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Biochemical changes in rice leaves due to rice leaf folder *Cnaphalocrocis medinalis* (Guenee) infestation

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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. The biochemical profile of rice leaves damaged by a leaf folder was studied for quantitative and qualitative variations in chlorophyll, phenol and carotenoid using standard biochemical procedures. The tests were carried out on a rice variety called TN1 that is susceptible to the insect. The result revealed that with increase in infestation by *C. medinalis* resulted in decreased chlorophyll a, chlorophyll b and total chlorophyll content in rice leaves. The similar trend was observed for carotenoid content with increase in infestation. The phenol was found increased amount in infested leaves as it was a component of plant resistance.

Keywords: Chlorophyll, leaf folder, rice, biochemical

Introduction
Rice is the only crop that can properly describe the lives and cultures of Asian countries (*Oryza sativa* L.). For more than half of humanity, rice is life. Good numbers of technical and socioeconomic impediments stand in the way of increasing rice production. A dozen or more insect species are considered major pests in India. Each year, diseases and insect pests cause farmers to lose an estimated 37 per cent of the rice crop (Mohapatra *et al.*, 2021) [12]. Rice leaf folder *Cnaphalocrocis medinalis* (Guenee) [Lepidoptera: Pyralidae] is one of the significant leaf feeding insects of rice causing substantial yield loss. This insect, which was previously thought to be a minor pest, has become a serious problem in several regions of India, including Odisha (Shah *et al.*, 2008) [17]. Although moths are observed throughout the years in the tropics, they are usually more frequent during the rainy season. The insect is active between May and October in cool areas, which are four to five generations throughout these periods; later generations usually overlap. The crucial parameters for the abundance of the insect are the high humidity and optimum temperature (Mohapatra *et al*., 2019) [13]. A single *C. medinalis* larva may defoliate or remove the chlorophyll from several rice leaves, which will disrupt photosynthesis. Feeding frequently causes plant green leaves to become stunted, curled, or yellow (Dash *et al.*, 2020) [5]. The larva feeds by scraping the green mesophyll tissue from within the folded leaves after stitching the leaf borders together to fold the leaves lengthwise. A thin, horizontal white stripe is the outcome of this feeding. The damage causes membranous patches to develop. Early second-instar larvae are mostly gregarious in character. The larva feeds on the folded leaves starting in the late second instar. It becomes solitary. Due to the leaf folder epidemic scenario, the yield loss ranges from 30 to 80 per cent (Tanwar *et al.*, 2019) [18]. Leaf folder infestations can occur at any time during the rice crop's life cycle; however, infestations are most common during the reproductive and ripening stages (Litsinger *et al.*, 2006) [10]. Leaf folder feeding significantly reduces the overall vigour and photosynthetic capacity of an infected rice plant. Additionally vulnerable to bacterial and fungal infestation are infected plants (Bashir *et al.*, 2004) [4].
Due to the infestation of leaf folder in rice, there is change in various biochemical parameters such as leaf chlorophyll, phenol as well as carotenoid in leaves of rice according to the extent of damage. Usha Rani and Jyothsna (2010) [19] concluded that proteins, phenols and carbohydrates were enhanced in leaf after infestation of *C. medinalis*. Kumar *et al*, (2010) [8] observed 50% chlorophyll was reduced than un-infested mustard leaf with aphid infestations. Punithavalli *et al.*, (2013) [16] reported an increase in 23.85 per cent in total phenol content in TN1 rice variety by after infestation of *C. medinalis*.

Anjali *et al.*, (2017) [2] reported that the carotenoid content was decreased with increase in infestation of *Rhopalosiphum maidis* in sorghum. The results from the biochemical analysis of TN1 variety confirmed that there was increase of total phenol by 13.11 per cent after stem borer infestation (Amsagowri *et al*., 2018) [1].

The aim of the particular study was to evaluate the change in above mentioned biochemical components in rice leaves based on differential leaf folder damage. The tests were carried out on the sensitivity to all insects TN1 rice variety.

Methodology

Experiments were conducted in a rice field during the, *Rabi* 2018-19 at the ICAR - National Rice Research Institute, Cuttack (28°36′ N, 77°13′ E). Twenty-two-day-old seedlings of rice variety TN 1 were transplanted in micro-plots, each measuring 1 m x 1 m, to create different damage levels by leaf folder which were replicated four times. The crop field was maintained with the recommended agronomic practices. Variable numbers of males and females of rice leaf folders were released in different plots to create differential leaf folder damage levels. The infested leaves were collected after 7 days after infestation and the differential infestation rate (in per cent) of individual leaf were calculated. The infested leaves were grouped into five different grades based on the percentage of infestation (Table 1) according to INGER 1996 [6].

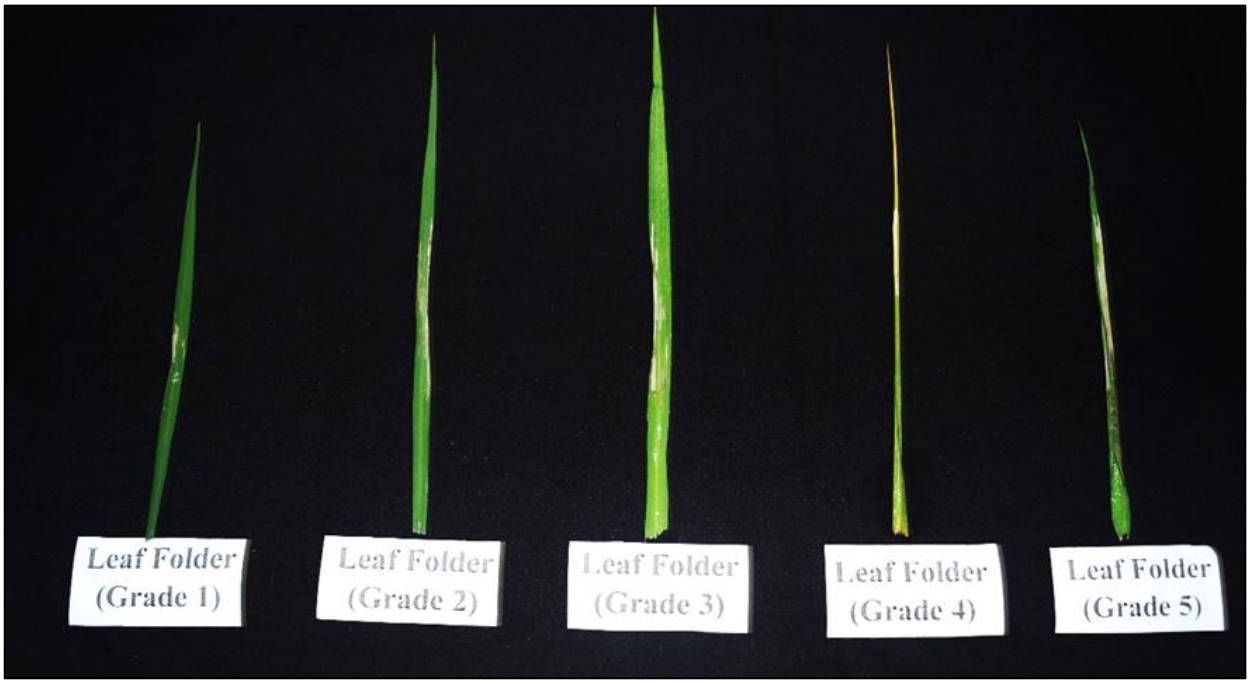

Fig 1: Differential leaf folder infestation in rice leaves

Table 1: 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

After grouping the leaves based on their damage level, the representative samples from each group were processed for analysis of biochemical parameters. Chlorophyll will be measure by the method suggested by Arnon, (1949) [3]. Likewise, Phenol was measured by Folin-Ciocalteu method suggested by Malik and Singh (1980) [11]. The carotenoid measurement was done by the procedures as recommended by Litchenthaler and Wellburn (1983) [9].

Result and Discussion

Insect pests produced feeding stress in rice plants, which resulted in apparent reactions. The nutritional composition of the plant changed, and the amount of its biochemicals and enzymes also altered. The observed biochemical parameters were represented in graph.

Chlorophyll concentration

The amount of *chlorophyll a* in rice leaves significantly decreased as the infestation rate grew due to rice leaf folder feeding and destruction. The degree of damage was directly correlated with the decrease in *chlorophyll a* content. The amount of chlorophyll gradually reduced as the percentage of damaged leaves grew. The average amount of chlorophyll a per gram of fresh leaf weight was 2.95 mg in healthy, uninfested leaves. The grade 5 leaves, or those with an infection rate more than 50%, had the lowest concentration (1.11 mg/g fresh weight leaf). The *chlorophyll a* content was discovered to be 2.61, 2.39, 1.88 and 1.28 mg per gram fresh weight leaf for grades 1, 2, 3 and 4, respectively.

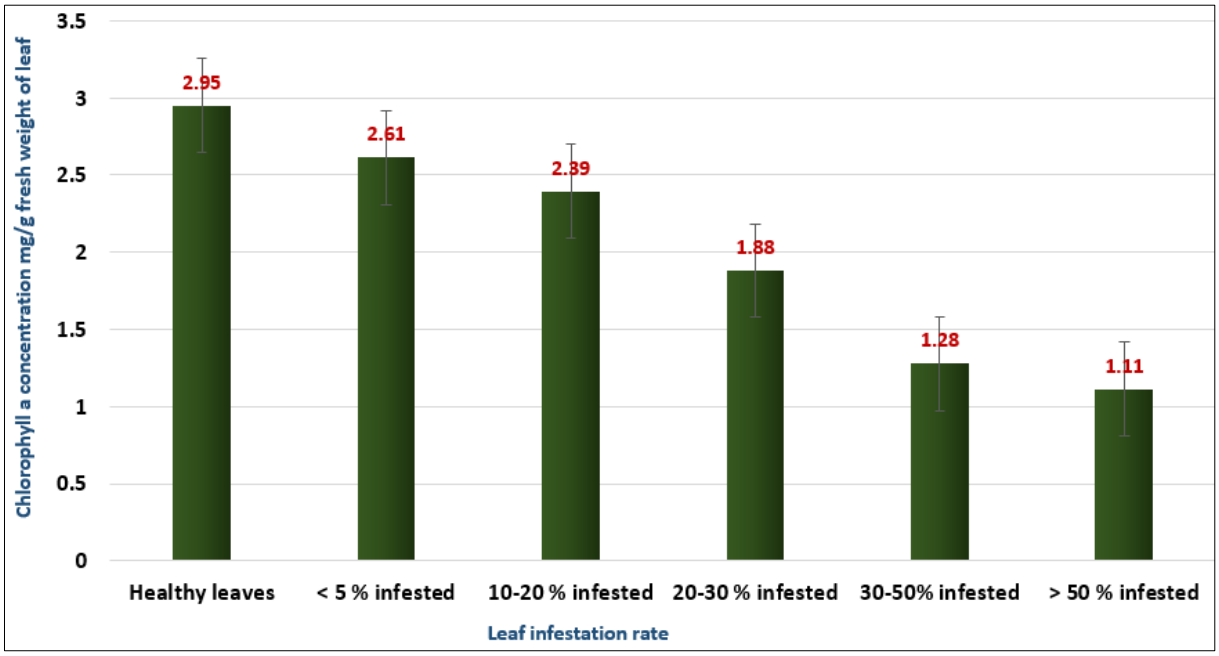

Fig 2: Concentration of *chlorophyll a* in relation to differential infestation of *C. medinalis* in TN 1

In contrast to *chlorophyll a*, chlorophyll b concentration was slightly affected by insect feeding causing up to 20% damage compared to the control (healthy, grade 1 and grade 2). The concentration of *chlorophyll b* again fell dramatically as the infection rate increased. The highest concentration of *chlorophyll b*, 1 mg per gramme of fresh leaf weight, was identified in leaves that were not infested with leaf folders, and the lowest concentration of *chlorophyll b* was discovered in leaves that had more than 50% infestation (0.35 mg per gramme fresh weight leaf). Similarly, the *chlorophyll b* concentrations in grades 1, 2, 3, and 4 were determined to be 0.87, 0.82, 0.65, and 0.45 mg per gram fresh weight leaf, respectively.

On the other hand, the amount of total chlorophyll decreased significantly as the infection rate rose. The highest chlorophyll content, 3.96 mg per gram of fresh leaf weight, was identified in leaves that were not infected with leaf folder, and the lowest chlorophyll content, less than 50% infection, was discovered in leaves (1.47 mg per gram fresh weight leaf). Chlorophyll concentrations in Grades 1, 2, 3, and 4 were discovered to be 3.49, 3.21, 2.53, and 1.72 mg per gram of fresh weight leaf, respectively.

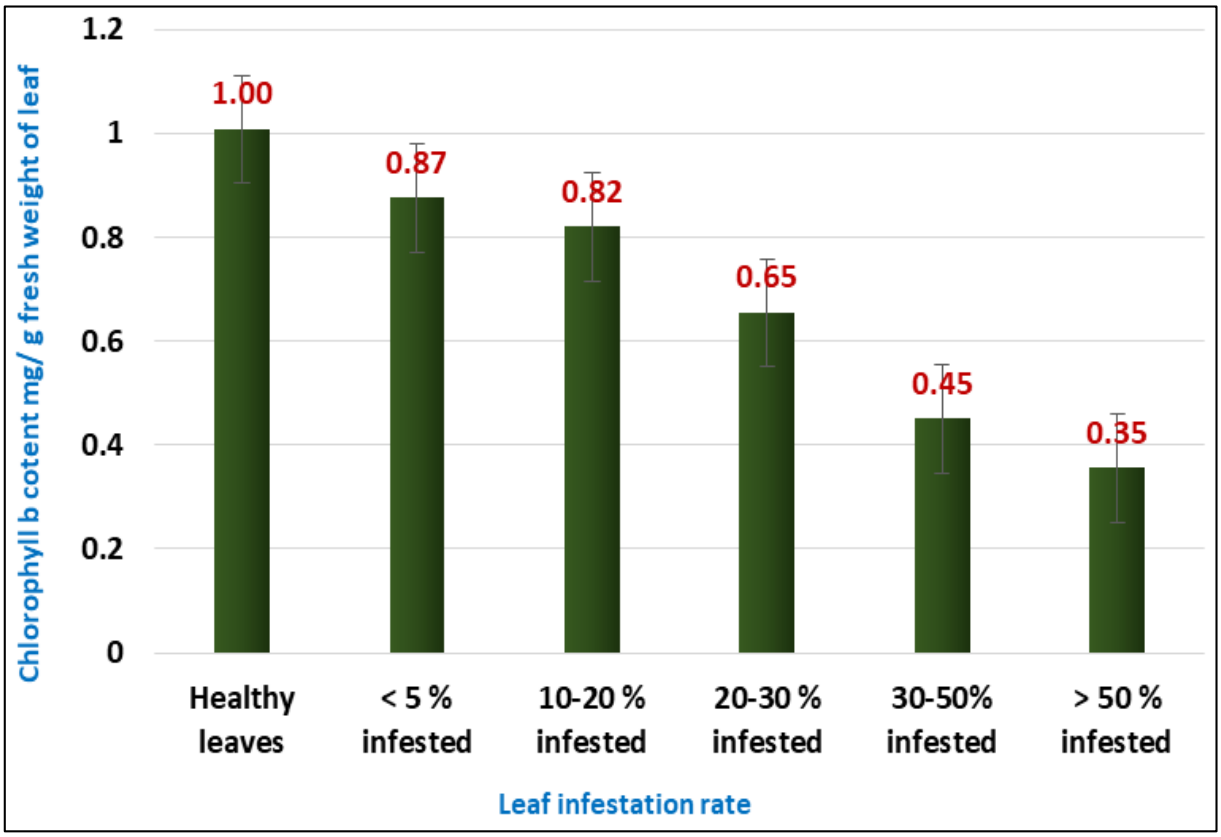

Fig 3: Chlorophyll b content in relation to differential infestation of *C. medinalis* in TN 1

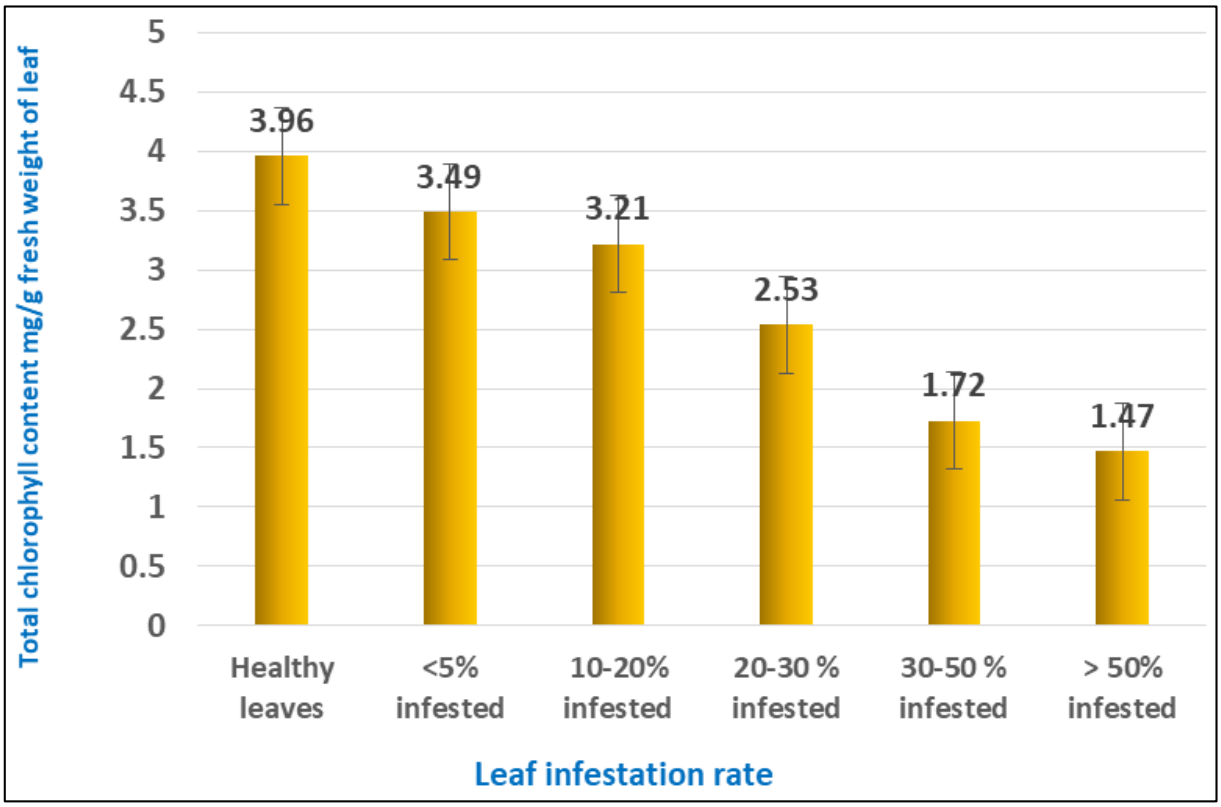

Fig 4: Total chlorophyll content in relation to differential infestation of *C. medinalis* in TN 1

Phenol concentration

The total phenol content in the leaves increased as the infestation percentage increased, according to the results of the experiment. The rate of phenol content augmentation was lower because the test variety TN 1 was a susceptible variety for rice leaf folder infestation. The phenol concentration of healthy leaves was 3.25 mg per grams, and it increased gradually with the percentage of infestation. The leaves with the highest phenol concentration (4.79 mg per gram) were those with a leaf folder infestation of more than 50%. Similarly, the phenol content of Grade 1, Grade 2, Grade 3, and Grade 4 were 3.73, 3.93, 4.15, and 4.32 mg per gram, respectively.

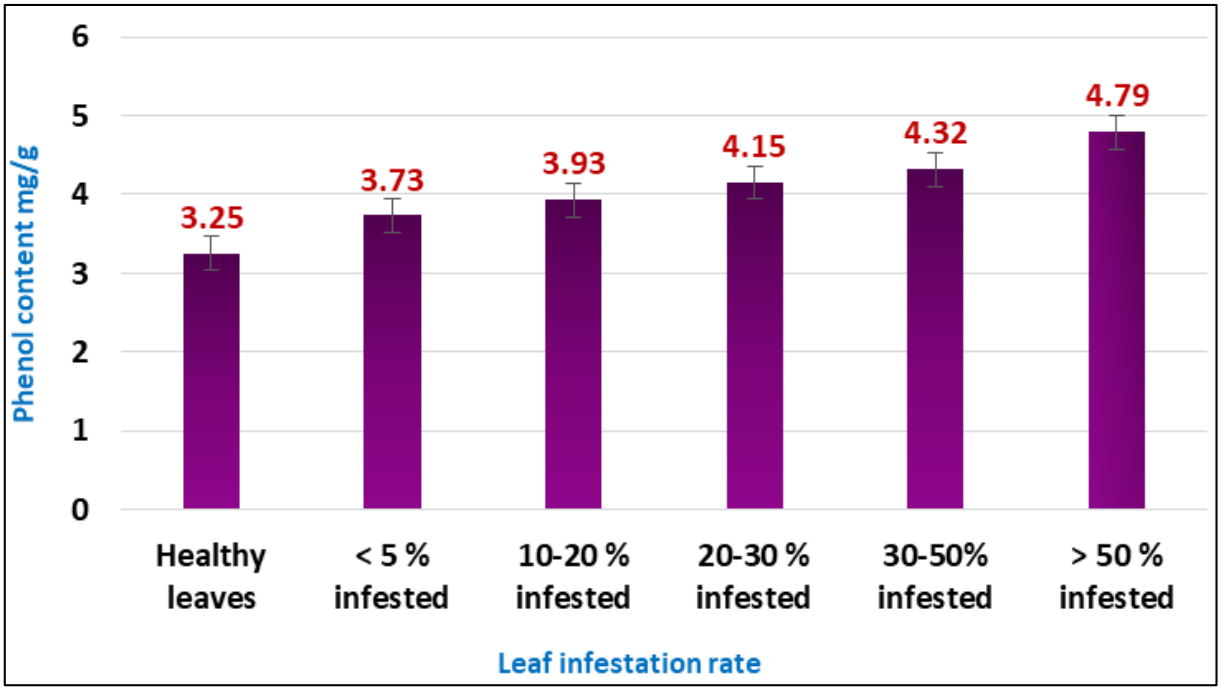

Fig 5: Effect of differential leaf folder damage on phenol concentration of leaf of TN 1

Carotenoid concentration

Similar to chlorophylls, the concentrations of leaf carotenoids decreased slightly when compared to control plants but did not alter dramatically as a result of insect feeding. Carotenoids play two important roles in plants: they protect chlorophyll from light deterioration and they capture light

energy for use in photosynthesis. Uninfected leaves had the highest carotenoid concentration (0.36 mg/g fresh weight leaf), whereas leaves with more than 50% infestation had the lowest carotenoid content (0.21 mg/g fresh weight leaf). Grades 1, 2, 3, and 4 have carotenoid concentrations of 0.33, 0.30, 0.27, and 0.24 mg/g fresh weight leaf, respectively.

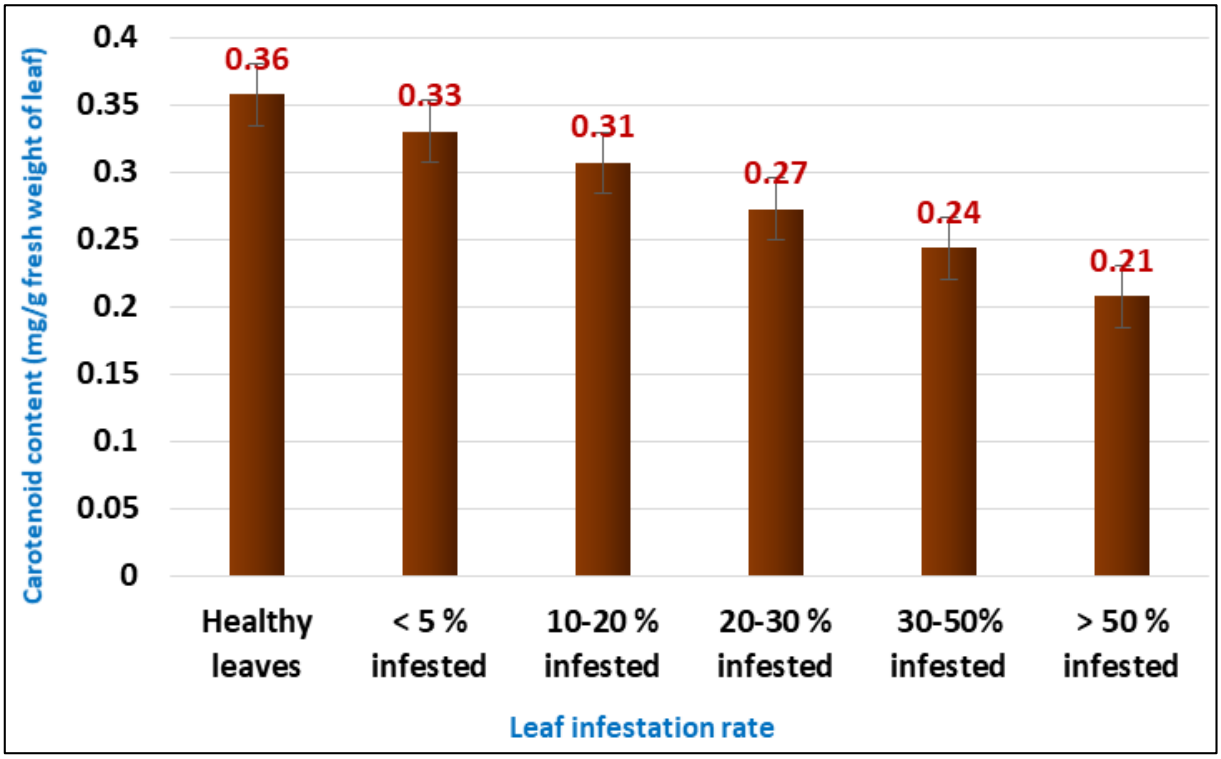

Fig 6: Effect of differential leaf folder damage on carotenoid concentration of leaf of TN1

The findings of above research are supported by experimental results of many scientists. Insect infestation generally lowers the chlorophyll content in leaf and stem. This conclusion of above research is strongly supported by Jayasimha *et al.*, (2015) [7] who discovered the reduced concentration of soluble protein and sugar after infestation of brown plant hopper. However, Usha Rani and Jyothsna (2010) [19] conducted a laboratory investigation to determine the influence of feeding by various pests on the biochemical components of rice plants. Yellow stem borer (YSB), leaf roller (LR), and brown plant hopper (BPH), were the test insects which were examined for the quantitative and qualitative changes in the metabolic profile that take place when the plant's defensive reactions are analyzed. It was found that quantity of biochemicals such as proteins, phenols, and carbohydrates was increased following a laboratory investigation on the influence of BPH, YSB, and LF infestation on biochemical parameters of rice plant. But from the present findings it can be concluded that the phenol content of leaf enhanced with increase in infestation.

Experiments were carried out by Watanabe and Kitagawa (2000) [20] to see how BPH feeding affected photosynthesis and assimilate translocation in rice plants. Matured japonica rice was used. Infested plants had a lower photosynthesis rate and leaf nitrogen concentration than control plants. BPH lowered the chlorophyll concentration and total plant dry weight after flowering. According to the findings, BPH has the largest impact on rice plant development and productivity by removing assimilates and reducing photosynthesis. The present investigation is strongly supported by the experimental results of Nayak *et al.*, (2019) [14] who conducted experiment to show the change in biochemical parameters by feeding of insects such as yellow stem borer (YSB), white backed plant hopper (WBPH), brown plant hopper (BPH) and leaf folder (LF). The results showed that insect infestation considerably decreased the amount of leaf starch, which was highest in the control group (Control followed by YSB, WBPH, BPH and LF damaged plants respectively). Total soluble sugar content was highest in control plants, and it was more than halved by insect feeding. The amount of chlorophyll a and b in the leaf was not affected by insect feeding, in contrast to biochemicals, however both levels were decreased. There was a little reduction in leaf carotenoids as compared to control plants.

Padmavathi *et al.*, (2013) [15] investigated the effect of leaf folder damage on chlorophyll of rice leaves which gives a strong support to recent findings. In comparison to the control, it was discovered that leaf folder damage caused a 57 per cent loss in chlorophyll content, a 23 per cent decrease in PS II activity, and a 23 per cent decrease in relative water content. When the rice leaf folder larva folds a leaf, it scrapes away the green tissue from within the fold, scorching and drying the leaf. Different changes in larvae density were made to the effective leaf area of the rice crop. The results from present findings are also supported by the experiment conducted by Punithavalli *et al.*, (2013) [16] which concluded that, after infestation of leaf folder in TN 1 rice variety the total phenol content was increased by 23.85 % (3.48 mg/g in healthy and 4.31 mg/g in leaf folder infested plants) due to a considerable upsurge in defence mechanisms based on antibiosis, which persistently prevent rice leaf folder oviposition, population growth, survival, and adult emergence. The results from the biochemical analysis of TN1 variety by Amsagowri *et al.*, (2018) [1] confirmed that there was increase of total phenol by 13.11% after YSB infestation.

Conclusion

With the exception of phenol, all biochemical measures measured in insect pest-infested plants had lower values. This assumption suggests that insects' herbivorous nature causes significant damage to plant growth by restricting many metabolic processes involved in carbon uptake and development. The insects function as parasites, focusing on photosynthesis' primary production. It's worth noting that, while insect feeding reduces leaf photosynthesis, it doesn't have the same impact on products like starch and soluble carbohydrates, which the insect depends on for survival. As one of the most important leaf-feeding insects, the leaf folder has a direct impact on chlorophyll and carotenoid levels. Plant defense is symbolized by an increase in phenol, and because TN1 is a susceptible variety for insects, the rate of increase in phenol is lower than in other resistant varieties.

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