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Uptake of nitrogen, phosphorous and potassium content in bitter gourd, *Momordica charantia* varieties infected by root knot nematode, *Meloidogyne incognita*

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Abstract

Seventeen bitter gourd varieties were collected and screened under greenhouse condition for both susceptibility and biochemical reactions to root-knot nematode (RKN) *Meloidogyne incognita*. Only one variety was resistant with 7-10 number of galls per plant while others were moderately resistant to highly susceptible with > 10 numbers of galls per plant. In order to understand the basis of nematode resistance six varieties namely Sundargarh local-1, Amatalla Beejghar Karala long green, Ankur hybrid (Moderately resistant) and Nakhara local, Indojapane hybrid, Rajsunakhala local-1 (Highly susceptible) were sown in earthen pots in the greenhouse maintaining four replications in Completely Randomized Design. This experiment was terminated at 45 days after sowing of seeds or 30 days after inoculation of nematodes. The Nitrogen content (%) in shoot was significantly lower by 11.628 to 81.819 in the resistant varieties as compared to that of susceptible varieties but increased by 5.54 per cent in root system of resistant variety in contrast to susceptible one (37.107%). The infected plants had decreased per cent of phosphorus content in shoots and increased per cent of roots in highly susceptible and moderately resistant varieties. Significant increase of potassium content of both susceptible and resistant infected plants which increase was more pronounced in both shoots and roots of resistant varieties as compared to the susceptible varieties.

Keywords: Bitter gourd, *Meloidogyne incognita*, nitrogen, phosphorus, potassium

1. Introduction

Bitter gourd (*Momordica charantia*), a tropical and subtropical vine of the family Cucurbitaceae, is a commercially important vegetable crop growing in India. From the recent report of Horticultural Statistics, Govt. of India 2018 [6], it has been found that, there is a gradual increase in the area and production of bitter gourd in India. The most prominent states in bitter gourd production are Chhattisgarh, Telangana, Andhra Pradesh, Odisha, Madhya Pradesh. In Odisha the production is 106.81 metric tons which shares about 10.21% of the total production of the country [6]. The *Meloidogyne* species are polyphagous and endoparasitic plant nematodes responsible for causing the root-knot disease by infecting the roots of vegetable crops of mostly all families. *Meloidogyne spp.* are the most prevalent economical crop pests worldwide (Oka *et al.*, 2000; Sasser, 1989) [12, 14] and they interfere with anchorage and absorption of crop plants. The cells associated with *Meloidogyne* infection, there is establishment of giant cells which proliferate and undergo hyperplasia and hypertrophy to form characteristic root galls. The morphological response of plants to nematode infection resulted in severe stunting, chlorosis, wilting and drooping of leaves, delay in flowering, fruit formation and yield, aggregation of nutrient deficiencies and retardation of growing point of shoot and root systems. In general, nematode pests affect host plants both quantitatively by reducing yield about 20.6% worldwide (Sasser, 1989) [14] and qualitatively by reducing the quality of the crops. Such damage varied mainly depending on host suitability, nematode genera and or species. Root-knot nematodes potentially alter the host metabolism by disturbing the physiological and biochemical mechanisms of the host plants (Ganguly *et al.*, 1991; Zinov'eva *et al.*, 2004) [3, 16]. There was a considerable interference in the metabolism of protein, nitrogen and carbohydrate in the nematode infected plants. Nematode species decrease the host content of total carbohydrates, total protein, total soluble sugars and the minerals Ca, Fe, Mg, N, P, K and Zn (Pathak *et al.*, 1983) [13]. The rates of chemical reductions are generally higher in roots than in shoots which depend on nematode species, nematode density and host plant. The root-knot nematodes achieve higher rates of chemical reduction on infected plants. Total proteins, total soluble sugars, Zn, Mg, Fe, and P are most affected (Hofmann and

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Grundler, 2007; Hofmann *et al.*, 2008) [4, 5]. This study was primarily focused on analyzing the change in nutrient status *i.e.* macro nutrients like N, P, K both in susceptible and resistant varieties of bitter gourd infected with root knot nematode, *Meloidogyne incognita*.

2. Materials and Methods

In order to understand the basis of nematode resistance, six varieties namely Sundargarh local-1, Amatalla Beejghar Karala long green, Ankur hybrid (Moderately resistant), Nakhara local, Indojapane hybrid and Rajsunakhala local-1 (Highly susceptible) were grown in earthen pots in the greenhouse of Department of Nematology, College of Agriculture, OUAT. The earthen pots of 15 cm height x 15 cm diameter were sterilized with formaldehyde solution (1%) and were filled with autoclaved soil (15lbs/ 20 min). Pots were arranged inside the green house condition with Completely Randomized Design for maintaining four replications. The water used for irrigation purpose was passed through a 500-mesh sieve before use.

2.1 Estimation of nitrogen and crude protein content

Crude protein and nitrogen content of shoot were estimated by the procedure of Mahadevan and Sridhar, 1986 [10]. Two hundred mg of powdered plant parts were taken in 100 ml micro Kjeldahl digestion flasks. About 200 mg of digestion mixture ($K_2SO_4:CuSO_4 = 5:1$) and 4 ml of concentrated H_2SO_4 were added. These flasks were kept as such for about 1 hour and then heated slowly till frothing occurred. Two crystals of sodium thio-sulphate were added to each digestion flask to check the frothing. Thereafter, digestion was continued until the contents of the flask became completely clear blue syrupy liquid without any bubbling. Then the flask was cooled and the content was diluted with 25 ml distilled water. Then 10 ml of diluted sample extract was transferred into micro Kjeldahl distillation unit. Thereafter, 10 ml of 40% NaOH was added and distillation was continued for 10 minutes. During distillation period, liberated ammonia was absorbed by 150 ml conical flask containing 2 drops of mixed indicator. After completion of distillation, distillate was titrated against 0.05 NH_2SO_4 .

Calculation

$$N_2 \% \text{ in sample} = \frac{(\text{Sample titer} - \text{blank titer}) \times N_2 \text{ of } H_2SO_4 \times 14 \times 100 \times 2.5}{\text{Sample weight (g)} \times 1000}$$

2.2 Estimation of phosphorus and potassium content

2.2.1 Digestion of samples

Powdered plant samples (0.2 g) were taken in 100 ml conical flasks. Fifteen ml of concentrated HNO_3 was added to each flask. The flasks were kept as such for overnight. Then the flasks were heated with hot plates till brown fumes evolved. Then 5ml of di-acid mixture ($HNO_3: HClO_4$ (70%) = 3:2) was added to each flask. Again, the flasks were heated till white fumes evolved reducing the volume of content to about 2ml. Thereafter, conical flasks were taken out from hot plate and

allowed to cool. One ml of 6N HCL was added and flasks were heated gently for 1 minute. Then 15 ml of warm distilled water was added to each flask. The content of the conical flask was transferred to a 50 ml volumetric flask followed by twice rinsing with distilled water. Then the volume was made up to 50 ml with distilled water and the aliquot was filtered through Whatman No.42 filter paper. The filtered extract was kept for the estimation of phosphorus and potassium.

2.2.2 Estimation of phosphorus content

Phosphorus present in plant samples was estimated by adopting the procedure of Jackson, 1973 [9]. Standards of 0, 2.5, 5.0, 7.5 and 10.0 ml of 25 ppm phosphorus solution and 2 ml of digested sample extracts were taken in 25 ml volumetric flasks. Five ml of 2 $NHNO_3$ solution was added to each flask. Then required amount of distilled water was added to each flask to make the final volume 15 ml. Thereafter, 2.5 ml molybdate-vanadate solution was added. Final volume was made up to 25 ml with distilled water and flasks were shaken well. Absorbance was measured by a colorimeter at 420nm after 20 minutes of shaking. The phosphorus content of plant samples was calculated in percentage by using the standard curve.

2.2.3 Estimation of potassium content

One ml digested sample extract of shoot and root were taken in 25 ml volumetric flasks and the volume was adjusted to 25 ml with distilled water. Similarly, 1, 2, 3, 4 and 5 ppm standard K solution (*i.e.* 0.1907 g KCL/lit) were taken in 100 ml volumetric flasks with water. The readings for standards and samples were taken in a digital flame photometer. As per the standard curve, the ppm of potassium present in extracting solution was calculated. Then the percentages of potassium present in shoot samples were calculated.

3. Result and Discussion

3.1 Nitrogen content influenced by the nematodes

The total nitrogen content was decreased in the shoot system of infected varieties of bitter gourd, Sundargarh local-1, Amatalla Beejghar Karala long green Ankur hybrid, Nakhara local, Indojapane hybrid and Rajsunakhala local-1 by 1.254, 2.068, 2.178, 2.013, 0.605, 0.572 per cent respectively over control on dry weight basis. Whereas in the root, nitrogen content was increased by 1.943, 2.589, 2.453, 2.163, 2.398 per cent in all the above mentioned varieties accordingly. The total nitrogen content decreases in shoot system of bitter gourd varieties due to the infection of *M. incognita* to the extent of 81.819 per cent in Rajsunakhala local (HS) to 11.628 per cent in Sundargarh local-1 (MR) over control (Table 1). Whereas in the roots the nitrogen content increases in all the varieties. The nitrogen content (%) both in root and shoot were significantly higher in the susceptible varieties as compared to that of resistant variety. The increase of total nitrogen content of nematode infected root samples is in confirmative with the findings of the earlier workers (Mohanty and Pradhan, 1990; Zaki and Bhatti, 1986 [11, 15]).

Table 1: Variation in nitrogen content in healthy (H) and root-knot infected (I) varieties of bitter gourd

Variety	Nitrogen content (%) on dry weight							
	Shoot				Root			
	Infected	Healthy	Mean	Change over (%)	Infected	Healthy	Mean	Change over (%)
Sundargarh local-1 (MR)	1.254	1.419	1.337	-11.628	1.943	1.841	1.892	5.540
Amatalla Beejghar Karala long green (MR)	2.068	2.464	2.266	-16.071	2.589	2.406	2.497	7.605
Ankur hybrid (MR)	2.178	2.805	2.491	-22.352	2.453	2.255	2.354	8.780
Nakhara local (HS)	2.013	3.047	2.531	-33.935	2.163	1.952	2.058	10.81
Indojapane hybrid (HS)	0.605	1.837	1.221	-67.065	2.398	2.039	2.219	17.607
Rajsunakhala local-1(HS)	0.572	3.146	1.859	-81.819	2.398	1.749	2.073	37.107
SE(m)+	0.302	0.283			0.094	0.102014		
CD(P=0.05)	0.29	0.087			0.06	0.09		

3.2 Phosphorus content influenced by nematodes

The phosphorus content was decreased in the shoot system of infected varieties of Rajsunakhala local-1, Indopanae hybrid, Nakhara local, Ankur hybrid, Amatalla Beejghar Karla long green and Sundargarh local-1 by 44.868, 36.988, 24.419, 8.738, 5.516 and 1.545 per cent respectively and increased in infected root by 0.819, 0.729, 0.649, 0.633 and 0.571 percent respectively over control on dry weight basis. Whereas phosphorus content was recorded highest as 55.408% in infected roots of variety Rajsunakhala local-1 followed by

42.424% in Indojapane hybrid, 24.615% in Nakhara local, 23.384% in Ankur hybrid, 15.301% in Amatalla Beejghar Karala long green and 8.144% in Sundargarh local-1 (Table 2). The results of the present investigation revealed that the infected plants had decreased percentage of phosphorus content in shoots with increased percentage in roots of susceptible and resistant varieties. Similar trend was also observed by (Chakraborti and Mishra, 2002; Hunter, 1958) ^[1, 7] in root-knot nematode infected plants.

Table 2: Variation in phosphorous content in healthy (H) and root-knot infected (I) varieties of bitter gourd

Variety	Phosphorous content (%) on dry weight							
	Shoot				Root			
	Infected	Healthy	Mean	Change over (%)	Infected	Healthy	Mean	Change over (%)
Sundargarh local-1 (MR)	0.446	0.453	0.450	-1.545	0.571	0.528	0.550	8.144
Amatalla Beejghar Karala long green (MR)	0.531	0.562	0.547	-5.516	0.633	0.549	0.591	15.301
Ankur hybrid (MR)	0.376	0.412	0.394	-8.738	0.649	0.526	0.588	23.384
Nakhara local (HS)	0.325	0.430	0.378	-24.419	0.729	0.585	0.657	24.615
Indojapane hybrid (HS)	0.523	0.830	0.677	-36.988	0.705	0.495	0.600	42.424
Rajsunakhala local-1(HS)	0.419	0.760	0.590	-44.868	0.819	0.527	0.673	55.408
SE(m)+	0.033	0.073			0.035	0.012		
CD(P=0.05)	0.040	0.034			0.025	0.019		

3.3 Potassium content influenced by nematodes

The root-knot nematode inoculated plants measured an increase in K₂O content to the tune of 59.542, 42.029, 31.422, 29.136, 10.754 and 3.183 per cent in shoots and 38.772, 24.191, 17.297, 14.738, 7.274 and 2.618 per cent in roots of varieties, Sundargarh local-1, Amatalla Beejghar Karala long green, Ankur hybrid, Nakhara local, Indojapane hybrid and Rajsunakhala local-1 respectively. Table 3 revealed that there was significant increase of potassium content of both

susceptible and resistant infected plants which was more pronounced in shoots of resistant varieties as compared to the susceptible variety. A decrease in potassium content due to infection of *Xiphinema americanum* and *M. incognita* in sour cherry and chickpea respectively reported by Chakraborti and Mishra, 2002 ^[1]. In the nematode infected plant tissues various compounds relating to ion-exchange may be decreased by the reduction of potassium content in nematode infected plant.

Table 3: Variation in potassium content in healthy (H) and root-knot infected (I) varieties of bitter gourd

Variety	Potassium content (%) on dry weight							
	Shoot				Root			
	Infected	Healthy	Mean	Change over (%)	Infected	Healthy	Mean	Change over (%)
Sundargarh local-1 (MR)	1.881	1.179	1.530	59.542	1.514	1.091	1.303	38.772
Amatalla Beejghar Karala long green (MR)	1.764	1.242	1.503	42.029	1.612	1.298	1.455	24.191
Ankur hybrid (MR)	1.719	1.308	1.514	31.422	1.458	1.243	1.351	17.297
Nakhara local (HS)	1.569	1.215	1.392	29.136	1.487	1.296	1.392	14.738
Indojapane hybrid (HS)	1.411	1.274	1.343	10.754	1.342	1.251	1.297	7.274
Rajsunakhala local-1(HS)	1.394	1.351	1.373	3.183	1.215	1.184	1.200	2.618
SE(m)+	0.081	0.026			0.057	0.032		
CD(P=0.05)	0.291	1.125			0.223	0.118		

4. Conclusion

All the six infected varieties taken for biochemical studies exhibited significant reduction in different growth parameters due to nematode infection. The single generation of *M. incognita* showed the changes in both concentration and total content of different elements in the bitter gourd varieties to establish relationship between nutrients status and physiological processes. Plant nutrients profile *viz.* macro nutrients are considered to be the essential supplement for the better crop yield. Root-knot nematode infection leads to the reduced photosynthetic ability of the plants by combining reduction in leaf chlorophyll contents^[3]. The present study showed that leaf NPK contents of the root-knot nematode infected plants had decreased compared with healthy uninfected leaves. The present study showed that it had significant impact in reducing the nutritional value of crops. During biochemical studies it was observed there was variation between healthy and infected plant parts (leaves, roots) which showed significant changes with response to contents. The data indicate that a change in the concentration of the nutrient elements in plant is probably one of the first effects of the nematode on host physiology. These changes in nutrient concentration after host metabolism contributes directly or indirectly to the chlorosis of infected plants. These effects on the host increase with level and duration of infection and, along with changes in other physiological problems such as photosynthesis appear to be the main cause of a lower yield in nematode infected plants. The change in potassium concentration seems to be important because of its effect on photosynthesis either by affecting carbon dioxide uptake or by altering other key physiological processes such as osmotic potential.

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