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Biochemical basis of resistance in rice against rice sheath mite, *Steneotarsonemus spinki* Smiley

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Abstract
An experiment was conducted during *Kharif*-2019 at Main Rice Research Centre, Navsari Agricultural University, Navsari to studies mechanism of bio-chemical basis of resistance in rice against rice sheath mite, *Steneotarsonemus spinki* Smiley. At peak stage (100 DAT), the minimum phenol content (4.67±0.10 mg/g) was found in rice variety GR-11 and the maximum phenol content (7.38±0.08 mg/g) was found in variety GR-15. The presence of higher amount of total phenols provided resistance by acting as a barrier to rice sheath mite, *S. spinki* from utilizing the plant nutrients. It is quite possible that endogenous high levels of phenols rendered resistance against *S. spinki*. At peak stage (100 DAT), the minimum protein content was recorded 15.40±4.71 mg/100 g in rice variety GR-15 and the maximum protein content was recorded 22.30±6.84 mg/100 g in variety GR-11. Rice varieties, GNR-4, TN-1 and GR-11 with higher protein content evidently supported higher rice sheath mite population. The protein content had been reported to indirectly influence resistance against mite in rice plants. At peak stage (100 DAT), the minimum content of total sugar was recorded 16.65±4.13% in rice variety GR-15 and the maximum content of total sugar was recorded 30.79±4.49% in variety GR-11. The reduction of the total sugar content in rice plant at the critical stages might have adversely affected the rice sheath mite population. At peak stage (100 DAT), the minimum chlorophyll content was recorded 20.71±4.33 (SPAD value) in rice variety GR-15 and the maximum chlorophyll content was recorded 32.92±7.67 (SPAD value) in variety GR-11. The reduction in chlorophyll was less in the rice varieties GR-15, GNR-7 and GNR-3 recording low population of *S. spinki*.

Keywords: Rice sheath mite, biochemical, phenol, protein, total sugar and chlorophyll

Introduction
Rice (*Oryza sativa* L.), is the one of the oldest and second most intensively grown cereal crops in the world next to wheat and ranks third in grain production. It has two cultivated species as well as 22 wild species. Rice is the staple food of nearly half of the humanity and is mainly grown and consumed in Asian countries. Asia is considered to be a 'rice bowl' of the world, where more than 90% of world's rice is produced and consumed. Rice was first domesticated in India and South-East Asia; people have been growing it for thousands of years. India is considered as an important country for rice cultivation. The area of rice cultivated in India in the year 2017-2018 was 42.949 million hectares, production 112.905 million tonnes and productivity 2629 kg/ha. In Gujarat, rice is cultivated in an area of 0.805 million hectares with a total production of 1.762 million tonnes and productivity 2189 kg/ha. There are different factors responsible for the lower productivity of rice. The rice plant is subject to attack more than 100 species of insects. Out of 100, 20 species are more destructive rice pests such as rice yellow stem borer, leaf folder, gall midge, plant hoppers, gundhi bug and rice sheath mite consider as major pest of rice in India as well as Gujarat.
Considering non-insect pests, a number of mite species have been reported from rice (Rao *et al*. 1993; Rao and Prakash, 1995) [5, 10] of which the tetranychid mite, *Oligonychus oryzae* Hirst infests rice leaves causing yellowing and drying of leaves. Among other mite species, rice sheath mite, *Steneotarsonemus spinki* Smiley was observed for the first time as a serious pest of rice in West Bengal affecting in leaf sheath during season. In India, it has been reported from Orissa (Rao and Das 1977; Rao and Prakash, 1992) [8, 6] and from East and

West Godavari districts of Andhra Pradesh (Rao *et al*., 2000) [8]. The yield losses due to rice sheath mite, *S. spinki* ranged from 4.9 to 23.7 per cent (Rao and Prakash, 1996) [7]. Incidence of *S. spinki* was noticed first time in 1993 from paddy field in South Gujarat (Rai *et al*., 1998) [4]. Rice sheath mite, *S. spinki* cause extensive damages in flag leaf sheath stage of the crop and causes brown discoloration and also its infestation on panicle causes chaffy grains with discoloured or ill-filled grains (Fig. 1 and 2). Feeding of these mites on reproductive parts of flowers results into grain sterility and this mite has also been reported as vector of pathogenic fungi like *Fusarium moniliforme*, *Helminthosporium oryzae*. Nearly 13 species of tarsonemid mites have been reported to infest rice (Tseng and Lo, 1980) [12]. Low temperature and high relative humidity were congenial for higher incidence of sheath mite in rice (Fang, 1980) [2]. Recently, rice sheath mites have achieved its position in the category of major insect-pests of rice followed by yellow stem borer and leaf folder in South Gujarat condition. The mites are causing considerable economic damage to the crop and significant reduction in yield of rice crop is also being observed. Therefore, it became necessary to study the biochemical basis of resistance in different varieties of rice with respect to sheath mite population at different crop growth stage.

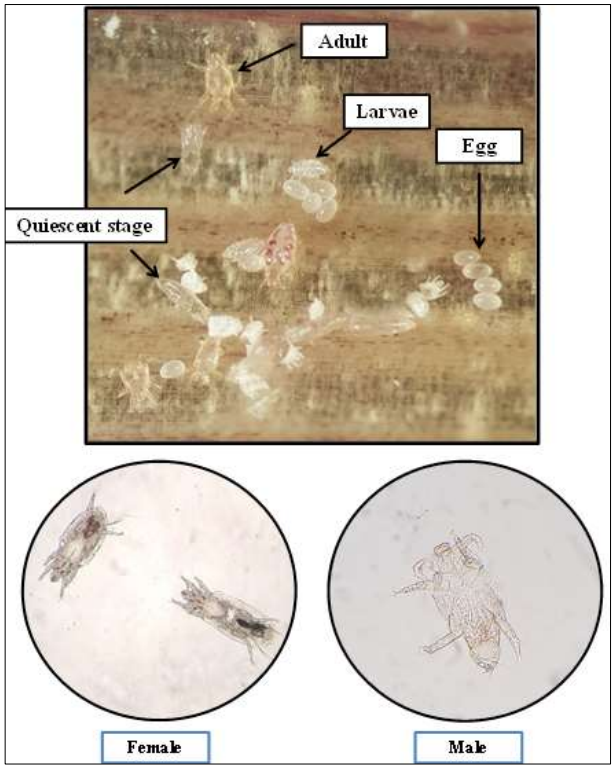

Fig 1: Colony and microscopic view of steneotarsonemus spinki smiley with different life stage on flag leaf sheath

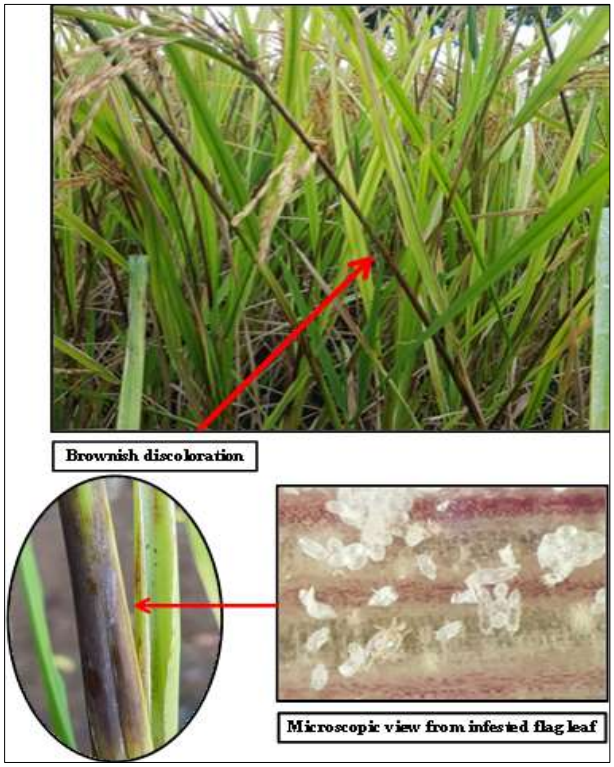

Fig 2: Brownish discoloration on flag leaf sheath caused due to *Steneotarsonemus spinki* smiley

Materials and Methods

An experiment was conducted during *Kharif*-2019 at Main Rice Research Centre farm, Navsari Agricultural University, Navsari. The experiment was laid out in randomized block design (RBD). Plot size was 5.4 m × 3.6 m and the total experimental area was 840.1 sq. meters. There were total ten different rice varieties *viz*., T_1: NAUR-1, T_2: GNR-2, T_3: GNR-3, T_4: GNR-4, T_5: GNR-5, T_6: GNR-6, T_7: GNR-7, T_8: GR-15, T_9: GR-11(SSC), T_{10}: TN-1(NSC) used for the experiment. Transplanting of rice seedlings were done in *Kharif*-2020 at 20 cm × 15 cm spacing. All the recommended agronomic packages of practices were followed to raise the crop. Total 3 plants were selected randomly from each rice plot and were pulled out and placed into separate polythene bag and rice sheath mite populations were recorded. The observations of rice sheath mite were recorded during initial stage (*i.e.* 70 days after transplanting at flag leaf initiation) and during peak stage (*i.e.* 100 days after transplanting). Leaf sheath of these rice plants were cut into 2 cm length by sterilized scissors and total sheath mite population was taken from 2cm length of leaf sheath under stereo binocular microscope. Numbers of mobile and adult stages were recorded accordingly at initial stage and peak stage.

The laboratory investigations on biochemical aspects *viz*., phenol, protein, total sugar and chlorophyll content were carried out at initial stage (70 DAT) as well as at peak stage (100 DAT) of infestation of rice sheath mite to know role of biochemical parameters in imparting resistance. Samples of fresh leaves of each variety from each replication were taken from 3 randomly selected plants in each treatment for studying biochemical variations of rice leaves with respect to the damaged by rice sheath mite. Total phenol content of the flag leaf was estimated by Folin-Ciocalteau method (Bray and Thorpe, 1954) [1]. Protein content of the flag leaf was estimated by Lowry's method (Lowry *et al*., 1951) [3]. Total soluble sugar was estimated by using Anthrone reagents as given by Thimmaiah (Thimmaiah, 2004) [11]. Chlorophyll content from the flag leaf of rice was determined by using instrument (SPAD value).

I. Phenol: Total phenol content of the flag leaf was estimated by Folin-Ciocalcalteau method (Bray and Thorpe, 1954) [1].

Reagents
- Ethanol (80%)
- Folin-Ciocalteu reagent (FCR)
- Sodium bicarbonate (Na_2CO_3) (20%)
- Standard (100 mg catechol in 100 ml of water) and diluted 10 times for a working standard.

Procedure
1. Weight 1g of the sample and grind it with a pestle and mortar in 80 per cent ethanol.
2. Centrifuge at 10,000 rpm for 20 minutes. Save the supernatant, repeat with residues.
3. Evaporate the supernatant to dryness by putting on hot water bath and dilute in double distilled water by adding 5 ml double distilled water.
4. Pipette out 2 ml different aliquots and volume make up to 3 ml in each test tubes.
5. Add 0.5 ml FCR and after 3 minutes, add 2 ml of 20 per cent Na_2CO_3 to make it homogeneous.
6. Place the test tubes in a boiling water for one minute, cool and measure the absorbance at 650 nm against a reagent blank in spectrophotometer and prepare a standard curve.

II. Protein

Protein content of the flag leaf was estimated by Lowry's method (Lowry *et al*. 1951) [3].

Reagents
- **Solution A:** 2 per cent Sodium carbonate (Anhydrous) in 0.1N NaOH.
- **Solution B:** 0.5 per cent Copper sulphate ($CuSO_4.5H_2O$) in 1 per cent sodium potassium tartrate (prepare fresh).
- **Solution C:** Mix 50 ml of solution A with 1 ml of solution B just prior to use.
- Folin-Ciocalteu reagent (FCR).
- **Stock standard protein solution:** 50 mg of bovine serum albumin/50 ml of water.
- **Working standard solution:** Dilute 10 ml of the stock solution to 50 ml with water to obtain 200 µg protein/ml.

Procedure
1. Weight 0.5 g of the sample and grind it with a buffer in a pestle and mortar.
2. Centrifuge at 10,000 rpm for 20 minutes and take supernatant for protein estimation.
3. Pipette out 0.2 ml different sample extract into different test tubes and volume make up by 1 ml along with blank.
4. Add 5 ml of solution C, mix well and incubate at room temperature for 10 minutes and after add 0.5 ml of FCR, mix well immediately and incubate at room temperature in dark for 30 minutes.
5. Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard solution into series of test tubes. Read the absorbance at 660nm against the blank in spectrophotometer.
6. Draw a standard graph and calculate the amount of protein in the sample.

III. Total Sugar: Total soluble sugar was estimated by using Anthrone reagents as given by Thimmaiah (2004) [11].

Reagents
- 2.5 N Hydrochloric acid (HCl).
- Anthrone reagent: Dissolve 200 mg Anthrone in 100 ml of ice cold 95 per cent H_2SO_4. Prepare fresh before use.
- Standard glucose: Stock-Dissolve 100 mg in 100 ml water, working standard-10 ml of stock diluted to 100 ml with distilled water. Store refrigerated after adding a few drops of toluene.

Procedure
1. Weight 100 mg of oven-dried leaf powder into a boiling tube.
2. Hydrolyze by keeping it in a boiling water bath for 3hours with 5 ml of 2.5 N HCl, cool to room temperature.
3. Neutralize it with solid sodium carbonate until the effervescence ceases.
4. Make up the volume to 100 ml and centrifuge.
5. Collect the supernatant and take 1 ml aliquots.
6. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard.
7. Make up volume to 1 ml in all the tubes including the sample tubes by adding distilled water.
8. Then add 4 ml of Anthrone reagent and heat for 8 minutes in a boiling water bath. Cool rapidly, read the dark green color at 630 nm in spectrophotometer. Draw a standard graph and calculate amount of sugar.

Calculation

$$\text{Total sugar in sample (\%mg)} = \frac{\text{Sugar value from graph (µg)}}{\text{Aliquot sample used}} \times \frac{\text{Total vol. of extract (ml)}}{\text{wt. of sample (mg)}} \times \frac{1}{1000}$$

IV. Chlorophyll

Chlorophyll content from the flag leaf of rice was determined by using instrument (SPAD value).

Procedure
1. The SPAD-502, a hand-held chlorophyll meter (Minolta corporation, Ramsey, N. J.) was used for rapid non-destructive estimation of extractable chlorophyll in green leaves.
2. This instrument used a silicon photo-iodide to detect transmittance of light emitted by two light emitting diodes- through a leaf sample, one with peak emmitance at 650 nm, where absorbance by chlorophyll is high and relatively unaffected by carotene and another with peak emmitance at 940 nm, where absorbance by chlorophyll is negligible.
3. Chlorophyll content from the flag leaf of rice was determined from rice varieties by using Chlorophyll meter SPAD-502 plus.
4. Flag leaf of randomly selected rice plant was cleaned to remove dust particles by gently rubbing tissue paper on leaves.
5. After that leaf of different rice plant were used to determine Chlorophyll by using Chlorophyll meter SPAD-502 plus.

Results and Discussion

Phenol content

At initial stage (70 DAT), the minimum (0.73±0.08 mg/g) phenol content was found in rice variety GR-11 (Table 1 and Fig. 3). Whereas, the maximum (2.49±0.08 mg/g) phenol content was found in variety GR-15. The phenol content (r = -0.865**) had a significant negative correlation

with the mean rice sheath mite population at 1 per cent level of significance (Table 2). At peak stage (100 DAT), the minimum (4.67±0.10 mg/g) phenol content was found in rice variety GR-11 (Table 1 and Fig. 4). Whereas, the maximum (7.38±0.08 mg/g) phenol content was found in variety GR-15. The phenol content (r = -0.971**) had a significant negative correlation with the mean rice sheath mite population at 1 per cent level of significance (Table 2). The results of present study revealed that amount of total phenol was significantly increased after infestation of *S. spinki*. The presence of higher amount of total phenols provided resistance by acting as a barrier to rice sheath mite, *S. spinki* from utilizing the plant nutrients. It is quite possible that endogenous high levels of phenols rendered resistance against *S. spinki*. Thus, it can be said that phenols were associated with the chemical defense of plants against insects and non-insects' pests. The reason for reduction in sheath mite population with increase in phenol content might be due to its toxic molecules which interrupted mite metabolism.

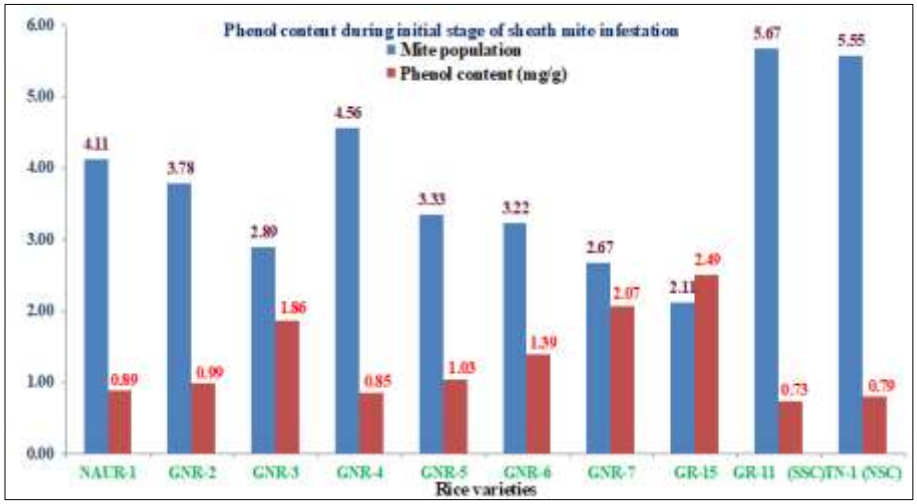

Fig 3: Phenol content and mean population of rice sheath mite in different rice varieties during initial stage

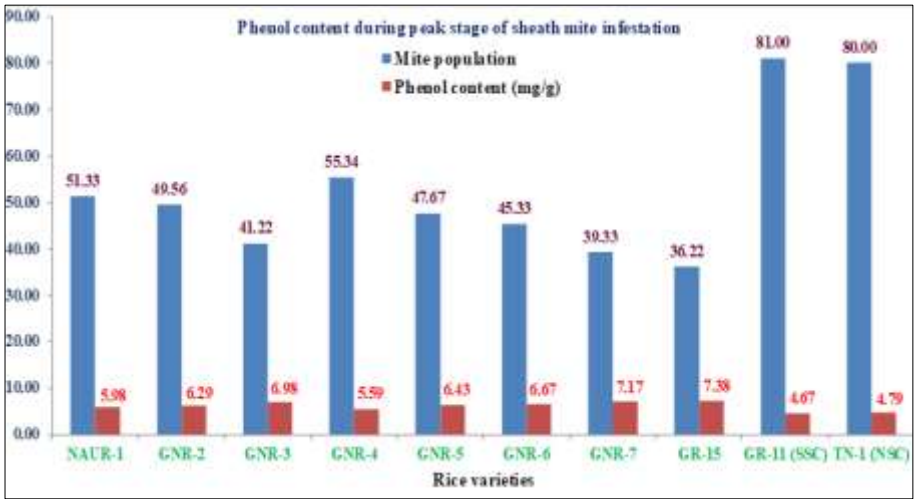

Fig 4: Phenol content and mean population of rice sheath mite in different rice varieties during peak stage

Protein content

At initial stage (70 DAT), the minimum protein content was recorded 21.48±4.68 mg/100 g in rice variety GR-15 (Table 1 and Fig. 5). Whereas, the maximum protein content was recorded 27.15±3.37 mg/100 g in variety GR-11. The protein content (r = 0.990**) had a significant positive correlation with the mean rice sheath mite population at 1 per cent level of significance (Table 2). At peak stage (100 DAT), the minimum protein content was recorded 15.40±4.71 mg/100 g in rice variety GR-15 (Table 1 and Fig. 6). Whereas, the maximum protein content was recorded 22.30±6.84 mg/100 g in variety GR-11. The protein content (r = 0.934**) had a significant positive correlation with the mean rice sheath mite population at 1 per cent level of significance (Table 2). The result showed that amount of protein content was decreased at peak stage. In almost all the rice varieties (GR-15, GNR-7 and GNR-3), protein content presented a decreasing trend with the age of crop. Rice varieties, GNR-4, TN-1 and GR-11 with higher protein content evidently supported higher rice sheath mite population. The protein content had been reported to indirectly influence resistance against mite in rice plants.

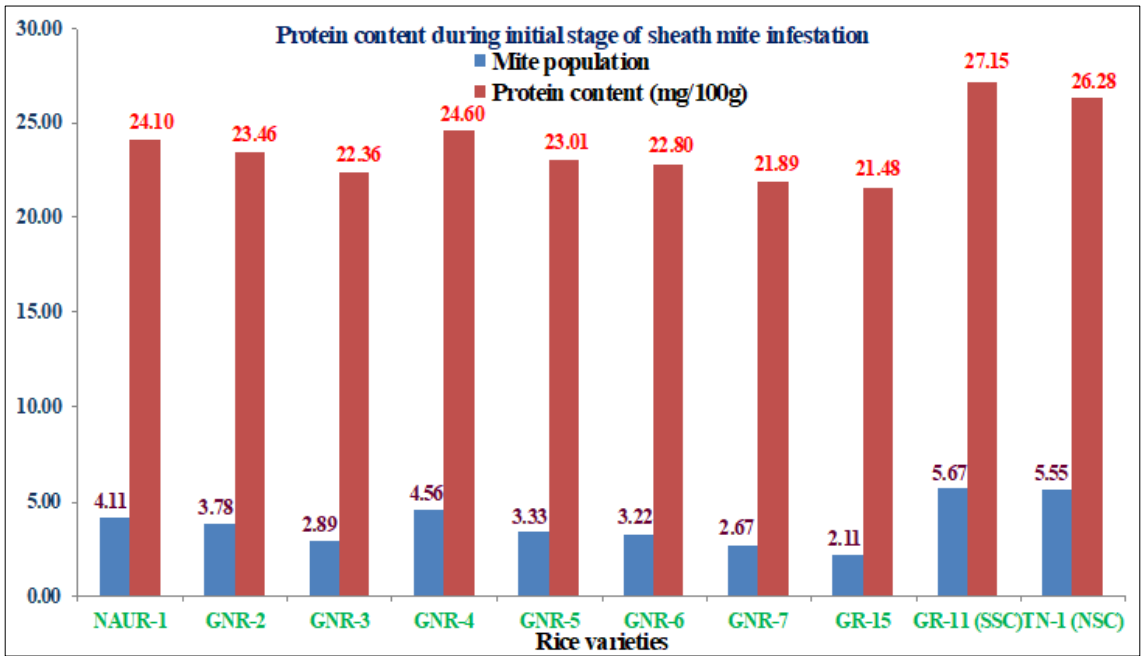

Fig 5: Protein content and mean population of rice sheath mite in different rice varieties during initial stage

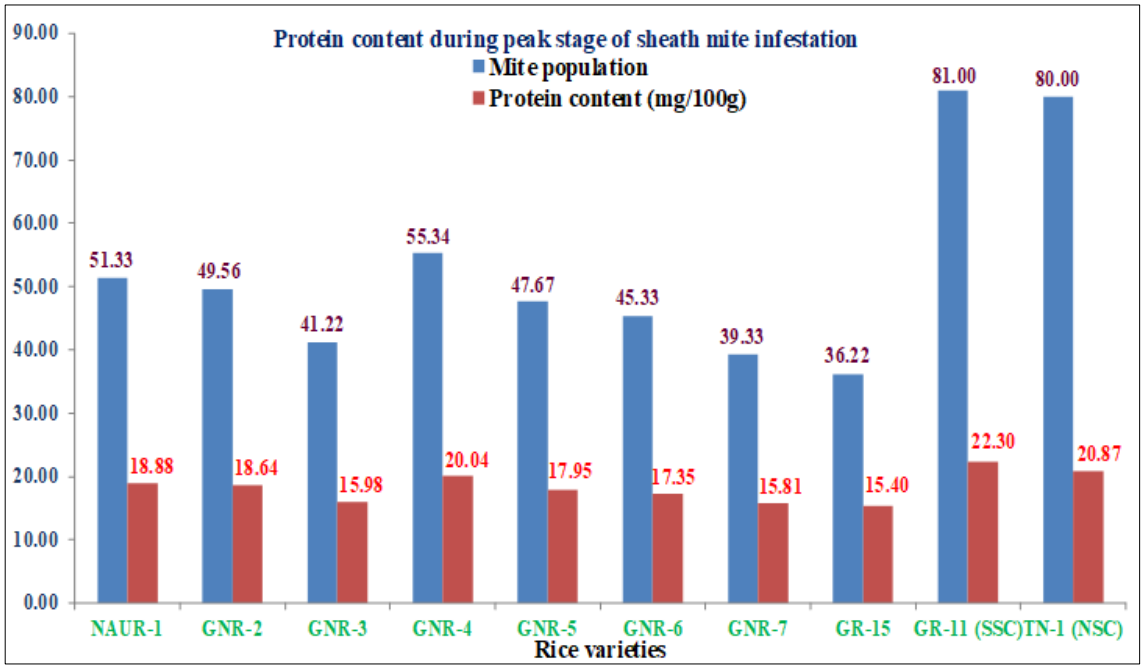

Fig 6: Protein content and mean population of rice sheath mite in different rice varieties during peak stage

Total sugar content

At initial stage (70 DAT), the minimum content of total sugar was recorded 22.19±2.61% in rice variety GR-15 (Table 1 and Fig. 7). Whereas, the maximum content of total sugar was recorded 33.89±2.34% in variety GR-11. Total sugar content (r = 0.962**) had a significant positive correlation with the mean rice sheath mite population at 1 per cent level of significance (Table 2). At peak stage (100 DAT); the minimum content of total sugar was recorded 16.65±4.13% in rice variety GR-15 (Table 1 and Fig. 8). Whereas, the maximum content of total sugar was recorded 30.79±4.49% in variety GR-11. Total sugar content (r = 0.943**) had a significant positive correlation with the mean rice sheath mite population at 1 per cent level of significance (Table 2). The reason for increased rice sheath mite population with increase in total sugar content might be due to its phago-stimulant nature which increased growth and survival rate of rice sheath mite in rice plants. The reduction of the total sugar content in rice plant at the critical stages might have adversely affected the rice sheath mite population.

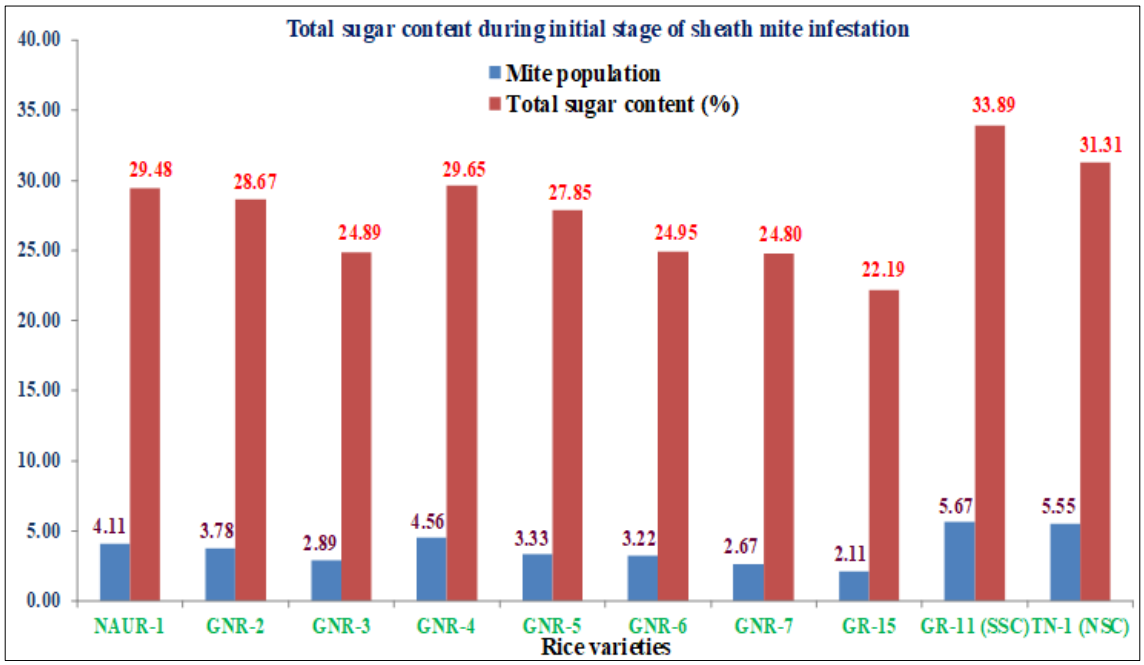

Fig 7: Total sugar content and mean population of rice sheath mite in different rice varieties during initial stage

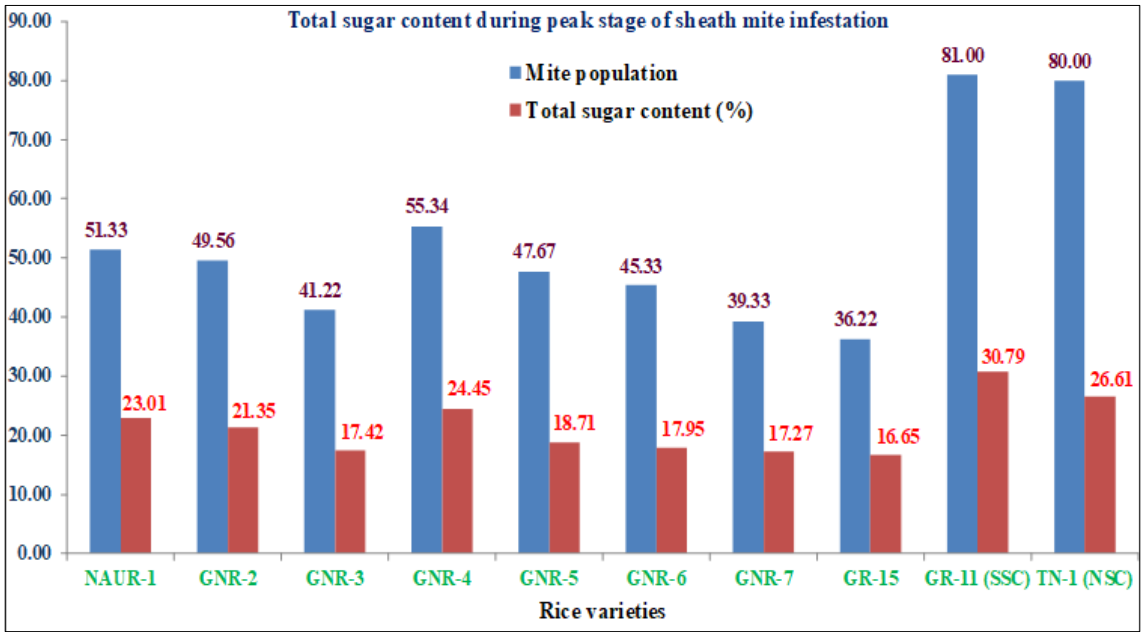

Fig 8: Total sugar content and mean population of rice sheath mite in different rice varieties during peak stage

Chlorophyll content

At initial stage (70 DAT), the minimum chlorophyll content was recorded 34.32±4.75 (SPAD value) in rice variety GR-15 (Table 1 and Fig. 9). Whereas, the maximum chlorophyll content was recorded 60.07±5.44 (SPAD value) in variety GR-11. The chlorophyll content (r = 0.977**) had a significant positive correlation with the mean rice sheath mite population at 1 per cent level of significance (Table 2). At peak stage (100 DAT), the minimum chlorophyll content was recorded 20.71±4.33 (SPAD value) in rice variety GR-15 (Table 1 and Fig. 10). Whereas, the maximum chlorophyll content was recorded 32.92±7.67 (SPAD value) in variety GR-11. The chlorophyll content (r = 0.969**) had a significant positive correlation with the mean rice sheath mite population at 1 per cent level of significance (Table 2). Reduction in chlorophyll content increased as the sheath mite infestation level increased across different rice varieties. Hence, reduction in chlorophyll was less in the rice varieties GR-15, GNR-7 and GNR-3 recording low population of *S. spinki*.

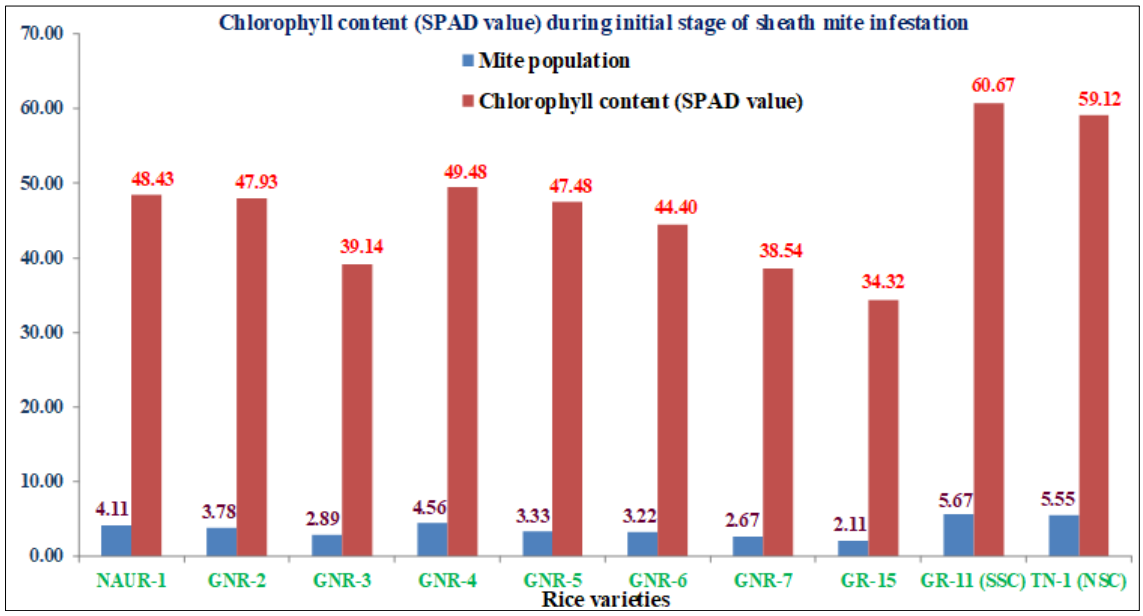

Fig 9: Chlorophyll content and mean population of rice sheath mite in different rice varieties during initial stage

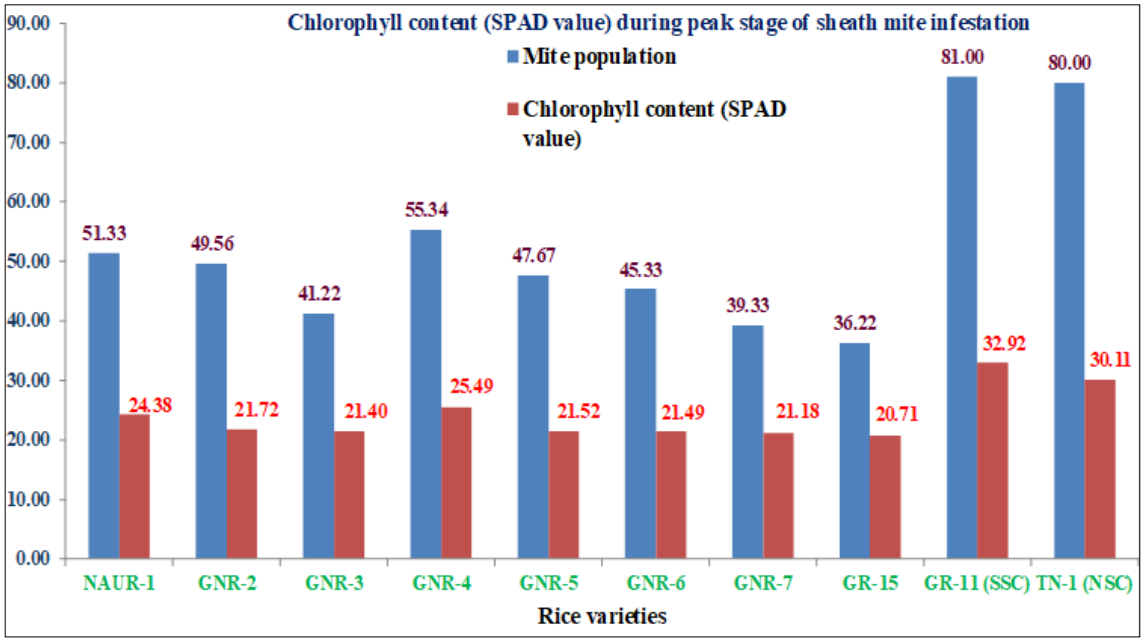

Fig 10: Chlorophyll content and mean population of rice sheath mite in different rice varieties during peak stage

Table 1: Different Biochemical parameters from rice leaves during initial stage (70 DAT) and peak stage (100 DAT) of rice sheath mite infestation

Sr. No.	Treatments	Mean No. of Mite population (Mobile stages)/ 2 cm length of leaf sheath at		Phenol content (mg/g)		Protein content (mg/100 g)		Total Sugar content (%)		Chlorophyll content (SPAD value)	
		70 DAT*	100 DAT	70 DAT Mean ± S.D.	100 DAT Mean ± S.D	70 DAT Mean ± S.D.	100 DAT Mean ± S.D	70 DAT Mean ± S.D.	100 DAT Mean ± S.D	70 DAT Mean ± S.D.	100 DAT Mean ± S.D
1.	NAUR-1	2.15 (4.11)	7.20 (51.33)	0.89±0.16	5.98±0.07	24.10±4.04	18.88±8.02	29.48±2.85	23.01±5.58	48.43±4.05	24.38±3.37
2.	GNR-2	2.07 (3.78)	7.07 (49.56)	0.99±0.12	6.29±0.08	23.46±3.54	18.64±3.08	28.67±1.23	21.35±5.66	47.93±1.67	21.72±0.96
3.	GNR-3	1.84 (2.89)	6.46 (41.22)	1.86±0.04	6.98±0.13	22.36±2.89	15.98±2.70	24.89±3.15	17.42±2.90	39.14±4.02	21.40±2.08
4.	GNR-4	2.25 (4.56)	7.47 (55.34)	0.85±0.08	5.59±0.11	24.60±1.36	20.04±6.67	29.65±3.65	24.45±7.18	49.48±6.02	25.49±4.04
5.	GNR-5	1.96 (3.33)	6.94 (47.67)	1.03±0.14	6.43±0.12	23.01±2.48	17.95±2.30	27.85±5.89	18.71±7.05	47.48±8.76	21.52±5.76
6.	GNR-6	1.93 (3.22)	6.77 (45.33)	1.39±0.03	6.67±0.14	22.80±4.26	17.35±2.04	24.95±4.36	17.95±5.08	44.40±4.62	21.49±3.58
7.	GNR-7	1.78 (2.67)	6.31 (39.33)	2.07±0.12	7.17±0.09	21.89±3.44	15.81±4.57	24.80±1.94	17.27±5.24	38.57±1.31	21.18±6.10
8.	GR-15	1.61 (2.11)	6.05 (36.22)	2.49±0.08	7.38±0.08	21.48±4.68	15.40±4.71	22.19±2.61	16.65±4.13	34.32±4.75	20.71±4.33
9.	GR-11 (SSC)	2.48 (5.67)	9.03 (81.00)	0.73±0.08	4.67±0.10	27.15±3.37	22.30±6.84	33.89±2.34	30.79±4.49	60.07±5.44	32.92±7.67
10.	TN-1 (NSC)	2.46 (5.55)	8.97 (80.00)	0.79±0.08	4.79±0.21	26.28±3.25	20.87±3.07	31.31±1.21	26.61±6.49	59.12±3.11	30.11±6.58

*DAT= Days After Transplanting, SSC= State Susceptible Check, NSC= National Susceptible Check
* Figures in the parenthesis are retransformed value of (√x+0.5) transformation

Table 2: Correlation coefficient of mean rice sheath mite population with biochemical parameters (n=10)

Sr. No.	Parameters	Duration	Correlation Coefficient (r)
1.	Phenol content (mg/g)	70 DAT Mean ± S.D.	-0.865**
		100 DAT Mean ± S.D	-0.971**
2.	Protein content (mg/100 g)	70 DAT Mean ± S.D.	0.990**
		100 DAT Mean ± S.D	0.934**
3.	Total Sugar content (%)	70 DAT Mean ± S.D.	0.962**
		100 DAT Mean ± S.D	0.943**
4.	Chlorophyll content (SPAD value)	70 DAT Mean ± S.D.	0.977**
		100 DAT Mean ± S.D	0.969**

Here, ** Significant at 1% level of significance

Conclusion

At peak stage (100 DAT), the minimum phenol content (4.67±0.10 mg/g) was found in rice variety GR-11 and the maximum phenol content (7.38±0.08 mg/g) was found in variety GR-15. The amount of total phenol was significantly increased after infestation of *S. spinki*. The presence of higher amount of total phenols provided resistance by acting as a barrier to rice sheath mite, *S. spinki* from utilizing the plant nutrients. It is quite possible that endogenous high levels of phenols rendered resistance against *S. spinki*. At peak stage (100 DAT), the minimum protein content was recorded 15.40±4.71 mg/100 g in rice variety GR-15 and the maximum protein content was recorded 22.30±6.84 mg/100 g in variety GR-11. Rice varieties, GNR-4, TN-1 and GR-11 with higher protein content evidently supported higher rice sheath mite population. The protein content had been reported to indirectly influence resistance against mite in rice plants. At peak stage (100 DAT); the minimum content of total sugar was recorded 16.65±4.13% in rice variety GR-15 and the maximum content of total sugar was recorded 30.79±4.49% in variety GR-11. The reduction of the total sugar content in rice plant at the critical stages might have adversely affected the rice sheath mite population. At peak stage (100 DAT), the minimum chlorophyll content was recorded 20.71±4.33 (SPAD value) in rice variety GR-15 and the maximum chlorophyll content was recorded 32.92±7.67 (SPAD value) in variety GR-11. The reduction in chlorophyll was less in the rice varieties GR-15, GNR-7 and GNR-3 recording low population of *S. spinki*.

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