# Quantitative Estimation of Different Organic Metabolites in Root Knot Nematode (*Meloidogyne Incognita*) Infested and Organically Treated Spinach

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***Abstract*** **- The phytonematodes are mostly responsible for treacherous disease symptoms in different crops frequently and extensively resulting in huge losses. Nematodes may damage plants directly or indirectly. In general, the nematode infestation in fields is poly specific, however, depending upon the agro-climatic conditions, one or two species dominates over the rest. *Meloidogyne incognit*a is universally destructive to almost all the plants including vegetables, fruits and some cereal crops. Spinach (Spinacea oleracea) is also found infested by the same. Biocontrol with peels of *Citrus aurantifoli*a (kaghzi neemboo) proved beneficial in controlling the infestation. Various organic metabolites were estimated in root knot nematode infested spinach. Amongst organic metabolites chlorophyll, total carbohydrates, total free amino acids were estimated. Root knot nematode infested spinach was treated with peels of lemon which proved beneficial in terms of increased chlorophyll content. Altered total carbohydrate and total free amino acid content was found with S/4 of lemon peels treated spinach plants.**

**Key Words**: Chlorophyll content, Total carbohydrates, Total free amino acids, Root knot nematode (*Meloidogyne incognita),* Spinach (*Spinacea oleracea*), peels of kaghzi neemboo (*Citrus aurantifolia)*

## I. INTRODUCTION

The phytonematodes are mostly responsible for treacherous disease symptoms in different crops frequently and extensively resulting in huge losses. Nematodes may damage plants directly or indirectly In general, the nematode infestation in fields is poly specific, however, depending upon the agro-climatic conditions, one or two species dominates over the rest.Accordingly, control efforts have been made for prevention of nematode entry, suppression of its population, reduction in its effects on the crops or combination of these principles. Literature reveals that root knot nematodes *(Meloidogyne incognit*a) are universally destructive to almost all the plants including vegetables, fruits and some cereal crops. Economically important species in India are *M. javanica, M. incognita,* M. g*raminicola* & *M. exigua.* The principal symptoms, galls or knot produced on roots are diagnostic of the root knot nematode (M. incognita) infection. With this idea the present study had been taken to observe and control the root knot infestation on spinach *Spinacea oleracea* (spinach) belongs to Chenopodiaceous family and is extensively cultivated in India for its nutritious leaves. It is unique among vegetable crops because of its extremely high yield in a relatively short period of time. Besides an important source of Vitamin K, spinach is a good source of minerals, 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, which affect its overall growth followed by production. Amongst various nematodes viz. *Tylenchorynchus sp. Tylenchus sp., Heterodera sp., Meloidogyne sp.,* root knot nematode (*M. incognita)* and found to parasitize roots of spinach exhibiting heavy gall formation and loss to this crop.

In nematology, new control technology tools are being worked out on the pattern of those developed for insect pest control. Research in these areas is picking up and possible efforts may yield some useful alternatives. These practices include pheromone communication, steroid or hormone activity, sensory stimuli, use of avermectins which have potent anthelminthic and insecticidal activities & are in wide spread use, especially as agents affecting parasitic nematodes. However, it is universally realized that integrated nematode management is the best option for keeping the population levels of the pests below economic threshold by combined use of different control practices.

A solution to this problem is also by the use of phytotherapeutic substances, through which nematode management is expected to be highly practicable from the point of view of cost effectiveness, environmental safety and socio-economic viability. Aqueous and organic extracts of many plants have been reported to contain nematicidal or nematostatic compounds [10] : [13] : [5] : [9] : [16] : [2] and [18] 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.*

## II. MATERIALS AND METHODS

Seeds of spinach were seeded in three replicas each of Normal-Control, Infested Infected–control, and chopped peels of *Citrus aurantifolia* (lemon) and were amended in 100%, 50% and 25% w/v of autoclaved soil and named as S, S/2 and S/4 respectively. After 60 days plants were uprooted and following parameters were estimated-

### *A. Total Carbohydrate Content*

Total carbohydrate content was determined as:

1. 100 mg of plant sample was neutralized with 5 ml of 2.5 N HCl in water bath for 3 hrs.
2. Neutralized further with $Na_2CO_3$.
3. The volume was made up to 100 ml and centrifuged.
4. 4 ml of Anthrone reagent was added to 1 ml supernatant.
5. The test samples were kept along with control in water bath for 8 minutes.
6. It was cooled and optical density was measured at 630 nm against glucose as 'blank'.
7. A standard curve was drawn using different concentrations of standard glucose (0.2, 0.4, 0.6, 0.8 and 1 ml respectively).

The results were expressed as the amount of total sugar present in 100gm of plant sample.

### *B. Total Free Amino Acid:*

Method of [6] was followed for the estimation of free amino acid:

1. Ca++, Mg++, Na+ ions1. 500 mg of plant sample was extracted with 10 ml of 80 % ethanol.
2. After centrifugation, 0.1 ml of supernatant was taken and in it 0.1 ml distilled water and 2 ml of ninhydrin solution was added.
3. It was kept in water bath for 15 minutes.
4. Test samples were cooled and 2 ml of ethanol was added.
5. Purple color develops.
6. Optical Density of sample was measured at 575 nm against leucine as 'blank'. Total free amino acid was expressed as percent equivalent to leucine.

### *C. Chlorophyll Content:*

1. Total chlorophyll was estimated by the method of [4].
1. 1 gm of finely cut leaves were ground in 20 ml of 80 % ethanol and centrifuged at 5000 rpm for 5 minutes.
2. Supernatant was separated and residue was again ground with 80% acetone till it become colorless and again centrifuged at 5000 rpm.
3. The volume of supernatant was made up to 100 ml with 80 % acetone.
4. The absorbance of solution was read at 645, 663 and 652 nm against 80 % acetone as blank.

## III. RESULTS AND DISCUSSION

Total Chlorophyll Content:

The data presented in Table I revealed that chlorophyll content got altered in the normal, infested and treated spinach.

### *A. Chlorophyll content in spinach after amendment of peel of Citrus aurantifolia.*

Total chlorophyll content in spinach leaves amended with peels of *Citrus aurantifolia* showed increase in chlorophyll content as compared with infested-control (I-C). Spinach treated with concentrations of S, S/2, S/4 contained 1.34, 1.29, 0.88 mg chlorophyll/gm tissue when compared to 0.79 mg chlorophyll/gm tissue of I-C and 0.94 mg chl/gm in normal-control (N-C). However, amendment with S and S/2 concentration showed more increase over infested-control and normal-control spinach. Data presented in Table I revealed that amendment of peels alter chlorophyll content of spinach.

Total chlorophyll deteriorated in infested-control which have 0.45 mg chl/gm tissue as compared to 0.82 chl a/gm of normal-control spinach S, S/2 and S/4 have 0.75, 0.82 and 0.84 mg chl/gm tissue S/4 contains more chlorophyll than S and S/2. Chlorophyll b in I-C, N-C spinach is 0.18, 0.45 mg chl b/gm tissue. S, S/2, S/4 contain 0.43, 0.44, 0.45 mg chl b/gm tissue. S/4 showed increase over S, S/2. Treatments showed much increase Chl b content over infested-control.

### *B. Total Carbohydrate content*

Increased content of total carbohydrate had been recorded in the diseased roots of spinach as compared to normal-control spinach (Table II). Infested spinach showed 137.5% carbohydrate content over normal. Kaghzi neemboo amended spinach contains lower carbohydrate than normal-control. 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. [15] reported

increased sugar content in the root knot nematode inoculated roots, which may be due to the movements of various metabolites towards the infection site from the other parts of plants. [13] also found increase in total sugar content on increasing concentrations of nutrients in root knot nematode infested cotton.

However, several other workers [19-21], and [22] reported decrease carbohydrate content in the diseased root as compared to normal. [11] , [12] and [17] agreed with the increase sugar levels to high metabolic activity in diseased tissues.

### *C. Total free amino-acids*

Increased total free amino acid had been found in the infested-control spinach as compared to normal-control spinach. Infested-control spinach contained 3.70 mg/ml whereas normal spinach had showed only 0.38 mg/ml total amino acid content, S, S/2, S/4, kaghzi neemboo treated spinach contained 0.51, 3.30 and 4.87 mg/ml total amino acid content.

Similar conditions had been met by several workers, ([3]; [14], [15]. [8] noticed increased amino acid content due to enhanced turnover for the benefit of nematode into easily assailable form of amino-acid. They also co-related the increased level of soluble proteins and amino acids with high protease activity in infected tissue.

The proteases are secreted by the nematode into host tissue for such a proteolytic degradation. [1] also observed similar changes that increase level of protein content as a result of inhibition of rootknot infestation in Okra and brinjal plants. [7] also reported increased protein concentration at initial stage of infection.

TABLE I
ESTIMATION OF CHLOROPHYLL (MG/GM) 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/gm | 0.94 | - | 0.79 | -15.95 | 1.34 | +42.53 | 1.29 | +37.23 | 0.88 | -6.81 |
| 3 | Cholorophyll a mg/gm | 0.38 | - | 0.31 | -18.42 | 0.57 | +50.00 | 0.56 | +47.36 | 0.35 | -7.89 |
| 4 | Cholorophyll b mg/gm | 0.60 | - | 0.48 | -0.20 | 0.98 | +63.33 | 0.93 | +55.00 | 0.56 | -6.66 |

TABLE II
QUANTITATIVE ESTIMATION OF DIFFERENT METABOLITES IN THE ROOTS OF SPINACH

| **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 |

## REFERENCES

[1] M. W. Abbasi,, N. Ahmed, M. J. Zaki and S.S. Shaukat, "Effect of Baleria acanthoides VAHL., on Root Knot Nematode Infection and Growth of Infected Okra And Brinjal Plants", Pakistan Journal of Botany, Volume 40, Issue 21, pp. 93-98, 2008

[2] M. Akhtar and M. M. Alam, "Effect of Bare Root Dip Treatment with Extracts of Castor on Root Knot Development and Growth of Tomato", Nematol. Medit, Volume 18, pp. 53-54, 1989

[3] I. J. Singh, J. Sharma, and R. Sharma, "Biochemical Alterations Induced by M. incognita in Brinjal", Indian Journal of Nematology, Volume 8, pp. 122-126, 1978

[4] D. I. Arnon, "Copper Enzymes in Isolated Chloroplasts, Polyphenol Oxidase in Beta Vulgaris", Plant Physiol, Volume 24, pp. 1-5, 1949

[5] D. S. Bhatti and S. C. Dhawan, "Effect of Crushed Seeds of Carrot and Coriander on Wheat Plant, Growth and Multiplication of Heterodera Avenae", Haryana Agriculture University Journal of Research Volume 10, pp. 419-420, 1980

[6] J. R. Spies, "Colorimetric Procedures For Amino Acids: Methods of Enzymology", Academic Press, pp. 464-471, 1957

[7] S. K Gautam, Poddar and Aditi, "Study on Protein and Sugar Content in Meloidogyne Incognita Infested Roots of Bitter Gourd", International Journal of Current Microbiology and Applied Science, Volume 3, Issue 5, pp. 470-478, 2014

[8] M. S. Tayal and M. L. Agarwal, "Biochemical Alterations in Galls Induced By M. Incognita: Some Hydrolyzing Enzymes and Related Chemical Metabolites", Indian Journal of Nematology, Volume 12, pp. 379-382, 1982

[9] D. C.Gupta and K. Ram, "Studies on the control of Meloidogyne Javanica Infecting Chickpea in Different Types of Soil", Indian Journal of Nematology, Volume 11, pp. 77-80, 1981

[10] S. N. Hameed, "Notes on the Effect of Some Organic Additives on the Incidence of Root Knot Nematodes in Tomato", Indian Journal of Agriculture Science, Volume 40, pp. 207-210, 1970

[11] T. Hofmann, K. Wieczorek, A. Blochl and F. M. W. Grundler, "Sucrose Supply to Nematode Induced Syncytia Depend on the Apoplasmic and Symplasmic Pathways", Journal of Experimental Botany, Volume 58, pp. 1591-1601, 2007

[12] J. Hofmann, D. Szakasites, A. Blochl, M. Sobczak, C. K. Daxbo, S. Hormth, W. Golinowski, H. Bohlmann and F. M. W. Grendler, "Starch Serves as a Carbohydrate Storage in Nematode Induced Syncytica", Plant Physiology, Volume 146, pp. 228-235, 2008

[13] I. Yuhara, "Effect of Soil Treatment, Dry Organic Matter Powders on the Population of Meloidogyne Hapla Attacking Sugarbeets", Bulletin of Sugarbeet Research, Volume 13, pp. 201-205, 1971

[14] A. H. Khan, A. Masood and S. K. Saxena, "Effect of Water Soluble Extracts of Oilcakes on Incognita", Indian Journal of Nematology, Volume 10, pp. 105-106, 1980

[15] K. C. Mohanty, P. K. Mohanty and T. Pradhan, "Effect of Meloidogyne Incognita on Root Biochemistry and Functioning of Nodules in Green Gram", Indian Journal of Nematology, Volume 27, Issue 1, pp. 1-5, 1997

[16] S. N. Nandal and D. S. Bhatti, "Preliminary Screening of Some Weeds/Shrubs for Their Nematicidal Activity against Meloidogyne Javanica", Indian Journal of Nematology, Volume 13, pp. 123-127, 1988

[17] D. K. Nayak and R. C. Mohanty, "Biochemical Changes in Brinjal Induced by Root Knot Nematode Meloidogyne Incognita", Indian Journal of Nematology Volume 40, Issue 1, pp. 43-47, 2010

[18] R. Pandey, A. Kalra and S. Kumar, "Nematicidal Activity in Flowers of Some Medicinal and Aromatic Plants", Indian Journal of Nematology, Volume 31, Issue 1, pp.79-98, 2001

[19] R. Saxena and R. Singh, "Efficacy of Botanicals, Efficacy of Botanicals Against Root Knot Nematode Meloidogyne Incognita on Sponge Gourd", Luffa cylindrica, L. International Journal Mendel, Volume 18, Issues 1-2, pp. 43-46, 2001

[20] W. Sharma and P. C. Trivedi, "Evaluation of Various Metabolites as Influenced by Root Knot Nematode in Abelmoschus Eesculentus Biochemical", Indian Journal of Nematology, Volume 26 (2), pp. 152-157, 1996

[21] Z. A. Siddiqui, R. A. Mir, M. Irshad and I. Mahmood, "Effects of Meloidogyne Incognita, Fusarium Oxysporum, Rhizobium Species and different Soil types on Growth, Chlorophyll and Carotenoid Pigments of Pea", Israel Journal of Plants Science. Volume 47, Issue 4, pp. 251-256, 1999

[22] R. Singh, "Evaluation of Some Natural Plant Extracts against Root Knot Nematode, Meloidogyne Species on Cucurbitaceae", Ph.D. Thesis, M. J. P. Rohilkhand University, Bareilly, 1999