Indian Journal of Entomology Online published Ref. No. e24944 DoI. No.: 10.55446/IJE.2024.1944

ESTIMATION OF BIOCHEMICAL PARAMETERS IN BROWN PLANTHOPPER *NILAPARVATA LUGENS* (STÅL) INFESTED RICE PLANTS

Vidya Madhuri E[1], Subhash Chander[2], Rabi N Sahoo[3], Rupali J S[1] and Rajna S[1]*

[1]Division of Entomology; [3]Division of Agricultural Physics,
ICAR-Indian Agricultural Research Institute, New Delhi 110012, India
[2]ICAR-National Centre for Integrated Pest Management, New Delhi 110068, India
*Email: rajnasalim@gmail.com (corresponding author): ORCID ID 0009-0001-9729-4759

ABSTRACT

Biochemical analysis of healthy and brown planthopper (BPH), *Nilaparvata lugens* damaged rice plants across three varieties, viz., Pusa 1509 and Pusa 1121 (Basmati varieties prevalent in cultivation in North India) and the susceptible variety TN-1 revealed substantial variations in the chlorophyll, carotenoid, protein, flavonoid contents and relative water content in infested plants. These alterations serve as vital indicators of pest severity, aiding in cost-effective pest management. Assessing the impact of BPH over 20 and 40 days after infestation (DAI) highlighted significant reductions in chlorophyll, carotenoid, and protein levels. Flavonoid content initially increased after infestation but decreased with high BPH stress after 40 DAI. Relative water content decreased, indicating sustained adverse effects of BPH on rice plants. These findings underscore the susceptibility of physiological traits to BPH stress, urging further research for sustainable pest management to ensure global food security.

Key words: Carotenoid, chlorophyll, differential infestation, flavonoid, *Nilaparvata lugens*, protein, Pusa 1509, Pusa 1121, relative water content, TN1

Rice (*Oryza sativa* L.) is a crucial global staple food, feeding over three billion people and playing a key role in global food security. Despite its significance, rice faces substantial threats from insect infestation, with the brown planthopper, *Nilaparvata lugens* (Stal), standing out as a major pest, causing significant yield losses of up to 60% (Srivastava et al., 2009). The feeding habits of *N. lugens*, primarily targeting rice leaf sheaths, result. This damage manifests as premature yellowing, circular patches of plant drying, and weakened stems, ultimately impacting grain production. Understanding the intricate dynamics between insect infestation and plant biochemical responses is crucial to mitigate the effects on rice productivity. Numerous studies have delved into the changes in biochemical parameters induced by insect infestations, shedding light on alterations in chlorophyll, carotenoids, proteins and other vital constituents (Jayasimha, 2015; Nayak, 2019). These investigations provide valuable insights into how insect feeding patterns affect plant physiology, causing shifts in nutrient uptake, gas exchange, and cell structure, ultimately influencing the biochemical profiles of rice plants.

Research has explored different pest-feeding modes, focusing on yellow stem borer, *Scirpophaga incestulas* (Walker), leaf folder *Cnaphalocrocis medinalis* (Guenee, 1854), and *N. lugens* revealing a decline in protein activities within infested rice plants (Usha Rani et al., 2010). Nayak et al. (2019) expanded this exploration, investigating the effects of various pests on starch, nitrogen, soluble sugar, protein, total chlorophylls, and carotenoids in rice. While starch concentration peaked in control plants, *N. lugens* and white-backed plant hopper (Sogatella furcifeia) (Horrath, 1899) led to more pronounced reductions in total nitrogen concentration. However, there were no significant impacts on chlorophyll A, chlorophyll B, or carotenoid concentrations. Jabeen et al. (2017) demonstrated a substantial decline in chlorophyll A, chlorophyll B, total chlorophyll, and carotenoid levels in susceptible rice cultivars compared to resistant varieties. Furthermore, insights into rice resistance against *N. lugens* have been elucidated through flavonoid compounds like schaftoside (Hao et al., 2018). This research aims to investigate the biochemical alterations induced by *N. lugens* infestation, with a specific focus on chlorophyll, carotenoids, proteins, and key components. By building on prior research into various pest-feeding modes, the study aims to contribute essential insights into the interaction between pest infestation and biochemical responses of the plant that are pivotal for developing targeted strategies to mitigate the impact of BPH.

MATERIALS AND METHODS

The research work was undertaken in the Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi (28° 36'36''N,77° 13'48" E). Two Basmati rice varieties (Pusa 1121 and Pusa 1509) were selected based on their popularity in North India and TN1 was used as a susceptible check. Thirty-day-old rice seedlings of varieties Pusa 1509, Pusa 1121, and TN1 were kept in the glasshouse and exposed to varying levels of BPH infestation, ranging from 0 to 200 second instar nymphs, following a Completely Randomized Design (CRD). Additionally, a water stress treatment was included as abiotic stress for comparison. Samples for biochemical analysis were collected at 20 and 40 days after infestation as this enables us to know the immediate and sustained biochemical responses to *N. lugens* infestation, while also allowing the assessment of damage caused by the progeny over time for comprehensive analysis. The biochemical parameters analyzed were chlorophyll, carotenoid, flavonoid, protein, and relative water content. The treatments were control (0), T1 (5 nymphs), T2 (10 nymphs), T3 (20 nymphs), T4 (40 nymphs), T5 (80 nymphs), T6 (100 nymphs), T7 (200 nymphs) and T8 (water stress). All experiments were performed in two biological and three technical replicates, giving six replications for each treatment.

Estimation of chlorophyll and carotenoid content was done following the non-maceration method using dimethyl sulfoxide (DMSO) described by Hiscox and Israelstam (1979). 50 mg fresh rice leaf samples were finely chopped and placed in test tubes with 10 ml DMSO. Covered with aluminium foil, the tubes were heated at 65°C for 4 hrs and cooled to room temperature. After 1 hour, chlorophyll (A and B) and carotenoid absorbance were measured at 645, 663 and 480 nm respectively in a microplate reader. DMSO served as the blank. Chlorophyll content (A and B) was calculated using Arnon's formula (1949), and total carotenoid content was calculated using the formula given by Lichtenthaler (1987) and expressed as mg/g/leaf sample. Protein estimation was done spectrophotometrically by the Bradford method (Bradford, 1976). 100 mg of the rice leaf sample was crushed using liquid nitrogen and homogenized in 1 ml of 0.1 M potassium phosphate buffer pH (7.5) containing 1mM EDTA, 1% PVP and 10 mM mercaptoethanol. The homogenized sample was centrifuged at 10,000 rpm for 12 min at 4°C. The supernatant collected was used for protein estimation using Bradford reagent. The absorbance was measured at 595 nm and the total protein content was calculated using BSA standard curve. The flavonoid in the leaf samples were estimated using aluminium chloride colorimetric method (Chang et al., 2002). 500 mg of fresh leaf sample were soaked in 10 ml methanol solvent for 60 min. Methanol extracts (0.4 ml) of the samples, 3 ml $NaNO_3$ (5% w/v), 2.5 ml distilled water were added and incubated for 3 min. Then 0.3 ml $AlCl_3$ (10%) was added and the reaction mixture was kept for 0 min and 2 ml of 1M NaOH was added and made up to 10 ml. The solutions were mixed thoroughly and incubated at room temperature for 60 min to allow the complex formation. After the incubation period, the absorbance was measured at 415 nm. The flavonoid content was determined by comparing their absorbance values with the calibration curve obtained from the known concentrations of quercetin standard solutions.

Relative Water Content (RWC) serves as a crucial indicator of a plant water status, reflecting the physiological consequences of cellular water deficit (Barrs and Weatherley, 1962). 250 mg of fresh leaf samples of the fully expanded top most leaves were taken. Immediately after sampling, the samples were placed in a polythene bag and sealed properly to minimize leaf water loss. The leaves were cut into 5-10 cm length mid-leaf sections or 5-10 leaf discs of around 1.5 cm in diameter. The fresh weight of the samples were measured and samples were hydrated at room temperature by dipping them in double distilled water for 4 hrs until the leaves were completely turgid. After removing excess water, the turgid weight was measured, and the samples were kept in butter paper cover with holes. The samples were dried in a hot air oven at 55- 60°C for 2-3 days until the dry weight became constant. The relative water content was estimated by using the following formula given by Lugojan et al. (2011). The estimated biochemical data were subjected to statistical analysis using analysis of variance (ANOVA). This analytical process was facilitated through the utilization of Statistical Package for Social Science (SPSS)software (Version 20.0). To distinguish between the different treatment means, Tukey's test was applied at a significance level of p=0.05.

RESULTS AND DISCUSSION

The study investigated the impact of *N. lugens* infestation on various biochemical parameters in three rice varieties: Pusa 1509, Pusa 1121, and TN-1. Our findings revealed a consistent trend across the varieties and enriched our understanding of the intricate dynamics of biochemical responses to BPH infestation. Chlorophyll levels significantly decreased in infested plants, indicating the sensitivity of chlorophyll to pest damage (Table 1). This decline was observed

Table 1. Biochemical parameters in *N. lugens* infested rice plants

Treatment (No. of nymphs released)	Pusa 1509 (mg g^{-1} of fresh weight)		Pusa 1121 (mg g^{-1} of fresh weight)		TN1 (mg g^{-1} of fresh weight)	
	20 DAI	40 DAI	20 DAI	40 DAI	20 DAI	40 DAI
Chlorophyll content in the rice varieties after BPH infestation						
Control (0 nymph)	4.50± 0.09^{a}	4.07± 0.04^{a}	4.54± 0.07^{a}	4.36± 0.10^{a}	4.26± 0.07^{a}	4.06± 0.07^{a}
T1 (5 nymphs)	4.28± 0.05^{a}	3.30± 0.04^{b}	4.27± 0.03^{b}	2.88± 0.22^{b}	3.98± 0.09^{a}	3.20± 0.05^{b}
T2 (10 nymphs)	3.77± 0.09^{b}	3.02± 0.11^{b}	3.68± 0.03^{c}	2.61± 0.18^{b}	3.23± 0.05^{b}	2.95± 0.04^{c}
T3 (20 nymphs)	3.15± 0.03^{c}	2.05± 0.15^{c}	3.39± 0.04^{d}	2.05± 0.11^{c}	2.86± 0.04^{b}	2.31± 0.02^{d}
T4 (40 nymphs)	2.88± 0.08^{cd}	1.82± 0.11^{c}	2.74± 0.04^{e}	1.64± 0.08^{c}	2.53± 0.10c	1.74± 0.06^{e}
T5 (80 nymphs)	2.68± 0.05^{d}	1.67± 0.10^{c}	2.53± 0.02^{f}	1.50± 0.07^{cd}	2.49± 0.10^{c}	1.50± 0.03^{f}
T6 (100 nymphs)	1.98± 0.03^{ef}	0.88± 0.05^{d}	1.94± 0.04^{g}	0.91± 0.04^{d}	1.74± 0.09^{d}	0.73± 0.03^{g}
T7 (200 nymphs)	1.68± 0.04^{f}	0.69± 0.05^{de}	1.51± 0.01^{h}	0.67± 0.01^{d}	1.43± 0.11^{e}	0.56± 0.01^{gh}
T8 (water stress)	1.94± 0.08^{ef}	0.43± 0.01^{e}	1.85± 0.07^{g}	0.40± 0.09^{d}	1.89± 0.31^{d}	0.39± 0.03^{h}
F	313.14	217.98	874.73	91.59	75.86	850.92
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Carotenoid content in the varieties studied after BPH infestation						
Control (0 nymph)	0.97± 0.02^{a}	0.87± 0.02^{a}	0.65± 0.02^{a}	0.63± 0.02^{a}	0.64± 0.01^{a}	0.67± 0.01^{a}
T1 (5 nymphs)	0.80± 0.02^{b}	0.66± 0.01^{b}	0.59± 0.00^{ab}	0.53± 0.01^{b}	0.64± 0.03^{a}	0.51± 0.01^{b}
T2 (10 nymphs)	0.70± 0.01^{c}	0.47± 0.02^{b}	0.53± 0.01^{b}	0.42± 0.03^{bc}	0.58± 0.03^{ab}	0.47± 0.01^{bc}
T3 (20 nymphs)	0.68± 0.01^{c}	0.44± 0.01^{c}	0.46± 0.02^{c}	0.40± 0.03^{cd}	0.51± 0.01^{b}	0.45± 0.02^{cd}
T4 (40 nymphs)	0.63± 0.01^{c}	0.34± 0.01^{c}	0.43± 0.03^{d}	0.33± 0.01^{d}	0.47± 0.01^{b}	0.40± 0.01^{d}
T5 (80 nymphs)	0.45± 0.03^{d}	0.32± 0.01^{d}	0.34± 0.01^{e}	0.31± 0.01^{de}	0.33± 0.01^{c}	0.35± 0.01^{e}
T6 (100 nymphs)	0.33± 0.00^{e}	0.24± 0.01^{d}	0.26± 0.02^{f}	0.28± 0.01^{ef}	0.24± 0.01^{cd}	0.23± 0.02^{f}
T7 (200 nymphs)	0.23± 0.02^{f}	0.19± 0.01^{e}	0.25± 0.02^{fg}	0.20± 0.01^{fg}	0.18± 0.01^{e}	0.17± 0.01^{g}
T8 (water stress)	0.14± 0.01^{g}	0.02± 0.006^{f}	0.18± 0.002^{g}	0.09± 0.007^{g}	0.19± 0.05^{e}	0.02± 0.009^{h}
F	276.31	250.97	93.94	65.42	57.832	182.46
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Protein content in the varieties studied after BPH infestation						
Control (0 nymph)	39.05± 1.17^{a}	38.65± 0.49^{a}	36.23± 1.10^{a}	35.86± 0.43^{a}	38.24± 1.81^{a}	36.81± 0.48^{a}
T1 (5 nymphs)	37.06± 0.66^{ab}	30.90± 0.11^{b}	32.29± 0.41^{b}	25.52± 0.31^{b}	31.18± 1.08^{b}	21.33± 0.20^{b}
T2 (10 nymphs)	32.60± 0.67^{bc}	24.34± 0.57^{c}	30.90± 0.58^{bc}	23.10± 0.25^{c}	28.32± 1.43^{b}	19.68± 0.45^{b}
T3 (20 nymphs)	31.61± 1.04^{d}	19.88± 0.46^{d}	27.93± 1.37^{c}	17.64± 0.28^{d}	22.52± 0.15^{c}	12.41± 0.45^{c}
T4 (40 nymphs)	26.14± 2.24^{e}	14.26± 0.13^{e}	21.60± 0.82^{d}	12.81± 0.36^{e}	18.54± 0.81^{cd}	8.52± 0.97^{d}
T5 (80 nymphs)	22.36± 1.30^{fg}	10.63± 0.33^{f}	18.79± 0.86^{e}	7.18± 0.29^{f}	15.69± 0.31^{de}	2.32± 0.38^{e}
T6 (100 nymphs)	17.82± 0.48^{gh}	7.60± 0.67^{g}	14.89± 0.36^{f}	4.60± 0.39^{g}	13.02± 0.45^{ef}	1.03± 0.15^{ef}
T7 (200 nymphs)	14.62± 0.85^{gh}	4.10± 0.36^{h}	12.18± 0.29^{f}	2.12± 0.16^{h}	10.90± 0.55^{ef}	0.13± 0.05^{ef}
T8 (water stress)	17.86± 0.78^{h}	2.08± 0.48^{i}	13.75± 0.45^{f}	1.39± 0.28^{h}	13.37± 0.68^{g}	0.48± 0.09^{g}
F	62.19	842.29	132.17	841.42	97.61	792.92
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Flavonoid content in the varieties studied after BPH infestation						
Control (0 nymph)	4.38± 0.06^{a}	4.23± 0.02^{b}	4.25± 0.04^{a}	3.53± 0.08^{b}	3.66± 0.08^{a}	3.44± 0.03^{b}
T1 (5 nymphs)	4.72± 0.08^{ab}	4.35± 0.02^{ab}	4.60± 0.11^{ab}	3.65± 0.1^{b}	3.63± 0.05^{ab}	3.44± 0.03^{b}
T2 (10 nymphs)	4.85± 0.20^{abc}	4.45± 0.06^{ab}	4.71± 0.10^{ab}	3.81± 0.04^{ab}	3.71± 0.06^{be}	3.48± 0.05^{ab}
T3 (20 nymphs)	5.04± 0.15^{abc}	4.5± 0.06^{a}	4.81± 0.06^{bc}	3.96± 0.1^{a}	3.80± 0.09^{be}	3.57± 0.02^{ab}
T4 (40 nymphs)	5.14± 0.09^{abc}	4.42± 0.05^{ab}	4.93± 0.08^{bc}	3.74± 0.02^{ab}	3.93± 0.07^{cd}	3.74± 0.02^{a}
T5 (80 nymphs)	5.32± 0.33^{bc}	3.62± 0.11^{c}	5.28± 0.04^{de}	3.8± 0.05^{ab}	4.16± 0.06^{d}	3.51± 0.8^{ab}
T6 (100 nymphs)	5.53± 0.08^{cd}	2.52± 0.02^{d}	5.42± 0.07^{df}	2.88± 0.04^{c}	4.58± 0.07^{e}	2.76± 0.04^{c}

(contd.)

(contd. Table 1)

T7 (200 nymphs)	6.14± 0.19^{d}	2.09± 0.07^{e}	5.76± 0.11^{f}	1.79± 0.05^{d}	4.81± 0.04^{ef}	2.2± 0.03^{d}
T8 (water stress)	5.07± 15^{abc}	1.93± 0.06^{e}	5.26± 0.20^{cde}	1.67± 0.06^{d}	5.09± 0.04^{f}	1.84± 0.08^{e}
F	9.074	326.05	21.107	183.79	69.59	141.05
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Relative water content in the varieties studied after BPH infestation						
Control (0 nymph)	0.97± 0.02^{a}	0.87± 0.02^{a}	0.65± 0.02^{a}	0.63± 0.02^{a}	0.64± 0.01^{a}	0.67± 0.01^{a}
T1 (5 nymphs)	0.80± 0.02^{b}	0.66± 0.01^{b}	0.59± 0.00^{ab}	0.53± 0.01^{b}	0.64± 0.03^{a}	0.51± 0.01^{b}
T2 (10 nymphs)	0.70± 0.01^{c}	0.47± 0.02^{b}	0.53± 0.01^{b}	0.42± 0.03^{bc}	0.58± 0.03^{ab}	0.47± 0.01^{bc}
T3 (20 nymphs)	0.68± 0.01^{c}	0.44± 0.01^{c}	0.46± 0.02^{c}	0.40± 0.03^{cd}	0.51± 0.01^{b}	0.45± 0.02^{cd}
T4 (40 nymphs)	0.63± 0.01^{c}	0.34± 0.01^{c}	0.43± 0.03^{d}	0.33± 0.01^{d}	0.47± 0.01^{b}	0.40± 0.01^{d}
T5 (80 nymphs)	0.45± 0.03^{d}	0.32± 0.01^{d}	0.34± 0.01^{e}	0.31± 0.01^{de}	0.33± 0.01^{c}	0.35± 0.01^{e}
T6 (100 nymphs)	0.33± 0.00^{e}	0.24± 0.01^{d}	0.26± 0.02^{f}	0.28± 0.01^{ef}	0.24± 0.01^{cd}	0.23± 0.02^{f}
T7 (200 nymphs)	0.23± 0.02^{f}	0.19± 0.01^{e}	0.25± 0.02^{fg}	0.20± 0.01^{fg}	0.18± 0.01^{e}	0.17± 0.01^{g}
T8 (water stress)	0.14± 0.01^{g}	0.02± 0.006^{f}	0.18± 0.002^{g}	0.09± 0.007^{g}	0.19± 0.05^{e}	0.02± 0.009^{h}
F	276.31	250.97	93.94	65.42	57.832	182.46
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

The means indicated by the different letters in lower case are significantly different ($p< 0.05$)

consistently across all the three varieties. Control plants exhibited higher chlorophyll content than infested ones at both 20 and 40 days after infestation (DAI), underscoring the impact of *N. lugens* on photosynthetic pigments. In Pusa 1509, control plants had a chlorophyll content of 4.50 mg/ g fresh weight (FW), while infested ones had 1.68 mg/ g FW at 20 DAI and 0.69 mg/ g FW at 40 DAI. Similar trends were observed in Pusa 1121 and TN-1, indicating a consistent decline in chlorophyll levels post- infestation. Recent studies by Adhikari et al. (2022) support our findings, emphasizing a consistent reduction in total chlorophyll content with increasing *N. lugens* infestation severity. The significant decrease in chlorophyll content underscores the vulnerability of rice plants to pest stress, impacting photosynthetic efficiency.

Similar to chlorophyll, carotenoid content significantly decreased in infested plants across all varieties and time points compared to controls (Table 1). Control plants consistently displayed higher carotenoid content than infested ones at both 20 and 40 DAI. In Pusa 1509, control plants had a carotenoid content of 0.97± 0.02 mg/ g FW, while infested ones had 0.23± 0.02 mg/ g FW at 20 DAI and 0.19± 0.01mg/ g FW at 40 DAI. The findings of Nayak et al. (2019) align with the present result, highlighting the impact of *N. lugens* on photosynthetic pigments. The substantial reduction in carotenoid content underscores the severity of *N. lugens* infestation and its consequences on essential plant pigments. Protein levels notably decreased in response to BPH infestation across all varieties. Control plants consistently displayed higher protein content than infested ones at both 20 and 40 DAI. For instance, in Pusa 1509, control plants had a protein content of 39.05± 1.17mg/g FW, while infested ones had 14.62± 0.85mg/ g FW at 20 DAI and 4.10± 0.36 mg/ g FW at 40 DAI as in (Table 1). This reduction aligns with studies reporting decreased soluble protein and sugar levels post- infestation (Jayasimha et al., 2015).

Flavonoid content exhibited a dynamic response to *N. lugens* infestation (Table 1). While initially increasing from control of 4.38± 0.06 mg/ g to infested ones of 6.14± 0.19 mg/ g at 20 DAI, this trend reversed by 40 DAI for control of 4.23± 0.02 mg/g and for infested ones of 2.09± 0.07 mg/ g suggesting higher *N. lugens* stress leading to reduced flavonoid levels as plants experienced drying. This result is supported by recent literature, regarding flavonoid content variation in different rice varieties (Hao et al., 2018). Shen et al. (2009) found that flavonoid levels in red rice generally exhibited higher flavonoid levels compared to white rice, but certain red rice accessions still displayed lower levels. The significant changes in flavonoid content emphasize the intricate adjustments in plant defence mechanisms under varying BPH infestation levels. Relative water content (RWC) significantly decreased in infested plants compared to controls in all three varieties at both 20 and 40 DAI (Table 1). Control plants consistently displayed higher RWC than infested ones at both time points, emphasizing the sustained impact of *N. lugens* infestation over the study duration. In Pusa 1509, control plants had an RWC of 0.97± 0.02 mg/ g FW, while high *N. lugens* -infested plants had 0.14± 0.01 mg/ g FW at 20 DAI and 0.02± 0.006 mg/ g FW at 40 DAI. Recent literature underscores the susceptibility

of rice plants to dehydration due to pest infestation and found substantial losses in chlorophyll content, PS II activity, and relative water content due to *C. medinalis* damage (Rashid et al., 2016; Padmavathi et al., 2013). The significant reduction in RWC highlights the enduring detrimental impact of *N. lugens*.

The biochemical analyses conducted in the rice varieties (Pusa 1509, Pusa 1121, TN1) subjected to brown planthopper, *N. lugens*, revealed significant variations in chlorophyll, carotenoid, protein, flavonoid, and relative water content, serving as crucial indicators of infestation severity. Over 20 and 40 days after infestation, a consistent decline in chlorophyll, carotenoid, and protein levels underscores the vulnerability of these parameters to *N. lugens* induced by stress, impacting photosynthetic pigments and vital metabolic processes. These findings not only contribute essential insights into the biochemical responses but also emphasize the need for further research to understand genotypic differences and specificities in pest-plant interactions.

ACKNOWLEDGEMENTS

The authors thank the Indian Agricultural Research Institute, New Delhi, for providing the facilities for conducting the research.The authors thank ICAR-Network Program on Precision Agriculture for the support provided.

FINANCIAL SUPPORT

The financial support for conducting the experiments was obtained from ICAR-NePPA Program.

AUTHOR CONTRIBUTION STATEMENT

RS, SC and RNS designed the research, EVM conducted the experiment and wrote the manuscript. RS has reviewed the manuscript, RJS helped in statistical analysis of data. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

Adhikari B, Mohapatra L N, Senapati R, Mohapatra M, Muduli L, Mohapatra S D. 2022. Biochemical changes in rice leaves due to rice leaf folder *Cnaphalocrocismedinalis* (Guenee) infestation. The Pharma Innovation 11(8): 1463-1468.

Arnon D I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology 24(1): 1 pp.

Barrs H D, Weatherley P E. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian Journal of Biological Sciences 15(3): 413-428.

Bradford M M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72(1-2): 248-254.

Chang C C, Yang M H, Wen H M, Chern J C. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 10(3): 3 pp.

Hao P Y, Feng Y L, Zhou Y S, Song X M, Li H L, Ma Y, Ye C L, Yu X P. 2018. Schaftoside interacts with NlCDK1 protein a mechanism of rice resistance to brown planthopper *Nilaparvata lugens*. Frontiers in Plant Science 9: 710 pp.

Hiscox J D, Israelstam G F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany 57(12): 1332-1334.

Jabeen A, Kiran T V, Subrahmanyam D, Lakshmi D L, Bhagyanarayana G, Krishnaveni D. 2017. Variations in chlorophyll and carotenoid contents in tungro infected rice plants. Journal of Research and Development 5(1): 1-7.

Jayasimha G T, Nalini R, Chinniah C, Muthamilan M, Mini M L. 2015. Evaluation of biochemical constituents in healthy and brown planthopper *Nilaparvata lugens* (Stal.) (Hemiptera: Delphacidae) damaged rice plants. Current Biotechnology 9(2): 129-136.

Lichtenthaler H K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology 148: 350-383.

Lugojan C, Ciulca S. 2011. Evaluation of relative water content in winter wheat. Journal of Horticulture, Forestry and Biotechnology 15(2): 173-177.

Nayak A, Baig M J, Mohapatra P K, Behera K S. 2019. Effect of insect feeding on biochemical changes in rice plant. Journal of Entomology and Zoology Studies 7: 138-142.

Padmavathi C, Katti G, Padmakumari A P, Voleti S R, Subba Rao L V. 2013. The effect of leaf folder, *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae) injury on the plant physiology and yield loss in rice. Journal of Applied Entomology 137(4): 249-256.

Rashid M M, Jahan M, Islam K S. 2016. Impact of nitrogen, phosphorus and potassium on brown planthopper and tolerance of its host rice plants. Rice Science 23(3): 119-131.

Shen Y, Jin L, Xiao P, Lu Y, Bao J. 2009. Total phenolics, flavonoids, antioxidant capacity in rice grain and their relations to grain color, size and weight. Journal of Cereal Science 49(1): 106-111.

Srivastava C, Chander S, Sinha S R, Palta R K. 2009. Toxicity of various insecticides against Delhi and Palla population of brown plant hopper *Nilaparvatalugens*. Indian Journal of Agricultural Sciences 79(12): 55-58.

Usha Rani P, Jyothsna Y. 2010. Biochemical and enzymatic changes in rice plants as a mechanism of defense. Acta Physiologiae Plantarum 32: 695-701.

(Manuscript Received: January, 2024; Revised: May, 2024;
Accepted: June, 2024; Online Published: July, 2024)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e24944