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Effect of organic amendment on organic metabolites in root knot nematode (*Meloidogyne Incognita)* infested spinach

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ABSTRACT: The Nematodes are roundworms that are found in every environment of the earth. While some species are harmful parasites, others play a vital role in nutrient cycle and medical research. Nematode infestation in the fields is poly-specific; however, depending on the agro-climatic conditions, one or two species are dominant over the rest. The present studies attempts to observe and control the root knot infestation on spinach (*Spinacea oleracea),* which belong to Chenopodiaceous family and is extensively cultivated in India for its nutritious leaves. Various organic metabolites have been estimated in root knot nematode infested spinach, including chlorophyll, total carbohydrates, and total free amino acids. Spinach plants, infested with root knot nematode, have been treated with peels of lemon, which proved beneficial in terms of increased chlorophyll content. Altered total carbohydrate and total free amino acid content have been found with S/4 of lemon-peel-treated spinach plants. It has been found that the infested spinach shows 137.5% carbohydrate content over the normal plants. The kaghzi neemboo amended spinach contains lower carbohydrate than normal-control. Rate of carbohydrate contents has been found to be inversely proportional to the rate of extracts concentrations as S/4, S/2, and S show 92.5%, 55%, and 37.5% increase over normal-control spinach. The bio-amendment of citrus aurantifolia, (kaghzi neemboo) helps controlling root knot nematodes, which is more beneficial than using chemicals to control the same, as the chemical fertilizer causes pollution, exerting negative impacts on flora and fauna.

Keywords: chlorophyll content, infested and treated spinach, normal, peels of kaghzi neemboo, root knot nematode, total-free amino acids.

INTRODUCTION

Nematodes are roundworms that are found in every environment on Earth. While some species are harmful parasites, others play a vital role in nutrient cycle as well as medical research. Nematode infestation in the fields is poly-specific; however, depending on the agro-climatic conditions, one or two species dominate over the rest. Accordingly, there have been control efforts to either prevent nematode entry, limit its population, or reduce its effects on the crops, or to apply a combination of these principles. The literature reveals that root knot nematodes *(Meloidogyne incognita*) are universally destructive to almost all plants, including

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vegetables, fruits, and some cereal crops. Economically important species in India are *M. javanica, M. incognita,* M. g*raminicola*, and *M. exigua.* The principal symptoms, galls or knots, produced on the roots are diagnostic of the root knot nematode (M. *incognita*) (García et al., 2015) infection.

Aqueous and organic extracts of many plants have been reported to contain nematicidal compounds, e.g. Akhtar and Alam (1989), Pandey et al. (2001) and Ahmad et al. (2013) reported that flower extracts of *Bauhinia variegata*, *Ixora parviflora*, *Moringa oleifera*, *Tagetes eracta*, *Argemone maxicana* & others were highly toxic against J2's of *M. incognita* (Jang et al., 2016). Amongst various nematodes viz. *Tylenchorynchus sp., Tylenchus sp., Heterodera sp., Meloidogyne sp.,* root knot nematodes (M. *incognita*) are found to parasitize spinach roots (exhibiting heavy gall formation and loss to this crop. *Spinacea oleracea* (spinach) is unique among vegetable crops, thanks to its extremely high yield in a relatively short period of time. A good source of minerals, Vitamin K, Vitamin B complex, ascorbic acid, and carotene, it is being attacked by various other agents viz. bacteria, fungi, etc. besides nematodes' causing necrosis, curling, and patches on its leaves.

The present research work aims to observe and control the root knot infestation on spinach *Spinacea oleracea* (spinach). New control technologies and tools are being worked out on the pattern for nematode control. Research in these areas is picking up and possible efforts may yield some useful alternatives, like pheromone communication, steroid or hormone activity, sensory stimuli, use of avermectins that have potent anthelminthic, and insecticidal activities. However, it is universally realized that integrated nematode management is the best option for keeping the levels of pest population below economic threshold via combined use of different control practices. The solution to this problem is also through phytotherapeutic substances, with which nematode management is expected to be highly practicable from the point of view of cost effectiveness, environmental safeness, and socio-economic viability.

MATERIALS AND METHODS

Spinach seeds were sown in three replicas, namely normal-control, infested–control, and chopped peels of *Citrus aurantifolia* (lemon) (Figs. 1, 2). They were amended in 100%, 50%, and 25% w/v of autoclaved soil and named *S, S/2, and S/4,* respectively. After 60 days, the plants were uprooted and the estimation was performed by the given method.

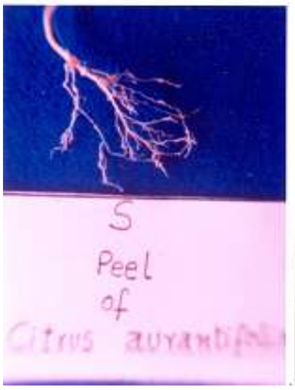

Fig. 1. Peel of Citrus Aurantifolia

Fig. 2. Infected control spinach

An amount of 100 mg of the plant sample was neutralized with 5 ml of 2.5 N HCl in water bath for 3 hrs, to be further neutralized with Na_2CO_3. The volume was adjusted up to 100 ml and centrifuged.

Now, 4 ml of anthrone reagent was added to 1 ml supernatant. The test samples were kept along with control in water bath for 8 minutes. They were cooled down and the optical density was measured at 630 nm against glucose as 'blank'. A standard curve was drawn, using different concentrations of standard glucose (0.2, 0.4, 0.6, 0.8, and 1 ml, respectively). Results were expressed as the amount of total sugar present in 100gm of plant sample. The total carbohydrate content was determined with the method, presented by Then, from 1.500 mg of the plant sample, ions of Ca^{++}, Mg^{++}, Na^{+} were extracted with 10 ml of 80% ethanol. After centrifugation, 0.1 ml of supernatant was taken and received 0.1 ml of distilled water along with 2 ml of ninhydrin solution. It was kept in water bath for 15 min. The test samples were cooled and had 2 ml of ethanol added to them. The color of the obtained solution was purple. And the sample's optical density was measured at 575 nm against leucine as 'blank'. The total free amino acid was expressed as percent equivalent to leucine. Then in order to estimate free amino acid, the method, given by Spies (1957) was followed, where 1 gm of finely cut leaves were ground in 20 ml of 80% ethanol to be centrifuged at 5000 rpm for 5 min. The supernatant was separated and the residue was ground again, this time with 80% acetone, until it became colorless and was centrifuged at 5000 rpm, once more. The supernatant's volume reached 100 ml with 80% acetone. The absorbance of solution was read at 645, 663, and 652 nm against 80% acetone as blank.

RESULTS AND DISCUSSION

Table 1 shows the measured data in chlorophyll contents for both normal infested and treated spinach. The total chlorophyll content in spinach leaves, amended with peels of Citrus aurantifolia, shows increased chlorophyll content as compared with infested-control (I-C). The spinach treated with concentrations of S, S/2, S/4 (discussed above in the Materials and Methods section) contains 1.34, 1.29, and 0.88 mg chlorophyll/g tissue, compared to 0.79 mg chlorophyll/g tissue of I-C and 0.94 mg chlorophyll/m of normal-control (N-C).

Table 1. Estimation of chlorophyl (mg/g) of *Spinacea oleracea* (spinach)

Sr. No.	Amendment	Normal control	% I/D	Infested control	% I/D	S	% ID	S/2	% ID	S/4	% ID
1.	Peels of *Citrus aurantifolia*		--								
2	Total cholorophyll mg/g	0.94	-	0.79	-15.95	1.34	+42.53	1.29	+37.23	0.88	-6.81
3	Cholorophyll a mg/g	0.38	-	0.31	-18.42	0.57	+50.00	0.56	+47.36	0.35	-7.89
4	Cholorophyll b mg/g	0.60	-	0.48	-0.20	0.98	+63.33	0.93	+55.00	0.56	-6.66

Values are signified at $P \leq 0.05$, s-stock; I/D: increase / decrease

However, amendment with S and S/2 concentration is much higher than both infested-control and normal-control spinach. The data, presented in Table 1, reveals that peel amendment alters chlorophyll content of spinach. The total chlorophyll deteriorated in infested-control, which has 0.45 mg chlorophyll/gm tissue, compared to 0.82 chlorophyll/g of normal-control spinach. S, S/2, and S/4 have 0.75, 0.82, and 0.84 mg chlorophyll/g tissue, thus S/4 contains more chlorophyll than S and S/2. The chlorophyll b/g tissue in I-C and N-C spinach is 0.18 and 0.45 mg respectively. S, S/2, and S/4 contain 0.43, 0.44, and 0.45 mg chlorophyll b/g tissue, respectively. Based on the results, S/4 shows an increase over S and S/2. The treatments shows greater amount of chlorophyll b content than infested-control.

Higher content of total carbohydrate was recorded in the diseased roots of spinach, compared to normal-control spinach (Table 2), where the infested spinach showed 137.5% carbohydrate content over the normal. The kaghzi neemboo amended spinach contains lower carbohydrate than normal-control. The rate of carbohydrate contents were found to be inversely proportional to the rate of extracts concentrations as S/4, S/2, and S showed 92.5%, 55% and 37.5% increase over normal-control spinach.

Table 2. Quantitative estimation of different metabolites in spinach roots

Sr. No.	Concentrations	Organic			
		Total carbohydrate content (mg/ml)	% I/D	Total free amino acid (mg/ml)	% I/D
	Normal-control	0.40	------	0.38	---
	Citrus aurantifolia treatment				
1.	S	0.55	+37.5	0.51	+34.21
2.	S/2	0.62	+55.0	3.30	+768.4
3.	S/4	0.77	+92.5	4.87	+1181.5
	Infested Control	0.95	+137.5	3.70	+873.0

Values are signified at P≤0.05, s-stock; I/D: increase / decrease

Nayak and Pandey (2016) reported higher sugar content in the root knot nematode inoculated roots, which may be due to various metabolites' tendency to move the infection site from other parts of the plants. Gautam and Poddar (2014) also found higher total sugar content upon increasing the concentration of the nutrients in root-knot-nematode-infested bitter gourd. However, several other workers viz. Sharma and Trivedi (1996), and Singh (1999) reported less carbohydrate content in the diseased root, compared to the normal sample. Hofmann et al. (2007; 2008), and Nayak and Mohanty (2010) agreed that high metabolic activity in diseased tissues increased sugar levels.

It has been found that total free amino acid was increased in the infested-control spinach, compared to the normal-control one. The former contained 3.70 mg/ml, while the latter showed only 0.38 mg/ml of total amino acid content. In addition, S, S/2, and S/4 variants of kaghzi-neemboo-treated spinach contained 0.51, 3.30, and 4.87 mg/ml total amino acid content, respectively. These findings are in accordance with the conditions, met by several researchers (Sharma & Trivedi, 1996; Mohanty et al., 1997; Singh, 1999). Wenefrida et al. (2013), & Tayal and Agarwal (1982) who noticed higher amino acid content due to enhanced turnover for the benefit of nematode into easily assimilable form of amino-acid). They also co-relate the increased level of soluble proteins and amino acids with high protease activity in infected tissue. The proteases are secreted by the nematode into the host tissue for such a proteolytic degradation. Abbasi et al. (2008) also observed similar changes that increased level of protein content, as a result of inhibiting root knot infestation in Okra and brinjal plants. Both Gautam and Poddar (2014), and Nayak (1995) reported increased protein concentration at initial stage of infection as well.

CONCLUSION

According to the results, it is concluded that the total chlorophyll content in spinach leaves, amended with peels of Citrus aurantifolia, show higher content of chlorophyll in comparison to infested-control; however, in case of amendment with S and S/2, this becomes even greater than infested-control and normal-control spinach. It has also been found that S/4 shows an increment over S and S/2. The treatments show much higher content of chlorophyll b than infested-control. The rate of carbohydrate contents has been found to be inversely proportional to the rate of extracts concentrations. It is also found that total free amino acid rise in the infested-control spinach, compared to normal-control spinach. The bio-amendment of citrus aurantifolia, (kaghzi neemboo) helps controlling root knot nematodes, which proves more beneficial than using chemicals for the same purpose, as chemical fertilizer is supposed to spread pollution and cause negative impacts on flora and fauna. The bio amendment also serves the purpose of recycling the bio-waste of citrus aurantifolia from industries, restaurants, hotels etc.

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