
*Journal of Agrometeorology*

*ISSN : 0972-1665 (print), 2583-2980 (online)*
*Vol. No. 26 (1) : 92 - 98 (March - 2024)*
*https://doi.org/10.54386/jam.v26i1.2519*
*https://journal.agrimetassociation.org/index.php/jam*

***Research Paper***

# Rice brown planthopper, *Nilaparvata lugens* (Stål) feeding behavior in relation to elevated $CO_2$ and temperature

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**ABSTRACT**

Feeding behavior of brown planthopper, *Nilaparvata lugens* (Stål) populations collected from different geographical regions Ludhiana, Nalgonda and West Godavari at three generations 1, 5 and 10 fed on rice plants grown under different $CO_2$ and temperature levels 1) Ambient $CO_2$@ 380±25ppm + ambient temperature ($aCO_2$+aT), 2) Elevated $CO_2$@500±25ppm + ambient temperature ($eCO_2$+aT) and 3) Elevated $CO_2$@500±25ppm + elevated temperature ($eCO_2$+eT) in closed $CO_2$ chambers was studied. Elevated $CO_2$ + elevated temperature increased feeding rate of BPH nymphs while BPH adults showed declined feeding rate. BPH nymphal feeding rate increased with progressive generations while it decreased in BPH adults. Ludhiana adult BPH population showed higher honeydew excretion compared to West Godavari and Nalgonda populations. $CO_2$ + temperature levels at progressive generations have varying effects on adults and nymphs of three BPH populations. Biochemical composition of rice plants grown under different $CO_2$ levels revealed increased rate of soluble sugars, phenols and decreased rate of reducing sugars, soluble proteins, free amino acids, nitrogen, potassium, phosphorous in elevated $CO_2$ + elevated temperature levels compared to ambient conditions. Increased feeding rate of BPH under elevated $CO_2$ levels may be to compensate changes in host plant quality *i.e*., high soluble sugars and low nitrogen.

***Keywords***: Elevated $CO_2$ and temperature, honeydew excretion, *Nilaparvata lugens*, feeding behavior, Brown planthopper populations.

The current atmospheric $CO_2$ concentration is at an average of approximately 421.37 parts per million (ppm). According to Prather *et al.,* (2001), $CO_2$ concentration is predicted to rise to 550 µl l-1 by 2050. Rice (*Oryza sativa* L.) is one of the most important staple food crops in Asia and the world. The brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) is one of the most serious insect pests of rice and has become problematic over the past few years (Vailla *et al.,* 2017) which is known for its seasonal migration and '*r*' strategy pattern of life. It directly damages the plant by sucking phloem sap, causing hopper burn, and also by transmitting viral diseases such as rice grassy stunt and rice ragged stunt (JhansiLakshmi *et al*., 2019). It causes yield losses as high as 60% under epidemic conditions. In India, during *kharif,* 2017, several rice fields were damaged due to BPH attack. Rising temperatures and humidity were also favourable for BPH outbreaks.

Doubling of air $CO_2$ concentration results in an increase of vegetative growth of rice plants and faster biomass accumulation (Raj *et al.,* 2016). Elevated $CO_2$ treatment results in lower nitrogen, protein and total amino acid content and increased carbohydrate concentration in the C3 plants like rice due to accumulation of non-structural carbohydrates compared to ambient $CO_2$ treatment. Changes in N and carbohydrate levels correlate with the performance of insects, altering their fecundity, population size, food consumption and development time and increased Carbon to Nitrogen ratio results in negative impacts on phloem feeding insects (Stiling and Cornelissen 2007). To meet the amino acid requirement, phloem feeders increase ingestion of assimilates from the phloem, leading to an increase in crop damage (Sun *et al.,* 2009). Temperature directly affects the insects, while $CO_2$ affects them through host plants. Temperature influences insect behaviour, distribution, development, survival, and reproduction. Rise of $CO_2$ and temperature directly affects the food grain production and

***Article info - DOI***: *https://doi.org/10.54386/jam.v26i1.2519*
*Received:29 January 2024; Accepted: 5 February 2024; Published online : 1 March 2024*

indirectly affects through its effect on crop pests. Under elevated $CO_2$ (570-700 ppm) and elevated temperature (^3$^0$C higher) BPH had shorter nymphal duration, lower female longevity and higher fecundity (36.1%) without effecting honeydew excretion of adults (Shi *et al.,* 2014). The information available on interactive effects of elevated $CO_2$ and temperature on the biology of different BPH populations collected from different ecosystems is scanty. In this study, effect of different levels of $CO_2$ and temperature on feeding of three complete generations of three BPH populations reared on rice plants in three $CO_2$×temperature combinations, along with biochemical changes in the rice plant were observed.

## MATERIALS AND METHODS

### *Insect populations*

BPH populations were collected from three different regions of the country *viz*., Ludhiana (Punjab state-North West India) with hot semi-arid climate where rice crop is grown only during May to September (Wet season); West Godavari District (Andhra Pradesh state) with hot sub-humid to semi-arid climate and Nalgonda district (Telangana state- Deccan Plateau) with hot semi-arid climate in South India where rice is grown in two seasons (Dry and Wet). From Ludhiana, BPH populations were collected from Shergarh, Chuharpur, Machhiwara, GarhiTarkhana, Ramgarh and Samrala; from West Godavari district, the BPH populations were collected from Tanuku, Penumantra, Iragavaram, Poduru and Achanta; from Nalgonda district, the BPH populations were collected from Tipparthi, Peddavoora, Nidamanur, Tripuraram, Miryalaguda and Huzurnagar. The populations were separately reared on young rice seedlings (cvTN1) using modified Japanese method (Sunil *et al.,* 2018) in flexi cages to avoid mating and intermixing of the three populations in the $CO_2$ chambers at ICAR-Indian Institute of Rice Research, Hyderabad, India.

### *$CO_2$ chambers*

A specially designed closed climate controlled chamber was constructed at ICAR-Indian Institute of Rice Research, Hyderabad (Latitude-17$^o$10'' N, Longitude-78$^o$E, Altitude -542m) with a total dimension of 10'x8'x10'. It had three chambers each of 2.5'x 8'. The chambers were maintained at three levels of $CO_2$ concentration and temperature combinations continuously as given below

1. Ambient $CO_2$@ 380±25ppm + ambient temperature (a$CO_2$+aT),

2. Elevated $CO_2$@500±25ppm + ambient temperature (e$CO_2$+aT) and

3. Elevated $CO_2$@500±25ppm + elevated temperature (e$CO_2$+eT).

The aforesaid climate variables were maintained throughout the study through fully automatic control and monitoring system with $CO_2$ sensors, PLC and SCADA programme for PC within the chambers in an anteroom. The $CO_2$ levels, temperature and humidity were recorded continuously during the experiment. A temperature of 26-30°C and relative humidity of 70-80% was recorded in ambient temperature chambers and 29-33°C and relative humidity of 60-70% was recorded in elevated temperature chambers. The BPH populations were reared for 10 generations in the $CO_2$ chambers and the feeding studies were conducted in 1$^{st}$, 5$^{th}$ and 10$^{th}$ generations. The susceptible rice variety TN1 was grown for 30 days in $CO_2$ chambers and used for honeydew excretion test. The amount of honeydew excreted by the adults and nymphs of BPH on rice plants exposed to different $CO_2$ and temperature levels in 1, 5 and 10 generations was measured by following methodology described by Nanthakumar *et al.,* (2012). Whatman No.1 filter paper was dipped in bromocresol green solution in ethanol and allowed to dry for one hour and dipped again till the filter paper turned yellowish orange. The treated paper was then placed on the wooden plank with a central hole kept at the base of 30-days old plant and a single stem was inserted into the hole of the plastic cup placed over the filter paper. Five 1day old brachypterous female adults pre-starved for 1 hour were released into the plastic cup and the hole was closed with cotton and the experiment was repeated with 3$^{rd}$ instar nymphs. The BPH adults and nymphs were allowed to feed for 24 hours on the rice stem. The honeydew droplets excreted by the BPH when come in contact with bromocresol green turn into blue spots. The area of blue spots on filter paper was measured by using ImajeJ software and the data was analyzed by Factorial Completely Randomized Design.

### *Estimation of plant nutrients*

TN1 rice seedlings were grown in plastic pots in $CO_2$ chambers for 30 days and the leaf sheaths were sampled after 30 days and samples were dried at 32$^0$C in a hot-air oven for 24-48 hours and powdered using pestle and mortar. The powdered samples were sieved through a 100 mesh screen and stored in the sealed containers at 4$^0$C, for estimation of biochemical constituents. The total soluble proteins, soluble sugars, reducing sugars and total free amino acid content in the samples were estimated by the standard procedures suggested by Bradford (1976). Nitrogen, potassium and phosphorous were estimated by using micro Kjeldahl distillation method (Yoshida *et al.,* 1976).

### *Statistical analysis*

The effect of $CO_2$ levels, generations and populations on honeydew excretion was statistically analyzed by three $3^3$ factorial ANOVA.

## RESULTS AND DISCUSSION

### *Honeydew excretion at different $CO_2$ levels*

Honeydew excretion (feeding rate) of brown planthopper adults was highest at elevated $CO_2$ levels with increasing temperature (e$CO_2$+eT) (188.8 mm$^2$) compared to that of e$CO_2$+aT (138.6 mm$^2$) and a$CO_2$+aT (123.4 mm$^2$). Brown planthopper nymphs excreted highest honeydew at elevated $CO_2$ levels with increasing temperature (e$CO_2$+eT) (57.4 mm$^2$) compared to that of e$CO_2$+aT (47.8 mm$^2$) and a$CO_2$+aT (24.7 mm$^2$). Brown planthopper adults excreted more honeydew compared to nymphs (Table 1).

**Table 1:** Honeydew excretion ($mm^2$) of female adults and 3rd instar nymphs of BPH populations, at $CO_2$ levels and progressive generations

| Populations/$CO_2$ levels/ generations | Honeydew excretion ($mm^2$) (Mean ± SE) | |
|---|---|---|
| | Adults | 3rd instar nymphs |
| **BPH Populations** | | |
| West Godavari BPH | 140.9±14.0$^b$ | 44.9±6.2 |
| Ludhiana BPH | 177.9±19.4$^a$ | 38.6±4.5 |
| Nalgonda BPH | 131.9±15.0$^b$ | 46.3±6.2 |
| S.Em (±) | 8.84 | 3.42 |
| C.V. (%) | 21.63 | 29.0 |
| CD (0.05) | 17.73 | NS |
| **$CO_2$ levels** | | |
| $eCO_2$+aT | 138.6±17.1$^b$ | 47.8±5.0$^b$ |
| $eCO_2$+eT | 188.8±14.4$^a$ | 57.4±6.9$^a$ |
| $aCO_2$+aT | 123.4±16.0$^b$ | 24.7±1.9$^c$ |
| S.Em (±) | 8.84 | 3.42 |
| C.V. (%) | 21.63 | 29.0 |
| CD (0.05) | 17.73 | 6.85 |
| **Generations** | | |
| 1 | 237.3±10.9$^a$ | 24.5±1.7$^c$ |
| 5 | 101.5±14.0$^b$ | 43.0±3.6$^b$ |
| 10 | 112.0±9.4$^b$ | 62.4±7.4$^a$ |
| S.Em (±) | 8.84 | 3.42 |
| C.V. (%) | 21.63 | 29.0 |
| CD (0.05) | 17.73 | 6.85 |

$eCO_2$+aT- elevated $CO_2$ & ambient temperature; $eCO_2$+eT- elevated $CO_2$ & elevated temperature; $aCO_2$+aT- ambient $CO_2$ & ambient temperature;

Means in a column followed by same letter are not significantly different from each other

***Honeydew excretion of different BPH populations***

Among the BPH populations, adult honeydew excretion was more in Ludhiana BPH population (177.9 $mm^2$) compared to that of West Godavari (140.9 $mm^2$) and Nalgonda BPH populations (131.9 $mm^2$). In 3rd instar nymphs, honeydew excretion was highest in Nalgonda BPH population (46.3 $mm^2$) compared to West Godavari (44.9 $mm^2$) and Ludhiana (38.6 $mm^2$) brown planthopper populations but there was no significant difference in the honeydew excretion in these three populations (Table 1).

***Honeydew excretion in different generations***

The adult honeydew excretion decreased with advanced generations whereas in 3rd instar nymphs, the honeydew excretion was increased with the increasing generations (Table 1). At all the $CO_2$ levels, the honeydew excretion of brown planthopper adults was highest in the 1st generation (237.4 $mm^2$) and it decreased in the 5th generation (101.5 $mm^2$) and again increased in 10th generation (112.0 $mm^2$). In brown planthopper nymphs, the honeydew excretion at all $CO_2$ levels increased with increasing generations *viz*., 24.5 $mm^2$ in 1st generation; 43.0 $mm^2$ in 5th generation and 62.3 $mm^2$ in 10th generation (Table1).

Honeydew excretion of adults decreased at all $CO_2$ levels with increase in number of (progressive) generations but in 3rd instar nymphs increased rate of honeydew excretion was noticed at all $CO_2$ levels with advanced generations (Table 2). The interaction of BPH populations and $CO_2$ levels revealed higher feeding rate of adults and nymphs at $eCO_2$+eT followed by $eCO_2$+aT. However, lower honeydew excretion was noticed in $aCO_2$+aT level. The feeding rate of BPH populations was significantly reduced in adults in advanced generations but the feeding rate increased in 3rd instar nymphs (Table 3).

The three BPH populations exhibited varying correlations with different generations and $CO_2$ levels. In West Godavari BPH population, adult honeydew excretion was negatively correlated with generations and positively correlated with $CO_2$ levels (r= -0.54 and 0.99 respectively). In 3rd instar nymphs, honeydew excretion was positively correlated with generations (r= 0.64) and $CO_2$ levels (r= 0.99). In Ludhiana BPH population, adult and 3rd instar nymphal honeydew excretion was positively correlated with $CO_2$ levels (r=0.96 and 0.99 respectively) and negatively correlated with generations (r=-0.60 and -0.46 respectively). In Nalgonda BPH population, adult honeydew excretion was significantly and negatively correlated with generations (r=-0.69) but positive correlation was there with $CO_2$ levels (r=0.314). In 3rd instar nymphs, honeydew excretion was positively correlated with generations (r= 0.51) and $CO_2$ levels (r= 0.89) (Table 6).

The interaction of BPH populations at different $CO_2$ levels with increasing generations revealed that honeydew excretion of adults was significantly decreased with increasing generations and elevated $CO_2$ levels. On the other hand, honeydew excretion of 3rd instar nymphs increased at elevated $CO_2$ levels compared to that of ambient $CO_2$ level ($aCO_2$+aT) (Table 3).

***Biochemical composition of plants grown at different $CO_2$ levels***

30 days old TN1 rice plants grown under different $CO_2$ levels showed increased rate of soluble sugars (10.53mg/g), total phenols (0.07 mg/g) and decreased rate of reducing sugars (1.19 mg/g), total soluble proteins (1.19 mg/g), total free amino acids (42.73 mg/g), nitrogen (1.08%), phosphorous (0.15%) and potassium (1.19%) in elevated $CO_2$ and temperature conditions ($eCO_2$+eT) compared to those grown in elevated CO2 and ambient temperature ($eCO_2$+aT) (8.92 mg/g, 0.06 mg/g, 1.50 mg/g, 1.46 mg/g, 81.56 mg/g, 2.11%, 0.29% and 1.31% respectively) and in ambient $CO_2$ and ambient temperature ($aCO_2$+aT) (8.20 mg/g, 0.06 mg/g, 2.16 mg/g, 3.03 mg/g, 121.11 mg/g, 2.33%, 0.22% and 1.38% respectively) (Table 4).

Correlation between $CO_2$ levels and biochemical components indicates that reducing sugars (r=-0.987), soluble proteins (r=-775) and free amino acids (r=-0.504) were negatively correlated with increased $CO_2$ levels whereas total sugars (r=0.292)

**Table 2:** Honeydew excretion ($mm^2$) of adults and 3rd instar nymphs of BPH populations at different $CO_2$ levels and progressive generations

| $CO_2$ levels | Honeydew excretion ($mm^2$) (Mean ± SE) | | | | | |
|---|---|---|---|---|---|---|
| | Adults | | | 3rd instar nymphs | | |
| | Generations | | | | | |
| | 1 | 5 | 10 | 1 | 5 | 10 |
| $eCO_2$+aT | 237.6±26.7[a] | 73.2±6.4[ef] | 105.1±13.6[d] | 31.5±3.6[de] | 41.0±6.5[d] | 70.9±9.2[b] |
| $eCO_2$+eT | 248.5±10.2[a] | 176.4±27.7[b] | 141.5±19.8[c] | 19.6±1.9[f] | 55.2±6.1[c] | 97.3±6.7[a] |
| $aCO_2$+aT | 226.0±17.4[a] | 54.8±5.4[f] | 89.4±10.5[c] | 22.3±1.5[ef] | 32.9±4.0[de] | 18.8±2.3[f] |
| S.Em (±) | | 15.32 | | | 5.92 | |
| C.V. (%) | | 21.63 | | | 29.0 | |
| CD (0.05) | | 30.71 | | | 11.86 | |
| BPH Populations | $CO_2$ levels | | | | | |
| | $eCO_2$+aT | $eCO_2$+eT | $aCO_2$+aT | $eCO_2$+aT | $eCO_2$+eT | $aCO_2$+aT |
| West Godavari | 149.4±26.6[c] | 180.4±19.0[b] | 93.0±19.0[d] | 48.5±10.0[b] | 61.3±13.7[a] | 24.9±2.2[c] |
| Ludhiana | 162.8±41.5[bc] | 231.8±21.1[a] | 139.1±30.1[c] | 36.0±4.7[c] | 54.9±10.6[ab] | 25.0±2.9[c] |
| Nalgonda | 103.7±12.7[d] | 154.2±28.5[bc] | 138.0±32.4[c] | 58.9±9.5[ab] | 55.9±12.8[ab] | 24.1±4.9[c] |
| S.Em (±) | | 15.32 | | | 5.92 | |
| C.V. (%) | | 21.63 | | | 29.0 | |
| CD (0.05) | | 30.71 | | | 11.86 | |
| BPH Populations | Generations | | | | | |
| | 1 | 5 | 10 | 1 | 5 | 10 |
| West Godavari | 213.3±13.8 | 89.8±15.5 | 119.7±21.4 | 22.4±1.6[d] | 39.7±4.5[bc] | 72.7±13.8[a] |
| Ludhiana | 274.4±16.2 | 131.3±37.7 | 128.0±14.9 | 22.6±3.8[d] | 44.4±8.0[b] | 48.9±8.5[b] |
| Nalgonda | 224.4±20.8 | 83.3±9.0 | 88.2±8.7 | 28.4±3.0[cd] | 45.0±6.4[b] | 65.5±15.3[a] |
| S.Em (±) | | 15.32 | | | 5.92 | |
| C.V. (%) | | 21.63 | | | 29.0 | |
| CD (0.05) | | NS | | | 11.86 | |

$eCO_2$+aT- elevated $CO_2$ & ambient temperature; $eCO_2$+eT- elevated $CO_2$ & elevated temperature; $aCO_2$+aT- ambient $CO_2$ & ambient temperature; Means in a column followed by same letter are not significantly different from each other

**Table 3:** Honeydew excretion ($mm^2$) of female adults and 3rd instar nymphs of brown planthopper at different $CO_2$ levels in progressive generations

| BPH Populations & $CO_2$ levels | Honeydew excretion ($mm^2$) (Mean ± SE) | | | | | |
|---|---|---|---|---|---|---|
| | Generations | | | | | |
| | Adults | | | 3rd instar nymphs | | |
| | 1 | 5 | 10 | 1 | 5 | 10 |
| West Godavari BPH- $eCO_2$+aT | 244.9±7.9[b] | 74.9±12.9[hi] | 128.3±26.8[cdef] | 26.3±3.1[ij] | 32.0±3.1[hij] | 87.3±6.5[bc] |
| West Godavari - $eCO_2$+eT | 228.5±21.8[b] | 147.0±8.2[cd] | 165.7±44.5[c] | 18.4±1.1[ij] | 56.2±4.8[efg] | 109.3±11.3[a] |
| West Godavari - $aCO_2$+aT | 166.3±3.0[c] | 47.6±3.0[i] | 65.1±14.2[ghi] | 22.4±1.8[ij] | 31.0±0.9[hij] | 21.3±4.8[ij] |
| Ludhiana BPH- $eCO_2$+aT | 323.0±18.7[a] | 59.9±4.1[hi] | 105.4±23.7[defgh] | 32.8±8.7[hij] | 27.9±5.7[hij] | 47.3±7.4[fgh] |
| Ludhiana- $eCO_2$+eT | 252.8±16.9[b] | 277.5±29.3[ab] | 165.1±26.5[cdefg] | 13.8±0.9[j] | 73.3±7.1[cde] | 77.5±4.8[cd] |
| Ludhiana- $aCO_2$+aT | 247.4±27.1[b] | 56.5±12.4[hi] | 113.4±19.0[cdefg] | 21.2±1.5[ij] | 31.9±7.4[hij] | 21.9±2.4[ij] |
| Nalgonda BPH- $eCO_2$+aT | 144.7±11.6[cd] | 84.9±12.5[fghi] | 81.4±19.4[fghi] | 35.5±6.8[hi] | 63.1±9.6[def] | 78.2±21.7[cd] |
| Nalgonda- $eCO_2$+eT | 264.2±12.3[b] | 104.7±12.8[def] | 93.6±18.1[efghi] | 26.5±1.2[ij] | 36.2±4.9[ghi] | 105.1±9.4[ab] |
| Nalgonda- $aCO_2$+aT | 264.2±12.3[b] | 60.3±12.1[ghi] | 89.5±12.9[fghi] | 23.0±4.6[ij] | 35.8±11.2[ghi] | 13.2±2.9[j] |
| S.Em (±) | | 26.53 | | | 10.25 | |
| C.V. (%) | | 21.63 | | | 29.0 | |
| CD (0.05) | | 53.20 | | | 20.55 | |

*Significant at 0.05; $eCO_2$+aT- elevated $CO_2$ & ambient temperature; $eCO_2$+eT- elevated $CO_2$ & elevated temperature; $aCO_2$+aT- ambient $CO_2$ & ambient temperature. Means in a column followed by same letter are not significantly different from each other

**Table 4:** Biochemical composition of 30 days old TN1 plants grown in different $CO_2$ levels

| $CO_2$ level | Biochemical parameters | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Soluble sugars (mg/g) | Reducing sugars (mg/g) | Phenols (mg/g) | Soluble proteins (mg/g) | Free amino acids (mg/g) | Nitrogen (%) | Phosphorous (%) | Potassium (%) |
| $eCO_2$+aT | 8.92±0.25[b] | 1.50±0.04[b] | 0.06±0.0[b] | 1.46±0.02[b] | 81.56±0.50[b] | 2.11±0.05[b] | 0.29±0.02[a] | 1.31±0.02[a] |
| $eCO_2$+eT | 10.53±0.03[a] | 1.19±0.02[c] | 0.07±0.0[a] | 1.19±0.02[b] | 42.73±0.59[c] | 1.08±0.02[c] | 0.15±0.02[b] | 1.19±0.04[b] |
| $aCO_2$+aT | 8.20±0.12[c] | 2.16±0.08[a] | 0.04±0.0c | 3.03±0.19[a] | 121.11±1.09[a] | 2.33±0.02[a] | 0.22±0.03[ab] | 1.38±0.03[a] |
| S.Em | 0.2 | 0.03 | 0.01 | 0.15 | 1.1 | 0.02 | 0.032 | 0.04 |
| CD 0.05 | 0.6 | 0.08 | 0.01 | 0.40 | 2.70 | 0.06 | 0.08 | 0.1 |

**Table 5:** Correlation between $CO_2$ levels and biochemical content of 30 days old rice plant

| Biochemical component | Correlation Coefficient (r ) |
|---|---|
| Soluble sugars | 0.292[NS] |
| Reducing sugars | -0.987*[0.1] |
| Soluble Proteins | -0.775[NS] |
| Free amino acids | -0.504[NS] |
| Total Phenols | 0.451[NS] |
| Nitrogen | -0. 125[NS] |
| Phosphorous | -0.456[NS] |
| Potassium | -0.302[NS] |

Significant at $p < 0.1$

lower honeydew excretion. In the brown planthopper nymphs also, reduced feeding and honeydew excretion was noticed and negative correlation was observed with increased levels of reducing sugars (r=-0.999), proteins (r=-0.98), free amino acids (r=-0.97), Nitrogen (r=-0.82), Phosphorous (r=-0.28) and potassium (r=-0.92) (Table 6).

In our study, it was observed that 30 days old rice plants grown under elevated $CO_2$ levels had higher sugars, higher phenols, lower reducing sugars, proteins, free amino acids, nitrogen, phosphorus and potassium content. Nitrogen limiting rice plants grown under elevated $CO_2$ accumulates more carbohydrates, produce more phenolic based compounds and low total soluble proteins, total amino acids (Rashid *et al.,* 2017). Lower concentrations of N,

**Table 6:** Correlation between honeydew and $CO_2$ levels, generations and biochemical components

| Biochemical component | Honeydew excretion of Correlation Coefficient (r ) between biochemical components and honeydew excretion | | | | | |
|---|---|---|---|---|---|---|
| | WG Nymphs | W G adults | Nalgonda Nymphs | Nalgonda adults | Ludhiana nymphs | Ludhiana adults |
| $CO_2$ level | 0.99*[0.1] | 0.99*[0.1] | 0.89[NS] | 0.31[NS] | 0.99[NS] | 0.96[NS] |
| Generations | 0.64*[0.1] | -0.54*[0.1] | 0.51[NS] | -0.69*[0.1] | -0.46[NS] | -0.60*[0.1] |
| Sugars | -0.51[NS] | 0.47[NS] | -0.02[NS] | 0.18[NS] | -0.18[NS] | -0.27[NS] |
| Reducing sugars | -0.28[NS] | -0.86[NS] | -0.49[NS] | 0.78[NS] | -0.65[NS] | -0.37[NS] |
| Proteins | 0.04[NS] | -0.81[NS] | -0.36[NS] | 0.33[NS] | -0.40[NS] | -0.02[NS] |
| Free amino acids | 0.36[NS] | -0.64[NS] | -0.16[NS] | 0.01[NS] | -0.05[NS] | 0.27[NS] |
| Phenols | -0.24[NS] | 0.64[NS] | 0.04[NS] | -0.05[NS] | -0.01[NS] | -0.19[NS] |
| Nitrogen | 0.59[NS] | -0.38[NS] | 0.11[NS] | -0.39[NS] | 0.26[NS] | 0.54[NS] |
| Phosporous | 0.42[NS] | 0.05[NS] | 0.38[NS] | -0.75[NS] | 0.45[NS] | 0.73[NS] |
| Potassium | 0.27[NS] | -0.43[NS] | -0.26[NS] | -0.22[NS] | -0.04[NS] | 0.39[NS] |

- Significant at $p < 0.1$

and total phenols (0.451) were positively correlated with increased $CO_2$ levels. Nitrogen (r=-0.125), Potassium (r=-0.301) and Phosphorous content (r=-0.456) contents decreased with increasing $CO_2$ levels (Table 5).

***Correlation between honeydew excretion and biochemical composition of rice plants***

The honeydew excretion of brown planthopper adults and nymphs increased when the sugar levels (r=0.99 and r=0.89 respectively) and phenols (r=0.88 and r=0.99 respectively) in the rice plants were more. The increased levels of reducing sugars (r=-0.87), proteins (r=-0.77), free amino acids (r=-0.95), Nitrogen (r=-0.99), Phosphorous (r=-0.73) and potassium (r=-0.9) have negative impact on the feeding of brown planthopper adults and resulted in

P and K were observed in rice plants grown at elevated $CO_2$ which may be due to dilution of N due to extra carbohydrates (Seneweera, 2011). In our present study, it was observed that when BPH was continuously exposed to different levels of $CO_2$, the feeding rate of nymphs was increased than that of adults. Elevated $CO_2$ levels with increasing temperature had accelerated the feeding rate of brown planthopper nymphs and adults than ambient $CO_2$ and temperature.

Under elevated $CO_2$ levels, carbon to nitrogen ratio is increased in the phloem sap resulting in diminished nutritive value of host plants and it negatively affects the phloem feeding insects due to limitations in nitrogen supply (Stiling and Cornelissen 2007). Therefore, in order to meet the amino acid requirement, phloem feeders increase ingestion of assimilates from the phloem, leading to an increase in crop damage (Sun *et al.,* 2009). Changes in N and carbohydrate levels correlate with the performance of insects, altering their fecundity, population size, food consumption and development time. Shi *et al.,* (2014) also observed increased sap sucking by BPH under elevated $CO_2$ conditions.

Among the BPH populations, adult honeydew excretion was more in Ludhiana BPH population compared to that of West Godavari and Nalgonda BPH populations. In 3rd instar nymphs, honeydew excretion was highest in Nalgonda BPH population compared to West Godavari and Ludhiana brown planthopper populations. Honeydew excretion of adults decreased at all $CO_2$ levels with increase in number of (progressive) generations but in West Godavari BPH, adult feeding rate was not affected. However, 3rd instar nymphs positively responded to elevated $CO_2$ levels over generations but ambient $CO_2$ had not influenced the feeding rate of nymphs in all BPH populations over successive generations. Srinivasa Rao *et al.,* (2014) observed that *Spodoptera litura* larvae fed with peanut foliage exposed to *e*$CO_2$ exhibited increased consumption by 29-35% over the four generations. The average temperature range prevailing in Ludhiana is wider with minimum temperature of 9.5°C and maximum of 35.3°C while the temperature range in West Godavari and Nalgonda region is narrower with minimum temperature of 25.3°C and a maximum of 31.8°C over years. Ludhiana population responded positively to elevated $CO_2$ levels with higher feeding rate. The results of the present study indicated that populations brought from areas with a greater temperature range *i.e.* lower/higher temperatures are positively affected. Flynn *et al.,* (2006) reported that the responses of aphid populations to elevated $CO_2$ were highly species-specific. Neumeister, 2010 reported that the optimum temperature for surviving and reproduction varies between insect species and even different populations of each species.

## CONCLUSIONS

The present study revealed significant effects of elevated $CO_2$ + elevated temperature on biochemical composition of rice plants *viz*., soluble sugars, free amino acids, soluble proteins and nitrogen in rice plants. Feeding measured as honeydew excretion of brown planthopper nymphs was high under elevated $CO_2$ with increased temperature over successive generations. Ludhiana adult brown planthopper population showed higher feeding rate under elevated $CO_2$ and temperature conditions compared to other two BPH populations.

## ACKNOWLEDGMENT

The authors are thankful the Director, ICAR-Indian Institute of Rice Research for providing facilities.

***Funding****:* The research work was carried out under National Innovations on Climate Resilient Agriculture (NICRA) project funded by ICAR,

***Conflict of interest statement*****:** The author(s) declare(s) that there is no conflict of interest.

***Data availability*****:** Data available on reasonable request from the authors.

***Authors contribution:*** **V. Sunil**: Conducting the experiment, data collection, sample analysis, and manuscript writing; **V. Jhansi Lakshmi**: Conceived and planned the experiment, interpretation of data, manuscript writing and editing the manuscript; **K. Chiranjeevi**: Conducting the experiment, data collection, sample analysis; **D. Sanjeeva Rao**: Helped in the biochemical analysis of rice plants; **M. Sampath Kumar**: Facilitating the experiment;

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