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Variation in nutrient status of susceptible and resistant bitter gourd varieties infected by root knot nematode, *Meloidogyne incognita*

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Abstract
Seventeen bitter gourd genotypes/ seeds 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 plants while others were moderately resistant to highly susceptible with > 10 numbers of galls per plants. 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 11.92-69.34 in the resistant varieties as compared to that of susceptible variety but increased by 5.94% in root system of resistant variety in contrast to susceptible one (37.07%). The crude protein of the infected shoot decreased in the similar trend like nitrogen content of the infected shoot. The infected plants had decreased percent of phosphorus content in shoots of highly susceptible and moderately resistant varieties except Sundargarh local-1 with increased percent of phosphorus content in roots. 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. In shoot and root the micronutrients were observed decrease percentage in Zinc by 19.00-21.76 and 9.53-23.93, Iron by 3.11-4.1 and 14.35-33.85, Copper by 5.91-48.04 and 15.10-25.08 respectively, except Manganese by 4.10-18.5 in shoot and an increase trend of percentage of 6.35 to a decrease trend of 41.02percent in infected plants over control in varieties of bitter gourd.

Keywords: Bitter gourd, *Meloidogyne incognita*, nitrogen, crude protein, phosphorus, potassium, zinc

1. Introduction

Bittergourd (*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 [8], 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 bittergourd 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 [10].

The *Meloidogyne* species are polyphagous and endoparasitic plant nematodes responsible for causing the root-knot disease by attacking the roots of vegetable crops of mostly all families. *Meloidogyne spp.* are the most prevalent economical crop pests worldwide [17, 19] and they interfere with anchorage and absorption of crop plants. The cells surrounding the developing juvenile and giant cells proliferate and enlarge to form a gall, because of hyperplasia and hypertrophy condition of the infected cells. 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 [19] 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 [4, 22].

Pathak *et al*. 1983 [18] concluded that there was 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; however total phenol and tannins are increased. 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 [6,7]. This study was primarily focused on analyzing the change in nutrient status e.g. (macro nutrients like N, P, K and micro nutrients like (Fe, Zn, Cu, Mn) both in susceptible and resistant varieties of bittergourd, 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 Dept. of Nematology, C.A, OUAT. The earthen pots of 15 cm height x 15 cm diameter were sterilized with formaldehyde solution (1.0%) and were filled with autoclaved soil (15lbs/20min). 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) [12]. 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 one hour and then heated slowly till frothing occurred. Two crystals of sodium thiosulphate 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

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

Crude protein

Percentage of protein present in shoots and roots were determined by multiplying the N_2% with 6.25. This protein is called crude protein.

2.2 Estimation of phosphorus and potassium content

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.

2.2.1 Estimation of phosphorus present in plant samples

Phosphorus present in plant samples was estimated by adopting the procedure of (Jackson 1973) [11]. 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 $2NHNO_3$ 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.

2.2.2 Estimation of potassium

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.

2.3 Estimation of micro-nutrients influenced by root knot nematode in bitter gourd plant samples

Mineral acids like of diacid (HNO_3 - $HClO_4$) digestion [11] by SS method through model GCBAwanta. Powdered samples (0.2g) were taken in a 100 ml conical flask. To each flask 10 ml of concentrated HNO_3 was added. The flasks were kept undisturbed overnight. Then the flask containing samples were heated on a hot plate till brown fumes evolved. Five mlof di-acid mixture (HNO_3: $HClO_4$ (70%)::3:2 by volume) was added to each flask. Again, the flasks were heated till white fumes evolved reducing the volume of content to about 2 ml Thereafter, conical flasks were taken out from hot plate and allowed to cool. One ml then 15 ml warm distilled water was added to each flask. The content of 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. Then the filtrate was kept for estimation of mineral nutrients like potassium, sodium, phosphorus, calcium, magnesium and micro-nutrients like Fe, Zn, Cu, Mn by adopting the procedure of (Jackson 1973) [11].

Micro-nutrients (Fe, Zn, Cu, Mn) estimation

Digested sample was introduced to AAS for Fe, Zn, Cu, Mn analysis after standardizing the AAS with respective standards.

$$(\text{Fe, Zn, Cu, Mn})\ \text{mg/100g} = \frac{\text{AAS R x 50}}{\text{sample wt(g) x10}}$$

3. Result and discussion

3.1. Nitrogen content influenced by the nematode

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 1.14, 1.88, 1.98, 1.83, 0.55 and 0.52 percent respectively over control on dry weight basis. Whereas in the root's nitrogen content was increased by 2.02, 2.64, 2.18, 2.33, 2.63 and 2.18 percent in all the above-mentioned varieties accordingly. The total nitrogen content decreases in shoot system of varieties of bitter gourd due to the infection of *M. incognita* to the extent of 69.34 percent in Rajsunakhala local (HS)to 11.92 percent in Sundargard local-1 (MR) over control (Table 1, Fig 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 [14, 21].

Table 1: Percentage increase /decrease in Nitrogen content in Healthy (H) and root-knot infected (I) varieties of bitter gourd.

Sl. No.	Varieties	Nitrogen content% on dry weight basis							
		Shoot (leaf)				Root			
		Infected(I)	Healthy(H)	Mean	%increase (+)/ decrease (-) over control	Infected(I)	Healthy(H)	Mean	%increase (+)/ decrease (-) over control
01	Sundargarh local-1 (MR)	1.14.	1.29	1.21	-11.92	2.02	1.91	1.96	+5.94
02	Amatalla Beejghar Karala long green (MR)	1.88	2.24	2.06	-16.03	2.64	2.46	2.55	+6.95
03	Ankur hybrid (MR)	1.98	2.55	2.26	-22.31	2.18	1.99	2.09	+9.40
04	Nakhara local (HS)	1.83	2.77	2.30	-34.00	2.33	2.04	2.18	+13.82
05	Indojapane hybrid (HS)	0.55	1.67	1.10	-67.00	2.63	2.05	2.34	+28.57
06	Rajsunakhala local-1(HS)	0.52	2.86	1.11	-69.34	2.18	1.59	1.89	+37.07
	SE(m)±	0.0030	0.0029			0.0022	0.0033		
	CD(P=0.05)	0.0089	0.0087			0.0066	0.0099		

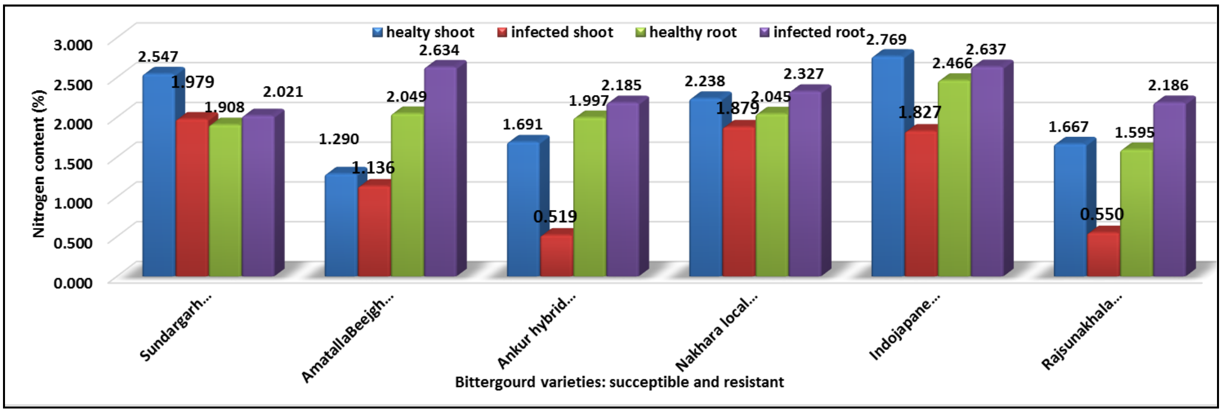

Fig 1: Percentage increase/ decrease in nitrogen content on dry weight basis

3.2. Phosphorus content influenced by the nematode

The phosphorus content was decreased in the shoot system of infected varieties of Rajsunakhala local-1, Indopanae hybrid, Nakhara local, Ankur hybrid and Amatalla Beejghar karla long green by 36.85, 18.80, 14.16, 10.41 and 9.76 percent respectively except Sundargarh local-1(3.88%) and increased in infected root by 0.55, 0.63, 0.77, 0.71, 0.71 and 1.02 percent in bitter gourd varieties like Sundargarh local-1, Amatalla Beejghar Karala long green, Ankur hybrid, Nakhara local, Indojapane hybrid and Rajsunakhala local-1 respectively over control on dry weight basis. Whereas phosphorus content was recorded highest as 67.73% in infected roots of variety Rajsunakhala local-1 followed by 54.99% in Indojapane hybrid, 31.54% in Nakhara local, 28.06% in Ankur hybrid, 27.18% in Amatalla Beejghar Karala long green and 5.20% in Sundargarh local-1(Table 2, Fig 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, 9] in root-knot nematode infected plants.

Table 2: Percentage increase /decrease in Phosphorus content in Healthy (H) and root-knot infected (I) varieties of bitter gourd.

Sl. No.	Varieties	Phosphorus content% on dry weight basis							
		Shoot (leaf)				Root			
		Infected (I)	Healthy(H)	Mean	%increase (+)/ decrease (-) over control	Infected (I)	Healthy(H)	Mean	%increase (+)/ decrease (-) over control
01	Sundargarh local-1 (MR)	0.52	0.50	0.51	+3.88	0.55	0.52	0.53	+5.20
02	Amatalla Beejghar Karala long green (MR)	0.39	0.44	0.42	-9.76	0.63	0.50	0.57	+27.18
03	Ankur hybrid (MR)	0.48	0.54	0.51	-10.41	0.77	0.60	0.68	+28.06
04	Nakhara local (HS)	0.48	0.56	0.52	-14.16	0.71	0.54	0.63	+31.54
05	Indojapane hybrid (HS)	0.42	0.52	0.47	-18.80	0.71	0.46	0.58	+54.99
06	Rajsunakhala local-1(HS)	0.52	0.83	0.67	-36.85	1.02	0.61	0.82	+67.73
	SE(m)±	0.013	0.011			0.006	0.008		
	CD(P=0.05)	0.040	0.034			0.019	0.025		

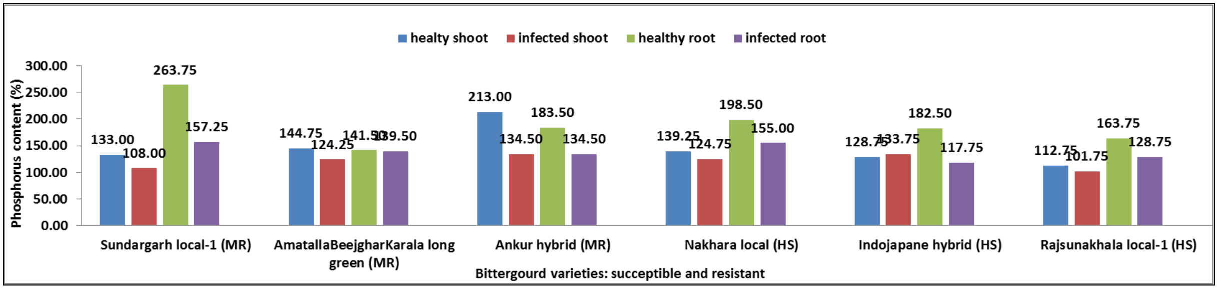

Fig 2: Percentage increase/ decrease in phosphorus content on dry weight basis

3.3. Influence of the nematode on potassium content

The root-knot nematode inoculated plants measured an increase in K_2O content to the tune of 35.96, 9.87, 8.59, 8.48, 4.48 and 1.42 percent in shoots and 27.03, 12.50, 8.76, 6.43, 2.94 and 0.59 percent 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 (Table 3, Fig 3). 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 'K.' content in nematode infected plant.

Table 3: Percentage increase /decrease in potassium content in healthy (H) and root-knot infected (I) varieties of bitter gourd.

Sl. No.	Variety	Potassium content% on dry weight basis							
		Shoot (leaf)				Root			
		Infected (I)	Healthy(H)	Mean	%increase (+)/ decrease (-)over control	Infected (I)	Healthy (H)	Mean	%increase (+)/ decrease(-)over control
01	Sundargarh local-1 (MR)	1.21	0.89	1.05	+35.96	1.41	1.11	1.26	+27.03
02	Amatalla Beejghar Karala long green (MR)	1.67	1.52	1.60	+9.87	1.89	1.68	1.74	+12.50
03	Ankur hybrid (MR)	1.39	1.28	1.34	+8.59	1.49	1.37	1.43	+8.76
04	Nakhara local (HS)	1.79	1.65	1.72	+8.48	1.49	1.4	1.45	+6.43
05	Indojapane hybrid (HS)	1.40	1.34	1.37	+4.48	1.40	1.36	1.38	+2.94
06	Rajsunakhala local-1(HS)	1.43	1.41	1.42	+1.42	1.70	1.69	1.70	+0.59
	SE(m)±	0.09	1.97			0.07	0.03		
	CD(P=0.05)	0.29	5.94			0.22	0.11		

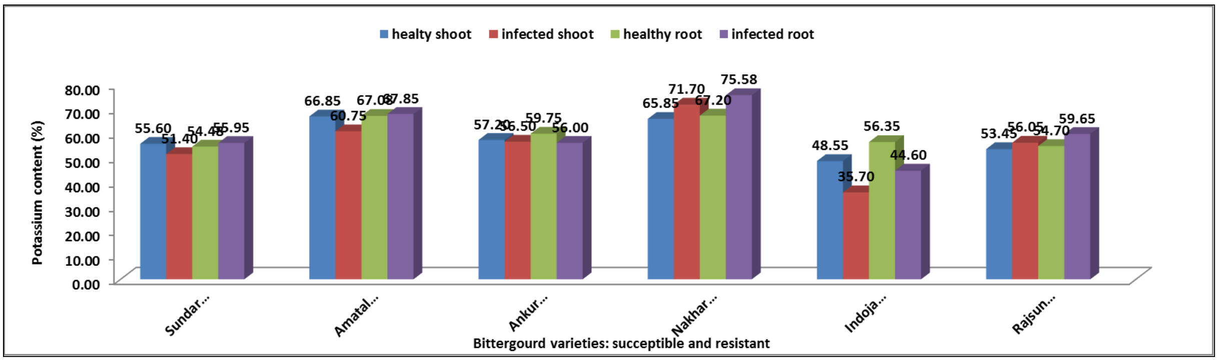

Fig 3: Percentage increase/ decrease in potassium content on dry weight basis

3.4. Effect of nematode infection on crude protein content

Due to infection of root-knot nematode, the percentage of shoot crude protein content was low (-69.34%) in variety Rajsunakhala local-1, -67% in Indojapane hybrid, -34% in Nakhara local, -22.31% in Ankur hybrid than that of Amatalla Beejghar Karala long green (-16.03%) and Sundargarh local-1 (-11.92%) whereas crude protein content in root was high in Rajsunakhala local-1(37.07%) than that of Indojapane hybrid (28.57%), Nakhara local (13.82%), Ankur hybrid (9.40%), Amatalla Beejghar Karala long green (6.95%) and Sundargarh local-1(5.94%) (Table 4, Fig 4). The crude protein of both the infected shoot decreased and infected root increased in the similar trend like nitrogen content of the infected shoot. The increased crude protein content in roots of nematode infected plants was reported in various works [2, 5, 16].

Table 4: Percentage increase /decrease in crude protein content in healthy (H) and root-knot infected (I) varieties of bitter gourd.

Sl. No.	Varieties	Crude protein content% on dry weight basis							
		Shoot (leaf)				Root			
		Infected(I)	Healthy (H)	Mean	%increase (+)/ decrease (-) over control	Infected (I)	Healthy (H)	Mean	%increase (+)/ decrease (-) over control
01	Sundargarh local-1 (MR)	7.10	8.06	7.58	-11.92	12.63	11.92	12.28	+5.94
02	Amatalla Beejghar Karala long green (MR)	11.75	13.99	12.87	-16.03	16.48	15.41	15.95	+6.95
03	Ankur hybrid (MR)	12.37	15.92	14.14	-22.31	13.66	12.48	13.07	+9.40
04	Nakhara local (HS)	11.42	17.30	14.36	-34	14.55	12.78	13.66	+13.82
05	Indojapane hybrid (HS)	3.44	10.42	6.93	-67	16.46	12.80	14.63	+28.57
06	Rajsunakhala local-1(HS)	3.24	10.57	6.91	-69.34	13.66	9.97	11.82	+37.07
	SE(m)+	0.018	0.018			0.014	0.020		
	CD(P=0.05)	0.055	0.054			0.041	0.061		

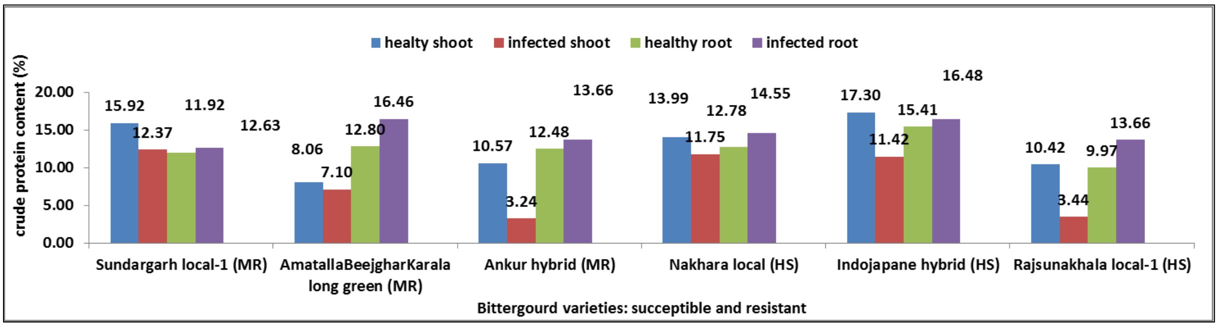

Fig 4: Percentage increase/ decrease in crude protein content on dry weight basis

3.5. Effect of nematode infection on micro-nutrient contents of plants

Root knot nematode infection often reduces plant growth and yield & decreases nutrient uptake in the infested plants showing the deficiencies of zinc, iron, copper, manganese due to root damage and subsequent prevention of water & nutrient uptake by the roots.

3.5.1. Action of nematode infection on zinc content

Table 5.a. revealed that zinc 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 3.70, 3.86, 4.39, 4.61, 4.77 and 5.38 mg/100g and in root system by 7.08, 7.75, 7.88, 8.8., 9.46 and 13.37 mg/100g respectively. Whereas zinc content was recorded highest in a decreased order as 23.93% in infected roots of variety Indojapane hybrid followed by 23.48% in Nakhara local, 22.08% in Rajsunakhala local-1, 14.14% in Amatalla Beejghar Karala long green, 9.53% in Ankur hybrid and 9.44% in Sundargarh local-1 over control on dry weight basis (Fig 5.a).

Table 5a: Percentage increase /decrease in zinc content in healthy (H) and root-knot infected (I) varieties of bitter gourd.

Sl. No.	Varieties	Zinc content mg/100g dry weight basis							
		Shoot (leaf)				Root			
		Infected (I)	Healthy (H)	Mean	%increase (+)/ decrease (-) over control	Infected (I)	Healthy (H)	Mean	%increase (+)/ decrease (-) over control
01	Sundargarh local-1 (MR)	3.70	4.59	4.15	-19.38	7.08	7.82	7.45	-9.44
02	Amatalla Beejghar Karala long green (MR)	3.86	4.76	4.31	-19.00	7.75	9.02	8.38	-14.14
03	Ankur hybrid (MR)	4.39	5.20	4.79	-15.58	7.88	8.71	8.29	-9.53
04	Nakhara local (HS)	4.61	5.56	5.09	-17.08	8.80	11.50	10.15	-23.48
05	Indojapane hybrid (HS)	4.77	6.09	5.43	-21.76	13.37	17.16	15.26	-22.08
06	Rajsunakhala local-1(HS)	5.38	6.27	5.82	-14.23	9.46	12.43	10.94	-23.93
	SE(m)+	0.23	0.10			0.13	0.92		
	CD (0.05)	0.71	0.31			0.39	2.76		

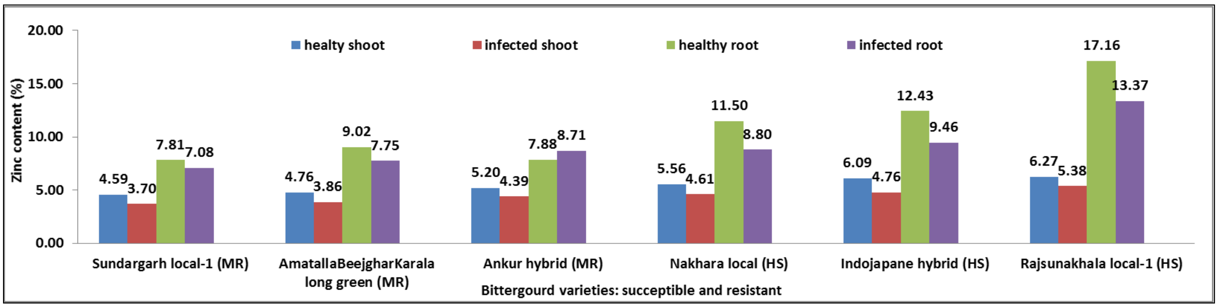

Fig 5a: Percentage increase/ decrease in zinc content mg/100g dry weight basis

3.5.2. Action of nematode infection on iron content

Table 5.b. depicted that iron 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 80.08, 96.33, 95.99, 79.10, 78.56 and 118.05 mg/100g and in root system by 314.22, 258.94, 293.41, 352.04, 280.51 and 235.25 mg/100g respectively. Whereas iron content was recorded highest in a decreased order as 33.85% in infected roots of variety Rajsunakhala local-1 followed by 25.95% in Indojapane hybrid, 20.16% in Amatalla Beejghar Karala long green, 19.39% in Ankur hybrid, 17.61% in Nakhara local and 14.35% in Sundargarh local-1 over control on dry weight basis (Fig 5.b).

Table 5b: Percentage increase /decrease in iron content in healthy (H) and root-knot infected (I) varieties of bitter gourd.

Sl. No.	Varieties	Iron content mg/100g dry weight basis							
		Shoot (leaf)				Root			
		Infected (I)	Healthy (H)	Mean	%increase (+)/ decrease (-) over control	Infected (I)	Healthy (H)	Mean	%increase (+)/ decrease (-) over control
01	Sundargarh local-1 (MR)	80.08	82.67	81.37	-3.13	314.22	366.89	340.56	-14.35
02	Amatalla Beejghar Karala long green (MR)	96.33	99.42	97.88	-3.11	258.94	324.31	291.62	-20.16
03	Ankur hybrid (MR)	95.99	100.17	98.08	-4.17	293.41	363.98	328.70	-19.39
04	Nakhara local (HS)	79.10	82.50	80.80	-4.13	352.04	427.31	389.68	-17.61
05	Indojapane hybrid (HS)	78.56	81.34	79.95	-3.41	280.51	378.83	329.67	-25.95
06	Rajsunakhala local-1(HS)	118.05	122.50	120.27	-3.63	235.25	355.62	295.43	-33.85
	SE(m)+	0.24	0.37			0.28	0.25		
	CD (0.05)	0.74	1.12			0.84	0.74		

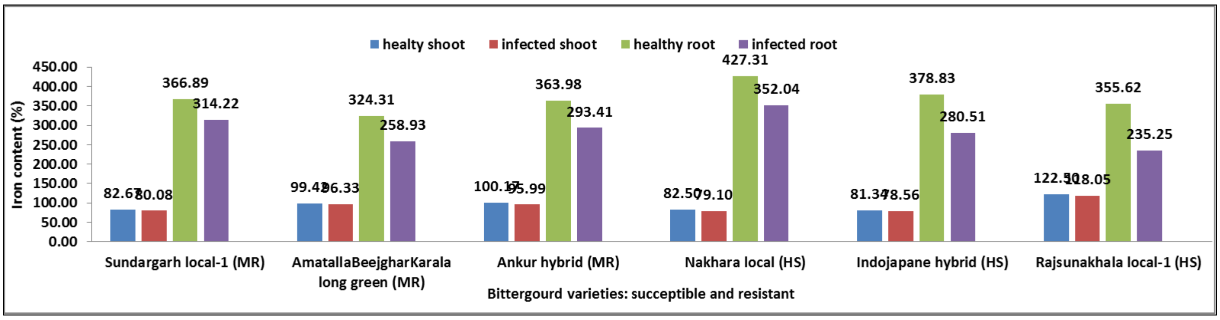

Fig 5b: Percentage increase/ decrease in iron content mg/100g dry weight basis

3.5.3. Action of nematode infection on copper content

As depicted in Table 5.c. copper 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 1.30, 1.68, 1.24, 1.31, 1.20 and 1.48 mg/100g and in root system by 0.26, 0.23, 0.24, 0.27, 0.23 and 0.29 mg/100g respectively. Whereas copper content was recorded highest in a decreased order as 25.08% in infected roots of variety Indojapane hybrid followed by 21.28% in Nakhara local, 18.84% in Sundargarh local-1, 18.41% in Rajsunakhala local-1, 17.36% in Amatalla Beejghar Karala long green and 15.10% in Ankur hybrid over control on dry weight basis (Fig 5.c).

Table 5c: Percentage increase /decrease in copper content in healthy (H) and root-knot infected (I) varieties of bitter gourd.

Sl. No.	Varieties	Copper content mg/100g dry weight basis							
		Shoot (leaf)				Root			
		Infected (I)	Healthy (H)	Mean	%increase (+)/ decrease (-) over control	Infected (I)	Healthy (H)	Mean	%increase (+)/ decrease (-) over control
01	Sundargarh local-1 (MR)	1.30	2.49	1.89	-48.0	0.26	0.32	0.29	-18.84
02	Amatalla Beejghar Karala long green (MR)	1.68	2.32	2.00	-27.40	0.23	0.27	0.25	-17.36
03	Ankur hybrid (MR)	1.24	1.42	1.33	-12.72	0.24	0.28	0.26	-15.10

04	Nakhara local (HS)	1.31	1.53	1.42	-14.38	0.27	0.34	0.30	-21.28
05	Indojapane hybrid (HS)	1.20	1.27	1.23	-5.91	0.23	0.30	0.26	-25.08
06	Rajsunakhala local-1(HS)	1.48	1.98	1.73	-25.32	0.29	0.35	0.32	-18.41
	SE(m)±	0.057	0.078			0.004	0.008		
	CD(P=0.05)	0.172	0.236			0.013	0.023		

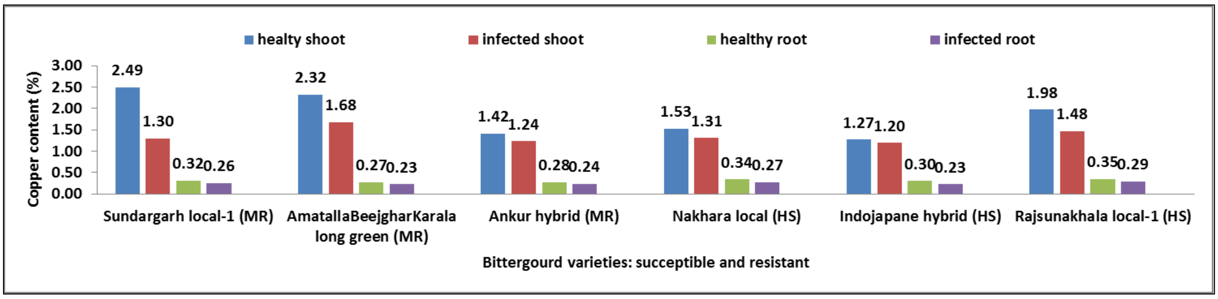

Fig 5c: Percentage increase/ decrease in copper content mg/100g dry weight basis

3.5.4. Action of nematode infection on manganese content

Table 5.d. revealed that manganese 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 4.67, 4.29, 5.52, 4.99, 4.35 and 7.13 mg/100g and in root system by 5.24, 5.31, 1.64, 6.68, 5.53 and 3.72 mg/100g respectively. Whereas manganese content was recorded highest as 6.35% in infected roots of variety Indojapane hybrid then 1.02% in Rajsunakhala local-1 followed by in a decreased order as 12.62% in Nakhara local, 16.32% in Amatalla Beejghar Karala long green, 22.67% in Sundargarh local-1 and 41.02% in Ankur hybrid over control on dry weight basis (Fig 5.d).

From the above discussion of results, it has been found that there is a decrease in the percentage of micronutrients like zinc, iron, copper & manganese content in both shoots and roots of susceptible and resistant varieties. More precisely, an increasing trend of Zn, Fe, Cu & Mn content from susceptible cultivars to resistant, except highly susceptible varieties like Indojapane hybrid and Rajsunakhala local-1in case of Mn. The above data is in confirmation with the research findings of Mohanty *et al*. 1999 [13] indicated that there was reduction of micro nutrients *viz*. Zn, Cu, Fe, Mn, Mo and B on cowpea when inoculated by *R. reniformis*. According to Nasar *et al.* 1983 [15], there was increase in concentration of calcium, magnesium, copper in both leaves and roots of bitter almond infested with root knot nematode. And Sher 1957 [20] also found chlorosis increased in the infected leaves due to deficiency of iron, copper, potassium infested by *Pratylenchus vulnus*.

Table 5d: Percentage increase /decrease in manganese content in healthy (H) and root-knot infected (I) varieties of bitter gourd

Sl. No.	Varieties	Manganese content mg/100g dry weight basis							
		Shoot (leaf)				Root			
		Infected (I)	Healthy (H)	Mean	%increase (+)/ decrease (-) over control	Infected (I)	Healthy (H)	Mean	%increase (+)/ decrease (-) over control
01	Sundargarh local-1 (MR)	4.67	5.58	5.13	-16.30	5.24	6.77	6.01	-22.67
02	Amatalla Beejghar Karala long green (MR)	4.29	5.27	4.78	-18.52	5.31	6.34	5.82	-16.32
03	Ankur hybrid (MR)	5.52	6.55	6.03	-15.69	1.64	2.79	2.21	-41.02
04	Nakhara local (HS)	4.99	5.47	5.23	-8.91	6.68	7.65	7.17	-12.62
05	Indojapane hybrid (HS)	4.35	4.66	4.50	-6.66	5.53	5.20	5.37	6.35
06	Rajsunakhala local-1(HS)	7.13	7.43	7.28	-4.10	3.72	3.68	3.70	1.02
	SE(m)±	0.046	0.113			0.045	0.079		
	CD (0.05)	0.138	0.339			0.136	0.237		

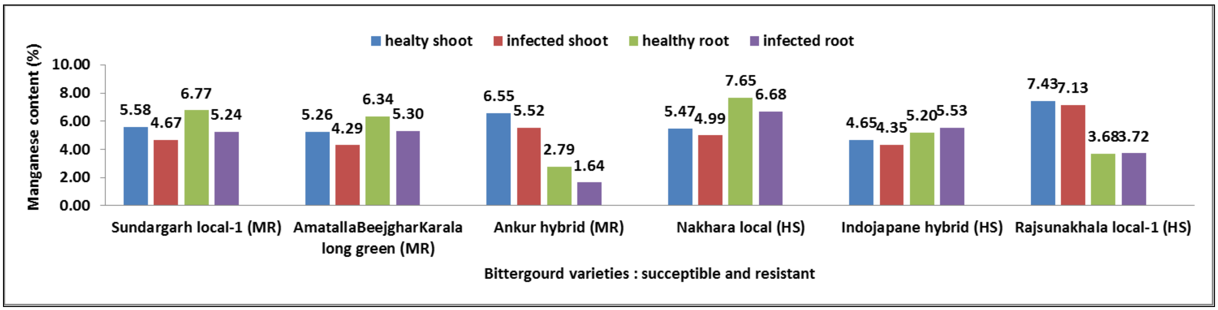

Fig 5d: Percentage increase/ decrease in manganese content mg/100g dry weight basis

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 including macro and micro 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. Over the life cycle of the nematode the trend of this varied from decreasing amounts of Zn, Cu and Mn maximum in shoots in all six tested entries except Fe content which was reversed i.e. more percentage decrease in roots than shoots. Mn, Cu and Zn changed more or less equally between shoots and roots. The data showed that these changes in host elemental concentration didn't affect nematode population but did affect nematode physiology. The changes of these elements such as Zn, Cu, Mn and Fe play an important role in synthesis of chlorophyll. The changes in the concentration of these nutrient elements in the plant, small as they may be appeared to have a profound effect on the host physiology. 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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