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Interaction effect of root-knot nematode, *Meloidogyne incognita* and vesicular arbuscular mycorrhizal fungus on chlorophyll and sugar content of resistant and susceptible cultivars of tomato

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Abstract
Study of biochemical characterization of shoots and roots of four tomato cultivars, Banki Local (Resistant), Kashi Aman (Moderately resistant), Utkala Dipti (Susceptible) and Utkala Kumari (Highly susceptible) grown under sterile, *Meloidogyne incognita* and *Glomus fasciculatum* inoculated soil both individually and in combinations revealed that post inflectional increase in chlorophyll 'a', 'b' and total in *G. fasciculatum* inoculated leaves and that of decrease in *M. incognita* and *M. incognita* + *G. fasciculatum* inoculated leaves over check. Decrease of chlorophyll content was maximum in highly susceptible variety Utkala Kumari (chl a-80.35%, chl b- 76.79%, Total chl- 78.19%) and that of minimum in resistant variety Banki local (chl a-10.98%, chl b- 8.48%, total chl- 9.62%) in nematode inoculated plant over check. There was decrease in total sugar content of shoot in T_1 having 10.57%, 22.49%, 43.63% and 52.73% & increase in root having 14.04%, 26.29%, 62.22% and 75.72% in Banki local, Kashi Aman, Utkala Dipti and Utkala Kumari respectively compared to untreated check (T_6). Percent decrease of starch content in shoot was maximum in T_3, followed by T_5, T_4, and that of T_2 over check. Maximum accumulation of starch was marked in the roots of T_3, T_4 and T_5 where both nematode and VAM were inoculated than T_1 over check. Comparing T_3, T_4 and T_5 maximum decrease of reducing sugar in shoot was observed in T_3 followed by T_5 and T_4 in all varieties over check. In T_1 maximum increase of reducing sugar in root was seen in Utkala Kumari (73.15%) followed by Utkala Dipti (65.53%), Kashi Aman (24.34%) and minimum in Banki Local (10.29%).

Keywords: *Glomus fasciculatum*, *Meloidogyne incognita*, chlorophyll, total sugar, starch, reducing sugar, tomato

Introduction
Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops, which is grown widely throughout the world, owing to its high nutritive value and diversified use. root-knot nematodes (RKN), *Meloidogyne* spp. are sedentary endoparasites that attack nearly all crops causing great yield losses [1] and also the major pest of tomato crop causing yield loss of 35% in India [2]. According to Trudgill and Block (2001) [3] *Meloidogyne incognita* is one of the most important apomictic species of root-knot nematodes in many temperate and tropical countries. RKN, *Meloidogyne incognita* also acts as mechanical wounding agent, host modifier, rhizosphere modifier and resistant breaker; inciting or aggravating fungi and bacteria producing disease complex on severe infestations can kill the tomato plant outright [4]. They interfere with anchorage, absorption of nutrients by the crop plants.
Among various obligate parasites, root-knot nematode (*Meloidogyne incognita*) and vesicular arbuscular mycorrhizal (VAM) fungus, *Glomus fasciculatum* are dependent on a common host for their nourishment, reproduction and concomitant infection on feeder roots. But in context to their behavior, when root knot nematode is regarded as the harmful pathogen, in contrast, VAM fungus establishes symbiotic association with the plant and confers resistance to nematodes [5]. The resistance is more or less localized to the mycorrhiza colonized roots but not systemic. The pre-establishment of VAM fungus reduces the reproduction of *M. incognita* and also disease severity in infected soil [6].
In the present context it is essential to relate chlorophyll, total sugar, starch and reducing sugar of tomato shoots and roots that received VAM (*Glomus fasciculatum*), root-knot nematode

(*Meloidogyne incognita*) and VAM plus root-knot nematode with the resistance to nematode infection in tomato roots.

Materials and Methods

In order to understand the basics of resistance to nematode, *Meloidogyne incognita* inoculated with VAM (*Glomus fasciculatum*) four tomato varieties Banki local (Resistant), Kashi aman (Moderately resistant), Utkala dipti (Susceptible) and Utkala kumari (Highly susceptible) were grown during 2017-18 in pots in the net house of Department of Nematology, College of Agriculture, Odisha University of Agriculture and Technology, Odisha, India in 15cm diameter clay pots surface sterilized in 1% formaldehyde solution and filled with aerated sterilized soil (autoclaved at 1.1kg/cm^2 pressure for one hour daily for two consecutive days) mixed with sand and FYM in the ratio of 2:1:1 following Complete Randomized Design (CRD) with six treatments, replicated four times. The water used for irrigation had a five hundred mesh screen before use. The treatments were as follows: T_1- Inoculation of Nematode (*Meloidogyne incognita*) alone @ 1000 J_2/ kg of soil, T_2- Inoculation of VAM (*Glomus fasciculatum*) alone @ 600 chlamydospores / kg of soil, T_3- Inoculation of VAM after 7 days of Nematode inoculation (N→V), T_4- Inoculation of Nematode after 7 days of VAM inoculation (V→N), T_5- Inoculation of both VAM and Nematode at a time (V+N), T_6- Untreated Check.

Recording of observations: At 30 days after nematode inoculation, inoculated plants were removed from the pot soil carefully and the following paameters were estimated by different methods:

Chlorophyll estimation of leaf: One hundred mg leaf sample of each treatment had been cut from the composite leaves and had been immersed in 10 ml of 80% acetone in a conical flask and kept for 24 hours in dark for extraction of chlorophyll from the leaf samples. Thereafter, the chlorophyll extracts were filtered through Whatman No.1 filter paper. Absorbance of the chlorophyll extract was measured at 645 nm and 663 nm using spectrophotometer. The amount of chlorophyll-a, chlorophyll-b and total chlorophyll had been calculated in mg/g fresh weight according to the following equations.

1. Chlorophyll –a (mg/g fresh weight of leaf) = $\{12.7(D\text{-}663) - 2.69(D\text{-}645)\} \times \frac{V}{1000 \times W}$
2. Chlorophyll-b (mg/g fresh weight of leaf) = $\{22.9\ (D\text{-}645) - 4.68\ (D\text{-}663)\} \times \frac{V}{1000 \times W}$
3. Total chlorophyll (mg/g fresh wt. of leaf) = $\{20.2\ (D\text{-}645) + 8.02\ x\ (D\text{-}663)\} \times \frac{V}{1000 \times W}$

Where

D –645 = optical density at 645 nm,
D-663 = optical density at 663 nm,
V = final volume of 80% acetone chlorophyll extract in ml,
W = Fresh weight in g of corresponding amount of fresh leaves used in the extraction of chlorophyll

Estimation of total sugar content

Extraction: One hundred mg of ground samples had been taken in 15 ml centrifuge tubes and 10 ml of 80% ethanol was added to it. Covering the mouth of the centrifuge tube with polythene paper kept in a water bath at 80-85ºC for 30 minutes. Then it was cooled and centrifuged for 15 minutes at 2000 rpm. Then the supernatant was decanted into a 25 ml. volumetric flask. This extraction procedure was repeated once again and the supernatant was collected in the previous 25 ml. volumetric flask. The final volume was made up to 25 ml with distilled water and was filtered through Whatman No.1 filter paper. This was the sugar extract kept for sugar estimation.

Estimation: Two ml of sugar extract was transferred into a 50 ml volumetric flask and volume was made up to 50 ml with distilled water. Five ml of this extract was taken in a 25 ml volumetric flask. Simultaneously standards of 0 ml, 1 ml, 1.5 ml and 2 ml of 100 ppm glucose solution were taken in 25 ml volumetric flasks. By adding distilled water volume of these standards was made up to 5 ml and then 2 drops of 80 per cent ethanol was added. Volumetric flasks containing samples and standards were kept in an ice-bath. To each volumetric flask, 10 ml of anthrone reagent (2 gm of anthrone in one litre of 95% H_2SO_4) was added allowing it to run down the side of the volumetric flask. The contents of the flasks were shaken slowly by swirling the flask and then shaken thoroughly. The volumetric flasks were kept in boiling water bath for exactly 7.5 minutes. Then immediately the flasks were cooled in ice. After cooling, absorbance was measured at 630 nm and sugar content was calculated by the help of standard curve.

Estimation of starch content: One ml of starch extract was taken in a 100 ml volumetric flask and diluted to 100 ml with distilled water. 5 ml of the above extract was transferred in a 50 ml test tube. Then all the standards and sample test tubes were kept in ice bath for cooling, and 10 ml of anthrone reagent was added to each test tube, allowing the reagent to run down the side of the flask. It was stirred slowly with a glass rod and then shaken thoroughly. The flask was kept in boiling water bath exactly for 7.5 minutes. Then the test tube was immediately cooled in ice-bath. After cooling, the O.D. at 630 nm was measured and the starch content was calculated by the help of standard curve, which was multiplied by 0.91 to get the exact value of the same.

Estimation of reducing sugar content: Weigh 100 mg of the sample and extract the sugars with hot 80% ethanol twice (5 ml each time). Collect the supernatant and evaporate it by keeping it on a water bath at 80°C. Add 10 ml water and dissolve the sugars. Pipette out 0.5 to 3 mL of the extract in test tubes and equalize the volume to 3 ml with water in all the tubes. Add 3 ml of DNS reagent. Heat the contents in a boiling water bath for 5 min. When the contents of the tubes are still warm, add 1 ml of 40% Rochelle salt solution. Cool and read the intensity of dark red colour at 510 nm. Run a series of standards using glucose (0–500 µg) and plot a graph. Calculate the amount of reducing sugars present in the sample using the standard graph.

Statistical analyses: Various observations recorded during the course of investigation were subjected to statistical analysis in a complete randomized design (CRD). Fisher's methods of analysis of variance at 5% level of significance were followed. Further, the comparison of the treatment means was done by calculating standard error of mean (S. EM) and least significant difference (C.D) in the following manner.

S.E (m)± for treatment = √EMS/R, CD (0.05)= √2× S.E (m) × t at error d.f. where, d.f.= degree of freedom, r= replication, EMS= Error means sum of square, S.E(m) = Standard error mean, CD (0.05) = Critical difference at 5% level.

The difference between two treatments means if greater than the CD value indicated the significant difference between the treatments.

Results and Discussion

Influence of nematode and VAM on chlorophyll content of leaves

The chlorophyll 'a', 'b' and total mg/g fresh weight) present in leaves were described in Table.1, 2, 3, 4.

Chlorophyll 'a' (Fig 1) was decreased in T_1 recorded 6.76, 6.27, 4.50, 1.81 mg/g fresh leaf in Banki local, Kashi Aman, Utkala Dipti and Utkala Kumari respectively over T_6 (Check). In T_2 where only VAM was inoculated there was increased in Chlorophyll 'a' in all varieties over check. The percent increase was 16.53% in Banki local, 29.29% in Kashi Aman, 23.94% in Utkala Dipti and 20.63% in Utkala Kumar over check. Compared with T_1 the reduction was seen minimum in T_3, T_4 and T_5 over check where combination of both nematode and VAM were inoculated in different time.

In case of chlorophyll 'b' (Fig 2) there was reduction in infected plant compared with healthy check. Maximum reduction was seen in T_1 of Utkala Kumari i.e. from14.02 to 3.25 mg/g having 76.79 percent over check and minimum reduction in Banki local variety from 9.03 to 8.27 mg/g having percent decrease of 8.48% over check (T_6). Less reduction of chlorophyll 'b' was seen in T_4 where VAM was applied before nematode inoculation having 4.19% in Banki local, 17.79% in Kashi Aman, 30.26% in Utkala Dipti and 25.26% in Utkala Kumari over check (T_6).

The data on total chlorophyll (Fig 3) content indicated decrease in all treatments except T_2 in all varieties over check. There was highest reduction in T_1 followed by T_3 and T_5 in descending order and the lowest being T_4 having 2.89%, 17.13%, 26.11% and 23.79% in in Banki local, Kashi Aman, Utkala Dipti and Utkala Kumari respectively over check. Also it was noticed that treatment where VAM was applied alone there was significant increase in total chlorophyll content in all varieties. Maximum increase was seen in Kashi Aman (34.79%) and minimum in Utkala Dipti (21.15%) over check.

Similar results were reported by Nayak and Pandey (2016) [7] in brinjal that the decreased chlorophyll content of both a, b and total was non-significant in resistant varieties than susceptible varieties by the nematode *Meloidogyne incognita* infection. Leaf pigment composition is sensitive to plant stress and nematode infection causes a loss of photosynthetic pigments i.e. chlorophylls [8]. Darade (2014) [9] reported that VAM fungi (*Glomus fasciculatum*) enhance the chlorophyll pigment in okra.

Table 1: Influence of *M. incognita* and *G. fasciculatum* either alone or in combination on chlorophyll content (mg/g) fresh leaf of tomato leaf var. Banki Local (R)

Treatments	Chlorophyll 'a'	% change over check	Chlorophyll 'b'	% change over check	Total Chlorophyll	% change over check
T_1 (N)	6.76	-10.98	8.27	-8.48	15.02	-9.62
T_2 (V)	8.84	+16.53	12.69	+40.42	21.52	+29.51
T_3 (N→V)	6.97	-8.12	8.38	-7.19	15.35	-7.61
T_4 (V→N)	7.49	-1.33	8.65	-4.19	16.14	-2.89
T_5 (V+N)	7.20	-5.16	8.54	-5.43	15.74	-5.31
T_6(Check)	7.59		9.03		16.62	
SE(m)±	0.24		0.69		0.63	
CD (0.05)	0.72		2.06		1.89	

(+) Increase, (-) Decrease

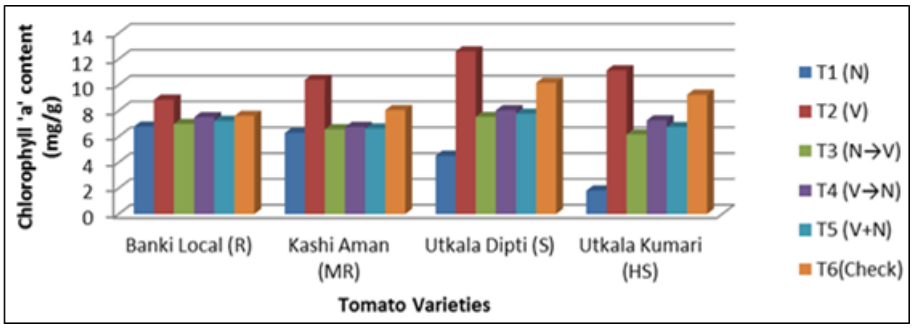

Fig 1: Influence of *M. incognita* and *G. fasciculatum* either alone or in combination on chlorophyll 'a' content (mg/g fresh leaf) of tomato

Table 2: Influence of *M. incognita* and *G. fasciculatum* either alone or in combination on chlorophyll content (mg/g) fresh leaf of tomato leaf var. Kashi Aman (MR)

Treatments	Chlorophyll 'a'	% change over check	Chlorophyll 'b'	% change over check	Total Chlorophyll	% change over check
T_1 (N)	6.27	-21.96	8.12	-22.46	14.39	-22.24
T_2 (V)	10.39	+29.29	14.55	+39.01	24.94	+34.79
T_3 (N→V)	6.55	-18.50	8.36	-20.14	14.91	-19.43
T_4 (V→N)	6.73	-16.29	8.61	-17.79	15.33	-17.13
T_5 (V+N)	6.61	-17.75	8.44	-19.36	15.05	-18.66
T_6(Check)	8.04		10.47		18.50	
SE(m)±	0.26		0.41		0.34	
CD (0.05)	0.76		1.23		1.02	

(+) Increase, (-) Decrease

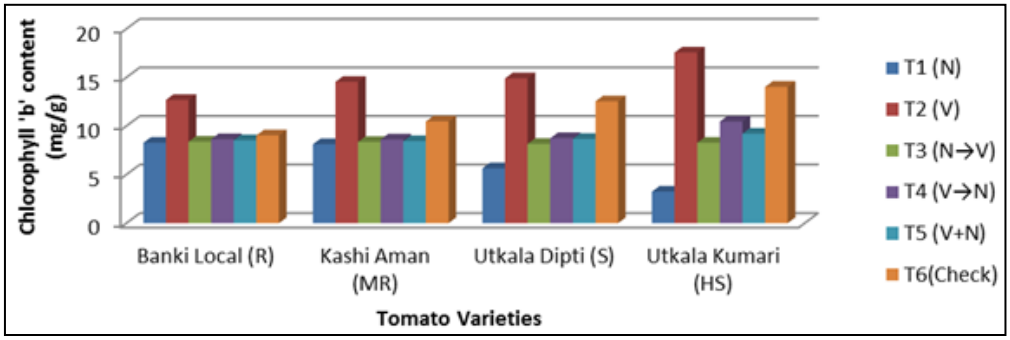

Fig 2: Influence of *M. incognita* and *G. fasciculatum* either alone or in combination on chlorophyll 'b' content (mg/g fresh leaf) of tomato

Table 3: Influence of *M. incognita* and *G. fasciculatum* either alone or in combination on chlorophyll content (mg/g) fresh leaf of tomato leaf var. Utkala Dipti (S)

Treatments	Chlorophyll 'a'	% change over check	Chlorophyll 'b'	% change over check	Total Chlorophyll	% change over check
T_1 (N)	4.50	-55.70	5.66	-54.85	10.16	-55.23
T_2 (V)	12.59	+23.94	14.90	+18.89	27.48	+21.15
T_3 (N→V)	7.52	-25.93	8.16	-34.90	15.68	-30.89
T_4 (V→N)	8.03	-20.98	8.74	-30.26	16.76	-26.11
T_5 (V+N)	7.76	-23.54	8.68	-30.72	16.44	-27.51
T_6(Check)	10.16		12.54		22.68	
SE(m)±	0.18		0.32		0.14	
CD (0.05)	0.52		0.94		0.41	

(+) Increase, (-) Decrease

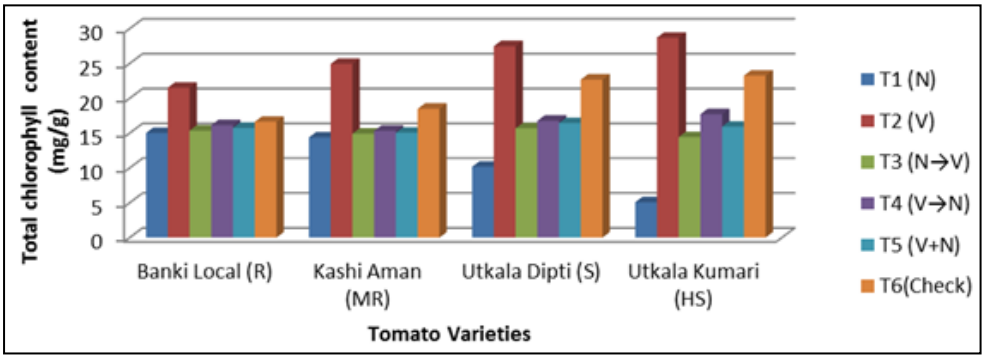

Fig 3: Influence of *M. incognita* and *G. fasciculatum* either alone or in combination on total chlorophyll content (mg/g fresh leaf) of tomato

Table 4: Influence of *M. incognita* and *G. fasciculatum* either alone or in combination on chlorophyll content (mg/g) fresh leaf of tomato leaf var. Utkala Kumari (HS)

Treatments	Chlorophyll 'a'	% change over check	Chlorophyll 'b'	% change over check	Total Chlorophyll	% change over check
T_1 (N)	1.81	-80.35	3.25	-76.79	5.07	-78.19
T_2 (V)	11.13	+20.63	17.57	+25.32	28.69	+23.46
T_3 (N→V)	6.16	-33.24	8.27	-41.02	14.42	-37.93
T_4 (V→N)	7.25	-21.49	10.46	-25.36	17.71	-23.79
T_5 (V+N)	6.72	-27.18	9.20	-34.36	15.92	-31.51
T_6(Check)	9.23		14.02		23.24	
SE(m)±	0.22		0.43		0.21	
CD (0.05)	0.66		1.27		0.62	

(+) Increase, (-) Decrease

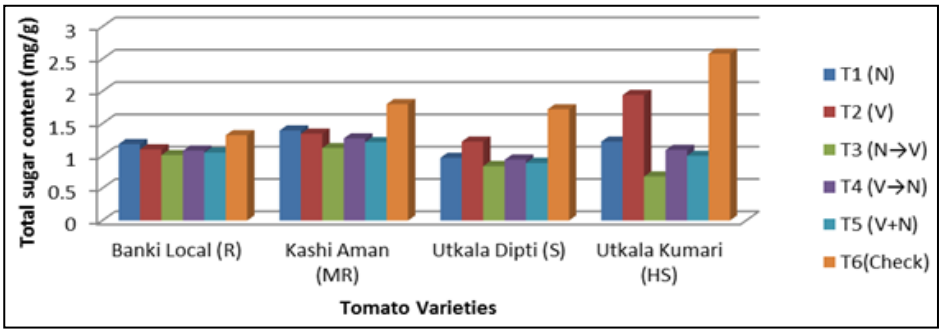

Fig 4: Effect of *M. incognita* and *G. fasciculatum* either alone or in combination on total sugar content (mg/g fresh weight) of tomato shoots

Influence of nematode and VAM on total sugar content

Total sugar content in shoot: From the data (Table 5 and Fig 4) on mean total sugar content in shoot, it was evident that T_1 exhibited the lowest total sugar content (1.39, 0.97 and 1.22 mg/g fresh weight in Kashi Aman, Utkala Dipti and Utkala Kumari respectively) except Banki local where the lowest being T_3 (1.18 mg/g). As compared to check (T_6), T_2 contributed decrease in total sugar content in all varieties having maximum decrease in Utkala Dipti (28.92%) and minimum in Banki Local (16.18%).

Total sugar content in root: Mean data (Table 5 and Fig 5) in various treatments in different varieties indicated that there was increase in total sugar content in all treatments over check. Among all treatments minimum amount of sugar accumulation was seen in T_1 of all varieties where only nematode was inoculated over untreated control (1.59 mg/g in Banki local, 2.03 mg/g in Kashi Aman, 2.65 mg/g in Utkala Dipti and 2.26 mg/g in Utkala Kumari). The highest accumulation of total sugars was observed in T_3 over their untreated check (34.56%, 39.53%, 73.88% and 84.88% in Banki local, Kashi Aman, Utkala Dipti and Utkala Kumari respectively).

Table 5: Effect of *M. incognita* and *G. fasciculatum* either alone or in combination on total sugar content (mg/g fresh weight) of tomato shoots and roots

Tr. No.	Banki Local (R)				Kashi Aman (MR)				Utkala Dipti (S)				Utkala Kumari (HS)			
	Shoot	% change over check	Root	% change over check	Shoot	% change over check	Root	% change over check	Shoot	% change over check	Root	% change over check	Shoot	% change over check	Root	% change over check
T_1 (N)	1.18	-10.57	1.59	+14.04	1.39	-22.49	2.03	+26.29	0.97	-43.63	2.65	+62.22	1.22	-52.73	2.26	+75.72
T_2 (V)	1.10	-16.18	1.75	+25.25	1.34	-25.63	2.07	+28.63	1.22	-28.92	2.12	+30.09	1.94	-24.83	1.62	+26.21
T_3 (N→V)	1.01	-23.59	1.88	+34.56	1.12	-37.48	2.24	+39.53	0.84	-51.29	2.84	+73.88	0.68	-65.90	2.38	+84.88
T_4 (V→N)	1.08	-18.27	1.78	+27.40	1.27	-29.55	2.13	+32.22	0.94	-45.28	2.74	+67.60	1.09	-57.76	2.30	+78.83
T_5 (V+N)	1.05	-20.55	1.82	+30.26	1.21	-32.75	2.22	+37.82	0.89	-48.51	2.80	+71.43	1.00	-61.42	2.33	+80.58
T_6 (Check)	1.32	-	1.40	-	1.80	-	1.61	-	1.72	-	1.63	-	2.58	-	1.29	-
SE(m)±	0.03		0.08		0.02		0.11		0.18		0.04		0.06		0.06	
CD (0.05)	0.09		0.24		0.07		0.31		0.53		0.11		0.18		0.16	

(+) Increase, (-) Decrease

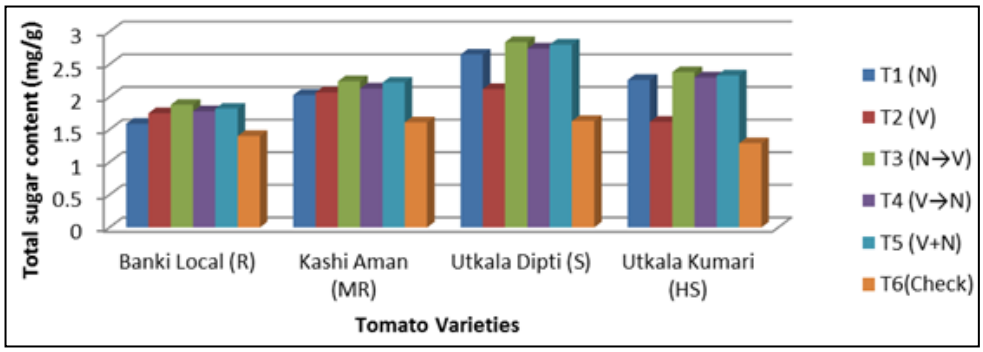

Fig 5: Effect of *M. incognita* and *G. fasciculatum* either alone or in combination on total sugar content (mg/g fresh weight) of tomato **roots**

Influence of nematode and VAM on starch content

Starch content in shoot: The amount of starch present in shoot (Table 6 and Fig 6) of the T_1 where only nematode was inoculated were recorded as 5.52, 4.35, 3.19, 1.93 mg/g of the varieties Banki local, Kashi Aman, Utkala Dipti and Utkala Kumari respectively on fresh weight basis over check. Percent decrease in starch content was maximum in T_3, followed by T_5, T_4, T_1 and that of T_2 it was also decreased over check (21.93, 20.67, 23.32 and 20.59 percent in Banki local, Kashi Aman, Utkala Dipti and Utkala Kumari respectively).

Starch content in root: The data (Table 6 and Fig 7) revealed that post inoculation of root-knot nematode of Banki local, Kashi Aman, Utkala Dipti and Utkala Kumari there was increase in starch accumulation of 11.84, 23.70, 67.68 and 74.87 percentages respectively. Maximum accumulation of starch content was observed in T_3, T_4 and T_5 where both nematode and VAM were applied than T_1 where only nematode was inoculated over check.

Table 6: Influence of *M. incognita* and *G. fasciculatum* either alone or in combination on starch content (mg/g fresh weight) of tomato shoots and roots

Tr. No.	Banki Local (R)				Kashi Aman (MR)				Utkala Dipti (S)				Utkala Kumari (HS)			
	Shoot	% change over check	Root	% change over check	Shoot	% change over check	Root	% change over check	Shoot	% change over check	Root	% change over check	Shoot	% change over check	Root	% change over check
T_1 (N)	5.52	-9.90	5.23	+11.84	4.35	-22.75	6.35	+23.70	3.19	-49.64	8.02	+67.68	1.93	-63.42	6.04	+74.87
T_2 (V)	5.21	-14.93	6.05	+29.39	4.47	-20.67	6.22	+21.01	4.87	-23.32	6.22	+30.05	4.19	-20.59	4.10	+18.65
T_3 (N→V)	4.66	-23.87	6.37	+36.06	3.88	-31.11	6.91	+34.48	2.45	-61.32	8.43	+76.20	1.47	-72.15	6.36	+84.06
T_4 (V→N)	5.10	-16.69	6.16	+31.63	4.18	-25.79	6.46	+25.82	3.02	-52.34	8.17	+70.71	1.71	-67.58	6.11	+76.66
T_5 (V+N)	4.89	-20.20	6.23	+33.18	4.15	-26.27	6.73	+30.98	2.74	-56.74	8.35	+74.63	1.61	-69.40	6.22	+80.06
T_6 (Check)	6.12	-	4.68	-	5.63	-	5.14	-	6.34	-	4.78	-	5.27	-	3.46	-
SE (m)±	0.18		0.22		0.26		0.22		0.24		0.33		0.20		0.17	
CD (0.05)	0.53		0.66		0.78		0.64		0.70		0.98		0.58		0.50	

(+) Increase, (-) Decrease

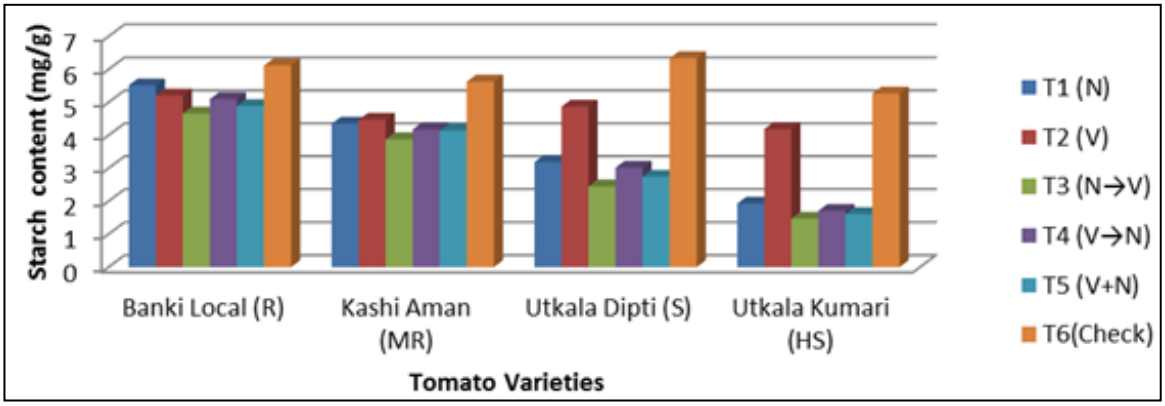

Fig 6: Effect of *M. incognita* and *G. fasciculatum* either alone or in combination on starch content (mg/g fresh weight) of tomato shoots

Influence of nematode and VAM on reducing sugar content

Reducing sugar content in shoot: The amount of reducing sugar present (Table 7 and Fig 8) in only nematode inoculated shoots are decreased in all varieties recorded as 6.06, 4.95, 3.78 and 2.98 percent in Banki local, Kashi Aman, Utkala Dipti and Utkala Kumari respectively. Comparing T_3, T_4 and T_5 maximum decrease was seen in T_3 followed by T_5 and T_4 in all varieties over check.

Table 7: Effect of *M. incognita* and *G. fasciculatum* either alone or in combination on reducing sugar content (%) of tomato shoots and roots

Tr. No.	Banki Local (R)				Kashi Aman (MR)				Utkala Dipti (S)				Utkala Kumari (HS)			
	Shoot	% change over check	Root	% change over check	Shoot	% change over check	Root	% change over check	Shoot	% change over check	Root	% change over check	Shoot	% change over check	Root	% change over check
T_1 (N)	6.06	-11.61	7.31	+10.29	4.95	-26.56	6.97	+24.34	3.78	-46.82	12.26	+65.53	2.98	-63.26	14.27	+73.15
T_2 (V)	4.82	-29.73	8.79	+32.60	5.43	-19.42	7.37	+31.53	5.46	-23.33	9.92	+33.96	6.48	-20.16	11.18	+31.07
T_3 (N→V)	4.27	-37.72	9.24	+39.47	3.85	-42.89	8.12	+44.94	2.72	-61.77	12.91	+74.27	1.79	-77.93	15.78	+84.99
T_4 (V→N)	4.72	-31.19	8.95	+35.02	4.54	-32.58	7.65	+36.55	3.34	-53.06	12.62	+70.36	2.74	-66.28	15.02	+76.08
T_5 (V+N)	4.51	-34.22	9.03	+36.34	4.28	-36.55	7.86	+40.21	2.29	-59.24	12.86	+73.63	2.31	-71.52	15.38	+80.30
T_6 (Check)	6.85	-	6.63	-	6.74	-	5.60	-	7.12	-	7.41	-	8.11	-	8.53	-
SE (m)±	0.22		0.38		0.41		0.26		0.53		0.45		0.20		0.22	
CD (0.05)	0.64		1.14		1.20		0.76		1.57		1.35		0.60		0.64	

(+) Increase, (-) Decrease

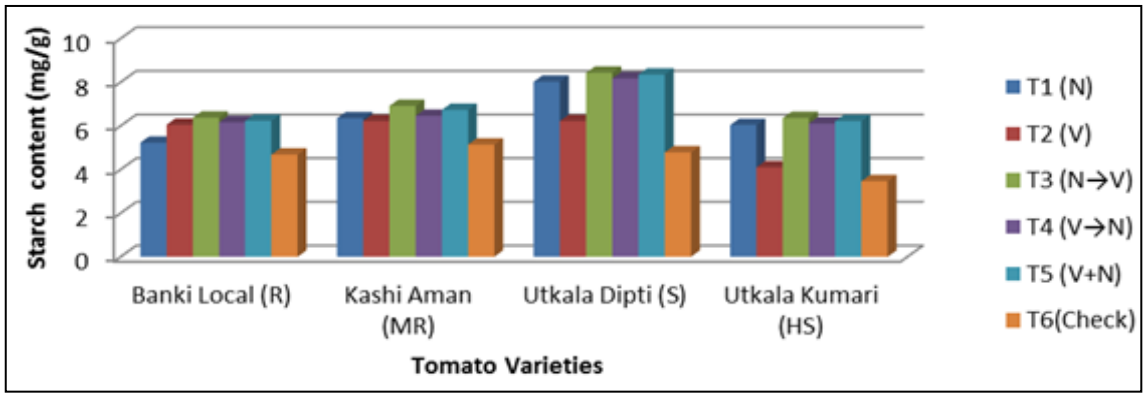

Fig 7: Effect of *M. incognita* and *G. fasciculatum* either alone or in combination on starch content (mg/g fresh weight) of tomato roots

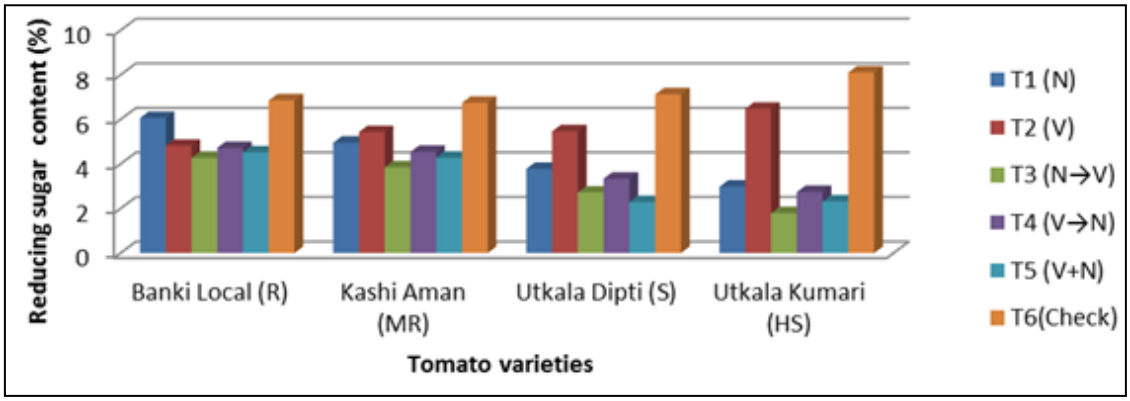

Fig 8: Effect of *M. incognita* and *G. fasciculatum* either alone or in combination on reducing sugar content (%) of tomato shoots

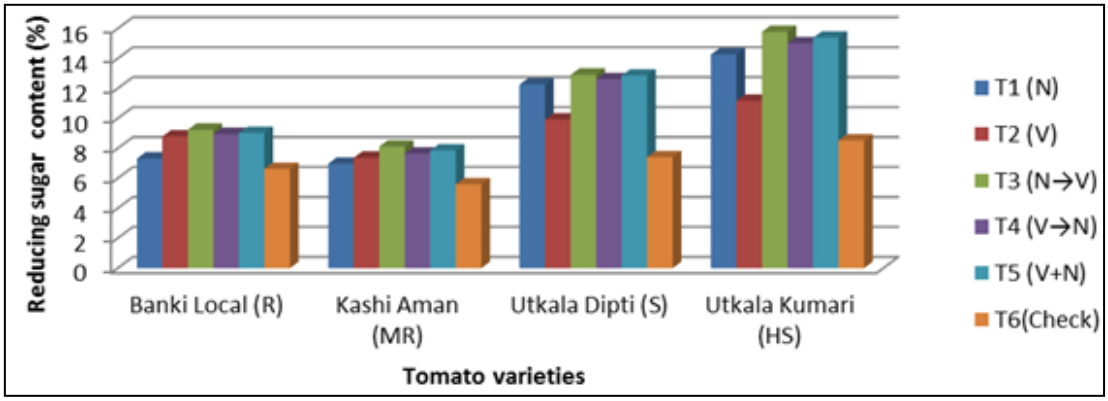

Fig 9: Effect of *M. incognita* and *G. fasciculatum* either alone or in combination on reducing sugar content (%) of tomato shoots

Reducing sugar content in root: There was increase in reducing sugar content in all treatment over check (Table 7 and Fig 9). In Banki Local and Utkala Dipti maximum increase was seen T_2 where only VAM was inoculated amounting 32.60 and 33.96 percentage respectively over check (T_6). In Utkala Dipti and Utkala Kumari maximum increase was seen in T_1 where nematode was inoculate alone recorded as 65.53% and 73.15% respectively over check.

Total sugar, starch and reducing sugar content in shoots of all varieties were decreased and that of increased in roots after nematode infection. The susceptible variety, Utkala dipti and highly susceptible variety Utkala Kumari recorded maximum increase in all of these content in roots compared to resistant varieties (Banki local and Kashi Aman). Minimum decrease in total sugar, starch and reducing sugar in shoot were seen in resistant varieties than susceptible varieties. The results were corroborating with the results reported by Nayak and Pandey (2016) [7] who reported that there was increase of total sugar in brinjal roots and decrease of the same in shoots marked in resistant varieties infected with *Meloidogyne incognita*. Pandey *et al.* (2017) [10] were studied that total sugar content of green gram shoot was maximum decreased in susceptible variety and starch content of shoot was least decrease in the resistant variety varieties infected with *Meloidogyne incognita*. Sobha *et al* (2017) [11] studied on ridge gourd varieties infected with *Meloidogyne incognita* and depicted that total sugar and reducing sugar content was more in infested roots of susceptible variety when compared to healthy roots. The increase in sugar in infected plants may be alteration in host metabolism due nematodes, so that the respiratory substrates move towards the site of infection from the other parts of plant in order that more carbohydrates are available for respiration and release of energy. A significant increase in total sugar, starch and reducing sugar in root were observed in the plants inoculated with VAM (*Glomus fasciculatum*), VAM prior to nematode (V→N), and VN simultaneously and nematode prior to VAM (N→V). In shoot these were decreased in all treatments except only VAM inoculated treatment over check. Brinjal plant inoculated with VAM alone had significantly increased the total sugar content compared to uninoculated plants. The plants inoculated with VAM+ Nematode after 10 days significantly increased total sugar in the roots of brinjal compared to nematode alone, nematode prior to VAM and VAM+nematode simultaneously [12]. Suresh and Bhagyaraj (1984) [13] also reported that *G. fasciculatum* increased the levels of total and reducing sugar in tomato plants. Similar results of increased total sugar, also reported by Nageswari and Sundarababu (1970) [14] in *G. fasciculatum* inoculated cowpea plants.

4. Conclusion

In conclusion our results indicate spectacular changes occur in susceptible and resistant cultivars of tomato after inoculating VAM (*Glomus fasciculatum*) and root-knot nematode (*Meloidogyne incognita*) either alone or in combination. Further we intended to screen some physiological indices which can be used for further study to develop nematode resistant tomato cultivars.

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