Soil amendment with halophytes induces physiological changes and reduces root-knot nematode infection in eggplant and okra

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Summary. The root-knot nematode *Meloidogyne javanica* (Treub) Chitwood is a soil-borne plant pathogen of roots. Nematode infection impairs plant growth and physicochemical processes due to gall formation. Many plants contain unique biochemicals that have biocidal properties and potentially offer a novel approach to suppressing nematode populations in the soil and improve the growth of crop plants. The effect of three indigenous halophytic plant species, *Tamarix indica* Willd, *Suaeda fruticosa* Forssk and *Salsola imbricata* (Schultz) Dandy, were tested against *M. javanica*. The halophytes significantly (*P*<0.001) reduced hatching and enhanced mortality of second-stage juveniles *in vitro*. When incorporated into the soil at 0.3, 0.5 and 1% (w:w), the halophytes markedly increased the growth of eggplant, *Solanum melongena* L. cv. Black beauty, and of okra, *Abelmoschus esculentus* (L.) Moench. cv. Arka anamika, and they controlled root-knot nematode infection at the higher doses (0.5 and 1%). The halophyte amended eggplants and okra showed a significant (*P*<0.001) increase in chlorophylls and a decrease in the chlorophyll a/b ratio. Protein concentration in the leaves of both plants was increased with a 1% amendment of *S. fruticosa* and *S. imbricata*, whereas the concentrations of nucleic acid varied with the treatment.

Key words: *Meloidogyne javanica,* galls, proteins, chlorophyll levels.

Introduction

Plant diseases can lead to serious losses in crop plants and can adversely affect the economy of agriculturally based countries. Plant pathogenic nematodes severely attack hundreds of economically important plants and reduce their yields. Species of the root-knot nematode *Meloidogyne* Goeldi are among the most widespread and polyphagous group of highly adapted obligate plant pathogens that reproduce and feed on plant roots and cause galls. Because of their endoparasitic mode of living and feeding nematodes disrupt the physiology of the plant. The above-ground symptoms on infected plants are suppressed shoot growth, nutritional deficiencies, temporary wilting and lower yield.

These symptoms are caused by *Meloidogyne* infection, which primarily impairs water and nutrient uptake, and upward translocation by the root system (Karssen and Moens, 2006).

Chemical nematicides are effective as controlling root-knot nematodes but they have environmental concerns (Giannakou *et al.*, 2004). However, there is an increasing interest in discovering nematicidal compounds in the plants themselves (Chitwood, 2002; Oka, 2010). Plant species contain unique biochemical compounds that have biocidal properties and they have the potential to suppress or impair rhizosphere fungal assemblages and the associated nematode populations (Shaukat and Siddiqui, 2001; Abbasi *et al.*, 2008). The nematicidal potential of many halophytes, as far as we know, has not been tested, but the leaves, stems, roots, rhizomes, flowers, etc. of several halophytes are reported to contain allelopathic substances (Horsley, 1977). Halophytes also have a peculiar biochemi-

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cal composition which may play an important role in changing the metabolic processes of crops. Zaheer *et al.*, (2007) reported that *Salsola imbricata* contained Salsolin A and B (triterpenes) and Sultanova *et al.*, (2004) reported that *Tamarix* sp. contained Isotamarixen (pentacyclic triterpenoids).

In this work, experiments were carried out to determine the nematicidal potential of three common halophytes, *Tamarix indica* Willd, *Suaeda fruticosa* Forssk. and *Salsola imbricata* (Schultz) Dandy, occurring in Pakistan, and to examine the physiological changes induced in eggplant, *Solanum melongena* L. cv. Black beauty, and in okra, *Abelmoschus esculentus* (L.) Moench. cv. Arka anamika infected with *Meloidogyne javanica*.

Materials and methods

Collection and extract preparation of halophytes

The three halophytes were collected from saline areas of southern Sindh, Pakistan: *T. indica* from Ghulam Ullah, *S. fruticosa* and *S. imbricata* from Gharo. Halophytes were air dried and the stems and leaves were powdered in an electric blender and kept at room temperature. Aqueous extracts (10%) were prepared by soaking the powders for 24 h in sterilized distilled water (SDW), straining through muslin cloth and filtering through Whatman No. 1 filter paper. An aqueous extract of halophytes diluted to 50% with SDW was prepared at the time of the *in vitro* experiment.

Hatching and mortality of second-stage juveniles

Eggs of *M. javanica* were collected from the roots of eggplant as described by Hussey and Barker (1973). One mL of egg suspension (40–60 eggs mL^{-1}) and 1 mL of aqueous plant extract was transferred to glass cavity blocks. Sterilized distilled water instead of plant extracts was used as the negative control. Hatching of the second-stage juveniles (J_2) was recorded after 72 h. To assess the effect of aqueous plant extracts on J_2 mortality, one mL of freshly hatched J_2 suspension (50–70 J_2 mL^{-1}) and one mL of plant extract (or SDW for the controls) was transferred to a glass cavity block, and mortality was recorded after 96 h of exposure. There were five replicates of each treatment for the hatching and the mortality tests. The toxicity of the plant extracts was assessed as the mean percentage of hatch and mortality of J_2 (Cayrol *et al.*, 1989).

Soil properties and treatments

Soil was dried in an oven at 105ºC for 24 h and passed through a 2 mm sieve to determine its properties. Soil texture and particle size were determined by the Bouyocos hydrometric method (Gee and Bauder, 1986). Plastic pots (diameter 8.1 cm) each containing 300 g sandy clay loam soil (sand 57.8%, silt 24.8% and clay 17.4%, bulk density 1.58 g cm^{-3}, porosity 40%, water holding capacity 31.2%) were amended with dried powder of halophytic plant species at 0, 0.3, 0.5 and 1% w:w. The pots were watered daily to decompose the plant material in the soil. Pots without amendment served as controls. Two set of pots were maintained, one for soil analysis and the other for the growth experiment. There were five replicates for each treatment. Two weeks after the soil treatments, the pH of the soil was determined in 1:5 soil water extract, while the electrical conductivity (EC) was recorded in 1:1 soil water paste. Available Na$^+$ and K$^+$ were also determined by a flame photometer (Moore and Chapman, 1986).

Plant growth experiment

Seedlings of eggplant were grown and maintained in large earthen pots each containing 5 kg of soil. One month after germination, two seedlings were transplanted to each plastic pot containing soil amended by one of the halophytes, while okra seeds were directly sown in the pots. Five days after transplantation of the eggplant seedlings and 10 days after the germination of the okra seedlings, approximately 2000 freshly hatched J_2 were introduced into four holes made around the roots of each plant, i.e. at all concentrations of halophytes, including 0%. Control plants had 0% halophyte but were inoculated with the nematode. Treatments were replicated five times and pots arranged in a randomized complete block design on a greenhouse bench. Six weeks after nematode inoculation, data on eggplant and okra growth, on nematode infection and any biochemical changes were recorded.

Chlorophylls

The chlorophylls in the fresh leaves were extracted in 80% acetone and its absorbance was recorded at 645 and 663 nm (Arnon, 1949). The chlorophyll contents were determined using the

extinction coefficient of Mackinney (1941). For chlorophyll a the extinction coefficient at 645 and 663 nm was 16.75 L g^{-1} cm^{-1} and 82.04 L g^{-1} cm^{-1} respectively, and for chlorophyll b at 645 and 663 nm it was 45.6 L g^{-1} cm^{-1} and 9.27 L g^{-1} cm^{-1} respectively.

Proteins and nucleic acids

The extracts for estimation of the protein and nucleic acids were prepared according to the method described by Nieman and Poulsen (1963) with minor modifications. Fresh leaves were placed in 80% hot ethanol and the extract was precipitated using 5% trichloroacetic acid (TCA). After centrifugation, the residue was washed three times with absolute ethanol:chloroform (3:1, v:v) and ethanol:ether (3:1, v:v), and incubated in 0.5 N NaOH for 16 h at 37°C. The supernatant was separated into two parts, one for proteins and the other for the nucleic acids. Protein was quantified using the method of Bradford (1976). For determination of the nucleic acids, the extract was acidified with 15% perchloric acid (PCA), incubated at 4°C for 40 min, centrifuged, and the supernatant was collected as a RNA fraction. The sediment was heated in 0.5 N PCA to 70°C for 15 min, centrifuged and the supernatant recovered as a DNA fraction. The nucleic acid (RNA and DNA) contents were determined by the method of Nieman and Poulsen (1963).

Statistical analysis

Data were analyzed and subjected to either one-way analysis of variance (ANOVA) or facto-rial analysis of variance (FANOVA). The follow up of ANOVA or FANOVA included Fisher's Least Significant Difference (LSD) and Duncan's Multiple Range Test (DMRT) to compare the treatment means. Percentage data were arcsine transformed (Sokal and Rohlf, 1995). The program package developed by one of us (SSS) in FORTRAN-77 was employed and is available on request.

Results

Nematicidal activity

Aqueous extracts of the halophytic plants (*S. imbricata, S. fruticosa* and *T. indica*) significantly reduced (*P*<0.001) *in vitro* hatching and increased the mortality (*P*<0.001) of *Meloidogyne javanica* second-stage juveniles (Table 1). The effect on nematode mortality was greater than that on hatching. Compared to the controls, *S. fruticosa* caused the greatest reduction in hatching and increase in mortality of J2, while *T. indica* and *S. imbricata* had a smaller effect. Electrical conductivity was highest for *S. fruticosa* and least for *T. indica* (Table 1).

Effect of amendments on soil properties

The properties of the soil were investigated in treated and untreated pots at the time of sowing and of seedling transplantation. The data suggested that amendment with halophytes slightly increased the pH in the soil at the highest concentration (1%). The EC of the soil also slightly increased in all the treated soils (Table 2). However, the EC remained below the salinity threshold for

Table 1. Effect of three halophytes (2.5%) on hatching and mortality of *Meloidogyne javanica* second-stage juveniles.

Treatment	pH	EC (dS m^{-1})	Hatching (%)	Mortality (%)
Control	7.2	0.60	59±3.7	9±2.4
Tamarix indica	7.9	4.50	40±2.9	52±2.4
Suaeda fruticosa	8.2	5.05	24±1.8	64±1.8
Salsola imbricata	8.1	4.52	34±3.6	52±3.5
LSD $_{0.05}$	–	–	4.9	4.2

both crop plants. Sodium ion concentrations were also significantly (P<0.05) higher in the soils after amendment. *Suaeda fruticosa* at all concentrations (0.3, 0.5 and 1%) showed the highest Na^+ ion concentration. The K^+ ion concentration was also higher (P<0.05) in halophyte treated soil. The K^+ ion concentration was highest in soil amended with *T. indica* at 1% (Table 2).

Plant growth and root-knot nematode infection

Growth of the crop plants was greater on soil amended with dried powder of halophytes, with a few exceptions (Table 3). The shoot length of eggplant was greater with most amendments; however, with the 1% *S. fruticosa* amendment the increase in shoot length was not significant. The greatest shoot length in eggplant was observed

Table 2. Effect of three halophytic amendments on soil pH, EC_e, Na^+ and K^+ ion concentration.

Treatment	% [a]	pH	EC (dS m^{-1})	Na$^+$ (μg g^{-1})	K$^+$ (μg g^{-1})
Control	0	6.90±0.02	0.50±0.003	50±1.2	25±0.3
Tamarix indica	0.3	7.00±0.03	0.58±0.003	56±0.9	34±0.7
	0.5	7.15±0.04	0.64±0.020	57±1.2	41±0.6
	1.0	7.10±0.06	0.78±0.014	60±0.3	45±0.6
Suaeda fruticosa	0.3	7.10±0.04	0.62±0.012	71±2.5	36±0.9
	0.5	7.15±0.03	0.66±0.008	90±5.7	41±1.2
	1.0	7.30±0.07	0.75±0.004	102±1.5	41±0.7
Salsola imbricata	0.3	7.00±0.02	0.54±0.002	55±0.8	31±0.5
	0.5	7.00±0.04	0.58±0.002	64±1.0	37±1.5
	1.0	7.25±0.04	0.63±0.017	62±0.9	40±1.2
LSD $_{0.05}$		0.13	0.03	4.18	2.63

[a] Dried powder of treatment (%, w:w).

Table 3. Effect of three halophytes on plant growth and *Meloidogyne javanica* infection in eggplant and okra.

Treatment	% [a]	Eggplant [b] Shoot length (cm)	Shoot weight (g)	Galls/root system	Okra [b] Shoot length (cm)	Shoot weight (g)	Galls/root system
Control [c]	0	14.0 e	0.94 c	205 c	20.6 d	1.32 c	172 bc
Tamarix indica	0.3	15.6 cd	1.22 bc	219 bc	21.8 cd	1.62 c	171 bc
	0.5	18.4 a	1.66 ab	247 ab	27.0 a	2.01 bc	103 e
	1.0	17.3 ab	1.58 ab	198 c	27.4 a	3.27 a	63 f
Suaeda fruticosa	0.3	16.7 bc	1.32 bc	273 a	23.8 bc	1.96 bc	261 a
	0.5	16.4 bcd	1.66 ab	179 c	26.6 a	2.86 a	178 b
	1.0	15.0 de	1.60 ab	80 d	25.7 ab	2.51 ab	143 cd
Salsola imbricata	0.3	16.0 bcd	1.70 ab	199 c	22.3 cd	1.59 c	189 b
	0.5	15.6 cd	1.18 bc	60 d	26.8 a	2.75 ab	124 de
	1.0	18.5 a	2.10 a	56 d	27.7 a	2.40 ab	102 e
LSD $_{0.05}$		1.3	0.48	37	2.1	0.77	28.5

[a] See table 2.
[b] Similar letters in each column are not significant according to the DMR test.
[c] Plants inoculated with *M. javanica* but without halophytes.

with 1% *S. imbricata* amendment, followed by 0.5% *T. indica,* with respectively, a 32 and 31%, increase over the controls. Amendment with the halophytes also led to a significant (*P*<0.05) increase in the shoot length of okra, with the exception of the 0.3% amendments with *T. indica* and *S. imbricata*.

The shoot weight of both plants was also significantly (*P*<0.001) enhanced by halophyte amendment, with the exception of 0.3% *T. indica* and *S. fruticosa* (eggplant and okra), 0.3% S. *imbricata* (eggplant), 0.5% *T. indica* (okra) and 0.5% *S. imbricata* (eggplant). The greatest shoot weight (2.1 g) was found in eggplant treated with 1% *S. imbricata* (Table 3).

The higher concentrations *of S. imbricata* (0.5 and 1%) significantly (*P*<0.001) reduced root-knot nematode infection by reducing gall formation in both plants (Table 3). At 1% amendment with *S. fruticosa* significantly reduced (*P*<0.001) infection in eggplant but not in okra. By contrast, the 1% *T. indica* amendment had no significant effect on eggplant. However, galls formed in both crop plants when they were treated with 0.3% *S. fruticosa* (Table 3).

Chlorophylls

Chlorophyll a and b increased significantly (*P*<0.001) in both plants with all the treatments as compared with the controls, with the exception of okra treated with 0.5 and 1% of *S. fruticosa,* in which chlorophyll b was lower (Tables 4 and 5). Although chlorophyll b levels increased in soils amended with halophytes, the differences were not significant. Chlorophyll b was highest with the 0.3% treatment in both plants, with the exception of *S. imbricata* in eggplant, where the greatest chlorophyll b contents was recorded with a 1% amendment.

The total chlorophyll of eggplant leaves increased (*P*<0.001) as the concentration of *S. imbricata* increased in the soil (Table 4). Amendments with other halophytes, however, gave contrasting results, with the lowest concentrations of halophyte extract causing the highest levels of chlorophyll, though even with the other less effective halophyte concentrations. The chlorophyll content was still higher than that of the controls. The greatest chlorophyll increase in eggplant was recorded with 1% *S. imbricata*, followed by 0.3% *T. indica*. In okra, the highest concentrations of chlorophylls were recorded with the 0.3% amendments of all plants, and declined when the concentration of the amendments was increased (Table 4). The highest chlorophyll levels occurred in plants treated with 0.3% *S. imbricata*, followed by 0.3% *S. fruticosa*. In eggplant, the chlorophyll a/b ratio declined significantly (*P*<0.001) with all treatments. The highest chlorophyll a/b ratio was recorded in the controls, and the minimum chlorophyll a/b ratio in plants

Table 4. Effect of three halophytes on the chlorophyll contents of eggplant infected with *Meloidogyne javanica*.

Treatment	% [a]	Chl a (μg g^{-1}) [b]	Chl b (μg g^{-1}) [b]	Chl a+b (μg g^{-1}) [b]	Chl a/b [b]
Control[b]	0	256 e	132 f	388 g	1.95 a
Tamarix indica	0.3	325 a	277 b	602 b	1.17 de
	0.5	321 ab	260 c	581 d	1.23 de
	1.0	310 c	244 d	554 e	1.27 cd
Suaeda fruticosa	0.3	317 abc	278 b	596 bc	1.14 e
	0.5	314 bc	262 c	576 d	1.20 de
	1.0	317 abc	270 bc	587 cd	1.17 de
Salsola imbricata	0.3	292 d	165 e	457 f	1.78 b
	0.5	323 a	237 d	560 e	1.36 c
	1.0	323 a	313 a	636 a	1.03 f
LSD $_{0.05}$		7.7	10.5	12.7	0.09

[a] See Table 2.
[b] See Table 3.
[c] See Table 3.

Table 5. Effect of three halophytes on the chlorophyll contents of okra infected with *Meloidogyne javanica*.

Treatment	% [a]	Chl a (μg g^{-1}) [b]	Chl b (μg g^{-1}) [b]	Chl a+b (μg g^{-1}) [b]	Chl a/b [b]
Control [c]	0	237 c	247 f	484 f	0.96 b
Tamarix indica	0.3	247 ab	301 d	548 d	0.82 d
	0.5	247 ab	279 e	526 e	0.88 c
	1.0	247 ab	279 e	526 e	0.88 c
Suaeda fruticosa	0.3	250 a	326 b	576 b	0.77 e
	0.5	249 ab	238 g	487 f	1.05 a
	1.0	249 ab	238 g	487 f	1.05 a
Salsola imbricata	0.3	245 b	428 a	673 a	0.57 f
	0.5	247 ab	316 c	563 c	0.78 e
	1.0	247 ab	312 c	559 c	0.79 de
LSD $_{0.05}$		7.7	7.5	7.9	0.03

[a] See Table 2.
[b] See Table 3.
[c] See Table 3

treated with 1% *S. imbricata*. Treatments with *S. imbricata* showed a gradual decline with increasing concentrations of this halophyte as a powder, whereas *T. indica* and *S. fruticosa* amendments produced a lower chlorophyll a/b ratio than did the controls, but between these treatments (halophyte species) the differences were not significant. In okra, the highest chlorophyll a/b ratio occurred with the 0.5 and 1.0% amendments of *S. fruticosa*, while with all the other treatments the ratio was below that of the controls with all the halophytes. The chlorophyll a/b ratio went up as the concentration of the amendments increased (Table 4).

Total proteins

In okra the total protein content was significantly higher ($P<0.001$) with amendments of *S. fruticosa* and *S. imbricata* compared with the controls. *T. indica,* on the other hand, at all concentrations in eggplant, and at 0.3 and 0.5% in okra significantly reduced the protein content as compared with the controls. Treatments with *S. fruticosa* and *S. imbricata* at all concentrations significantly increased the protein content in okra, but only at 1% concentration was the protein content increased in eggplant (Tables 6 and 7).

Nucleic acids

With a few exceptions RNA contents were decreased in both plants compared to the controls (Tables 6 and 7). Only the treatment with *S. fruticosa* in eggplant, and with *S. imbricata* in okra, both at 1% produced high levels of RNA. Similarly, the DNA contents decreased with most treatments in eggplant (as compared with the control) but it increased with a 1% concentration of *S. fruticosa* and *S. imbricata*. In okra, the DNA increased with halophyte amendments at all concentrations except 0.3% *T. indica* and 1% *S. fruticosa* (Table 7).

Discussion

Halophytes have some unique features enabling them to survive at high salinity (Abdelly *et al.*, 2008; Munns and Tester, 2008), and as far as we know the nematicidal activity of many halophytic plants has not been assessed. Three halophytes were tested for their nematicidal potential. Aqueous extracts of all three halophytes were effective in limiting *M. javanica* hatching and mortality. The EC and the pH of aqueous extract also increased (Table 1), as halophytes ac-

Table 6. Effect of three halophytes on total proteins and nucleic acid contents of eggplant infected with *Meloidogyne javanica*.

Treatment	% [a]	Total proteins (mg g^{-1}) [b]	RNA (μg g^{-1}) [b]	DNA (μg g^{-1}) [b]
Control [c]	0 %	3.41 c	98 b	76 cd
Tamarix indica	0.3 %	2.89 ef	78 d	43 h
	0.5 %	2.93 e	88 c	49 g
	1.0 %	3.28 d	99 b	57 f
Suaeda fruticosa	0.3 %	2.41 g	89 c	81 bc
	0.5 %	2.95 e	47 f	70 de
	1.0 %	3.56 b	104 a	86 b
Salsola imbricata	0.3 %	2.21 h	63 e	67 e
	0.5 %	2.79 f	75 d	71 de
	1.0 %	3.86 a	91 c	101 a
LSD $_{0.05}$		0.10	4.7	5.8

[a] See Table 2.
[b] See Table 3.
[c] See Table 3.

Table 7. Effect of three halophytes on total proteins and nucleic acid contents of okra infected with *Meloidogyne javanica*.

Treatment	% [a]	Total proteins (mg g^{-1}) [b]	RNA (μg g^{-1}) [b]	DNA (μg g^{-1}) [b]
Control [c]	0	1.96 f	148 b	16 ef
Tamarix indica	0.3	1.51 h	128 d	13 f
	0.5	1.86 g	126 d	26 c
	1.0	1.96 f	103 g	23 cd
Suaeda fruticosa	0.3	3.14 b	135 c	48 b
	0.5	2.12 e	96 h	50 b
	1.0	3.81 a	114 e	20 de
Salsola imbricata	0.3	2.26 d	108 f	58 a
	0.5	2.14 e	114 e	60 a
	1.0	2.71 c	158 a	61 a
LSD $_{0.05}$		0.08	4.0	4.3

[a] See Table 2.
[b] See Table 3.
[c] See Table 3.

cumulated NaCl and other compounds in their tissues, including granules of silicon and copper, such as was reported for *S. fruticosa* by Ahmad (1968), which may be another cause of the nematicidal activity of these halophytes *in vitro*. In pot experiments, halophytes at 0.3, 0.5 and 1% (w:w) were used as soil amendments. At these concentrations the salinity of the soil is not expected to rise to levels harmful to the crop plant because the high salt levels of halophytes were leached out during irrigation. This was indicated by the Na$^+$ ion content of the soil. The treatments slightly increased the EC of the soil, but the EC remained below the threshold level for the crops tested; for eggplant, 1.2 dS m^{-1} (Mass, 1986) and for okra, 1.1 dS m^{-1} (Heuer *et al*., 1986). The reduction of nematode infection is thought to be caused, at least in part, by an increase in the natural enemies of the nematodes. In addition, the decomposing organic materials in the soil apparently provide the host plants with a certain degree of tolerance to nematode attack by increasing the nutrients required for plant growth (Karssen and Moens, 2006; Oka *et al*., 2007). The decomposition of plant residues may also be detrimental, directly or indirectly, to the nematodes. The control of plant parasitic nematodes using toxic extracts from certain plants has been widely tested and is well documented (Ali *et al*., 2001; Shaukat *et al*., 2004; Abbasi *et al*., 2008). Cuevas (1997) found that the nutrient contents of halophytes used as organic amendments ultimately increased the growth of infected plants.

Root-knot nematodes cause severe damage to the roots and reduce the supply of water and nutrients from the soil to the upper parts of the plants by the formation of giant cells. This causes a shortage of nutrients in the above-ground parts of the plants that may alter the biochemical processes of plants. Chlorophyll a and b levels were higher in the treated plants than in the control plants. High levels of chlorophyll may increase the photosynthetic rate and thereby increase shoot growth, as was detected. During normal conditions, chlorophyll a is converted to chlorophyll b by chlorophyllide a oxygenase (CAO) activity (Tanaka *et al*., 1998). Under stressed conditions, CAO activity is impaired and further synthesis of chlorophyll b declines. The rise in the level of chlorophyll b on the other hand can be attributed

to a tolerance response to nematode infection induced by the treatments. This would account for the decrease in chlorophyll a/b ratio caused by the treatments as compared with the control plants. In the controls, chlorophyll b was converted to chlorophyll a on account of the activity of the enzymes chlorophyll b reductase and 7-hydroxymethyl chlorophyll reductase (Tanaka and Tanaka, 2006), leading to a greater chlorophyll a/b ratio.

The contents of total protein and of nucleic acids varied with the treatment and this variation may be attributed to the severity of the infection caused. It is reported that the concentration of proteins is reduced when infection is more severe and that this depends on how susceptible the plants are to *M. javanica* (Ahmed *et al*., 2009 a,b). The protein concentration probably decreases because the giant cells utilize the amino acid pool which is required for protein synthesis. Alternatively, the decrease in protein concentration is caused by the proteolysis of proteins under stress conditions. The level of proteins increased when the infection was cured. A similar result was reported by Abbasi *et al*., (2008) who examined *M. javanica* infection in eggplant and okra amended with *Barleria acanthoides*. Similarly, low concentrations of nucleic acids in infected plants were due to enhanced ribonuclease activity. The activity of this enzyme is possibly increased in susceptible plants due to the growth and multiplication of the nematodes in the roots.

The study clearly suggests that halophytes contain a variety of chemicals that have nematicidal activity when they are released through decay or leaching into the soil. Furthermore, the rhizosphere microflora including fungi and bacteria may also undergo changes, as demonstrated by Shaukat and Siddiqui (2001), and may play a role in reducing of root-knot nematode populations. Halophytes added to the soil can provide organic material to the soil and thereby improve plant growth, and they have a great potential for the control of root-knot nematodes of crop plants.

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