al.*[51] concluded that, total carbohydrate was significantly promoted in sunflower seeds infected with *M. arenaria*. In addition, Bali *et al.*[14] showed that several osmolytes (glycine betaine, proline and total carbohydrates) contents were stimulated during nematode infection. Metabolic pathways and synthesis of secondary metabolites were promoted in plants subjected to infection with endophytic fungi[52].

Connectively, CCNs relate to the host root for nutrition, alters in TSS contents demonstrate the survival requirements of it at different growth stages[53]. So, the assembling of soluble sugars in the plant leaves is parallel to the transport activity from the source (leaves) to the sink (roots)[54]. Also, reported that, a TSS increment was attributed to the induction of metabolic activity in infected tissues. Consistently, the results approved the value of TSS in fulfilling CCN energy requirements throughout initial infection in infected tomato plants[54]. They also concluded that, the TSS was promoted in tomato leaves and roots throughout initial infection by RKNs as a result of the hydrolysis of starch promoted by environmental changes, while an improvement in carbohydrates in the roots could be parallel to carbohydrate translocation from tissues of leaves to roots through transporters.

The increase in protein contents of infected root and leaves of the wheat plant may be attributed to the activation of several enzymes and different metabolites to tolerate the stress of CCN. This result was in agreement with those recorded by Yang *et al.*[55] who reported that, protein increased in biotic stressed plants due to improves redox homeostasis by modulating the activities of several antioxidant enzymes. Some proteins exempt naturally in the exudates of soybean seeds were found to have nematocidal properties against *M. incognita*[56]. These proteomic approaches and *in vitro* activity assays indicated the existence of 63 exuded proteins that are related to plant defence and able to reduce the hatching of nematode eggs and to cause 100 (%) mortality of second-stage juveniles (J_2). Recently, the results revealed that alterations in nitrogen metabolism consequently protein contents found in beet plants infected with the cyst nematode[57].

The stimulatory effect of nematode infection on the phenolic compound of both root and leaves of the wheat plant may be attributed to the regulatory role of these compounds under biotic stress conditions. In this regard, Yang *et al.*[58] postulated that, phenolic compounds were stimulated in grapevines plants infected with RKN and consequently alter root physiology of infected plants. The phenolic metabolism participates in obstructing pathogen growth and promoting host cell tolerance under unfavourable

stress conditions[14]. Additionally, some phenolic compounds have nematicidal effects to manage up the infections with RKN. Also, promotion in the phenols contents directly paralleled to the stress degrees as it gets participates on the cell wall to become more tougher against a pathogen attack so triggered the immune responses of plants to combat the mechanical injuries caused by pathogens[59,60]. Moreover, the synthesis of phenolic compounds restricts the nematodes movement and become the main reason for their morbidity[61].

It is worthy to mention that, ROS are included in several nematode physiological processes[62]. They have a role in cell signaling, at lower levels, whereas at higher levels they can be toxic via the promotion of oxidative stress causing oxidative damage to the cell-building molecules. MDA is considered an important stress trait for determining lipid peroxidation in plants grown under biotic or abiotic stresses and is accumulated due to the degradation of polyunsaturated fatty acids[63]. Our results cleared that, infection with CCN increased markedly Malondialdehyde (MDA) in both roots and leaves compared to non-infected wheat plants (Fig. 4). The same results were obtained at previous studies[14,64]. An overabundance of TBARs (MDA) under stress conditions indicates the damage caused as lipid peroxidation in leaves of barley plants were invaded with RKN. These give a direct impact on increased barley defense mechanisms against RKN[36,61].

Reactive oxygen species harm the plant tissues through stimulating signalling pathways in the infected plants. These ROS are affecting the membrane proteins, carbohydrates, photosynthetic apparatus, lipids and causing severe damage or even the death of cells. Plants have developed several defence strategies that induce immune responses through complex signalling networks and molecules, including defence related genes, Reactive Oxygen Species (ROS) and phytohormones[38]. Various antioxidative enzymes and non-enzymatic antioxidants are activated in infected plants to counteract the ROS effects. Antioxidative enzymes include Peroxidase (POX), Catalase (CAT) and Superoxide Dismutase (SOD)[61]. According to the impact of CCNs on the activities of some oxidative enzymes of wheat plants, (Fig. 4) showed that, the infection-induced significant increases in SOD activity in both wheat roots and shoots as compared to the non-infected plant. However, the infection-induced increase in POX activity in roots and showed a little decrease in shoots. The infection also decreased CAT activity in roots while, the opposite effect was recorded on wheat leaves as compared to the non-infected plant. In this study, the stimulation of the activities of SOD in both root and leaves, POX in roots and CAT in leaves are a good indication of the defence system of wheat plants to infection with the nematode. The decrease in the activities of POX in shoot and CAT in the root may be as a result of greater production of MDA at an infected plant which affects protein assimilation under such conditions. In this connection, the increases in the activities of different enzymes (APOX, CAT, GPOX, GST, PPO and SOD) in seedlings infected with *M. incognita* were reported by Gupta *et al.*[65] who concluded that, increase in the activity of SOD in infected plants, as a result of the internal immune response activation accompanied with the induction of systemic resistance. Also, Khajuria and Ohri[66] concluded that, the activities of CAT, GPOX, POD and SOD, etc., were promoted in nematode infected tomato plants.

In this connection, Lobna *et al.*[67] stated that, resistant cultivars have high POX and this increment may be related to lignification. The activity of CAT was significantly acted as a bifunctional enzyme, firstly, through catalysis decomposition of H_2O_2, secondly it can oxidize phenolic molecules with consumption of peroxides[68]. So, it can be summarized that both described systems (peroxidase-flavonoids and catalase-phenols) are involved in controlling MDA and H_2O_2 levels in barely leaves infected with the nematode. Moreover, stimulation of the activities of CAT, GuPOX, PPO and SOD were also found in tomato plants infested with nematodes[60]. These results could be due to the stimulation of protein content in plants after the augment of useful microbes, which leads to up-regulation of defence enzymes in the infected plants. Interestingly, the suggested function of the catalase-phenol activity is responsible for the variation of lipid peroxidation in infected plants that might partly be due to utilization of lipid peroxides by CAT[57] .

The mineral nutrients were fused in defence response to biotic stress generated by pathogen attack[67]. Regarding the mineral nutrition content of roots and leaves of the infected wheat plant, our results cleared that, the contents of all studied minerals (P, K, Fe, Mn, Zn and Cu) were decreased with infection as compared with non-infected plants. While, nitrogen content exhibited an opposite trend in the wheat shoot as compared with the non-infected plant. These results may be attributed to the unbalance of nutritional status due to alteration in mineral absorption and translocation under nematode conditions. The increase in N contents could be the natural result of different assimilation processes that occurred to ameliorate the stress of CCN. The same trend was observed in several infected plants[69], who concluded that, infection with nematodes cause various plant damages as alterations in the absorbent cells, with subsequent nutritional imbalance. Several nutrients had a direct role on plant diseases than others. K as Macro and Cu, Fe, Mn, Zn as micro-nutrients assumed the host susceptibility to pathogens: K[70], Cu[71], Fe[72], Mn[73] and Zn[74]. Moreover, Haase *et al.*[75] reported that, the

nematode-infection has a considerable effect on unsteady nutrients contents in roots via reducing the values of Cu, Fe, K, Mg, Mn and Zn. They also added that, nematode infection increased shoot biomass, N content of *H. vulgare*. These higher contents of N are thought to be related to nematode-induced leakage of plant-derived metabolites from damaged root cells. Moreover, the co-infection of pathogens reduced significantly the concentrations of K, Cu, Zn, Mn and Fe in the roots[67]. During infection, the accumulation of nutrients around the infection sites mineral was altered by which may be affected by susceptibility and resistance to pathogens. Recently, Abdel Sattar *et al*.[76] proved that, *M. incognita*-control treatments had significant effects on leaf petioles nutrient contents, they generally increased N, however, leaf potassium concentrations were below the optimum ranges.

Cereal cyst nematodes cause significant yield losses in many crops and also affect yield nutritional values[77]. Grain yields are often negatively correlated with the number of cereal cyst nematodes in soil[78]. Cereal cyst nematodes induced a reduction of yields in individual research trials or fields by about 20 (%) in Pakistan, 50 (%) in Australia, 50 (%) in Turkey and 90 (%) in Saudi Arabia[79,80]. Our results showed the infected plants decreased grain yield per plant by about 30 (%) compared to those of the control plant Table 6. Following our result, Ali *et al*.[81] reported that, nematodes induce 17-20 (%) yield losses in rice. Moreover, Dababat[82] documented that Egyptian populations of *H. avenae* are serious pests of Egyptian wheat cultivars. The loss in the grain yield ranged between 16-40 (%) under greenhouse conditions.

Our results also showed that, most nutritional values of yielded wheat decreased under biotic stress of CCN as compared with non-infected plants (Fig. 6). These results may be due to the inhibitory effects of CCN on the plant which start with the production of ROS as MDA (Fig. 4) that affect photosynthetic pigment production, chlorophyll a, chlorophyll b and carotenoids, (Fig. 1), altering endogenous phytohormones, IAA, ABA, GAs and Zeatin (Fig. 2), reconstruct the production of assimilates (TSS, Total carbohydrates and Protein) (Fig. 3). These variations stimulate the production of enzymatic antioxidants, SOD, CAT and POX (Fig. 4) and non-enzymatic antioxidant, phenol (Fig. 3). The infection also affects the absorption and translocation, production of minerals, P, K, Fe, Zn, Mn and Cu (Fig. 5). All of these changes reflected on the yield production and the nutritional values of yielded grain. In this regard, the possibility of yield reduction is attributed to the high population density of CCN attack root of tomato plants and impaired nutrient uptake and water absorption[83]. Also, wheat gall nematode causes significant yield losses and affects grain quantity and quality[84]. Moreover, Snider *et al*.[85] concluded that, the yield reductions are the result of anatomical and physiological changes that occur in the cotton root system as the nematode progresses through its lifecycle. In addition, Ali *et al*.[86] stated a strong correlation between nematode population and yield reduction, this correlation is affected by distribution and density of nematode at a particular location, crop variety, cultural techniques, prevailing climatic conditions and soil quality. Recently, Atia *et al*.[35] proved that infested plants with RKN decreased fruit yield and plant growth.

CONCLUSION

Generally, wheat plants infected with CCN exhibited several physiological and biochemical responses reflected not only on the yield production but also, lower the nutritional values of yielded grain. These responses started with high production of ROS and MDA in both roots and leave that parallel with lower production of photosynthetic pigment contents and alteration in the endogenous phytohormones, alteration in assimilate production and enzymatic and non-enzymatic antioxidant compounds. The infection also alters the absorption, translocation and production of minerals. Wheat plants use all of these responses to survive under these biotic stress conditions.

SIGNIFICANCE STATEMENT

This study discovers the effect of nematodes on wheat plants and several relations at physiological and biochemical levels and their effects on yield and nutritional values on produced grains. This can be useful in the biological control of nematodes that could be achieved through many biological and safe applications on the environment and on plants as well.

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