

# Original Research Article

## Mechanisms of Plant Resistance to Rice leaf mite, *Oligonychus oryzae* Hirst (Acari: Tetranychidae)

### Abstract

Due to the changing climatic scenario rice leaf mite, *Oligonychus oryzae* Hirst becoming one of the major pests of paddy and cause severe yield loss. In this context, field experiment was carried out at Tamil Nadu Agricultural University, Coimbatore to screen popular rice varieties for their resistance against *O. oryzae*. The results revealed that negative correlation existed between the mite population and the leaf morphological characters viz., the number and length of trichomes, thickness of the leaf lamina and leaf area. ADT 53 a resistant variety recorded 28.48 mg/g of total carbohydrates, while TN 1 susceptible variety recorded 79.59 mg/g. As compared to susceptible variety, the resistant varieties have higher phenol content. The resistant variety Ptb 33 (8.107 changes in OD value min/g of fresh leaf tissue) recorded increased peroxidase activity. Both healthy and infested leaves (0.567 and 0.611 changes in OD value/ min/ g of leaf tissue) from the resistant cultivar Ptb 33 had higher levels of Polyphenol oxidase.

**Keywords:** Host plant resistance, mechanisms, rice, leaf mite

### 1. Introduction

India is the second largest producer of rice in the world next to China with an average production of 95.32 million tonnes, grown in an area of 45.95 million ha. Across the world, rice is cultivated approximately in an area of 150 million hectares with an annual yield of about 610 million tonnes (<http://beta.irri.org/>). Rice is the main source of carbohydrates for almost half of the world population. Daily intake of 100g of rice portion provides 20 per cent of the daily energy and 15 per cent of the daily protein for an adult (Food and Agricultural Organization, 2013).

Rice productivity in India is very low of 2,373 kg /ha due to damage caused by pests, diseases and weeds. In Tamil Nadu, rice is cultivated in an area of 1.49 million ha with yield of about 4.05 million tonnes (Agricultural statistics, 2015).

Serious crop losses recorded due to insects, pathogens and mites has been accounted around 25 per cent worldwide (Oerke, 2006). Among different species of mites associated with rice crop, the sheath mite, *Steneotarsonemus spinki* Smiley (Acari: Tarsonemidae) and leaf mite, *Oligonychus oryzae* Hirst (Acari: Tetranychidae) are the most important species to cause appreciable damage in recent years (Gupta, 2012). Occurrence of the rice leaf mite has been noticed in Tamil Nadu due to changes in rainfall pattern, prolonged drought which provide congenial atmosphere for the multiplication of the mites.

This mite infests rice leaves, leading to yellowing and drying (Rao *et al.*, 1993; Rao and Prakash, 1995). The nymphs and adults are seen on the lower surface of leaves and cause damage by sucking the sap of leaves and inflict damage on mesophyll cells of the interveinal tissues. It results in characteristic whitish patches on leaves, which later turn to ash colour leading to drying from tip to down-wards (Nagarajan, 1957; Misra and Israel, 1968). Besides rice, several weeds found in rice ecosystems viz., *Panicum coloratum* L., *P. crusgalli* L. (Cherian, 1938; Nagarajan, 1957), *Cyanodon dactylon* L. and *Echinochloa colona* L. (Misra and Israel, 1968) have been reported as alternate hosts for *Oligonychus oryzae*.

Application of acaricides is the primary control tactics adopted by many farmers. But, the continuous use of acaricides leads to the development of resistance. Development of resistance in mites to wide range of acaricides and insecticides has been documented throughout the world. Therefore, better alternative would be utilization of resistant varieties which would go a long way in the management to protect the crop against mite damage. This resistance when combined with the use of indigenous natural enemies could form the basis of sustainable integrated pest management program for rice.

## 2. Materials and methods

### 2.1. Field trial

The field trial was carried out to screen rice varieties against leaf mite, *Oligonychus oryzae* Hirst on rice at Tamil Nadu Agricultural University, Coimbatore during Summer, 2021. Nine varieties viz., TN 1, PTB 33, ADT 51, ADT 52, ADT 53, ADT 54, CO 51, CO 52 and CO 53 were screened with three replications in randomized block design. Screening against leaf mite was done on 45, 60 and 75 days after transplanting. Ten plants were selected from each accession and from each plant top, middle and bottom leaves of 1 x 10 cm size were sampled to record the leaf mite population with the help of 10 X lens and expressed as numbers / 10 cm leaf length.

### Leaf damage rating

Leaf damage rating was done based on the rating scale (Archer, 1987) as mentioned below.

Chart 1 : Leaf area damage with gradation

Leaf area damage (%)	Grade
1-10	1
11-20	2
21-30	3
31-40	4
41-50	5

51-60	6
61-70	7
71-80	8
81-90	9
91-100	10

## Resistance rating

The level of resistance was graded (Radhakrishnan and Ramaraju, 2009) as per the rating given below

Chart 2 : Resistance level

Leaf damage Percentage	Mean leaf damage Rating	Level of resistance
0-40	1-4	Resistant ( R )
41-60	> 4-6	Moderately Resistant (MR)
61-80	> 6-8	Moderately Susceptible (MS)
> 80	> 8	Susceptible (S)

## 2.2 Mechanism of resistance:

### 2.2 Plant morphological characters

#### 2.2.1 Trichome density

Trichome density on the upper and lower surface of leaves of the five selected entries was estimated following the procedure of Maite *et al.* (1980). The **third fully expanded leaf was used for the estimation. Leaf samples were cut into 1 cm leaf bit and** boiled in 20 ml of water in small glass vials for 15 minutes in hot water bath (at 85<sup>0</sup> C). The water was then removed and 20 ml of 96 per cent ethyl alcohol was added and the leaf bits were boiled approximately for 20 minutes at 80<sup>0</sup> C. The alcohol was poured off and the boiling process with fresh alcohol was repeated until the chlorophyll content of the leaf bits were removed completely. Alcohol was removed from the boiling tube and 90 per cent lactic acid was added, the vials were stoppered and heated at 85<sup>0</sup> C until leaf segments cleared (approximately for 30-45 minutes). The vials were cooled and the leaf segments were taken and mounted on clean slides using a drop of lactic acid to observe the trichome density. The number of trichomes present in 1 cm leaf was counted under Leica ® stereo zoom binocular microscope.

#### 2.2.2 Length of trichomes

The length of the trichomes was measured on the same leaf bits using image analyser with digital scale in stereo zoom (Model: Leica EZ 4W) binocular microscope and expressed in mm.

#### 2.2.3 Leaf area

The maximum length and width of the third leaf of each entry was measured and multiplied with the leaf area constant 0.62 and expressed as  $\text{cm}^2$  (Palanisamy and Gomez, 1974).

#### **2.2.4 Leaf thickness**

Cross section of leaves was cut with the help of a fine razor and thickness of leaf lamina and midrib. For leaf lamina, the thickness was determined from three different places of each leaf with help of image analyser digital scale (Leica EZ 4W) and four observations were taken and expressed in millimeters.

### **2.3. Biochemical bases of resistance**

#### **2.3.1 Total carbohydrates**

Total carbohydrates content was determined by Anthrone method (Hedge and Hofreiter, 1962). One hundred milligram of leaf sample was weighed and kept in a boiling tube. The sample was hydrolysed by keeping it in a boiling water bath for 3 h with 5 ml of 2.5 N HCl and cooled to room temperature. The hydrolysate was neutralized with solid sodium carbonate until the effervescence ceased, after which the volume was made up to 100 ml with distilled water and centrifuged at 10,000 rpm for 10 minutes. To 0.5 ml of the supernatant, 0.5 ml of distilled water and 4 ml of anthrone reagent were added. The reaction mixture was heated for 8 minutes in a boiling water bath and cooled rapidly. The solution turned green to dark green, which was read at 630nm using spectrophotometer. D glucose was used as standard. Total carbohydrates content was calculated and expressed in terms of milligram of glucose equivalent per gram of leaf tissue on fresh weight basis.

#### **2.3.2 Reducing sugar**

Reducing sugar was determined following Somogyi (1952) method. Five hundred milligram of the leaf sample was weighed and the sugar was extracted using 10 ml hot 80 per cent ethanol twice. The supernatant was collected and evaporated by keeping on a water bath at  $80^{\circ}\text{C}$ . Ten millilitre of water was added to dissolve the sugars. Aliquot of 0.5 ml was pipetted out into a separate test tube and the volume was made up to 2 ml with distilled water. To this 1 ml of alkaline copper tartarate reagent was added to each test tube. The tubes were then placed in boiling water bath for 10 minutes, cooled and one ml of arsenomolybdic acid reagent was added and the volume was made up to 10 ml with water. A blank was run without the sample, following the other steps. Glucose solution was used as working standard.

The absorbance was read at 620 nm after 10 minutes. From the standard graph, the amount of reducing sugar present in the sample was determined and expressed in percentage.

#### **2.3.3 Phenols**

Method described by Malik and Singh (1980) was followed for the estimation of phenol. Five hundred milligram of the leaf samples was ground with a pestle and mortar in 80 per cent ethanol and centrifuged at 10000 rpm for 20 minutes, the supernatant saved, the residue was re

extracted with five times volume of 80 per cent ethanol and the supernatants were pooled and evaporated to dryness. The residue was dissolved in 5 ml of distilled water. The aliquot (0.2 ml) of was pipetted out into test tube and the volume was made up to 5 ml with distilled water, to this 0.5 ml of Folin – Ciocalteu reagent was added after 3 minutes. Two ml of 20 per cent  $\text{Na}_2\text{CO}_3$  solution was added to the test tube and mixed thoroughly. The tubes were placed in boiling water for exactly one minute, cooled and the absorbance were measured at 650 nm against a reagent blank. Standard curve was prepared using different concentrations of catechol. From the standard curve, the concentration of phenols in the leaf samples was determined and expressed as mg phenols / g material.

#### **2.3.4 Assay of enzymes**

The selected entries with respect to resistance / susceptible were used for the assay of enzyme studies. The leaf samples were drawn from each entry at different stages *viz.*, active tillering, panicle initiation and flowering for assay of enzymes on both healthy and infested leaves.

##### **2.3.4.1 Peroxidase (PO)**

Using pre-chilled pestle and mortar, 1 g of leaf sample was homogenized with 5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and to which a pinch of polyvinyl pyrrolidone (PVP) was added. The homogenate was centrifuged at 10,000 rpm for 10 minutes at 4<sup>0</sup> C and the supernatant was used as the enzyme extract for the assay of peroxidase activity.

The reaction mixture consisting of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1 per cent  $\text{H}_2\text{O}_2$  was incubated at  $28 \pm 1^0$  C. At the start of the enzyme reaction, the absorbance of the mixture was set to zero at 420 nm in spectrophotometer and changes in the absorbance were recorded at 30 sec. intervals for 3 minutes. Boiled enzyme preparation served as control.

The peroxidase activity was expressed as change in the absorbance of the reaction mixture per minute per gram on fresh weight basis (Hammerschmidt *et al.*, 1982).

##### **2.3.4.2 Polyphenol oxidase (PPO)**

Enzyme extract was prepared as per the procedure adopted for estimation of peroxidase. The polyphenol oxidase activity was assayed using the modified method of Mayer *et al.* (1965). Standard reaction mixture contained 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5), 0.5 ml of the enzyme preparation and 0.5 ml of 0.1 M catechol. The reaction mixture was incubated at  $28 \pm 1^0$  C and absorbance was set to zero at 495 nm in spectrophotometer. The changes in the optical density (OD) were followed at 30 sec. intervals for 3 min. and the PPO activity was expressed as change in the OD of the reaction mixture per minute per gram on fresh weight basis.

#### **2.4 Statistical analysis:**

The data on mite population in field experiments were subjected to square root ( $X + 0.5$ ) transformation, while the percentage data were subjected to arcsine transformation. The data obtained from field screening and laboratory experiments were subjected to statistical analysis using AGRES and the mean values separation was compared by LSD at 5 or 1 per cent level (Gomez and Gomez, 1984).

### **3. Results and discussion**

Among the nine varieties screened, ADT 52, ADT 53 and CO 52 recorded the mean leaf mite population of 7.21, 6.37 and 7.07 nos/ 10cm leaf length and were found to be resistant; ADT 51, ADT 54 and CO 53 recorded the leaf mite population of 11.34, 9.06 and 10.19 nos/ 10cm leaf length and were moderately resistant and CO 51 recorded 19.31 mites / 10 cm leaf and was moderately susceptible. Resistant check PTB 33 and susceptible check TN 1 recorded leaf mite population of 6.01 and 20.68 nos/10 cm leaf length (Table 1 & 2).

#### **3.1 Mechanism of resistance:**

##### **3.1.1 Plant morphological characters**

###### **3.1.1.1 Trichomes**

The number of trichomes per centimeter leaf length was counted. The resistant Ptb 33 recorded the maximum number of 623.66 / cm<sup>2</sup> leaf, whereas it was minimum of 63.00/cm in ADT 53. In other varieties, it varied from 238.66 to 471.33/ cm<sup>2</sup> (Table 3). Similar studies conducted by Ahmad *et al.* (2005) revealed that hair density, length of hairs, gossypol glands and thickness of leaf lamina played a significant and negative role towards resistance in cotton against jassids. The present study does not corroborate with investigations made by Rai *et al.*, (2011) who observed that the mite population was high in chilli leaves of high trichome density. Present finding is also supported by Radhakrishnan and Ramaraju (2009) who reported that the trichome density is negatively correlated with mite population.

###### **3.1.1.2 Length of the trichome**

The length of trichomes on the epidermis of Ptb 33 measured 0.56 mm and least trichome length of 0.135 mm recorded in susceptible TN 1 (Table 3).

###### **3.1.1.3 Thickness of leaf lamina and mid rib**

Thickness of rice leaf lamina was high in CO 52 (0.107 mm) and low in ADT 53 (0.067 mm). Midrib thickness was high in ADT 53 (Table 3). Similar findings of Hosamani (2007) who revealed that the *Polyphagotarsonemus latus* resistant accession had maximum leaf thickness and susceptible accession had minimum thickness.

###### **3.1.1.4 Leaf area**

The maximum leaf area of 37.00 cm<sup>2</sup> recorded in CO 52, while it was minimum of 13.49 cm<sup>2</sup> in ADT 53 (Table 3). The findings are in line with Karmakar (2008) who reported that leaf length and width are negatively correlated with *Steneotarsonemus spinki* population in rice.

### 3.1.2 Correlation of leaf morphological characters with mite population

Among the leaf morphological characters, the number of trichomes on leaf surface, length of trichomes, leaf lamina thickness, leaf midrib thickness and leaf area are negatively correlated with mite population (Table 4).

### 3.1.3 Biochemical analysis

The total carbohydrate content varied from 28.48 mg/g in ADT 53 to 79.59 mg/g in TN 1, whereas in infested leaves, it was less in CO 51 (15.89 mg/g) and highest in CO 52 (68.32 mg/g) (Table 5). The present findings agree with Palanisamy (1979) and Yadav myakala (2014) who reported that the resistant varieties contained higher amounts of carbohydrates than the susceptible varieties in bhendi and chilli crop for two spotted spider mite and yellow mite, respectively. Sanjib Ghoshal (2013) also observed reduction in total carbohydrate content in tulsi leaves due to two spotted mite feeding to an extent of 24.32 per cent.

Among the entries, healthy and infested leaves of ADT 53 recorded the maximum amount of reducing sugars of 5.17 mg/g and 3.53 mg/g, respectively (Table 5). The results are in conformity with Palanisamy (1984) on brinjal infested with two spotted mite.

As a result of the mite feeding, there was a rise in the content of phenols in all the varieties and in the infested leaves, the phenol content was significantly higher in the resistant entries than in the susceptible ones (Table 6). The healthy leaves of resistant ADT 53 had higher amount of phenols (17.84 µg /g), while it was less (9.47 µg /g) in CO 52. There was increase in phenol content to the tune of 26.06 per cent in Ptb 33 to 44. 52 per cent in TN 1. This explains that phenols played a vital role in imparting resistance against mite. The present study is in line with Sanjib Ghoshal (2013) and Kharbangar *et al.* (2015) who observed increase in phenolic compounds in tulsi and rice due to *T. neocaledonicus* and thrips infestation, respectively.

### 3.1.4 Enzyme activity

Peroxidase activity was estimated in selected five varieties. The entries responded differently in their peroxidase activity against the mite infestation and mite free plants (Table 7). N High peroxidase activity was noticed in resistant Ptb 33 (8.107 changes in OD value /min/g of fresh leaf tissue) followed by CO 52 (7.846 - changes in OD value /min/g of fresh leaf tissue) in the mite infested plants. The resistant varieties had a higher rate of peroxidase activity even under uninfested condition. However, the susceptible variety, TN 1 and moderately susceptible variety CO 51 under both infested and healthy condition recorded less peroxidase activity.

The polyphenol oxidase activity also exhibited significant differences among the test entries in healthy and infested plants. The maximum activity was recorded on the resistant Ptb 33 with 0.567 and 0.611 changes in OD value/ min/g of fresh leaf tissue in healthy and infested

leaves respectively. The less activity was observed on varieties *viz.*, ADT 53, CO 51 and TN 1 (Table 7). Antioxidant enzymes such as catalase, superoxide dismutase and peroxidase are known to be correlated with induced defenses to herbivory and mite attack (Grinberg *et al.*, 2005; Nachappa *et al.*, 2013). The peroxidase and polyphenol oxidase activities were studied in the healthy and infested selected rice accessions on different stages of crop. In the rice leaves, the *O. oryzae* infestation has led to increase in the peroxidase (PO) and polyphenol oxidase (PPO) activity, compared to the healthy mite free plants.

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## 5. Conclusion

The results of this experiment showed that the leaf morphological characters, the number of trichomes, length of the trichomes, leaf lamina thickness and leaf area are negatively significant with the mite population. The total carbohydrates due to mite infestation varies from ADT 53 (28.48 mg/g) in resistant variety to TN 1 (79.59 mg/g) susceptible variety. The phenol content was significantly higher in resistant variety compared to susceptible ones. Peroxidase activity was higher in the resistant variety Ptb 33 (8.107 changes in OD value min/g of fresh leaf tissue). Polyphenol oxidase was higher in the resistant variety Ptb 33 in both healthy and infested leaves (0.567 and 0.611 changes in OD value/ min/ g of leaf tissue). From the study it was concluded that leaf morphological traits and defense enzymes confer resistance to rice leaf mite in CO52 whereas the high phenol content contribute resistance in ADT 53.

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**Table 1. Screening of rice entries against leaf mite under Coimbatore condition (Summer, 2021)**

S.No.	Variety	Leaf mite population (no./10 cm leaf)		
		45 DAT	60 DAT	75 DAT
1	TN 1	17.77 (4.27)	39.00 (6.28)	5.2 (2.40)
2	PTB 33	5.88 (2.53)	10.89 (3.37)	1.27 (1.33)
3	ADT 51	10.56 (3.32)	21.33 (4.67)	2.13 (1.62)
4	ADT 52	6.44 (2.63)	12.11 (3.55)	3.07 (1.89)
5	ADT 53	5.33 (2.41)	10.78 (3.36)	3.00 (1.87)
6	ADT 54	7.12 (2.76)	16.33 (4.10)	3.73 (2.06)
7	CO 51	14.22 (3.84)	36.11 (6.05)	7.60 (2.85)
8	CO 52	6.33 (2.61)	12.22 (3.57)	2.67 (1.78)
9	CO 53	8.56 (3.01)	20.67 (4.60)	1.33 (1.35)
SE (d)		0.40	0.22	0.08
CD (p=0.05)		0.42	0.46	0.17

\*Mean of three replications

Figures in parentheses are square root transformed value

**Table 2. Mean leaf damage grading of rice entries under field condition**

S.No.	Variety	Mean mite population (no/10 cm)	Leaf damage (%)	Damage grade	Reaction
1	TN 1	20.68	82.6	9	S
2	PTB 33	6.01	24.4	3	R
3	ADT 51	11.34	47.9	5	MR
4	ADT 52	7.21	31.7	4	R
5	ADT 53	6.37	26.8	3	R
6	ADT 54	9.06	43.2	5	MR
7	CO 51	19.31	77.4	7	MS
8	CO 52	7.07	28.5	3	R
9	CO 53	10.19	58.2	6	MR

\*R - Resistant, MR - Moderately resistant, MS - Moderately susceptible, S – Susceptible

**Table 3. Leaf morphological traits in selected entries**

Variety	Trichome density (no/ cm <sup>2</sup> )	Length of the trichome (mm)	Thickness of leaf lamina (mm)	Thickness of midrib (mm)	Leaf area (cm <sup>2</sup> )
CO 52	395.33	0.548	0.107	0.483	37.00
TN 1	238.66	0.135	0.069	0.391	28.11
CO 51	471.33	0.320	0.100	0.283	14.96
PTB 33	623.66	0.565	0.094	0.269	27.47
ADT 53	63.66	0.538	0.067	0.522	13.49
SE(d)	2.84	0.05	0.003	0.005	0.72
CD	5.94	0.10	0.005	0.014	1.52

Mean of four replications

**Table 4. Correlation matrix of leaf morphological characters with mite population**

Parameters	Trichome density	Trichome length	Leaf lamina thickness	Leaf midrib thickness	Leaf area	Mean mite population
Trichome density	1					
Trichome length	0.19203	1				
Leaf lamina thickness	0.767583	0.364689	1			
Leaf midrib thickness	0.81053	0.23602	0.36288	1		
Leaf area	0.288838	0.189389	0.505339	0.212517	1	
Mean mite population	-0.05083	-0.95726	-0.16347	-0.36866	-0.26035	1

**Table 5. Total carbohydrates and reducing sugar in healthy and mite infested leaves of rice varieties**

Variety	Total carbohydrates (mg/g)			Reducing sugar (mg/g)		
	Healthy	Infested	Per cent reduction	Healthy	Infested	Per cent Increase
CO 52	69.6	68.32	16.21	5.07	3.02	40.52
TN 1	79.59	58.38	26.65	4.95	3.51	29.05
CO 51	23.8	15.89	33.24	4.04	2.59	35.93
Ptb 33	49.38	27.24	44.81	4.79	3.19	33.48
ADT 53	28.48	17.33	39.15	5.17	3.53	31.75
SE (d)	1.06	0.70		2.49	1.75	
CD (P=0.05)	2.27	1.51		5.33	3.75	

Mean of four replications

**Table 6. Total phenols content in healthy and mite infested leaves of rice varieties**

Variety	Total phenols ( $\mu\text{g/g}$ )		Per cent increase
	Healthy	Infested	
CO52	9.47	13.47	29.69
TN1	14.99	27.02	44.52
CO51	12.51	18.63	32.85
PTB33	14.94	21.06	29.06
ADT53	17.84	24.65	27.63
SE(d)	0.32	0.54	
CD	0.70	1.15	

Mean of four replications

**Table 7. Enzyme activity in healthy and mite infested leaves of selected rice varieties**

Variety	Peroxidase (Changes in OD values/min/g of leaf tissue)		Polyphenol oxidase (Changes in OD values/min/g of leaf tissue)	
	Healthy	Infested	Healthy	Infested
CO52	6.688	7.846	0.519	0.556
TN1	6.228	7.357	0.467	0.532
CO51	5.611	6.882	0.356	0.407
PTB33	7.253	8.107	0.567	0.611
ADT53	4.598	5.052	0.323	0.367