EFFECT OF ROOT-KNOT NEMATODE *MELOIDOGYNE INCognita* ON THE TOTAL PROTEIN, CARBOHYDRATE AND LIPID IN ROOTS AT DIFFERENT GROWTH STAGES OF *HIBISCUS ESCULENTUS*

BY

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Changes in the total protein, carbohydrate and lipid in the roots of *Hibiscus esculentus* resulting from infection with root-knot nematodes were studied at 15, 30, 45 and 60 days after inoculation. The effect of a nematicidal plant extract of *Anthocepalus cadamba*, applied 10 days after inoculation, on the test plants during their growth was also studied. The galls appeared on root within 15 days after inoculation and increased in number very rapidly as the plants grew. The treated plants had always fewer galls. The population of root-knot nematodes inside the roots rose to a peak in 45 days and declined thereafter. The treated plants had fewer nematodes at all the observed growth stages. The total protein, carbohydrate and lipid in roots increased in amount with the growth of test plants, both inoculated and uninoculated. The inoculated plants had always higher amount of protein but lower amount of carbohydrate and lipid in roots than the uninoculated ones. The effect of treatment could be measured with root-protein only. The root-carbohydrate and root-lipid, which are thought to be reduced due to feeding by nematodes, provide additional biochemical parameters for evaluating the intensity of infection with root-knot nematodes.

The total protein of galled roots of lady's finger, *Hibiscus esculentus* L. is proportional to the degree of infestation with root-knot nematodes measured 60 days after inoculation of plants (Chatterjee & Sukul, 1981). We tried to find whether root-protein content is a valid index for root-knot nematode infestation at different growth stages of test plants. In addition total carbohydrate and lipid in the roots of inoculated and un inoculated plants were also assayed to see whether these biochemical parameters could serve as additional indicators of root-knot nematode infestation. The development of root galls during the growth of host plants was also observed. Numbers inside roots were also estimated. Moreover, the result of treatment with a nematicidal plant extract on the development of root-knot disease in terms of the said parameters was observed.

MATERIALS AND METHODS

Pot test:

Seeds of *H. esculentus* were surface sterilized, germinated over moist filter papers and sown, one seed each in seventy-five 22 cm diameter clay pots
containing an autoclaved mixture 2:1 (v/v) of clay soil and composted manure. Fifty pots were inoculated with *Meloidogyne incognita* at 1012±58 juveniles/pot when the seeds were sown. Twenty-five pots served as uninoculated controls. Inoculation was with galled roots of *H. esculentus*, 2 g of fresh root choppings/pot. A sample of choppings contained 1012±58 juveniles/2 g roots (Christie & Perry, 1951). Twenty-five pots were treated 10 days after nematode inoculation with a water decoction of *Anthoccephalus cadamba* Miq. leaves at 250 ml extract per pot. The water extract was prepared by boiling 1 kg of leaves in 2.5 l water for 20 min (Sukul et al., 1974).

**Assay of root galls and nematode population:**

Five plants from each of the three batches, uninoculated, inoculated and treated, were randomly sampled and uprooted at intervals of 15 days during crop growth till the last harvest on 60th day. The root galls of the plants were counted. Samples of galled roots were analysed for the estimation of *M. incognita* population in them and an average per plant was determined (Christie & Perry, 1951). Data were analysed by analysis of variance.

**Assay of biochemical changes:**

Galled root pieces were thoroughly homogenized and a sample of 100 mg was taken for the quantitative determination of protein by the Folin-Ciocalteu reagent (Colowick & Kaplan, 1957). Five samples representing plants of each harvest were taken and an average expressed in terms of percentages root-protein by dry weight (wt of protein/dry wt of root × 103). Total carbohydrate in galled roots was determined colorimetrically by the method of Dische and Popper (Colowick & Kaplan, 1957). Total lipid in galled roots was estimated gravimetrically by the method of Folch et al., 1957. The last two were also expressed in terms of percentages by dry weight. All data were analysed statistically.

**RESULTS**

**Changes in root galls and nematode numbers:**

Numbers of root galls increased rapidly throughout the observed period of growth. They had appeared within 15 days of inoculation. Small galls (<2mm in diam) appeared first and were far more numerous than the larger ones. Treatment with the *A. cadamba* extract reduced root-galling significantly at all the four stages of growth (Table 1).

Within 15 days 73% of the *M. incognita* juveniles applied had entered the roots of untreated plants. In the treated plants the number was much smaller
TABLE I

Changes in root-galling and nematode population in roots at different growth stages of lady’s finger inoculated with Meloidogyne incognita.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Days of growth</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of galls</td>
<td></td>
</tr>
<tr>
<td>Inoculated</td>
<td>42.3 x</td>
</tr>
<tr>
<td>Treated</td>
<td>8.0 y</td>
</tr>
<tr>
<td>No. of juveniles</td>
<td></td>
</tr>
<tr>
<td>Inoculated</td>
<td>743.7 x</td>
</tr>
<tr>
<td>Treated</td>
<td>297.7 y</td>
</tr>
</tbody>
</table>

a Average of five plants.
b Inoculum given at the rate of 1012±58 juveniles per pot at the time of sowing of seeds.
c Plants were treated with the water decoction of Anthocephalus cadamba leaves. Same letters in vertical columns for each character are not significantly different from each other in the analysis of variance (P=0.05).

(29%). Nematode numbers increased in the roots for 45 days after which it decreased slowly. Numbers in the treated roots almost equalled the number added initially after 45 days of growth (Table I).

Biochemical changes:

Total protein, carbohydrate and lipid in the roots are in Fig. 1 (A, B and C). Root-protein increased steadily in all plants as they grew (Fig. 1A). Plants inoculated and untreated always contained most protein. Plants treated with nematicidal extract of A. cadamba did not much differ from the uninoculated controls except at the third harvest.

Root carbohydrate increased slowly with the growth of plants (Fig. 1B). Uninoculated controls always contained most root carbohydrate. Plants treated with extract of A. cadamba contained more root carbohydrate than those which had nematode inoculum at the fourth harvest only.

Root lipid, like root carbohydrate, was always significantly more in uninoculated controls than in inoculated ones (Fig. 1C). Lipid increased with plant growth. Among the nematode inoculated plants the ones treated with the decoction of A. cadamba differed significantly from the non-treated plants in having greater amount of root lipid at 45 and 60 days.
Fig. 1. (A, B, C). Changes in root protein (A), root carbohydrate (B) and root lipid (C) of lady's finger inoculated with *Meloidogyne incognita* at different growth stages. One batch was treated with the water decoction of *Anthocephalus cadamba* leaves. Lines in centre of bars represent standard deviations of mean (five replicates). Letters a, b and c on top of bars indicate significant differences among three batches of plants at a particular growth stage in the analysis of variance (P=0.05). Letters p, q, r and s indicate significant differences between various growth stages of a particular batch (uninoculated, inoculated or treated).

**DISCUSSION**

Table I shows that for a given inoculum the number of root galls depended on the age of the host. It is evident from Fig. 1A that the amount of root protein could serve as a reliable measure for evaluating nematode infestation as well as a nematicide at any stage of growth of test plants. This confirms our earlier observation that the total protein in roots is a good indicator of root-knot nematode infestation (Chatterjee & Sukul, 1981). There is a report that total proteins increased by 21 to 45% over the control in roots of *Solanum melongena* infected with *M. incognita* on days 30, 60 and 90 after inoculation (Singh *et al.*, 1978).
The decrease in root carbohydrate and root lipid in nematode-infected plants (Fig. 1 B, C) might be due either to rapid consumption of those substances by nematodes or to the metabolic shift caused by the secretions of invading nematodes. Infections by biotrophs often lead to a marked increase in the respiration rate of host tissues (Cooke, 1977). This might result in reduction of carbohydrate translocated to infected roots. Enzymes involved in carbohydrate metabolism are known to increase in roots due to root-knot nematode infection (Veech & Endo, 1970). Decrease in root carbohydrate resulting from nematode infection has been reported in other plants (Owens & Specht, 1966; Ramana & Rao, 1976; Singh et al., 1978; Nasar et al., 1980).

Root carbohydrate and root lipid could provide a measure for nematode infestation at any stage of growth of test plants. However, the efficacy of a nematicide cannot be evaluated by quantifying these substances (Fig. 1 B, C).

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REFERENCES


