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Impact of green leafhopper (*Nephotettix virescens*) infestation on protein and relative water content in rice

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Abstract

This study investigates the biochemical responses of three rice varieties, Pusa 1509, Pusa 1121 (Basmati varieties), and TN-1 (a susceptible variety), to infestation by the green leafhopper (GLH), *Nephotettix virescens*. Significant reductions in protein content and relative water content (RWC) were observed across all varieties following GLH infestation. These reductions serve as critical indicators of pest severity, providing valuable insights into the physiological stress experienced by rice plants under GLH attack. The study highlights the adverse effects of GLH infestation on rice plants, emphasizing the need for targeted pest management strategies. The findings underscore the importance of understanding the biochemical and physiological impacts of GLH on rice, which is vital for developing sustainable pest management practices to safeguard global rice production.

Keywords: Protein content, relative water content, *Nephotettix virescens*, green leafhopper, rice varieties, pest management

Introduction

Rice (*Oryza sativa* L.) is a crucial global staple food, providing sustenance to over three billion people and playing a key role in global food security. Despite its significance, rice production is significantly threatened by insect pests, particularly the green leafhopper (*Nephotettix virescens*), which can cause yield losses of up to 60% (Srivastava *et al.*, 2009) [16]. This pest is widespread in many Asian countries and primarily feeds on the phloem of susceptible rice varieties. In contrast, resistant varieties experience a shift in feeding behavior towards the xylem (Khan and Saxena, 1985) [9]. Studies have demonstrated that phloem feeding is more prevalent in susceptible varieties, while resistant ones show increased xylem feeding (Biswas and Chowdhury, 1997) [4]. The green leafhopper feeds mainly on the adaxial surface of the leaf blade, rarely targeting the leaf sheath. The direct damage from this pest includes sap extraction from the plant's vascular tissues, resulting in reduced plant vigor, fewer tillers, and substantial yield loss. In severe cases, rice plants may completely dry out. Additionally, the green leafhopper is a vector for several viral diseases, including the rice tungro virus, further exacerbating its impact on rice production (Hirao & Inoue, 1978) [6]. Research into the biology of *Nephotettix virescens* under controlled temperature conditions has provided insights into its life cycle and behavior (Muhammad Salim, 2002) [12]. Numerous studies have investigated the biochemical changes induced by insect infestations, highlighting alterations in chlorophyll, carotenoids, and other essential biochemical constituents (Nayak *et al.*, 2019; Jayasimha, 2015) [13, 7]. These investigations offer valuable insights into how insect feeding affects plant physiology, leading to changes in nutrient uptake, gas exchange, and cell structure, which ultimately influence the biochemical profiles of rice plants.

Further research into different pest-feeding modes, including yellow stem borer (YSB), leaf folder (LF), and brown planthopper (BPH), has shown a decline in protein activities within infested rice plants (Usha Rani and Jyothsna, 2010) [17]. Nayak *et al.* (2019) [13] expanded this research by examining the effects of various pests on starch, nitrogen, soluble sugar, protein, total chlorophylls, and carotenoids in rice. Their findings revealed that starch concentration was highest in control plants, while BPH and white-backed planthopper (WBPH) caused

more pronounced reductions in total nitrogen concentration. Additionally, research has elucidated rice resistance mechanisms against BPH through compounds like schaftoside, a flavonoid that inhibits BPH via distinct mechanisms (Hao *et al.*, 2018) [5].

This study aims to explore the biochemical alterations induced by green leafhopper (GLH) infestation, focusing on protein content and relative water content. The goal is to provide valuable insights into the interaction between GLH infestation and rice biochemical responses, which are critical for developing targeted strategies to mitigate the impact of GLH and ensure the sustainability of rice production.

Materials and Methods

The research was conducted at the Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi. The study involved two Basmati rice varieties, Pusa 1121 and Pusa 1509, alongside TN1, which served as a susceptible check. Thirty-day-old seedlings of these varieties were maintained in a glasshouse and subjected to varying levels of green leafhopper (GLH) infestation, ranging from 0 to 120 nymphs, using a Completely Randomized Design (CRD). Biochemical samples were collected post-infestation to assess both immediate and prolonged biochemical responses. The biochemical parameters analyzed included protein content and relative water content (RWC). The experimental treatments consisted of a control (0 nymphs), T₁ (20 nymphs), T₂ (40 nymphs), T₃ (80 nymphs), T₄ (100 nymphs), and T₅ (120 nymphs), with six replications for each treatment.

For protein estimation, rice leaves from different treatments were collected, transported in an icebox, and weighed fresh in the laboratory. The samples were then folded in aluminum foil and stored at -80°C. Protein content was quantified using the Lowry *et al.* (1951) [10] method, with absorbance measured at 660 nm against a standard curve.

Relative Water Content (RWC), which indicates plant water status and cellular water deficiency, was assessed as follows: 250 mg of fresh leaf samples from the uppermost fully mature leaves were collected and kept in polythene bags to prevent water loss. The leaves were then cut into sections and hydrated in double distilled water for four hours at room temperature to achieve full turgidity. Fresh weight was recorded immediately, followed by turgid weight measurement after removing excess water. The leaves were then dried in a hot air oven at 55-60°C for two to three days to obtain a consistent dry weight. The RWC was calculated using the formula provided by Lugojan *et al.* (2011) [11] and is expressed as mg/g/leaf sample.

Data were analyzed using R software with ANOVA and Duncan's multiple range test (DMRT) applied at a significance level of $p < 0.05$. Results are presented as mean \pm standard error (SE), with different superscript letters denoting significant differences between means.

Results

The study examined the impact of different infestation levels of green leafhopper (GLH) on protein content and relative water content (RWC) in three rice varieties: Pusa 1509, Pusa 1121, and TN-1.

Protein Content

Protein content decreased significantly with increasing GLH infestation across all three rice varieties. In Pusa 1509, protein content dropped from 38.05 \pm 1.17 mg/g in the

control group (0 nymphs) to 17.42 \pm 0.85 mg/g at the highest infestation level (120 nymphs). Similarly, Pusa 1121 showed a decline from 35.23 \pm 1.10 mg/g to 12.12 \pm 0.29 mg/g, and TN-1 from 36.24 \pm 1.81 mg/g to 11.80 \pm 0.55 mg/g under the same conditions. The F-values (62.19, 132.17, and 97.61 for Pusa 1509, Pusa 1121, and TN-1, respectively) indicate a highly significant effect of GLH infestation on protein content ($p < 0.001$) depicted in (Table 1).

Relative Water Content (RWC)

RWC also decreased as GLH infestation levels increased. In Pusa 1509, RWC fell from 0.97 \pm 0.02 in the control group to 0.23 \pm 0.02 at the highest infestation level. Pusa 1121 exhibited a similar reduction, from 0.65 \pm 0.02 to 0.25 \pm 0.02, and TN-1 from 0.64 \pm 0.01 to 0.18 \pm 0.01. The F-values (276.31, 93.94, and 57.832 for Pusa 1509, Pusa 1121, and TN-1, respectively) again indicate a highly significant impact of GLH infestation on RWC ($p < 0.001$) depicted in (Table 2).

These results demonstrate that GLH infestation leads to a significant reduction in both protein content and relative water content in rice leaves, reflecting the physiological stress imposed by the insect. As the number of nymphs increases, the protein content and water retention capacity of the leaves decrease, indicating severe cellular damage and dehydration. This reduction in protein content could affect the overall nutritional quality of the rice, while the decrease in RWC points to compromised water balance within the plant tissues, likely exacerbating the effects of environmental stress and reducing the plant's resilience to further pest attacks or drought conditions. The data suggest that higher GLH infestations lead to more pronounced physiological disruptions, with potential implications for yield and crop quality.

Table 1: Protein content in the varieties studied after GLH infestation

Treatment	Pusa 1509	Pusa 1121	TN-1
Control (0 nymph)	38.05 \pm 1.17 ^a	35.23 \pm 1.10 ^a	36.24 \pm 1.81 ^a
T ₁ (10 nymphs)	36.06 \pm 0.66 ^{ab}	31.29 \pm 0.41 ^b	30.18 \pm 1.08 ^b
T ₂ (20 nymphs)	31.50 \pm 0.67 ^{bc}	29.80 \pm 0.58 ^{bc}	27.32 \pm 1.43 ^b
T ₃ (40 nymphs)	25.34 \pm 2.24 ^d	20.30 \pm 0.82 ^c	17.54 \pm 0.81 ^c
T ₄ (80 nymphs)	23.36 \pm 1.30 ^{fg}	17.59 \pm 0.86 ^d	14.59 \pm 0.31 ^{cd}
T ₅ (100 nymphs)	19.92 \pm 0.48 ^{gh}	15.89 \pm 0.36 ^e	12.12 \pm 0.45 ^{de}
T ₆ (120 nymphs)	17.42 \pm 0.85 ^{gh}	12.12 \pm 0.29 ^f	11.80 \pm 0.55 ^{ef}
F- Value	62.19	132.17	97.61
p-value	<0.001	<0.001	<0.001

Table 2: Relative water content in the varieties studied after GLH infestation

Treatment	Pusa 1509	Pusa 1121	TN-1
Control (0 nymph)	0.97 \pm 0.02 ^a	0.65 \pm 0.02 ^a	0.64 \pm 0.01 ^a
T ₁ (10 nymphs)	0.80 \pm 0.02 ^b	0.59 \pm 0.00 ^{ab}	0.64 \pm 0.03 ^a
T ₂ (20 nymphs)	0.70 \pm 0.01 ^c	0.53 \pm 0.01 ^b	0.58 \pm 0.03 ^{ab}
T ₃ (40 nymphs)	0.68 \pm 0.01 ^c	0.46 \pm 0.02 ^c	0.51 \pm 0.01 ^b
T ₄ (80 nymphs)	0.45 \pm 0.03 ^d	0.34 \pm 0.01 ^d	0.33 \pm 0.01 ^c
T ₅ (100 nymphs)	0.33 \pm 0.00 ^e	0.26 \pm 0.02 ^e	0.24 \pm 0.01 ^{cd}
T ₆ (120 nymphs)	0.23 \pm 0.02 ^f	0.25 \pm 0.02 ^{ef}	0.18 \pm 0.01 ^e
F- Value	276.31	93.94	57.832
p-value	<0.001	<0.001	<0.001

The biochemical analysis of rice varieties (Pusa 1509, Pusa 1121, and TN1) exposed to green leafhopper (*Nephotettix virescens*) infestation demonstrated notable differences in protein content and relative water content (RWC), which are

key indicators of the severity of GLH impact (Singh *et al.*, 2014) [14]. Infested rice plants exhibited a marked reduction in both protein and RWC levels, highlighting their susceptibility to GLH-induced stress. Similarly, Amsagowri *et al.* (2018) [2] studied the increase in phenolic compounds in resistant rice accessions indicating a key defensive response against yellow stem borer infestation, contrasting with higher sugar content in susceptible genotypes, which suggests a susceptibility factor.

The reduction in starch content as leaf folder infestation increased is another critical finding. Starch, being a crucial storage carbohydrate, was found to diminish significantly in leaves with higher infestation levels. In healthy leaves, the starch content was 0.89 mg per gram of fresh weight, but this dropped to as low as 0.17 mg per gram in leaves with over 50% infestation. This reduction aligns with findings from Jood *et al.* (1993) [8], who reported similar decreases in carbohydrate content in cereal grains infested by insects. Specifically, similarly, Jood *et al.* (1993) [8] observed that the feeding activity of pests like the rice weevil and lesser grain borer led to a significant depletion of starch and sugars in infested grains. These findings underline the consistent impact that insect herbivory can have on plant carbohydrate reserves across different crop species.

Moreover, the trend observed in our study is also consistent with Soujanya *et al.* (2013) [15], who noted a decline in other biochemical parameters including starch and other carbohydrates, in maize infested by storage pests such as the maize weevil, and also found that pest infestation not only reduced the nutritional quality of the maize kernels but also triggered alterations in the plant's metabolic processes, further exacerbating the loss of vital biochemicals. This parallel between different studies highlights a common physiological response of plants to insect infestation, where the disruption of normal metabolic activities results in a marked decrease in energy reserves like starch and soluble sugars. This stress adversely affects photosynthetic pigments and essential metabolic functions. These results provide valuable insights into the biochemical effects of GLH infestation and underscore the necessity for further research to explore genotypic variations and the specifics of pest-plant interactions.

Conclusion

The study offers insightful information about the biochemical effects of three rice varieties- Pusa 1509, Pusa 1121, and TN-1 infested by green leafhoppers (GLH), *Nephotettix virescens*. The findings unequivocally show that for all three kinds, higher GLH infestation levels are associated with appreciable decreases in protein content and relative water content (RWC). These decreases are a reflection of the pest's physiological stress, which causes significant cellular damage and dehydration. The reduction in protein content impacts rice's nutritional value and suggests a disturbance in essential metabolic functions, whereas the drop in RWC indicates an imbalance in the water content that may make the plant more susceptible to environmental stressors like drought.

The results highlight the significance of these biochemical indicators in determining the extent of GLH infestation and highlight the need for additional study to investigate genotypic variations in response to pest stress. The development of focused, long-term pest management plans that will safeguard rice production and guarantee global food security depends on this kind of study.

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