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Ameliorative effects of vermicompost on the root-knot nematode, *Meloidogyne incognita* (Kofoid & White) with reference to organic constituents in the leaves of vegetable lady's finger plant, *Hibiscus esculentus* L. (var. COBh H1)

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Abstract

The organic constituents (such as total carbohydrate, protein, total free amino acid and chlorophyll ('a' 'b' and total) of leaves of vegetable plant, lady's finger, *Hibiscus esculentus* L (var COBh H1) grown (under pot culture set up) in control soil, vermicompost amended soil, nematode inoculated soil and nematode inoculated soil with vermicompost were investigated in order to understand the suppressive effect of vermicompost on the root knot nematode, *Meloidogyne incognita* (Kofoid and White). The higher magnitudes of reductions in total carbohydrate content of leaves of plants grown in NIV soil condition for 15 and 30 days probably indicated the compensatory effect of vermicompost on the parasitic effect by way of enhanced utilization of synthesized carbohydrates towards the increased growth of plants. The suppressive effect of vermicompost on the parasitic infestation is well pronounced by the higher (+36 percent) magnitude of increase in the leaf total protein content of plants grown in nematode inoculated soil with vermicompost after prolonged growth for 30 days. The significant higher magnitude of -75 percent reduction in the free amino acid content of plants grown in nematode inoculated soil with vermicompost compared to those grown in nematode inoculated soil for 15 days probably indicated the suppressive effect of vermicompost on the parasitic effect thereby enhancing the plant growth. The significant reductions in the free amino acid content of leaves of *Hibiscus esculentus* grown in all the three experimental conditions following 30 days of growth probably indicated elevated protein synthetic activity in plants upon prolonged periods. The overall significant reduction in the chlorophyll content of the plants grown in nematode inoculated soil with vermicompost for 15 days probably indicated the absence of suppressive effect of vermicompost on the parasitic effect, particularly during the early periods of growth. On the other hand, the vermicompost supplementation of the nematode inoculated soil appeared to restore (by suppressing the parasitic effect) an increased synthesis of leaf chlorophyll content in the plants following prolonged period of growth for 30 days.

Keywords: Lady's finger plant, *Hibiscus esculentus*, root-knot nematode, *Meloidogyne incognita*, vermicompost, organic constituents, total carbohydrate, protein, total free amino acid and chlorophyll.

Introduction

Vermicompost is known to suppress plant diseases caused by different fungi and insect pests (Edwards and Burrows, 1988 and Scott, 1988 Nakamura,1996; Szczech, 1999; Rodriguez *et al*. 2000; Chaoui *et al.*, 2002; Rao *et al.*, 2001 and Arancon *et al*., 2005a and b). The parasitic effect of the root-knot nematode, *Meloidogyne incognita* was studied in plants with reference to crop production (Swathi *et al*.,1998; Ribeiro *et al*., 1998; Arancon *et al*., 2002 and Arancon *et al.*, 2002; and Arancon *et al.*, 2003a, b, and c).

A perusal of literature revealed that the suppressive effect of vermicompost on the effect of the root-knot nematode *Meloidogyne incognita* was studied only with reference to crop yield. Hence, the present study includes the impact of vermicompost on the effect of root-knot *Meloidogyne incognita* with special reference to

the organic constituents such as total carbohydrate, protein, total free amino acid and chlorophyll ('a', 'b' and total) in the leaves of the vegetable lady's finger plant, *Hibiscus esculentus* L (var COBh H1).

Material and Methods

Under pot culture set up, seedlings of lady's finger plant, *Hibiscus esculentus* L (var COBh H1) were grown for 15 and 30 days in control soil, vermicompost amended soil, in nematode inoculated soil and nematode inoculated soil with vermicompost.

After 15 and 30 days of growth, the levels of organic constituents such as total carbohydrate, protein, total free amino acid and chlorophyll ('a', 'b' and total) were estimated in the leaves of *Hibiscus esculentus* of control plants and of the plants grown in amended soils for 15 and 30 days of growth.

Estimation of total carbohydrate content

The total carbohydrate contents of leaves collected from the plants grown in control and amended soils for 15 and 30 days were estimated separately by anthrone method of Hedge and Hofreiter (1962) and expressed as mg/g of wet wt of leaf.

Estimation of protein content

The total protein contents in the leaves of control and experimental plants were estimated by the Direct Biuret method as explained by Gornall *et al*.,(1949) and given as percentage.

Estimation of free amino acid content

The free amino acid content of leaves collected from plants grown in control soil, in soil amended with vermicompost, in nematode inoculated soil and in nematode inoculated soil with vermicompost was estimated by the ninhydrin method of Moore and Stein, (1948) and given as mg/g wet wt of leaves.

Estimation of chlorophyll content

The contents of chlorophyll 'a', chlorophyll 'b' and total chlorophyll in the leaves of plants grown in control and amended soil were estimated following the method of Arnon, (1949) and expressed as mg/g wet wt of leaves.

Statistical analysis

In the present study, all the investigations carried out for a particular parameter were repeated six times and mean values were calculated. The change (either increase or decrease) in a particular parameter of plants in amended soils from that of plants grown in control soil was calculated as percentage. The significance of the difference between the mean values of plants grown in control soil and of plants grown in amended soils were analyzed by Student's't' test (Steel and Torrie, 1960). The mean levels of control and experimental groups of a particular growth period were separately analyzed (for their significance among themselves) by Analysis of Variance (ANOVA or 'F' test) (Multiple 'F' test) and Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

Results and Discussion

Table 1 to 5 provide the data on the chemical analysis of selected organic constituents such as total carbohydrate, protein, total free amino acid and chlorophyll ('a', 'b' and total) in the leaves of plants grown for 15 and 30 days in control and amended soils.

Total carbohydrate

In the present study, *Hibiscus esculentus* showed different magnitudes of changes in the leaf total carbohydrate content of plants grown under different experimental soil conditions compared to control plants (Table -1).The reduction in the leaf total carbohydrate content in plants could be either due to reduced photosynthetic activity or due to utilization of synthesized carbohydrates for biological purposes in the plant. The observed reduction in total carbohydrate content in the leaves of plants grown in vermicompost amended soil and in the nematode inoculated and vermicompost amended soils following 15 and 30 days of growth could be taken to suggest that the synthesized carbohydrates are utilized for the vegetative growth of thickening of stems and roots which might help in the establishment of plants during the initial period of growth. The observed reduction in total carbohydrate content of leaves of plants grown in nematode inoculated soil for 15 and 30 days probably indicates the parasitic effect of the root-knot nematode, *Meloidogyne incognita* on *Hibiscus esculentus*.

Table- 1: Total carbohydrate content (as mg/g wet weight of leaf) in the leaves of *Hibiscus esculentus* grown (under pot culture) in control soil (C), in vermicompost amended soil (VA), in nematode inoculated soil (NI) and in nematode inoculated soil with vermicompost (NIV) for 15 and 30 days after sowing. Values are means of six observations ± S.E. Percent changes from control level are given in parenthesis.

Period of growth	Grown in Control soil	Grown in amended soils			'F' Value
		VA	NI	NIV	
15 days	8.60 ± 0.08^{d}	6.25 ± 0.13^{c} (-27) S	3.87 ± 0.16^{b} (-55) S	3.09 ± 0.04^{a} (-64) S	118.65 HS
30 days	7.56 ± 0.02^{d}	6.04 ± 0.01^{c} (-20) S	4.28 ± 0.03^{b} (-43) S	3.45 ± 0.01^{a} (-54) S	96.45 HS

(-) - Denotes per cent decrease from control level. S - Statistically significant, P<0.05.
H S - Statistically highly significant, P<0.01. In a row, mean values followed by a common letter are not significant at 5% level by DMRT.

Table- 2: Protein content (as percent wet weight of leaf) in the leaves of seedlings of *Hibiscus esculentus* grown (under pot culture) in control soil (C), in vermicompost amended soil (VA), in nematode inoculated soil (NI) and in nematode inoculated soil with vermicompost (NIV) for 15 and 30 days after sowing. Values are means of six observations ± S.E. Percent changes from control level are given in parenthesis.

Period of growth	Grown in Control soil	Grown in amended soils			'F' Value
		VA	NI	NIV	
15 days	12.65 ± 0.06^{d}	2.64 ± 0.08^{a} (-79) S	2.84 ± 0.07^{b} (-78) S	3.79 ± 0.19^{c} (-70) S	48.40 S
30 days	2.94 ± 0.01^{a}	3.11 ± 0.01^{b} (+6) S	3.49 ± 0.03^{c} (+19) S	3.99 ± 0.04^{d} (+36) S	125.80 HS

(+) - Denotes per cent increase from control level. (-) - Denotes per cent decrease from control level.
S - Statistically significant, P<0.05. H S - Statistically highly significant, P<0.01.
In a row, mean values followed by a common letter are not significant at 5% level by DMRT.

Table -3: Free-amino acid content (as mg/g wet weight of leaf) in the leaves of *Hibiscus esculentus* grown (under pot culture) in control soil (C), in vermicompost amended soil (VA), in nematode inoculated soil (NI) and in nematode inoculated soil with vermicompost (NIV) for 15 and 30 days after sowing. Values are means of six observations ± S.E. Percent changes from control level are given in parenthesis.

Period of growth	Grown in Control soil	Grown in amended soils			'F' Value
		VA	NI	NIV	
15 days	12.43 ± 0.06^{d}	9.16 ± 0.07^{c} (-26) S	5.56 ± 0.13^{b} (-55) S	3.08 ± 0.01^{a} (-75) S	42.65 S
30 days	9.10 ± 0.11^{d}	8.11 ± 0.04^{c} (-11) S	6.58 ± 0.08^{b} (-28) S	4.84 ± 0.04^{a} (-47) S	36.45 S

(-) - Denotes percent decrease from control level. S - Statistically significant, P<0.05.
In a row, mean values followed by a common letter are not significant at 5% level by DMRT.

Table – 4: Chlorophyll ('a', 'b' & total) content (as mg/g wet weight of leaf) in the leaves of *Hibiscus esculentus* grown (under pot culture) in control soil (C), in vermicompost amended soil (VA), in nematode inoculated soil (NI) and in nematode inoculated soil with vermicompost (NIV) for 15 days after sowing. Values are means of six observations ± S.E. Percent changes from control level are given in parenthesis.

Types of Chlorophyll	Grown in Control soil	Grown in amended soils			'F'Value
		VA	NI	NIV	
Chlorophyll 'a'	1.07 ± 0.01^{c}	1.03 ± 0.03^{b} (-4) NS	1.35 ± 0.04^{d} (+26) S	0.02 ± 0.01^{a} (-98) S	112.65 HS
Chlorophyll 'b'	0.47 ± 0.01^{b}	0.40 ± 0.02^{a} (-14) NS	0.67 ± 0.07^{d} (+42) S	0.48 ± 0.01^{c} (+2) S	108.45 HS
Total chlorophyll	1.52 ± 0.04^{b}	1.56 ± 0.02^{c} (+3) S	2.25 ± 0.04^{d} (+48) S	0.64 ± 0.01^{a} (-58) S	98.55 HS

(+) - Denotes per cent increase from control level. (-) - Denotes per cent decrease from control level.
S - Statistically significant, $P<0.05$. H S - Statistically highly significant, $P<0.01$.
In a row, mean values followed by a common letter are not significant at 5% level by DMRT.

Table- 5: Chlorophyll ('a', 'b' & total) content (as mg/g wet weight of leaf) in the leaves of *Hibiscus esculentus* grown (under pot culture) in control soil (C), in vermicompost amended soil (VA), in nematode inoculated soil (NI) and in nematode inoculated soil with vermicompost (NIV) for 30 days after sowing. Values are means of six observations ± S.E. Percent changes from control level are given in parenthesis.

Types of Chlorophyll	Grown in Control soil	Grown in amended soils			'F'Value
		VA	NI	NIV	
Chlorophyll 'a	0.07 ± 0.01^{a}	1.06 ± 0.02^{b} (+1414) S	1.48 ± 0.01^{d} (+2014) S	1.33 ± 0.07^{c} (+1800) S	116.50 HS
Chlorophyll 'b'	0.38 ± 0.01^{c}	0.39 ± 0.01^{d} (+2) S	0.32 ± 0.05^{b} (-15) S	0.21 ± 0.01^{a} (-44) S	128.40 HS
Total chlorophyll	0.73 ± 0.01^{a}	1.56 ± 0.04^{b} (+114) S	1.92 ± 0.03^{d} (+163) S	1.64 ± 0.01^{c} (+125) S	94.50 HS

(+) - Denotes per cent increase from control level. S - Statistically significant, $P<0.05$.
H S - Statistically highly significant, $P<0.01$.
In a row, mean values followed by a common letter are not significant at 5% level by DMRT.

Protein

The observed -79 percent reduction in the leaf protein content of *Hibiscus esculentus* grown in vermicompost amended soil probably indicates utilization of proteins as enzymes towards the synthesis of cellulose resulting in increased plant growth following 15 days. On the other hand, - 78 percent reduction in the protein content of leaves with the absence of corresponding increase in the plant growth parameters probably indicates the disruptive effect of root-knot nematode on the protein synthetic machinery in the plant following 15 days of growth. On the other hand, following 30 days of growth, the observed significant increase in the protein content of leaves in all the three conditions probably indicates elevated levels of protein

synthesis. The higher magnitude of increase in the leaf total protein content of plants grown in nematode inoculated soil compared to that in vermicompost amended soil probably indicates the insensitiveness of plant tissue towards the parasitic effect following prolonged growth. However, the suppressive effect of the vermicompost on the parasitic effect of root-knot nematode is well pronounced by a higher magnitude of +36 percent elevation in the leaf protein content of plants grown in nematode inoculated soil with vermicompost following 30 days of growth (Table -2).

Total free amino acid

Unlike the above two biochemical constituents of leaves, the free amino acid content in the leaves of *Hibiscus esculentus* showed different magnitudes of reduction in plants grown in different experimental soil conditions for 15 and 30 days. The observed reductions in the free amino acid content of leaves of *Hibiscus esculentus* grown in vermicompost amended soil, nematode inoculated soil and nematode inoculated soil with vermicompost following 15 days of growth (Table-3) together with the reduction in total protein content of leaves (Table - 2) probably indicates utilization of free amino acids in the production of cellulose synthesizing enzymes for enhanced growth of plants during early periods. The significant higher magnitude of -75 percent reduction in the free amino acid content of leaves of plants grown in nematode inoculated soil with vermicompost compared to those grown in nematode inoculated soil for 15 days further attests to the suppressive effect of vermicompost on the parasitic effect thereby probably enhancing the plant growth. The significant reductions in the free amino acid content of leaves of *Hibiscus esculentus* grown in all the three experimental soil conditions following 30 days further supports a possible increased protein synthetic activity in the plant tissues following prolonged growth for 30 days (Table - 3).

Chlorophyll content a, b and total

In the present study, the observations on chlorophyll contents ('a', 'b' and total) in the leaves of *Hibiscus esculentus* grown in control and different experimental soils after 15 and 30 days of growth (Tables - 4 and 5) showed highly variable magnitudes. The absence of significant changes in the chlorophyll contents of plants grown in vermicompost amended soil for 15 days (Table - 4) probably indicates that the vermicompost did not exert its influence on synthesis of chlorophyll during early periods of growth. On the other hand, the stimulatory effect of the vermicompost on the chlorophyll synthesis in the plants is quite significantly evident following 30 days of growth (Table -5).

The observed significant higher elevations in the chlorophyll content of plants grown in nematode inoculated soil for 15 and 30 days probably indicates the parasitic impact of the root-knot nematode, *Meloidogyne incognita* on the chlorophyll content of the leaves of *Hibiscus esculentus*. This stimulated increase in the chlorophyll content together with the reduction in total carbohydrate content (Table - 1) probably indicates the exhaustion of carbohydrates from the plants and subsequent utilization by the parasitic nematode under infested conditions.

The overall significant reduction in the chlorophyll content of leaves of plants grown in nematode inoculated soil with vermicompost for 15 days (Table - 4) probably indicates the absence of suppressive effect of the vermicompost on the parasitic effect particularly during early periods of growth. However, the vermicompost supplementation of the nematode inoculated soil appeared to restore (by suppressing the parasitic effect) an increased synthesis of leaf chlorophyll content in the leaves of plants following prolonged period of growth for 30 days (Table - 5).

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