The effect of differential pest load of leaf folder, *Cnaphalocrocis medinalis* (Guenee) on extent of leaf damage and alteration in leaf biochemical composition in rice

Abstract

The effect of feeding damages induced by the leaf folder, *Cnaphalocrocis medinalis* (Guenee) at various damage levels on the biochemical parameters of rice leaves was investigated along with the estimation of leaf roll rate. The leaf roll rate was shown to be higher as the pest population increased. Biochemical profile of rice leaves damaged by a leaf folder was studied for quantitative and qualitative variations in soluble soluble carbohydrate, starch and the ratio of chlorophyll a/b using standard biochemical procedures. The tests were carried out on a susceptible rice variety TN1. The result revealed that with increase in infestation by *C. medinalis* resulted in decreased soluble carbohydrates and starch content in rice leaves. But the trend was different for chlorphyll a/b and the ratio was found higher in pest infested vegetation.

(Key words: Chlorophyll, Leaf folder, rice, biochemical, carbohydrate, starch)

1. INTRODUCTION

Rice, *Oryza sativa* L. (Family: Graminae), the world's second most important cereal crop next to corn is mostly grown in hot and humid to subhumid regions of the globe under tropical and subtropical climate [1,2]. In recent times insect pests and disease problem are frequently threatening the rice production both qualitatively and quantitatively under the fluctuating climate change. The crucial parameters for the abundance of the insect are the high humidity and optimum temperature [3]. Although more than 800 insect species have been reported in rice crop worldwide, but only 20 insect species inflict significant damage in tropical Asia [4]. The average losses in rice yield due to insect pest attack in India is estimated to the tune of 28-30 percent [5,6].

The leaf folder or leaf roller, *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Crambidae), an important leaf eating pest of rice is observed in the rice-growing regions of Asia, Australia and Africa, between 48^0N and 24^0S latitude and 0^0E to 172^0W longitude [7]. Out of the eight species of leaf folder, the most widespread one is *C. medinalis* in rice ecosystem [8]. It is a migratory pest with 1 to 11 generations per year [9]. Rice is the most favourable host plants for *C. medinalis* among several other alternate hosts *viz.*, corn, sugarcane, wheat, sorghum, and several graminaceous weeds [10]. In many Asian nations, it was once regarded as a minor pest. However, there have been major outbreaks of the pest in various nations that cultivate rice, including India, China, Japan, Malaysia, Vietnam, Korea and Sri Lanka [11]. A single *C. medinalis* larva may defoliate or remove the chlorophyll from

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several rice leaves, which will disrupt photosynthesis. Feeding frequently causes plant green leaves to become stunted, curled, or yellow [12]. After folding the leaf margins, the larva feeds by scraping the green mesophyll tissue from within the folded leaves. This feeding results in a narrow, horizontal white stripe. Membranous patches appear as a result of the damage. The majority of early second-instar larvae are gregarious in nature. From the late second instar on, the larva feeds on the folded leaves. It moves into solitude. Although they can appear at any point of the rice crop's life cycle, leaf folder infestations tend to be more prevalent during the reproductive and ripening stages [13]. Leaf folder feeding significantly reduces the overall vigour and photosynthetic capacity of an infested rice plant.

Additionally, the plant becomes vulnerable to bacterial and fungal infestation are infected plants [14]. The biochemical components of rice leaves alter with the variation in the degree of infestation by the insect. As the insect is one of the most important leaf eating insects, the chlorophyll content decreases with the infestation. The different component of chlorophyll i.e., chlorophyll a, chlorophyll b generally decreases with the extent of leaf damage [15]. The ratio of chlorophyll a to b (Chl a/Chl b) is also the indicator of the extent of damage and the ratio has also the correlation with the infestation. Along with chlorophyll, the soluble carbohydrates as well as starch content of rice leaves alters with the degree of infestation and change in leaf roll rate [16, 17].

The aim of the particular study was to evaluate the change in soluble carbohydrates, starch along with the 'chlorophyll a / chlorophyll b' in rice leaves based on differential leaf folder damage. The tests were carried out on the sensitivity to all insects TN1 rice variety.

2. MATERIALS AND METHODS

Field experiment was conducted during Kharif 2018-19 and Rabi 2018-19 in the experimental field at the ICAR- National Rice Research Institute, Cuttack (28^{o}36′ N, 77^{o}13′ E). Twenty-two-day-old seedlings of rice variety TN 1 were transplanted in micro-plots of 1 m x 1 m, to create different damage levels by leaf folder. Each treatment was replicated four times. The crop field was maintained with the recommended agronomic practices. The micro-plots measuring 1m^2 (1m.x1m.) were separated from each other by 0.5m for intercultural operations as well as various experimental works. Drainage and irrigation facilities were adequately provided to the plots as per the requirement. Recommended agronomic packages of practices were followed for raising the crop during various seasons. Each micro-plot (1m X 1m) of rice plants was covered with small mosquito nets of approximately similar size (1.3 m X 1.3 m) (Fig 1) to avoid infestation of other pests and to maintain the damage level by *C. medinalis*.

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Fig 1: Microplots with rice vegetation covered by nylon nets

Adults of *C. medinalis* comprising of both males and females were collected from nearby rice field with the help of sweep net and insect collecting tubes. Equal numbers of male and female adult moths were then released into potted rice plants which were covered with mylar cages for avoiding the escape of the moths. The condition was made favourable for reproduction of the moths and the female moths laid minute eggs on the leaves after copulation. After seven to ten days some leaves were found folded by the hatched first instar larvae. The potted plants were observed regularly until the emergence of second and third instars which were used for the experiment. Some larvae were further reared in the laboratory to maintain the population by providing them natural leaf diet. Different numbers of 2nd–4th instar of *C. medinalis* were released into the plot at the rice jointing stage which resulted differential damage severity at the booting stage (Table 1).

Table 1. Treatments for the experiment at ICAR-NRRI, Cuttack:

Sl No	Treatments	Number of larvae of *C. medinalis* released per microplot	Damage by larvae
1	T_1	0	No damage (Untreated control)
2	T_2	10	Slight
3	T_3	20	Slight to moderate
4	T_4	40	Moderate
5	T_5	60	Moderate to severe
6	T_6	100	Severe

The leaf-roll rate by *C. medinalis* was worked out by counting the number of healthy and infested leaves and thereby the percentage of leaves infested was computed [18]. Six

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infestation scales (0–5) were defined in Table 2 according to the leaf-roll rate as per description of INGER (1996) [19] with some modifications. Thus, in each micro plot the desired level of infestation scale was maintained. The control plot was sprayed with pesticides on a regular basis to ensure insect pest or disease-free conditions.

Table 2. Infestation scales of *C. medinalis* as per INGER, 1996

Treatments (Incidence)	Insect level (Grade)	Leaf rolling ratio per unit area (Rolling leaves /total leaves) (%)
No damage	0	0
Slight	1	<5.0
Slight to moderate	2	5.0 - 20.0
Moderate	3	20.1 - 35.0
Moderate to severe	4	35.1 - 50.0
Severe	5	> 50.0

The effect of leaf folder damage on leaf biochemical composition was also investigated and the study was conducted taking the differentially infested leaves from the microplots during Rabi 2018-19. About sixty leaves were selected from the experimental microplots were selected as the representative samples on the basis of damaged area of individual leaf by *C. medinalis*. From the visual observations the damaged leaves were grouped into six different groups and from the individual representative leaves. the damage area of leaves of rice were determined by graphical method and validated with the visual observations taken for grouping into six different categories for discriminating the reflectance from each category. A damaged leaf was placed on the transparent sheet, and the damaged area's outline was drawn. The area was measured while being held up to a graph sheet with a 1 mm square grid. The total number of grids the outline covered was counted. An outline was considered to be occupied if it took up more than half of the grid. The grid count was used to compute the leaf folder damage, which was then represented as square millimetres [20].

Table 3: Assessment of leaf folder damage through scoring

Incidence	Grades	Percentage of leaf infestation
No damage	0	0
Slight	1	< 10
Slight to moderate	2	10-20
Moderate	3	20-30
Moderate to severe	4	30-50
Severe	5	> 50.0

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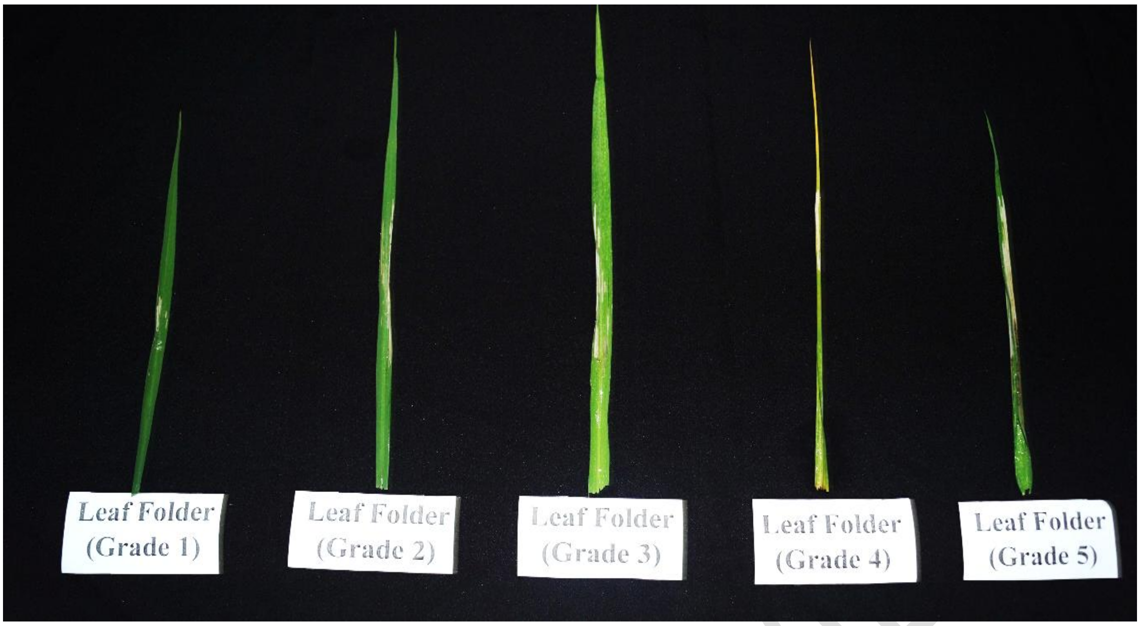

Fig.2. Differential leaf folder infestation in rice leaves

After grouping the leaves based on their damage level, the representative samples from each group were processed for analysis of biochemical parameters. Chlorophyll a/ chlorophyll b was measured after estimation of chlorophyll a and chlorophyll b by the method suggested by Arnon, (1949) [21]. Likewise, soluble carbohydrate was measured by Anthrone method suggested by Hedge and Hofreiter (1962) [22]. The starch measurement of differentially infested leaves was done by amyloglucosidase/ alpha amylase method as recommended by McCleary *et al.*, (1997) [23].

3. RESULTS AND DISCUSSION

3.1.Extent of leaf roll in response to various population level of *C. medinalis* in rice

Differential damage levels of *C. medinalis* in the crop canopy of rice (var TN1) were created artificially through manual release of larva in microplots during *Kharif* 2018-19 and Rabi 2018-19. All the treatments involving various levels of larval release showed significant difference in leaf roll rate by *C. medinalis* over untreated control (T_1) during various years of experimentation (Table 4). Significantly highest leaf roll rate of 60.37 per cent was observed in T_6 (100 larva/m^2) during *Kharif* 2018-19. The untreated microplot (unreleased plot) recorded no leaf roll in rice canopy. The leaf roll rate was reported to be increased with the number of leaf folder larva released in the microplot. The microplot with 10 larval release/ m^2 (T_2) had the leaf roll rate of 2.73 percent. Likewise, the leaf roll rate of 10.47, 21.4 and 37.87 percent were observed in T_3 (20 larva/m^2), T_4 (40 larva/m^2) and T_5 (60 larva/m^2) respectively.

A similar trend of leaf roll was also followed during Rabi 2018-19. All the treatments involving various levels of larval release showed significant difference in leaf roll rate by leaf folder over untreated control (T_1) during various years of experimentation. The leaf roll rate was significantly higher (66.87 percent) in T_6 (100 larva/m2) during Rabi 2018–19. There was no leaf roll observed in the rice canopy in the untreated microplot (unreleased plot). Microplots with 60 larva/m2 (T_5) had the second-highest leaf roll rate, at 42.6 percent.

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According to observations, as more leaf folder larva were released in the microplot, the rate at which leaves rolled up increased. The leaf roll rate in the microplot with 10 larval releases/m2 (T_2) was 2.45 percent. Similarly, T_3 (20 larva/m2) and T_4 (40 larva/m2) showed leaf roll rates of 10.2 and 28.4 percent, respectively. The results derived ample support from the findings of Huang *et al.* [18], Liu *et al.*, [24], Adhikari *et.al.*, [2] for the rice leaf folder infested rice vegetation during the investigation for the hyperspectral spectroscopy.

Table 4: Intensity of leaf roll at different population level of *C. medinalis* during *Kharif* 2018-19 and Rabi 2018-19.

Treatments	**Leaf roll rate in cropping seasons (%)**	
	Kharif 2018-19	**Rabi 2018-19**
T_1 (0 larvae / m^2)	0.00 (0.00)	0.00 (0.00)
T_2 (10 larvae / m^2)	2.73 (9.40)	2.45 (8.96)
T_3 (20 larvae / m^2)	10.47 (18.85)	10.2 (18.51)
T_4 (40 larvae / m^2)	21.4 (27.55)	28.4 (32.17)
T_5 (60 larvae / m^2)	37.87 (37.97)	42.6 (40.74)
T_6 (100 larvae / m^2)	60.37 (51.02)	66.87 (55.13)
SE(m) ±	0.943	1.717
CD (P=0.05)	2.84	5.17
CV %	7.82	13.25

Figures in parentheses are the transformed (Arc sine √%) values

3.2.Change in Chlorophyll a/b due to leaf folder damage in rice leaves

Chlorophyll is the indicator of healthiness of a vegetation. As rice leaf folder is one of the major leaf-eating pests of rice, it affects the leaves and reduce the chlorophyll content significantly. Different components of chlorophyll i.e., chlorophyll a, chlorophyll b as well as total chlorophyll were estimated following the standard procedures and the results showed a decreasing trend in all the above-mentioned parameters in leaf folder damaged rice leaves and results were discussed by Adhikari *et al.* [15]. But the ratio between chlorophyll a and b was estimated and presented in Fig 3. It is derived from the result that the ratio between chlorophyll a and b showed a reverse trend unlike the individual component of chlorophyll or the total chlorophyll with increase in infestation. The ratio (chl a/b) increased with increase in leaf damage by the insect. In healthy leaves the chl a/b was found to be 2.64 and the value increased with infestation. But up to the 30-50 percent damage the increase in the value of

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ratio was not significant. There was a sharp increase in chl a/b in the leaves damaged more than 50 percent (grade 5) and the value was 4.95. Similarly, chl a/b ratio of Grade 1, Grade 2, Grade 3, and Grade 4 were 2.96, 3.00, 3.07, and 3.28, respectively. The results concurred with the findings of Nayak et al. [16] and Usharani and Jyotsna [17] during the study of biochemical changes in rice leaves due to infestation of rice leaf folder. Ashraf and Harris [25] also confirmed the afore mentioned fact from the current findings in their research on the effect of different biotic and abiotic stress on the chl a/b in plants.

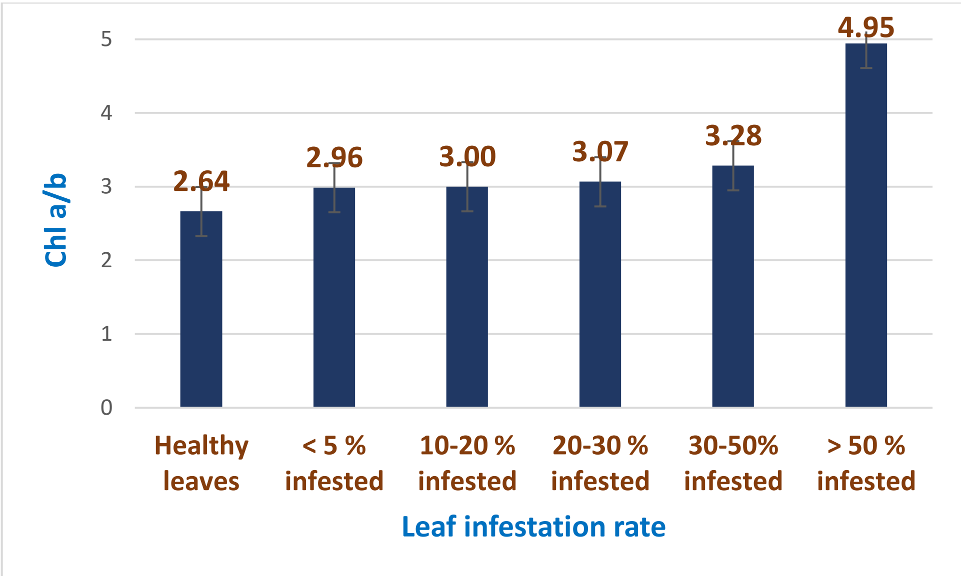

Fig 3: ***Chlorophyll a/ b* in relation to differential infestation of *C. medinalis* in TN 1**

3.3. Change in soluble carbohydrates and starch due to leaf folder damage in rice leaves

The amount of soluble carbohydrates in rice leaves significantly decreased with increase in leaf folder infestation. The damage extent was directly correlated with the decrease in soluble carbohydrate content. The average amount of soluble carbohydrates per gram of fresh leaf weight was 26.02 mg in healthy, uninfested leaves. The leaves with an infestation rate more than 50 percent (grade 5), had the lowest concentration (9.17 mg/g fresh weight leaf). The soluble carbohydrate content was found to be 21.85, 17.74, 14.29 and 10.90 mg per gram fresh weight leaf for grades 1, 2, 3 and 4, respectively (Fig 4).

Similar to soluble carbohydrates, the starch concentration in rice leaves was also affected by the differential leaf folder infestation. A significant difference between the starch concentration of healthy and severely infested leaves was found from the result. The decrease in the starch content of leaves was not significant for the leaves infested less than 5 percent (grade 1). The concentration of starch fell dramatically as the infection rate increased. The highest concentration of starch, 0.89 mg per gram of fresh leaf weight, was identified in uninfested leaves and the lowest concentration was reported in leaves that had more than 50 percent infestation (0.17 mg per gram fresh weight leaf). Similarly, the starch concentrations in grades 1, 2, 3, and 4 were determined to be 0.80, 0.61, 0.43, and 0.31 mg per gram fresh weight leaf, respectively (Fig 5).

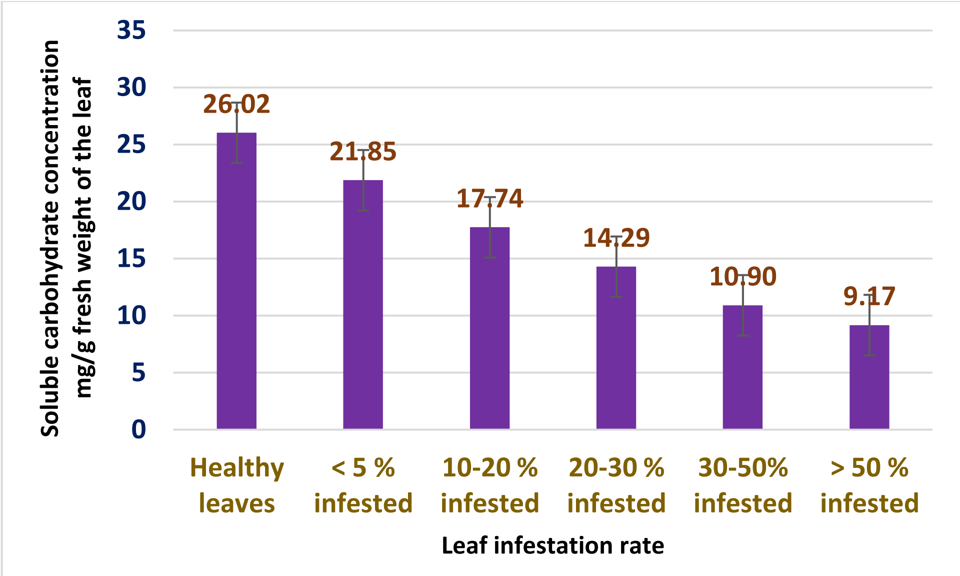

Fig 4: **Soluble carbohydrates in relation to differential infestation of *C. medinalis* in TN 1**

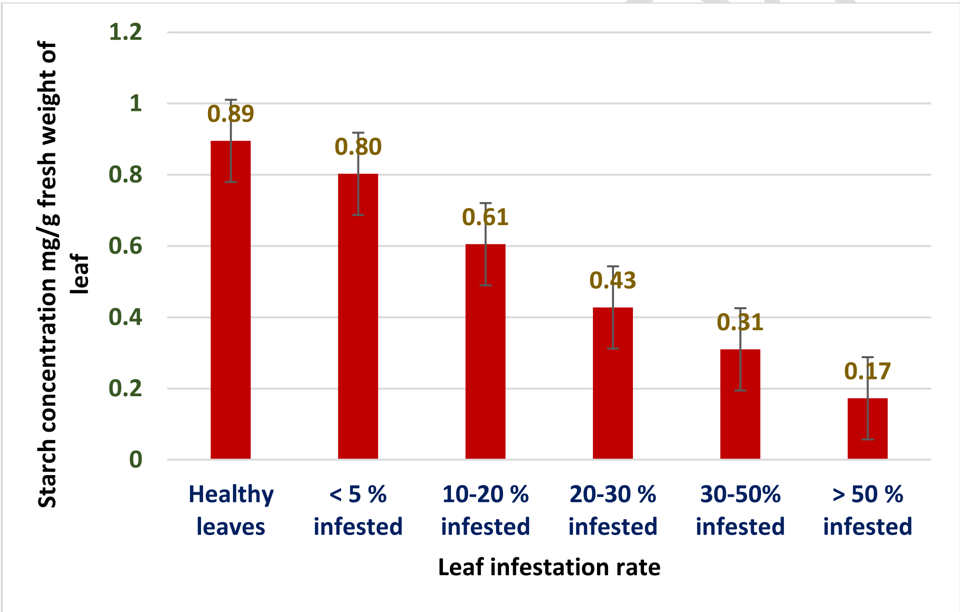

Fig 5: **Starch in relation to differential infestation of *C. medinalis* in TN 1**

Insect pests caused rice plants to suffer feeding stress, which led to visible reactions. The plant's nutritional content and the quantity of its biochemicals and enzymes both changed. The soluble carbohydrates and starch ae two important component of leaf they were found decreased with increase in infestation. The results derived substantial support from the findings of Nayak *et al.*, [16] for the leaf folder damaged rice leaves and Jood *et al.*, [26] in the study of variation in carbohydrate and starch in cereal grains due to insect infestation. Similar results were also found by Soujanya et al., [27] during the study of fluctuation of biochemicals in maize due to infestation of insect pests in storage conditions and carbohydrates was found decreased.

4. CONCLUSION

The number of larvae in the plot directly correlates with the leaf roll rate. The damage percentage likewise rises as the pest load does. In plants affected by insect pests, assessments of all biochemical elements, such as soluble carbohydrates and starch, revealed decreased levels. This supposition states that because numerous metabolic pathways necessary for the intake and development of carbon are restricted by insects' herbivorous nature, this damage to plant growth is severe. The parasitic insects concentrate on the initial stages of photosynthesis. The leaf folder, one of the most significant insects that eat on leaves, directly impacts on chlorophyll. However, it was observed that insect-infested leaves had a higher ratio of the two chlorophyll components, chlorophyll a/b.

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