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Impact of nematode infestation on nutritional quality of some underground vegetables in Pakistan

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Abstract

During the present investigation the nutritional quality of underground vegetables infected by root-knot nematode (*Meloidogyne incognita*) was evaluated. It was observed that physiological and biochemical changes occurred due to the invasion of root-knot nematodes in the studied vegetables. Results show that there was a significant difference in root-knot development and reproduction in infected and un-infected host plants. Data indicates that highest reproduction rate and root-knot index was observed in vegetable plants infected with root-knot nematodes after three months as compared to un-infected (control). The physiological parameters as well as biochemical contents showed significant difference in different growth criteria and amount of nutrients between infected host plants as compared to un-infected plants (control). Growth parameters of studied vegetable plants *viz*., fresh and dry weight and water content were decreased by the infection of root-knot nematode as compared to un-infected (control) plants. Similarly root-knot nematode decreased the host nutrients contents *viz*., total carbohydrates, total soluble sugars, total protein, total phenols and amino acids. Chlorophyll a, chlorophyll b and carotenoids also decreased in nematode infected plants as compared to control. This study gave the fair report towards nutritional quality because no significant work has been done so far on this aspect in Pakistan.

Key words: Nutritional quality, underground vegetables, root-knot nematode, Pakistan.

Vegetables crops are affected by nematode pests which cause varied amount of losses. Root-knot nematodes are economically important group of phytonematodes in vegetables around the world affecting both the quantity and quality of marketable yields. Besides RKN number of plant parasitic nematodes species have also been recorded from the rhizospheres and roots of underground vegetable crops causing sizeable damage. The estimated average crop yield losses due to root-knot nematode were about five percent in vegetables (Taylor & Sasser, 1978). Roots system of vegetables may be deformed and underground vegetables such as carrot, potato may be damaged and become unmarketable (Sikora & Fernandez, 2005; Bridge & Starr, 2007). Johnson (1992) reported eleven percent yield loss in vegetables in USA alone. Root-knot nematodes are member of the genus *Meloidogyne* (Goeldi, 1892). They are obligate plant parasites having worldwide distribution and parasitize nearly every species of higher plant (Moen *et al*., 2009). The *Meloidogyne* infection induces extensive galling and root damage; cause serious agricultural damage (Trudgill & Blok, 2001); affects water and nutritional uptake and alters upward translocation of minerals and photosynthates by the root system. By disrupting the host plant physiology, root-knot nematodes may not only reduce crop yield but also product quality and therefore are of great economic and social importance (Greco & Divito, 2009). As sedentary endoparasites, *Meloidogyne* spp., induce specialized feeding structures within their host roots known as giant cells, essential to the nutrition and development of the nematode (Bird, 1961).

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Materials and Methods

Test Plants: The underground vegetables *viz*., radish (*Raphanus sativus* L.), turnip (*Brassica rapa* L.), carrot (*Daucus carota* L.), and sugar beet (*Beta vulgaris* L.) were used in the present study.

Culturing Method: The isolated culture of root-knot nematode was maintained in pots by using brinjal (*Solanum melongena* L.) cv. Pusa purple as a host in sterilized soil in the green house. After two months of inoculation, the nematodes were extracted by harvesting brinjal roots. These harvested nematodes were used as starter inoculums for the experiment.

Experimental Location: Greenhouse experiments were conducted to evaluate the effect of root-knot nematodes on physiological and biochemical parameters of some underground vegetables. For this purpose experiments were laid out at green house of National Nematological Research Centre (NNRC), University of Karachi, Karachi. Experiments were conducted in 2014. The experiments were maintained weed free by regular hand weeding.

Experimental Method: Three seeds of plant were sown in earthen pots containing 5 kg of steam sterilized soil. After 15 days of sowing, seedlings were inoculated with 1000 2nd stage J_2 larvae of *Meloidogyne incognita* per pot, respectively, by drenching with water containing inoculum. Treatment has got 3 replicates. After 45 and 90 days, 10 randomly selected plants were uprooted gently from pots and observations were taken. Further, development and reproduction of *M. incognita* was also determined by examining number of galls, number of egg - masses, number of larvae and females per root system at above mentioned time intervals. The root-knot index graded on 0-5 scale (Taylor & Sasser, 1978) was also assessed at harvest. Uninoculated pots were served as control. Pots were watered as required. The initial juvenile population of *M. incognita* was recorded before sowing of seeds.

Experimental Design and Statistical Analysis: The experiment was arranged in complete randomized design (CRD) and the results were statistically analyzed using MSTAT-C (1988) software. The mean comparisons among treatments were determined by Duncan's multiple range tests at 5 % level of probability.

Physiological Parameters: Plants were gently uprooted at the end of experiment and following physical parameters were taken into account: i) length of shoot; ii) length of roots; iii) fresh and dry weights of shoot; iv) fresh and dry weights of roots; v) water content; vi) initial and final population of infective juveniles (J_2) of root-knot nematodes; vii) assessment of root-knots or galls: Root-knot index (RKI) was assessed by 0 to 5 scale (Taylor & Sasser, 1978), where, 0 = No galls, 1 = 1-2 galls; 2 = 3-10 galls; 3 = 11-30 galls; 4 = 31-100 galls and 5 = > 100 galls per root system.

Biochemical Parameters: Following methods were used for biochemical analyses:

- Estimation of carbohydrates (Yemm & Willis, 1954)
- Estimation of glucose (Riazi *et al*., 1985)
- Estimation of sucrose (Riazi *et al*., 1985)
- Estimation of total soluble sugar (Riazi *et al*., 1985)
- Estimation of total protein (Lowry *et al*., 1951)
- Estimation of amino acid (Moore & Stein, 1948)
- Estimation of proline (Bates *et al*., 1973)
- Estimation of chrolophyll a & b (Maclachlan & Zalik, 1963)
- Estimation of carotenoides (Maclachlan & Zalik, 1963)
- Estimation of Indole acitic acid (Larsen *et al*., 1962)
- Estimation of total phenolic compounds (Ainsworth & Gillespie, 2007)
- Estimation of lipid peroxidase (Predieri *et al*., 1995)
- Estimation of enzyme activities (Chen & Wang, 2006)

Results and Discussion

Present investigations were pertaining to the physiochemical and biochemical parameters of underground vegetables in relation to the pathogenecity of root-knot nematode, *Meloidogyne incognita*.

Radish

Physiological Parameters: Growth parameters of radish viz., fresh and dry weight and water content were non-significant by the Infection of root-knot nematodes as compared to healthy (control) plants; however, slight significant difference was observed in shoot and root length after 45 and 90 days though the difference was more pronounced after 45 days (Table 1). The severity of root-knot disease of underground vegetable crops was assessed by the presence of root-knots or galls on the plant root systems; egg-masses per root system; second stage juvenile population in soil and their reproduction factor. Data presented in Table (2) shows that there were significant variations in different growth criteria between infected and non infected plants and time period (after 45 and 90 days). Data indicates that highest reproduction rate (1.65) and root-knot index (3.0) was observed in plants infected with root-knot nematodes after 90 days.

The number of galls per plant was significantly increased in nematode infected plants as shown by the degree of root-knot index. Root-knot index (RKI) ranged from 2.5-3.0 after 45-90 days, respectively. J_2 population in 250 g soil was significantly increased in nematode infected plants; it was minimum (611) and maximum (828) after 45-90 days, respectively. The R factor ranged between 1.22-1.65 in radish after 45-90 days

Table 1. Physiological parameters of radish.

Parameters	45 days		90 days		LSD
	Infected	Control	Infected	Control	
Length (cm)					
Root length	2.06a	5.01b	2.55a	5.52b	0.98
Shoot length	11.0a	16.0b	12.27a	17.07b	1.65
Fresh weight (g)					
Shoot	7.28a	13.99a	8.92a	15.92b	5.56
Root (Bulb)	46.19a	41.74a	47.29a	44.64a	9.22
Dry weight (g)					
Shoot	0.58a	0.82a	1.28a	1.22a	0.12
Root	2.30a	2.29a	3.39a	2.59a	0.85
Water content					
Shoot	90.90a	93.26a	92.62a	94.93a	12.45
Root	92.63a	95.0a	94.34a	97.02a	13.43

Table 2. Development and reproduction of *Meloidogyne incognita* on radish.

Duration	No. of galls/ root system	No. of egg-mass/ root system	RKI*	J_2/250g soil	Rf=Pf/Pi*
45 days	10	52	2.56	611.3	1.22
90 days	20	71	3.0	828.0	1.65

*RKI = Root-knot index; Rf = Reproductive factor; Pf = Final population; Pi = Initial population.

respectively (Table 2). Occurrence of the root-gall disease in vegetables may cause variable growth parameters of plants as reported earlier by different workers (Anamika *et al*., 2011; Sikora & Fernandez, 2005).

Biochemical Parameters: The root-knot nematodes decrease the host contents of total carbohydrates, total soluble sugars, total protein, total phenols and amino acids. Generally rate of reduction was observed more in roots as compared to shoots which may depend on host plant, nematode species and its population in soil.

Carbohydrates: There was a significant difference in amount of carbohydrates in all parts viz., shoot and roots between infected plant and control at both time periods (45 and 90 days). Carbohydrate was more in shoot than root (Table 3). The decreased amount of carbohydrates in roots of nematode infected plants might be due to the rapid consumption of these substances by nematode (Basu & Sukul, 1983). Decrease amount of carbohydrates in roots resulting from nematode infection has been reported in other plants also (Owen & Specth, 1966; Singh *et al*., 1978; Nasr *et al*., 1980). Our results were in accordance with these earlier studies (Table 3). Parasitic plant pathogens depend on their roots for carbohydrates and other resources they have enormous potential to manipulate source-sink balance (Bockenhoff *et al*., 1996; McClure, 1977). According to Bird & Loveys (1975) and McClure (1977) *Meloidogyne* function as metabolic sink in infected plants and that is photosynthates are mobilized from shoot to roots particularly to giant cells. Giant cells are capable of absorbing nutrients but the plants with many giant cells cannot translocate adequate amount to vegetative organs. Thus a prolonged parasitism leads to reduced nutrient location (Ediz & Dickerson, 1976; Byrne *et al*., 1977; Finley, 1981). Suppression of photosynthesis by nematodes has been reported by earlier researches (Loveys & Bird, 1973; Schans, 1991). Decrease in photosynthesis of tomato occurred within two days following root parasitism by the root-knot nematodes, *Meloidogyne* spp. and 4000 nematodes per plant could significantly reduce photosynthesis (Bird, 1974).

Glucose: It was observed that amount of glucose (reducing sugars) decreased in shoot and there was a non significant difference between infected and control plants but significant difference was observed in *M. incognita* infected roots and control at different time periods (Table 3) whereas Sharma & Trevidi (1996) observed increasing trend of reducing sugars in infected roots than healthy plants. Similarly Farooqi *et al*., (1980) reported an increase in total sugar and reducing sugar content in root-knot nematode infected tomato plants. However, compared to sucrose, the low endogenous contents of reducing sugars indicate that they were quickly metabolized. High amount of reducing sugars might be due to increased root respirations. Root respiration increased has been observed with compatible and incompatible nematode plant interactions and was higher in the former (Poskuta *et al*., 1986).

Sucrose: Sucrose (non reducing sugars) decreased in infected roots over corresponding healthy (control) roots. Significant difference was observed in different parts *viz*., shoot and roots at 45 days and at harvest (90 days) (Table 3), whereas increased sucrose level was reported in giant cells by Hoffman *et al*., (2007).

Total Soluble Sugars: Total soluble sugars decreased considerably in shoot as a significant difference was observed; however, a non significant difference was observed in roots of infected and healthy plants after 45 and 90 days (Table 3). Our result was not in accordance with the earlier findings who stated that the infection of nematodes caused increase in sugar contents and it was also observed that increased sugar is helpful for the survival of nematodes. The reason for the increase in content of sugars in the infected shoot was may be due to the degradation of starch or inhibition of starch synthesis from sugar (Owens & Specht, 1966; Dropkin, 1969). Farooqi *et al*., (1980) reported an increase in total sugar and reducing sugar

content in root-knot nematode infected tomato plants. Increase sugar level enhanced nematode development (Grundler *et al*., 1991). Hoffman *et al*., (2007; 2008) and Nayak & Mohanty (2010) reported that the increased sugar and protein levels are due to high metabolic activity in discard tissues. High starch and total sugars due to higher metabolism was reported earlier (Jena & Rao, 1977); also reduction in uptake of nitrogen caused an increase in sugars (Prasad & Rao, 1981).

Protein: Amount of protein increased in all parts in infected plants as compared to control. There was a significant difference in shoot but non significant difference was observed in roots of infected and control plants (Table 3). Total protein increased over the control in roots infected by root-knot nematode after 30-90 days of inoculation (Singh *et al*., 1978). This biochemical parameter can therefore be used as an useful indicator to measure the level of root-knot infection of host plants (Chatterjee & Sukul, 1981). In healthy roots there was a gradual increase in protein till 60 days and later on decreased when observed after 90 days (Sharma & Trevidi, 1996) but we observed decreased amount of protein after 45 and 90 days. Changes in the amount of protein and structural components such as cellulose and hemicelluloses would be expected to be measureable and diagnostic (Hedin & Creech, 1998).

Amino Acid: Amount of amino acid decreased in shoot and root of healthy plants as compared to infected plants. There was a non significant difference in shoot and root of infected and control plants (Table 3). The lesser increase in nematode resistant root may be indicative of a lower rate of protein biosynthesis in response to infection of root-knot nematodes (Hedin & Creech, 1998). RKN cause measureable changes in the morphology and physiology of the host (Williamson & Gleason, 2003). Similar observations were also recorded earlier by many workers (Mohanty & Pradhan, 1990; Swain & Prasad, 1991; Mohanty *et al*., 2001; Mishra & Mohanty, 2007; Tripathy & Mohanty, 2008, 2009). The increase in various amino acids and amides following nematode infections may possibly due to progressive proteolysis of existing tissue protein or synthesis of new compounds through various metabolic sequences (Sireesha *et al*., 2014). Root damage from nematode resulted in stunted and chlorotic plants. A decreased amount of total carbohydrates, an increased amount of reducing sugars and total free amino acids have also been reported in root-knot nematode infected roots (Basu & Sukul, 1983; Sharma & Trivedi, 1996).

Total Phenols: There was a significant difference in total phenols in shoot and root between infected and control plants (Table 3). Infected plants showed lesser amount of phenols as compared to healthy ones. As plant increase there was a corresponding increase in phenol contents in both infected and healthy plants; the increase was more pronounced in 90 days old roots (Sharma & Trevidi, 1996). Phenolic compounds have been associated with nematode injury including browning of plant tissues (Acedo & Rhode, 1971) but the role of phenolic substances in both resistant and susceptible reactions is not well understood. Their accumulations in the cells damaged by nematode feeding have been reported by various workers (Acedo & Rohde, 1971; Khaberman, 1972; Valette *et al*., 1998). Increase in the concentration of free phenols following infection of *M. incognita* was also reported by Ganguly & Dasgupta (1984); Bajaj *et al*., (1985) and Gapasin *et al*., (1988). The increase in phenols helps in the formation of hypersensitive reaction towards the nematode infection (Shukla & Chakraborty, 1988; Mazzafera *et al*., 1989). The early accumulation of phenolic compounds at the infection site is reported as a result of the rapid hypersensitive death of cells (Fernandez & Heath, 1989).

Chlorophyll a & b and Carotenoids: Chlorophyll a, chlorophyll b and carotenoids of nematode infected plants have decreased in nematode infected plants as compared to control. Significant difference was observed between infected and healthy ones in all parts of plant (Table 4).

Table 3. Biochemical parameters of radish.

Parameters	45 days		90 days		LSD
	Infected	Control	Infected	Control	
Carbohydrate (mg/g)					
Shoot	22.53a	74.64b	24.57a	83.26b	20.33
Root (Bulb)	17.77a	31.85b	18.56a	32.61b	5.45
Glucose (mg/g)					
Shoot	3.12a	4.78a	4.12a	5.39a	1.33
Root	3.86a	6.34b	4.94a	7.22b	1.97
Sucrose (mg/g)					
Shoot	106.46a	132.66b	106.9a	133.28b	12.71
Root	77.4a	121.48b	78.31a	122.53b	16.87
Total Soluble Sugar (mg/g)					
Shoot	65.95a	95.75b	66.4a	96.01b	18.12
Root	78.84a	81.05a	79.09a	81.61a	11.28
Protein (mg/g)					
Shoot	86.96a	37.17b	90.18a	38.83b	13.32
Root	35.88a	34.08a	37.88a	36.40a	1.22
Amino acids (mg/g)					
Shoot	2.31a	1.08a	2.62a	1.27a	0.32
Root	0.36a	0.22a	0.42a	0.30a	0.04
Phenol (mg/g)					
Shoot	86.7a	95.36b	86.87a	96.95b	8.98
Root	95.51a	105.76b	97.48a	106.26b	17.23

Table 4. Pigment quantification of radish.

Pigments	45 days		90 days		LSD
	Infected	Control	Infected	Control	
Carotenoids	0.26a	0.89b	0.29a	0.92b	0.01
Chl a	0.16a	0.60b	0.23a	0.68b	0.00
Chl b	0.13a	0.47b	0.17a	0.705b	0.01

Turnip

Physiological Parameters: Growth parameters of turnip *viz*. shoot and root length, fresh and dry weight of shoot and root, respectively and water content of shoot have a significant difference between the infection of root-knot nematodes as compared to healthy (Table 5). The severity of root-knot disease of underground vegetable crops was assessed by the presence of root-knots or galls on the plant root systems, egg-masses per root system; second stage juvenile population in soil and their reproduction factor. (control) plants; however, no significant difference was observed in other parameters after 45 and 90 days though the difference was presented in Table (6). Data indicates that highest reproduction rate (1.67) and root-knot index (3.32) was observed in plants infected with root-knot nematodes after 90 days. The number of galls per plant was significantly increased in nematode infected plants as shown by the degree of root-knot index. Root-knot index (RKI) ranged from 3.0-3.3 after 45-90 days, respectively. Plants infected with root-knot nematodes; it was minimum (717) and maximum (836) after 45-90 days, respectively. The R factor ranged between 1.43 - 1 .67 after 45 - 90 days, respectively

(Table 6). Results (Table 5) show that water content in parts of plant viz., shoot and root (roots) hence plants infected with nematodes show the sign and symptoms similar to drought stress. of infected plants were reduced. The attack of root-knot nematode causes mechanical injury to the root tissues thereby reducing thewater absorption efficiency of the host roots (Alam *et al*., 1974). The growth suppressions may be due to osmotic reduction in water availability cause dehydration of the cells and less turgid roots show decrease water contents.

Table 5. Physiological parameters of turnip.

Parameters	45 days		90 days		LSD
	Infected	Control	Infected	Control	
Length (cm)					
Root length	4.1a	7.2b	5.26a	7.76b	1.11
Shoot length	17.0a	21.4b	18.06a	22.48b	0.98
Fresh weight (g)					
Shoot	21.56a	26.43a	24.45a	31.59a	2.39
Root (Bulb)	35.70a	32.0a	40.87a	36.22b	3.01
Dry weight (g)					
Shoot	2.10a	3.28a	2.45a	3.86a	0.33
Root	6.29a	0.68b	12.61a	1.05b	
Water content					
Shoot	90.22a	93.57a	91.10a	94.27a	11.69
Root	90.59a	94.2a	91.94a	95.36a	12.88

Table 6. Development and reproduction of *Meloidogyne incognita* on turnip.

Duration	No. of galls/ root system	No. of egg-mass/ root system	RKI*	J_2/250 g soil	Rf= Pf/Pi
45 days	19	76	3.0	717	1.43
90 days	34	89	3.32	836	1.67

Nutrient and water uptake are substantially reduced because of the damage root system resulting in weak and low yielding plants (Abad *et al*., 2003).

Biochemical Parameters: The data given in Table 7 regarding the biochemical parameters after 45 and 90 days of infection, all the parameters were decreased in shoot and root due to nematode infection in turnip plants except amino acid; significant difference was found between infected and healthy plants after 45 and 90 days which indicated stress induced by nematodes.

Carbohydrates: Amount of carbohydrates decreased in shoot and roots of infected plants as compared to control as significant difference was found between them (Table 7). Similar conditions had been met by other researchers; Sharma & Trevidi (1996) and Saxena & Singh (2001) also reported decrease carbohydrate content in the diseased root as compared to normal. In nematode infection there was a leakage of metabolites from roots (Wallace

1974). Nematodes act as nutrient sinker (Bird & Loveys, 1975). Several investigators have documented that infections of plants by plant parasitic nematodes initiates a rise in metabolic activities (Gangualy & Dasgupta, 1981; Patel & Patel, 1991). Reduction of carbohydrates was also observed in other plants susceptible to nematode infection (Nasr *et al*., 1980; Singh *et al*., 1980).

Glucose: Data presented in Table 7 showed that by the infection of root-knot nematode, there was a significant difference in the glucose content in both parts of the infected plants than control after 45 days. Amount of glucose (reducing sugar) showed an increasing trend in infected roots as compared to uninfected roots as reported by earlier researchers (Pandey & Trevidi, 1991; Sharma & Trevidi, 1996) (Table 7). However, Singh *et al*., (1978) and Sharma & Trevidi (1987) reported reduction in reducing sugars in the diseased tissues infected with *M. incognita*.

Sucrose: Significant difference was observed in sucrose (non reducing sugar) amount in infected plants at 45 days and 90 days over corresponding uninfected plants (Table 7). Such similar result was found by earlier researches (Sharma & Trevidi, 1996).

Total Soluble Sugars: Root-knot nematodes decrease the content of total soluble sugars in both parts (shoot and root) of turnip plants than control as significant difference was observed among them (Table 7). Kannan (1968) reported reduction in sugar values in the root-knot nematode infected plant as compared to the uninfected as observed in turnip plants. Tayal & Agrawal (1982) observed that diseased tissues showed increased activities of enzymes that cause low contents of total sugars. Reports of Kannan (1968) and Tayal & Agrawal (1982) were in accordance with the present study. Mohanty *et al*., (1997) reported increased sugar content in the RKN inoculated roots, due to the movements of various metabolites towards the infection site from the other parts of plants. Hofmann *et al*., (2008) and Nayak & Mohanty (2010) reported that the increase sugar levels were due to high metabolic activity in diseased tissues. Yuhara (1971) found increase in total sugar content on increasing concentrations of nutrients in RKN infected sugar beet.

Protein: Infected plants and control have significant difference in amount of protein in both parts; it decreased in shoot and root (root) after 45 days and 90 days (Table 7). This pattern is similar to control (Table 7). Results are in accordance with the earlier findings that there was a marked increase in protein and amino acids in galled roots (Alam *et al*., 1976). The giant cell cytoplasm contained up to 10 times more protein than normal cell cytoplasm (Bird, 1996). The galled root protein level was directly proportional to the root gall number as well as the population size of nematodes around the roots of infected plants. Protein increased in shoots and roots of nematodes infected plants, confirming earlier reports (Jena & Rao, 1977).

Amino acids: Significant difference in amount of amino acids was found in shoot only in infected and control plants at both time period (45 and 90 days) where as root showed non significant difference between infected and control after 45 and 90 days. Reduction in amount of amino acids was found in nematode infected plants as compared with control. Similar observations were also recorded earlier by many workers (Mohanty *et al*., 2001; Mishra & Mohanty, 2007; Tripathy & Mohanty, 2008, 2009; Sireesha *et al*., 2014). This increase in amino acids in nematode infected plants may possibly due to synthesis of new compounds through various metabolic sequences (Sireesha *et al*., 2014).

Total Phenols: Amount of phenols decreased in shoots more than in roots and it was increased in root as compared to control. Statistically significant difference was found in shoot only of infected as well as control

Table 7. Biochemical parameters of turnip.

Parameters	45 days		90 days		LSD
	Infected	Control	Infected	Control	
Carbohydrate (mg/g)					
Shoot	33.34a	76.04b	36.06a	38.26a	3.24
Root (Bulb)	29.74a	70.75b	31.15a	35.64a	2.92
Glucose (mg/g)					
Shoot	43.43a	36.45b	44.23a	36.8b	4.65
Root	46.48a	39.47b	47.53a	41.42b	4.56
Sucrose (mg/g)					
Shoot	96.35a	121.72b	96.5a	122.78b	13.28
Root	85.35a	88.94a	86.59a	90.87a	9.56
Total Soluble Sugars					
Shoot	82.87a	113.00b	83.17a	114.33b	
Root	110.29a	127.37b	111.59a	128.18b	12.76
Protein (mg/g)					
Shoot	182.13a	121.72b	183.03a	124.54b	15.34
Root	94.88a	69.38b	95.05a	70.19b	6.65
Amino acids (mg/g)					
Shoot	2.01a	0.88b	3.44a	0.95b	0.10
Root	0.39a	0.36a	0.47a	0.44a	0.11
Phenol (mg/g)					
Shoot	50.68a	75.56b	51.23a	76.70b	7.34
Root	90.48a	92.14a	90.91a	93.96a	11.98

plants after 45 and 90 days while there was a non significant difference in phenols between infected and control plants at both time periods (Table 7). The role of phenolic compounds in the defense mechanism of the plants has been reported by many workers (Acedo & Rohde, 1971; Vellete *et al*., 1998). Accumulation of phenol in plants has been reported as a possible resistance factor to nematode infection (Kerry, 2000). High amount of phenolic acids in infected plant due to stress induced by pathogens was reported by Singh *et al*., (2013). According to Mishra & Mohanty (2007) maximum phenolic acids were observed in susceptible and resistant cultivars as compared to healthy plants. Patel *et al*., (2001) reported that *Meloidogyne* spp. have ability to induce synthesis of peroxidase, polyphenol oxidase and total phenol in roots of chickpea (*Cicer arietinum*) but the leaf chlorophyll content was decreased. Rao *et al*., (1988) showed nutritional deficiencies such as total sugar, protein, IAA, cytokinin and thyamine and phenol due to infection by *Heterodera oryzicola* and *M. graminicola*.

Chlorophyll a, b and carotenoides: Chlorophyll a, b and carotenoides were reduced in all parts of infected turnip plant as compared to control and significant difference was found between them (Table 8). Results are in accordance with the findings of earlier researchers who stated that chlorophyll was reduced due to root-knot-nematode incidence (Korayem *et al*., 2012).

Table 8. Pigment quantification of turnip.

Pigment	45 days		90 days		LSD
	Infected	Control	Infected	Control	
Carotenoids	0.22a	0.62b	0.26a	0.65b	0.00
Chl a	0.13a	0.59b	0.15a	0.62b	0.00
Chl b	0.09a	0.32b	0.12a	0.34b	0.00

Carrot

Physiological parameters: Physiological parameters of carrot were examined of infected and healthy plants infected by RKN after 45 and 90 days. Significant results were obtained for root length after 45 and 90 days as compared to control whereas in shoot length difference was seemed to be negligible (Table 9).

Results for fresh weight of root have shown considerable difference between infected and control plants after 45 days and these results persist even after 90 days. Similar results were obtained for fresh weight of shoot after 45 days but after 90 days significant differences were found between infected and control plants (Table 9).

Similar pattern of dry weight data obtained for root and shoot after 45 and 90 days (Table 9). Data for reproduction rate and RKI were inversely proportional to the observed parameters of infected plants i.e. as the R_f and RKI increased the observed physical parameters were decreased after both time intervals (Table 10).

Biochemical Parameters: Plants have the potential to generate a high diversity of natural products with a defined function in the defense system against predators and pathogens. Pre-existing physical and chemical barriers are the part of these mechanisms, as well as inducible protection response in the form of induction of defense- related enzymes that become activated upon pathogen infection. In parasitic nematodes, they are particularly crucial for digestion of host tissues and evasion of host immune responses.

Carbohydrates: The quantity of carbohydrates was found to be decreased in shoot system of infected plants after 45 and 90 days whereas, reciprocal results were found for root system after 45 days but the difference between carbohydrate quantity was found to be negligible after 90 days in infected as compared to control plants (Table 11).

Glucose: There was a significant difference in the glucose content of both studied parts of carrot in infected plants than control in both time intervals (Table 11).

Sucrose: Data presented in Table 11 showed that by the infection of RKN, significant differences were found in sucrose level after 90 days (Table 11).

Protein: Similar trend was found for protein amount in infected plants as compared to the control one i.e. significantly decrease level was observed in infected plants (Table 11).

Phenol: Phenol amount was seemed to be non-significant between healthy and infected plant in root part whereas significant difference was observed for upper ground part of plant after 45 and 90 days (Table 11).

Chlorophyll, a, b and Carotenoids: Pigmentation assessing results were obtained after 45 and 90 days with significant differences between infected and control plants (Table 11).

Table 9. Physiological parameters of carrot.

Parameters	45 days		90 days		
	Infected	Control	Infected	Control	LSD
Length (cm)					
Root (Bulb)	11.09a	16.12b	15.89a	29.12b	0.89
Shoot	25.22a	29.43a	45.34b	49.32b	2.67
Fresh weight (g)					
Root	123.32a	176.23b	137.23a	215.22c	6.34
Shoot	121.32a	197.54b	267.34b	395.19c	10.33
Dry weight (g)					
Root	97.34b	98.34b	127.56c	193.23d	17.23
Shoot	75.96a	103.32b	112.18c	247.23d	23.48

Table 10. Development and reproduction of *Meloidogyne incognita* on carrot.

Duration	No. of galls/ root system	No. of egg-mass/root system	RKI*	J_2/250 g soil	Rf=Pf/Pi*
45 days	14	54	2.16	2344	2.3
90 days	23	110	2.45	3492	3.4

Table 11. Biochemical parameters of carrot (in 100g fresh wt).

Parameters	45 days		90 days		LSD
	Infected	Control	Infected	Control	
Carbohydrate (mg)					
Shoot	32.32a	43.04a	72.06b	93.49b	6.29
Root (Bulb)	65.14a	56.95a	185.15b	196.56b	10.36
Glucose (mg)					
Shoot	32.43a	43.45b	32.23a	47.8b	2.65
Root	76.48a	84.47b	146.53c	188.42d	5.16
Sucrose (mg)					
Shoot	14.35a	16.72a	44.53b	53.78b	0.28
Root	65.35a	78.94a	96.59a	117.87a	1.56
Protein (mg)					
Shoot	0.12a	0.56b	0.76a	1.54b	0.34
Root	0.98a	1.78b	1.25a	1.97b	0.65
Phenol (mg)					
Shoot	50.68a	75.56b	51.23a	76.70b	7.34
Root	90.48a	92.14a	90.91a	93.96a	11.98
Pigmentation					
Carotenoids	234a	422b	276a	345b	345
Chlorophyll a	654a	1243b	790a	1010b	1010
Chlorophyll b	134a	245b	234a	472b	472

Sugar beet

Physiological Parameters: The present study showed decreased overall growth of sugar beet evaluated under greenhouse conditions. Plant lengths (root and shoot) significantly affected by the infection of root-knot nematode at early stage i.e. after 45 days. After 90 days root length seems to be of equal length of infected and control plants whereas shoot length was under the influence of root-knot infection (Table 12). Fresh weight of root and shoot was determined for root-knot infection. Time interval has significant influence on infected and non infected plant except shoot weight after 45 days. Similarly, dry root weight of infected and control plants has shown non significant results whereas dry shoot weight has significant results after 45 and 90 days (Table 12). Previous studies *viz*., El-Sherif *et al*., (2014) and Chandra *et al*., (2010), have also shown that *M. incognita* infection causes decrease in different growth parameters like root and shoot length, weight of fresh and dry root and shoot in different plants. Our findings also confirms the decreased growth parameters and increased number of gall formation i.e., inversely proportional to each other as earlier stated by Khan *et al*., (2013). However, root and shoot weight showed gradual increase up to 45 days and after that it fell lower than the control plant. These findings are in agreement with the findings of Lynd & Ansuman (1989) and this may due to the formation of giant cells in the roots and lower part of the shoot regions. In the present study, we confined the inoculation level 1000 J_2/plant as the previous study by Kesba (2011) revealed that the density of *M. incognita* decrease after attaining a certain level of inoculation. It has been found that the number of galls per root system, number of egg-masses per root system, J_2 per g of soil was increased steadily after 45 and 90 days which could be easily predicted from RKI and R_f obtained values (Table 13).

Biochemical Parameters: Sugar beet plants considered to be the targeted victim of various diseases like wilt, leaf blight, viral infection and nematode infection among nematodes, root-knot [*Meloidogyne incognita* (Kofoid & White) Chitwood], is the most important and resulting in a substantial loss (Pandey *et al*., 2009). Nematode infection is measured one of the severe encounters facing the expansion of sugar beet. The second stage juveniles of *M. incognita* reach the roots and move towards the vascular cylinder, speed up metabolic activities in the roots, and boost nutrient transport program towards the induced root gall (Bowler *et al*., 1991; Cavalcanti *et al*., 2007). The galled roots are incapable to take up water and nutrients consequently show stunting of the plant and the severely affected plants and often wilt readily (Anwar & Din, 1986). The disruption in uptake pathway eventually decreases the host content of carbohydrates, glucose, sucrose, total protein and total phenols. In this study more reduction has been observed in the root content as compared to the shoot system which reflects the resistance capability of host towards its pathogen (root-knot species), and its population.

Carbohydrates: Significant difference has been found in amount of carbohydrates in parts viz., shoot and roots between infected plant and control. It has also been observed that the amount of carbohydrate increased non significantly after 45 and 90 days in all observed parts of plant. It shows that the root-knot infection effect the nutrient content after 45 days which constantly disrupted even after 90 days (Table 14). It is reported in other crops as well that decrease amount of carbohydrates in roots was due to nematode infection (Owen & Specth, 1966; Singh *et al*., 1978; Nasr *et al*., 1980).

Glucose: The amount of glucose decreased significantly in the upper part of plant (shoot) as compared to roots. The significant difference between infected and control plants was observed at both time periods, 45 and 90 days (Table 15). Sharma & Trevidi (1996) observed reciprocal results of reducing sugars in infected roots than healthy plants i.e., increase of reducing sugar in infected plants. Similarly Farooqi *et al*., (1980) reported an increase in

total sugar and reducing sugar content in root-knot nematode infected tomato plants.

Sucrose: The amount of sucrose in shoot and roots were traced with non-significant difference over corresponding healthy (control) shoot and roots (Table 14). In previous studied the raised sucrose level was reported due to giant cells (Hofman *et al*., 2006; Baldacci-Cresp *et al*., 2012). It was reported that the increase level of sugar has the potential to improve nematode development (Grundler *et al*., 1991).

Protein: Amount of protein increased in all parts in infected plants as compared to control. There was a significant difference in shoot but non significant difference was observed in roots of infected and control plants (Table 14). Total protein increased over the control in roots infected by root-knot nematode after 30-90 days of inoculation (Singh *et al*., 1978). This biochemical parameter can therefore be used as an useful indicator to measure the level of root-knot infection of host plant (Chatterjee & Sukul, 1981). In healthy roots there was a gradual increase in protein till 60 days and later on decreased when observed after 90 days (Sharma & Trevidi, 1996) but it was observed that decrease amount of protein after 45 and 90 days. Changes in the amount of protein and structural components such as cellulose and hemicelluloses would be expected to be measureable and diagnostic (Hedin & Creech, 1998). Infection caused by pathogen and various abiotic stresses that lead to over assemblage of reactive oxygen species (ROS). These are believed to be the source of damage to proteins, lipids, carbohydrates and DNA resulting in oxidative stress (Leng *et al*., 2009).

Chlorophyll a, b and Carotenoides: Chlorophyll a, b and carotenoides were reduced in all parts of infected sugar beet plant as compared to control and significant difference was found between them (Table 14). Our results are in accordance with the findings of earlier researchers who stated that chlorophyll was reduced due to root-knot-nematode incidence (Korayem *et al*., 2012).

Table 12. Physiological parameters of sugar beet.

Parameters	45 days		90 days		
	Infected	Control	Infected	Control	LSD
Length (cm)					
Root (Bulb)	16.31a	20.22b	25.89a	32.98b	3.89
Shoot	25.54a	34.41b	39.87a	50.33b	3.33
Fresh weight (g)					
Root	121.12b	125.88b	137.23a	215.22c	6.34
Shoot	175.35a	198.78a	267.34b	495.19c	10.33
Dry weight (g)					
Root	88.91b	98.34b	101.22c	189.93d	17.23
Shoot	145.32a	155.45b	202.38c	447.23d	23.48

Table 13. Development and reproduction of *Meloidogyne incognita* on sugar beet.

Duration	No. of galls/ root system	No. of egg-mass/root system	RKI*	J_2 / 250 g soil	Rf=Pf/Pi*
45 days	8	45	2.16	1500	1.5
90 days	17	63	2.45	3050	3.05

*RKI = Root-knot index; Rf = Reproductive factor; Pf = Final population; Pi = Initial population.

Chlorophyll is considered as one of an important biomolecule which directed to photosynthesis, and permits plants to absorb energy from light. Nematode infection reduces chlorophyll content and eventually the carbohydrate supply to the nodule that resulting in lower nitrogen fixation (Leng *et al*., 2009). The phenol, chlorophyll and peroxidase enzyme are the main factors for the possible defence mechanism in sugar beet against infection.

Total Indole Acetic Acid Content: Results showed that after the infection of RKN it disturb the amount of total IAA onto sugar beet. Significant increase was found for total IAA content in root after 45 and 90 days, as compared with non-infected beets. Roots of beet showed significant decrease in the total IAA content after 45 days, but in second evaluation after 90 days similar results were found in both infected and control plants (Table 15).

Total Phenolic Compounds Content: Results showed the variation in total phenolic compound of sugar beet in response to infection with nematodes (*M. incognita*). It was determined that, infection with nematodes induced significant increase in the level of TPC in roots even after 90 days. On the other hand, shoot showed significant reduction in total phenol content compared to non-infected plants after both time intervals (Table 15).

Lipid Peroxidation: Results showed that, malondialdehyde (MDA) in shoots and roots increased significantly in infected plants with *M. incognita* as compared to non-infected plants after 45 and 90 days. Whereas after 45 days the difference in infected and control seem to be non significant in roots (Table 15).

Enzyme Activities: The effect of infection with RKNs on activities of some oxidative enzymes of sugar beet has been clearly seen. The infection induced significant increase in PPO activity both in root and shoot of sugar beet plant after 45 and 90 days (Table 15).

In respect to the effect of RKNs on the activity of per-oxidase enzyme POX, the results showed that, the infection increased significantly in shoot with respect to enzyme activity after 45 days and it remained consistent after 90 days (Table 15). It might be explained the role of auxin in increasing root formation (Hirt, 2000) accordingly increasing number of galls/plant, gall index consequently R factor. Furthermore, the percent of increase in IAA content, phenolic compounds, and the increase in the oxidizing enzymes POX or PPO explains the regulatory roles of these compounds to protect plant against pathogen outbreak. The performance of infected sugar beet was accompanied with the low content of POX and high MDA as a result of lipid peroxidation. More or less same result obtained by Leng *et al*., (2009). So, it could be concluded that, the degree of infection that is estimated by reproduction rate of root- knot shows a correlation with changes of various biochemical parameters

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Table 14. Biochemical parameters of sugar beet (in 100g fresh).

Parameters	45 days		90 days		LSD
	Infected	Control	Infected	Control	
Carbohydrate (mg)					
Shoot	2.32a	3.04b	2.06a	4.49c	0.29
Root (Bulb)	5.14a	6.95b	5.15a	9.56c	0.36
Glucose (mg)					
Shoot	22.43a	27.45b	32.23a	47.8c	2.65
Root	46.48a	79.47b	46.53a	88.42b	5.16
Sucrose (mg)					
Shoot	2.35a	2.72a	4.53b	3.78a	0.28
Root	15.35b	18.94b	16.59b	17.87b	1.56
Protein (mg)					
Shoot	0.13a	0.72b	0.03a	0.54b	0.34
Root	0.88a	1.38b	1.05a	1.67b	0.65
Pigments					
Carotenoids	277a	410b	291a	398b	10.013
Chlorophyll a	772a	1400b	790a	1010b	151.019
Chlorophyll b	324a	550b	220a	467b	10.016

Table 15. Total indole acetic acid (IAA), Total phenolic compound (TPC) and lipid peroxidase LPO (Malodialdyhyde: MDA) amount in root and shoot (µg/fresh wt.) of sugar beet after 45 and 90 days.

Tested components	Plant parts	45 days		90 days		LSD
		Infected	Control	Infected	Control	
IAA	Root	3.74a	9.73b	6.33c	9.94b	1.31
	Shoot	20.15a	15.23b	27.42c	48.33d	3.46
TPC	Root	39.62a	52.60b	32.15a	54.32b	3.62
	Shoot	75.33a	42.50b	79.64a	45.61b	12.16
LPO	Root	13.83a	8.55a	27.63b	8.75a	0.34
	Shoot	23.73a	7.21b	36.27b	14.25a	0.73

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