Effects of elevated CO2 on resistant and susceptible rice cultivar and its primary host, brown plant hopper (BPH), *Nilaparvata lugens* (Stal)

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Research Article

Keywords: Plant emergence, defense enzymes, N. lugens, survival, nutritional indices.

Posted Date: December 10th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-118055/v1

Version of Record: A version of this preprint was published at Scientific Reports on April 26th, 2021. See the published version at https://doi.org/10.1038/s41598-021-87992-4.

Abstract

Elevated CO2 has positive response on plant growth and negative response on insect pests. As a contemplation, the feeding pattern of the brown plant hopper, Nilaparvata lugens Stål on susceptible and resistant rice cultivars and their growth rates exposed to elevated CO2 conditions were analyzed. The elevated CO2 treatment showed significant differences in percentage of emergence and rice biomass that were consistent across the rice cultivars, when compared to the ambient conditions. Similarly, increase in carbon and nitrogen ratio of leaves and alterations in defensive peroxidase enzyme levels were observed, but was non-linear among the cultivars tested. Lower survivorship and nutritional indices of N. lugens were observed in conditions of elevated CO2 levels over ambient conditions. Results were nonlinear in manner. We conclude that the plant carbon accumulation increased due to elevated CO2, causing physiological changes that decreased nitrogen content. Similarly, elevated CO2 increased insect feeding, but did not alter other variables such as their biology or reproduction.

Introduction

An increased amount of greenhouse gases due to human activities has been proposed to cause the global warming[1]. The magnitude of elevated CO_2 levels has seriously impacted our environment by imposing a change on global climate [2-4]. Atmospheric CO_2 is on an upsurge and has reached 409.46ppm this year, 2018, from 407.18 in 2017[5]. The increase CO_2 likely to alter the biology circuitously via climate change, and directly by creating changes in growth of plant growth, chemical composition of the plant tissue as well as influence on insect herbivory s life cycle [6]. Researchers have given much emphasis towards the effects of increased concentrations of CO_2 are likely to have more impact on global climate. The increasing CO_2 concentrations are also expected to have a direct effect on the growth, physiology, and chemistry of plants, independent of any effects on climate[7,-9]. The utmost adverse effects of elevated CO_2 on plants is an increase in the rate of photosynthesis, thereby increasing the carbon fixation by leaves. Higher photosynthetic rates eventually result in carbohydrate supply beyond demand for growth, that is stored in the plants, thus increasing the plant biomass, rather than their structural mass. In addition, the surplus carbon is engaged in the production of secondary metabolites[10] and plant tissues like cell walls and organelles[11]. Besides these effects, reduction in transpiration rate and stomatal conductance[12], suppression of dark respiration and photorespiration are also observed with higher CO_2 levels[13].

The quantity of food intake by insect on diverse host plants depends on the availability plant vigor which are known to be influenced by the carbon concentration in the surrounding environment that has direct effects on the plant quality factors such as plant biomass, water content and other plant traits such as leaf area, leaf thickness, chlorophyll content, carbon nitrogen (C and N) ratio in plant tissue and the secondary compounds[14,15]. The development, egg laying capacity, reproduction, adult longevity and population level of insect herbivores, may be affected due to the changes in host plant quality as well as quantity due to increased CO_2 concentration which impacts plant performance. The C/N ratio suggests that the distribution of resources to secondary compounds is controlled by the carbon-nutrient status of a plant[16].

The plants grown under increased CO_2 levels are often characterized by lower nitrogen content, but with increased carbon-based secondary compounds[17] without affecting the production of nitrogen-containing secondary compounds[16] which is an expensive process requiring high enzymatic activity[16,18]. Supportive studies of the above statements include the reduced dietary quality of leaves due to reduced nitrogen (N) by 10-30% in plants grown in enriched CO_2 conditions [19]. Lower foliar N content due to elevated CO_2 has also been shown to cause an increase in food intake by the insects up to 40%[20]. Both the plant nutrient content and secondary metabolites influence insect herbivore performance[21]. Hence, insect growth and development has often been shown to be negatively correlated with elevated CO_2 [11,15].

Many species of insects will meet less nutritious host plants under elevated CO_2, which may bring both prolonged developmental times, greater larval mortality, and lower fecundity[22]. Elevated levels of CO_2 increase plant growth but may also increase the injury caused by some pest insects through increased feeding[23].

Rice (*Oryza sativa* L. *Poaceae* Family) is the world's most significant crop for direct human consumption, feeding half of the world population. Recent studies indicated that elevated CO_2 increased rice photosynthetic rates, growth rate, biomass, and grain yield[24]. The direct reaction of rice plant physiological increases is considered to be 'positive' responses to increased CO_2 levels. The carbon-nutrient equilibrium recommends a premise that the carbon nutrient grade directly controls the secondary metabolite distribution in plants[16].

The brown planthopper (BPH) *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) and the white-backed planthopper, *Sogatella furcifera*, and are the important pests of rice in Asia [25-28]. Insect feeding patterns are varied among hopper species and showed either increased feeding rates or no feeding at all[14,15]. Herbivores may also influence plant productivity and the change ~~in~~ metabolism rates by feeding on them. Increased feeding by herbivores in elevated CO_2 regimes could potentially reduce plant productivity [29,30]. Under ambient ozone conditions, 5-35% reduction in crop yields was observed agriculturally important locations across South Asia which is about US$4 billion per annum for staple crops[31].

Insects that fed on plants grown under elevated CO_2 exhibited lower food utilization rates[22]. From this point of view, we investigated the response of susceptible and resistant rice varieties (IR 20 and ADT 46) under elevated and ambient CO_2 on (i) emergence, root and shoot weight length (ii) estimation of defensive enzymes on different rice varieties (iii) biology and reproduction of *N. lugens* and (iv) nutritional indices of *N. lugens* exposed to the plant varieties under elevate and ambient CO_2 conditions.

Results

Effect of ambient and elevated CO_2 condition on emergence.

Elevated CO_2 increased early emergence rates on susceptible and resistant rice cultivar *viz.,* IR 20 and ADT 46, that was significantly different when compared plants grown under ambient CO_2 conditions (χ^2=9.7, d.f=1, P=0.003) consistently (Fig. 1 A-B). A significant, 20% increase in the plant biomass (above and below ground) was observed as a result of plant exposure to elevated $CO_{2.}$ and was not influenced by the application of fertilizers.

Root-shoot ratio increased while grown in elevated CO_2 condition rather than ambient condition. However, the increase in plant biomass and root-shoot ratio was inconsistent among the cultivars used in this study (Fig. 2). The effect of elevated CO_2 on rice plants increased the shoot and root weight significant (P<0.01) among the cultivars (Fig. 2 A and B). For example, the IR 20 variety was influenced by elevated CO_2 levels as it exhibited maximum shoot weight when compared with control ($F_{1,8}$=15.89; P<0.004). Most of the measured root and shoot length characteristics differed among rice varieties in both ambient and elevated condition. The elevated CO_2 significantly increased shoot and tiller growth. The CO_2 treatment across all rice varieties increased shoot growth for the rice.

The variances in the effect of the ambient and elevated CO_2 effect on the root and shoot weight from glasshouse were analyzed (ANOVA) (Fig. 2) that showed a great deal of variation among ambience and elevation in speed and probability of seed germination. In general, elevated CO_2 levels had a positive effect on the germination.

Effect of ambient and elevated CO_2 condition on carbon: nitrogen ratio.

On average, elevated CO_2 increased the C/N ratio of leaves by 5 to 7%, but the effect was not steady among the rice varieties (Fig. 3). Also, the effect was consistent among the cultivars (Fig. 3). But in the case of nitrogen, conflicting results were observed. Overall, nitrogen content in plants decreased in elevated CO_2 condition compared to the ambient conditions. The nitrogen ratio shows greater change in rice strain ADT 46. We observed 1% of nitrogen decrease in rice strain ADT 46 when grown in elevated CO_2 condition while it was significantly greater when compared with ambient grown ADT 46 ($F_{1,8}$=7.10; P<0.029). Nitrogen level in elevated CO_2 condition was decreased in IR 20 rice leaves by 0.9% being significantly different ($F_{1,8}$=7.76; P<0.02) from ambient condition (Fig. 3A).

Effect of ambient and elevated CO_2 condition on rice plant growth.

Under elevated CO2 condition (725 ppm), both of the rice varieties grew faster and attained maturity quicker, that was evident by the presence of senescent yellow leaf blades, compared with rice plants grown in ambient condition. Elevated CO_2 influenced the rice plant physiology and growth, as yellow leaves per plant became greater than green leaves per plant, indicating changes in plant chemistry (data not shown).

Effect of ambient and elevated CO_2 condition on plant defense enzyme activity.

Peroxidase (POD) enzyme was activated more in elevated CO_2 condition than ambient condition, 24 d post planting (Fig. 4). Forty-five days post planting, the trend observed for elevated CO_2 grown rice varieties changed to mimic the condition with the least enzyme activity as in ambient grown rice varieties. IR 20 rice variety grown in elevated CO_2 had 6% more peroxidase activity than the same variety grown in ambient condition, after 45 days that was being significantly different ($F_{1,8}$=0.79; P<0.01). Similarly, ADT 46 rice varieties established increased peroxidase enzyme activity being 9% greater than rice grown in ambient conditions, after 24 d also being significantly different ($F_{1,8}$=9.33; P<0.012). However, at 45 days, ADT 46 rice variety was significantly different ($F_{1,8}$=6.42; P<0.005) from ambient grown rice plants at elevated CO_2 which produced an increase of 11% enhanced peroxidase activity.

Superoxide dismutase (SOD) enzyme was decreased in activity of elevated CO_2 condition than ambient condition, 24 d post-planting (Fig. 5). IR 20 rice variety grown in elevated CO_2 had 10.8% less superoxide dismutase activity than the same variety grown in ambient condition, after 45 days that was being significantly different ($F_{1,8}$=12.15; P<0.008). Similarly, ADT 46 rice variety established decreased superoxide dismutase activity being 9.8% less than rice grown in ambient conditions, at 45 days, ADT 46 rice variety was significantly different ($F_{1,8}$=13.94; P<0.006) from ambient grown rice plants at elevated CO_2 which produced a decrease of 11.8% of superoxide dismutase activity.

Effect of ambient and elevated CO_2 on *N. lugens* survival.

The *N. lugens* survivorship was significantly increased under elevated CO_2, compared with ambient CO_2. *N. lugens* longevity was significantly reduced when fed on rice varieties IR 20 (χ^2=3.81, df=1,P=0.039), IR 50 (χ^2=3.90, df=1, P=0.042), IR 64 (χ^2=3.98, df=1,P=0.046), ASD 16 (χ^2=4.10, df=1, P=0.043), ASD 19 (χ^2=4.21, df=1, P=0.051) and ADT 46 (χ^2=4.32, df=1,P=0.054) (Fig.6A-F). The survivability of *N. lugens* nymphs feeding on rice plants grown in ambient condition reached 50% in 13 days. In contrast, *N. lugens* nymphs feeding on rice plants grown in elevated CO_2 reached 50% survivability in 20 days (Fig. 6).

Effect of ambient and elevated CO_2 on nutritional indices of *N. lugens*.

The data presented in Fig. 7, revealed higher food assimilation and utilization rates of insects when grown in elevated CO_2 conditions rather than ambient conditions at 24 h. Planthopper nymphs fed on plants grown in increased CO_2 levels, exhibited increased food assimilation and ingestion. Efficiency of food conversion by females fed on rice leaves under elevated CO_2 concentrations were reduced significantly ($F_{5,10}$ =17.94, p < 0.001) (by 25%) than when fed on ambient condition plants.

Effect of ambient and elevated CO_2 on life cycle of *N. lugens*.

Nymph development time was positively influence by the elevated CO_2 and had positive influence on the development time of nymphs. However, the elevated CO_2 has negatively affecting their weight gain (Fig. 8 A-B). The results showed that elevated CO_2 condition produced direct effects on the growth and development rates of *N. lugens* being decreased overall when feeding under elevated CO_2 conditions. The same trend was observed in *N. lugens* fed on all two rice varieties. Adult longevity of *N. lugens* increased when reared under elevated CO_2 conditions (Fig. 8 C-D).

Discussion

Climate change resulting from increased carbon dioxide may play an important role in insect pest populations in several complex ways. The majority of research, reports that an increasing temperature will result in a change in development times of many insects[32]. Plant physiology will also be affected by temperature and CO_2 level changes, which can alter plant nutrition and disease tolerance, and pest resistance[33,34]. Temperatures also have a deliberate impact on the reproductive system of insects. Elevated CO_2 levels influence the temperature, thereby exerting a direct influence on insect physiology. This study reports on nutrient changes in carbon, nitrogen, peroxidase and super oxide dismutase of the rice varieties (IR 20 and ADT 46) when grown under increased CO_2 conditions in rice plants. Furthermore, there was a strong correlation between elevated CO_2, host plant with insect survival, weight gains, and development times[24]. Superoxide dismutase (SOD) helps as a defensive enzyme formed in the cell to defend the damage of reactive oxygen in the biological evolution process; it could remove potentially unwanted superoxide anions and hydrogen peroxide, discharge the impairment to plant cells, and control lipid oxidation. A higher SOD value meant the plant experienced higher stress levels[35]. The results of the current study showed that the general SOD action decreased significantly in the elevated CO_2 and was higher than that under ambient condition. This happened typically because the daily normal temperature increase in the heading stage was more than that of seedling and filling stages and the high temperature stress reduced the level of SOD activity[36].

Estimates predict that an increase in temperature from 1 to 3^{o}C leads to major changes in environmental conditions, which affect plant and insect physiology. These changes can increase the number of insect generations per season. However, if environmental conditions extend or prolong developmental times, there may be fewer generations per season, while a shorter developmental time can increase the number of insect generations per season[33].

Native rice from most of the rice-growing areas of the world identified as highly resistant to the *N. lugens*. Wu *et al.*[37] described that a high percentage of native rice strains were unaffected to the hopper papulation. It is well known that when plants are grown under elevated CO_2 condition, they display increased rates of photosynthesis as well as lower N levels in leaves due to increased plant growth rates[38].

These results augment to the literature, which reports positive effects of elevated CO_2 on the biomass of the rice. In this study the responses of the six rice varieties to elevated CO_2 levels from did not differ substantially[39]. The effects of elevated CO_2 and nutrient abundant on the aboveground biomass of the rice plants were additive[40]. This is in difference to the results of previous studies of Stöcklin & Körner[41] Matthies & Egli[42], they proved stronger effects from elevated CO_2 on plants receiving fertilizers.

Biochemical analysis of *Oryza sativa* revealed a substantial decrease (10%) of leaf carbon and nitrogen ratio under elevated CO_2 conditions as opposed to ambient. The general increase of carbon and nitrogen ratio and the reduction in chlorophyll amount of leaves under elevated CO_2 supports the results published in earlier studies[15]. However, our study showed that effects differed among plant species. In addition, most of the insect pests seems to be undesirably affected by elevated CO_2 because of the decrease in foliar nitrogen and increase in carbon and nitrogen ratio[15].

In our study, 15.9% increase in carbon and nitrogen ratio was observed under elevated CO_2 conditions. The CO_2 mediated changes in the rice foliage (i.e., decreased N and increased C) which affected the growth and development of *N. lugens*, causing higher consumption (55% in 350 ppm and 80% in 750 ppm CO_2 condition). The increased larval weight (25 and 35%) with higher excreta material release was experienced under both elevated CO_2 and ambient CO_2. It is also observed that most phloem feeding insects display compensatory increase in food intake[43]. Insects, when fed on elevated and ambient CO_2 grownup plants, were shown to increase their specific consumption due to the poor food quality of these plants[20,22]. In our study, the BPH development performance indices also significantly varied between elevated CO_2 and ambient conditions. The relative growth rate of larvae fed (food assimilation and ingestion) on elevated CO_2 plants was significantly reduced. Thus *N. lugens* consumed and assimilated more, but grew slower (lower relative growth rate), resulting in one to two days longer to reach pupation than when feeding on ambient plants. Food intake and digestion of the herbivore insects depend strongly on the nutritional quality of plant tissue[21].

However, our research was only aimed to explore the effects of changing food quality due to these factors on the feeding behavior and growth of insects as well as the defense strategies displayed by the host plants. *N. lugens*, when fed on resistant wild rice varieties were reported with various anomalies in settling, food consumption, absorption of ingested food, growth, lifespan, egg laying capacity and egg hatchability[35]. An increase in the rates of food ingestion and assimilation, irrespective of resistance and susceptible rice varieties were observed in N. *lugens* larvae fed with TN1, IR20 and ASD16. Other researchers made similar observations with *S. furcifera*, the white backed planthopper, which is a major, hemipteran pest of rice in Asia, evaluated on resistant cultivators[44].

The survivorship of *N. lugens* nymphs increased under CO_2 treated rice plants compared to control plants (Fig. 5). Elevated CO_2 condition of 750 ppm showed significant increase in the survival rate. Adverse effects of resistant rice *O. punctata,* a diploid, which belongs to the *O. officinalis* complex within the Oryzeae genome groups, is a member of the BB genome type, which have also reported reduced hatchability of *N. lugens* eggs on resistant rice varieties[35]. Increased hopper egg laying and growth under elevated CO_2 might also be allotted to advantageous microenvironment that results revealed from increased tillering and maximum plant growth under elevated CO_2 conditions. In other hemipterans, elevated CO_2 has been observed to increase egg lay like, cotton aphid, *Aphis gossypii*[45]; whitefly, *Bemisia tabaci* [46], and peach aphid, *Myzus persicae* [47,48]. Krishnan *et al.* [49] projected that increasing CO_2 levels would cause a reduction in yield, but an increase in CO_2 level at each temperature increased yields, based on estimates from a two-crop simulation model.

Conclusion

The effects of ambient (350 ppm) and elevated (725 ppm) CO_2 levels on plant chemistry were evaluated. An increase in CO_2 appears to be stimulate increases in plant growth under glasshouse conditions. Plant phytochemistry determined mainly by the independent effects of CO_2 on rice plants causes slow growth and a decrease in yields. The results of this study suggest that an increase in CO_2 causes an increase in rice growth, resulting in increases in biomass of the rice strains tested (IR 20 and ADT 46). However, the effect was not linear. Observations show that carbon content increased as nitrogen content decreased under increasing CO_2. The total carbon nitrogen ratio decreased in ambient grown rice varieties. Defense related plant enzymes, peroxidase (PO) and superoxide dismutase (SOD) activity was elevated initially, then decreased significantly, 25 days post planting. The responses of Brown Planthopper (BPH) *Nilaparvata lugens* (Stål) feeding under elevated CO_2 were variable and suggests that the effects on consumption and growth rate of this insect and other in the Hemiptera: Delphacidae, are not expected using the outline of the carbon-nutrient balance hypothesis. But the effects of CO_2 and nutrient availability on insect pest dependent upon species. Survival rate of *N. lugens* decreased significantly in elevated CO_2 as compared with ambient conditions. In elevated CO_2 conditions the herbivores feeding level increases which correlates to an increase in plant growth. Finally this study suggests that CO_2 increases the plant carbon accumulation while decreasing nitrogen content. Even though insect feeding increased, life span and reproduction rates were unchanged.

Methods

Laboratory mass culture of *N. lugens*

The *N. lugens* culture is maintained in the laboratory of the SPKCES, M.S University, Alwarkurichi without any prior exposure to insecticide. These insects were maintained on *O. sativa* (IR 20) L. seedlings (nine to eleven days after germination (DAG) for first to third instar nymph; 21 DAG for late third instar nymph to adult) in acrylic cages.

Glasshouse experiment

The glass house experiments were conducted for CO_2 studies. The chambers (1 m × 0.5 m × 1.0 m) were sustained at 25±2 °C and maximum 60% relative humidity. Daily daylight was supplemented by nine 400 W halide bulbs, positioned 0.3 m above the chambers (16-L: 8-D photoperiod). The level of CO_2 inside the chamber controlled by using an infrared gas analyzer and 12 chambers (six-ambient CO_2 at 350 ppm; six-elevated CO_2 at 725 ppm) were set to circulate air from an external source. Concentration of CO_2 was continuously monitored by the infra-red gas analyzer.

Experiment on the rice plant

Two rice varieties 'IR 20- susceptible, conventional, and ADT 46- resistant conventional, semi-dwarf long grain were used for this study. The rice varieties were grown in a soil, six seedlings per pot were sown in and exposed to ambient and elevated $CO_{2,}$ watered twice a week with tap water to ensure saturation. After germination, five pots per rice variety were used. In total, the experiment consisted of 30 pots per analysis and each pot contain two rice plants. Plants were randomly owed to glasshouse chambers of the same CO_2 treatment after watering. Every week, the placement of the pots within the chambers were re- arranged to provide uniform experimental conditions.

Effect of elevated CO_2 on rice plant

For the biomass experiments, the rice plants were removed from the chambers after 5 weeks (36 days) and harvested for dry and wet weights measurement. The leaves were collected and counted randomly from each treatment. The collected plants were individually oven dried at 80 °C for 7 d, to measure their dry weight percentage. The nitrogen content was analyzed by using Kjehldahl procedure and carbon content was analyzed by using a CHN analyzer (Model Vario EL III).

Percentage of emergence, root and shoot weight length and ratio estimation

The experiment was carried as described previously under 'experiment on the rice plant' in completely randomized design with alternate-day watering. Observations were recorded every day from the first day of germination after four days of sowing; 'the first true-leaf arose after six days'. Seedlings were counted, cleaned, and upper-part and root fresh weight (mg/plant) as well as the root length (cm/plant) and the greater leaf length (cm) were determined. Percentage of emergence (PE) was calculated according to the formula 1[51].

$$\mathrm{MGT} = \frac{\mathrm{Dn}}{\mathrm{N}}\, n \qquad (1)$$

'Where: *'n'* is the number of seeds that had germinated on day *D* and *D* is the number of days counted from the beginning of germination'.

Estimation of defense enzymes

Ambient and elevated CO_2 grown rice plants under glasshouse condition were examined. Five replications were sustained in each treatment; each replicate contain of five pots and in each pot four plants were maintained. For biochemical assays the leaf tissue was taken from the fifteenth day (15, 20, 25, 30, 35, 40 and 45d) after grown in ambient and elevated CO_2 condition.

Estimation of peroxidase (PO) activity

Hammerschmidt *et al.* [52] protocol was followed for estimating the PO activity. Konn weight (1 g) of the rice leaves was homogenized in 2 ml of 0.1M sodium phosphate buffer (pH 6.5) and centrifuged at 10,000 × g for 25 min at 4 ºC. The upperpart of the supernatant was used as an enzyme source. The enzyme extract (100µl) was taken along with 1.5ml of pyrogallol (0.05M). To initiate the response, 100ml of hydrogen peroxide (1%) (v/v) was supplemented to the sample cuvette and the absorbance was read at 420nm (Lambda 25, UV/Vis spectrometer, PerkinElmer). The enzyme activity was expressed as change in absorbance min^{-1} g^{-1} fresh tissue.

Estimation of Superoxide dismutase (SOD) activity

Giannopolitis and Reis[53] procedure was following to determine the SOD activity. Known quantity (1g) of the plant leaves was homogenized in 2 ml of 0.1M sodium phosphate buffer (pH 6.5) and centrifuged at 10,000 × g for 25 min at 4 ºC. The enzyme extract (100µl) was taken with nitro blue tetrazolium (NBT) in a reaction medium containing 50 mM potassium phosphate (pH 7.8), 14 mM methionine, EDTA 0.1 µM, NBT 75 µM and riboflavin 2 µM. The samples were illuminated for 7 min under 20 W. The spectrophotometer analysis was done in 560nm, where one unit of SOD was measured as the amount of enzyme able to inhibit by 50% the photoreduction of NBT under the experimental conditions. The SOD activity was expressed in U mg^{-1} of protein.

Biology and reproduction of *N. lugens*

Immature and mature insect of the *N. lugens* were fed on rice plants grown with ambient and elevated CO_2. Daily mortality and number of eggs laid by insects were noted every day [54,55].

To estimate the effects of CO_2 treatment on percentage of egg hatched, 5 pairs of newly hatched brachypterous males and females were caged on 20-day-old caged plants. Each treatment was replicated for five times. The total number of nymphs that emerged denoted the number of viable eggs produced by the females. At the end of nymphal emergence, unhatched eggs were recorded by separating leaf sheaths under stereo microscope [56]. Average lifetime number of eggs laid by the female and average daily eggs laid by the female was analyzed by analysis of variance (ANOVA) using Minitap® 16 statistical software package (Minitap, State College, PA).

Food utilization of BPH

To determine the intake of ingested and assimilated food, newly hatched BPH females that had been starved for three hours were evaluated individually on a microbalance. Each BPH was placed within a sealed parafilm sachet on the stem of twenty-five-day-old test plants. After 24 h, the BPH female and excreta weighed. The following formula (2) was used to estimate the food utlilization[44].

$$\text{Food assimilated} = IW \times \frac{IC - FC}{IC} + FW - IW \quad (2)$$

“where: IW = initial weight of test insect, FW-finial weight of test insect, IC-initial weight of control insect FC = final weight of control insect; and food ingested = food assimilated + weight of excreta”. There were four replications for each treatment including the control and the experiments were repeated three times for accuracy.

Population growth index

The population growth index was estimated by following method. Twenty-five-day-old caged rice plants of control and CO2 treated were infested with five pairs of BPH per experimental cage. Each treatment was replicated five times. Nymphs and adults were counted 30 days after infestation [57].

Mature and immature insects life span were analyzed using a log-rank $\chi 2$ test of equality over strata (PROC LIFE Table) along with formula (3) with Minitap®16 statistical software package (Minitab® 17, State College, PA).

$$\text{Nymph/Adult growth index} = \frac{\text{Percent survival of nymph/adult}}{\text{Duration of nymph/adult}} \quad (3)$$

Statistical analysis

Biology and nutritional indices were recorded as the mean of four replications and normalized by arcsine-square root transformation of percentages. The transformed percentages were undergone to analysis of variance (ANOVA). Differences between the five treatments were determined by Tukey’s−Kramer HSD test (P=0.05) by using Minitab 17® software package. Population growth index data’s were analyzed using a log-rank χ2 test (PROC LIFE Table) with Minitab 17® statistical software package (Minitab, State College, PA).

Declarations

Acknowledgments

This study was supported by grants from the following source: Council of Scientific and Industrial Research (CSIR), Grant No- 38 (1253)10/EMR-II. The author would like to thank Dr. W.B. Hunter for his comments over the first draft of the manuscript. The author would thank his scholars Dr. S. Sathish-Narayanan, Dr. R. Chndrasekaran and Dr. K. Revathi for helping during the experiment.

Author contributions statement

S.S.N, designed and conducted the experiments along with statistical analysis. S.S.N wrote the manuscript and approved

Competing Financial Interests Statement

The author has no competing financial interests to report.

I declare that I don't have any competing interests as defined by Nature Research, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are not publicly available due to funding agency agreement and intellectual properties but are available from the corresponding author on reasonable request with permission of funding agency.

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Figures

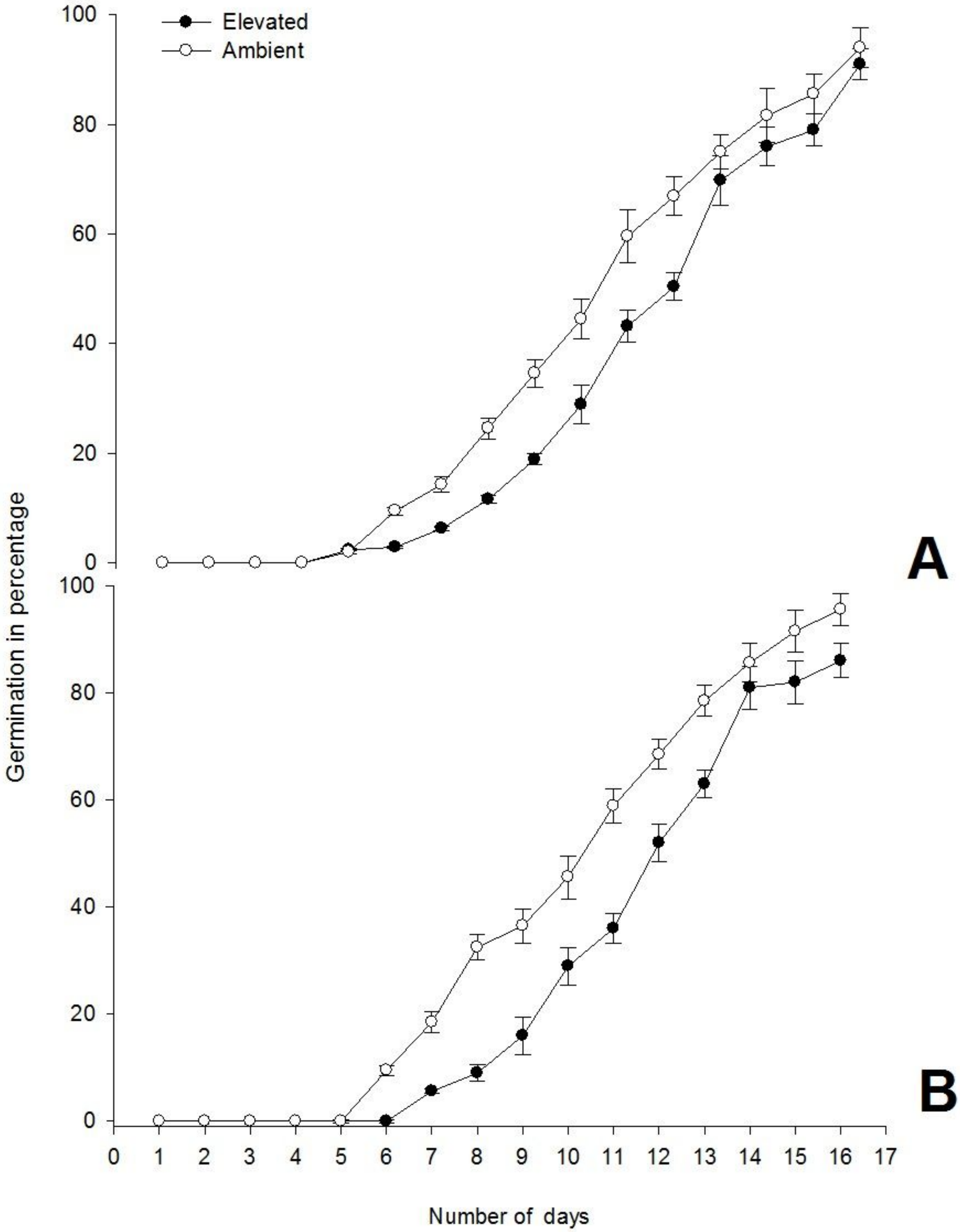

Figure 1

Percentage of different rice seed emergence under ambient and elevated condition. (A-IR 20; B-IR 50; C-IR 64; D-ASD 16; E-ASD 19 and F-ADT 46).

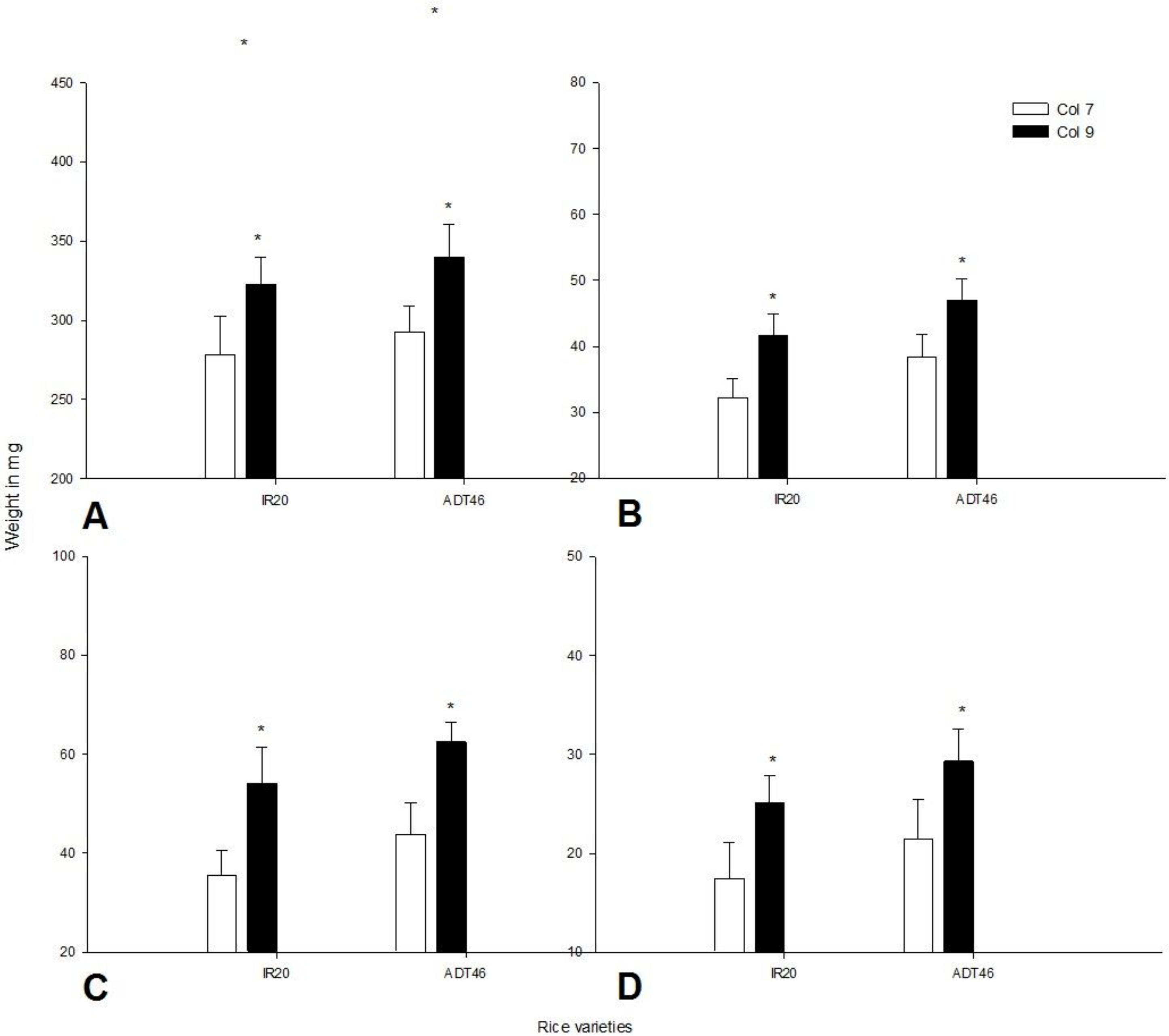

Figure 2

Effect of elevated CO2 on shoot (A-B) and root (C-D) weight of different rice varieties (Values are means ± SEM of five replications) (*Significant; ns-non significant).

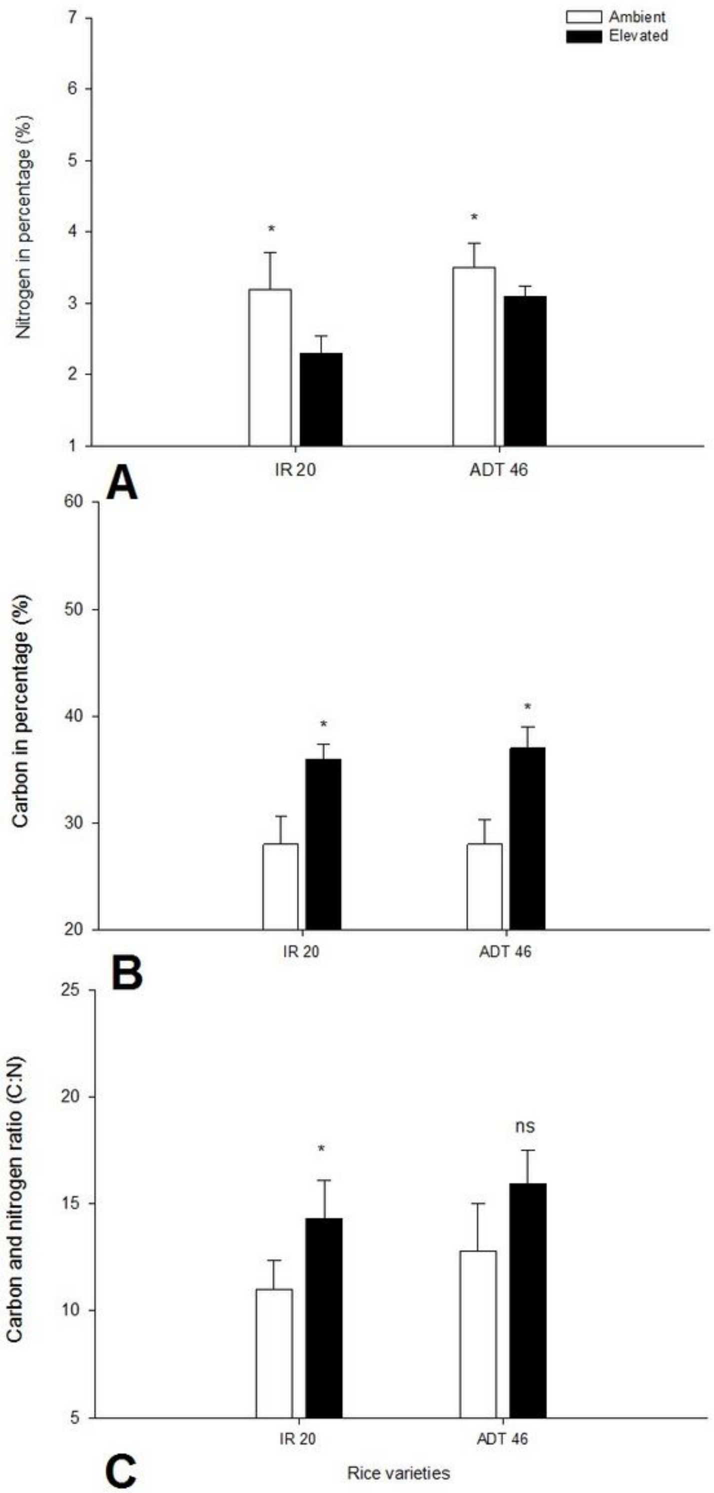

Figure 3

Effect of elevated CO2 on biochemical profile of different rice varieties (A- Nitrogen, B- Carbon, C- Nitrogen and Carbon ratio) (Values are means ± SEM of five replications) (*-Significant; ns-non significant).

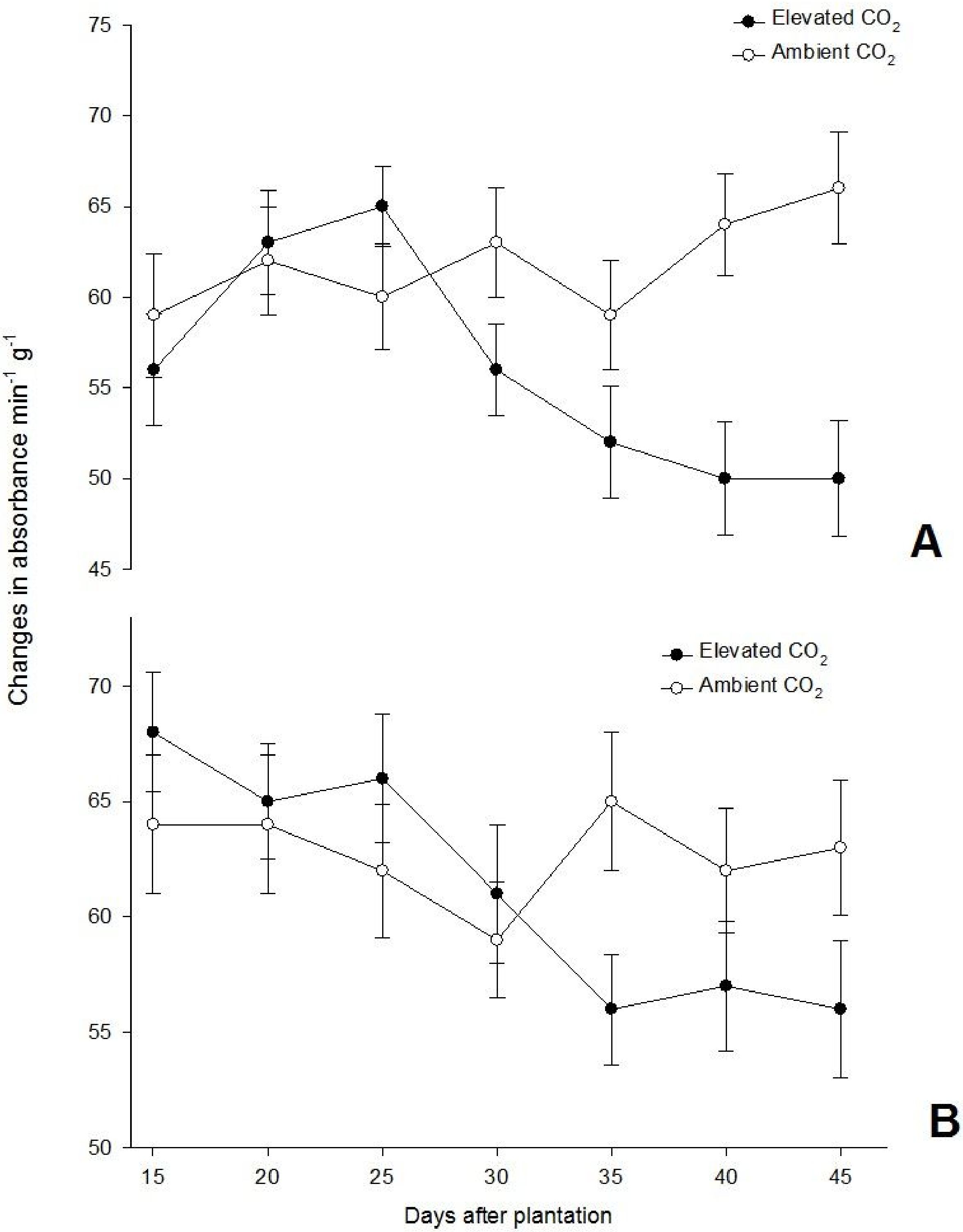

Figure 4

The peroxidase enzyme (PO) content in rice leaves grown in ambient and elevated CO2 (Values are means ± SEM of five replications).

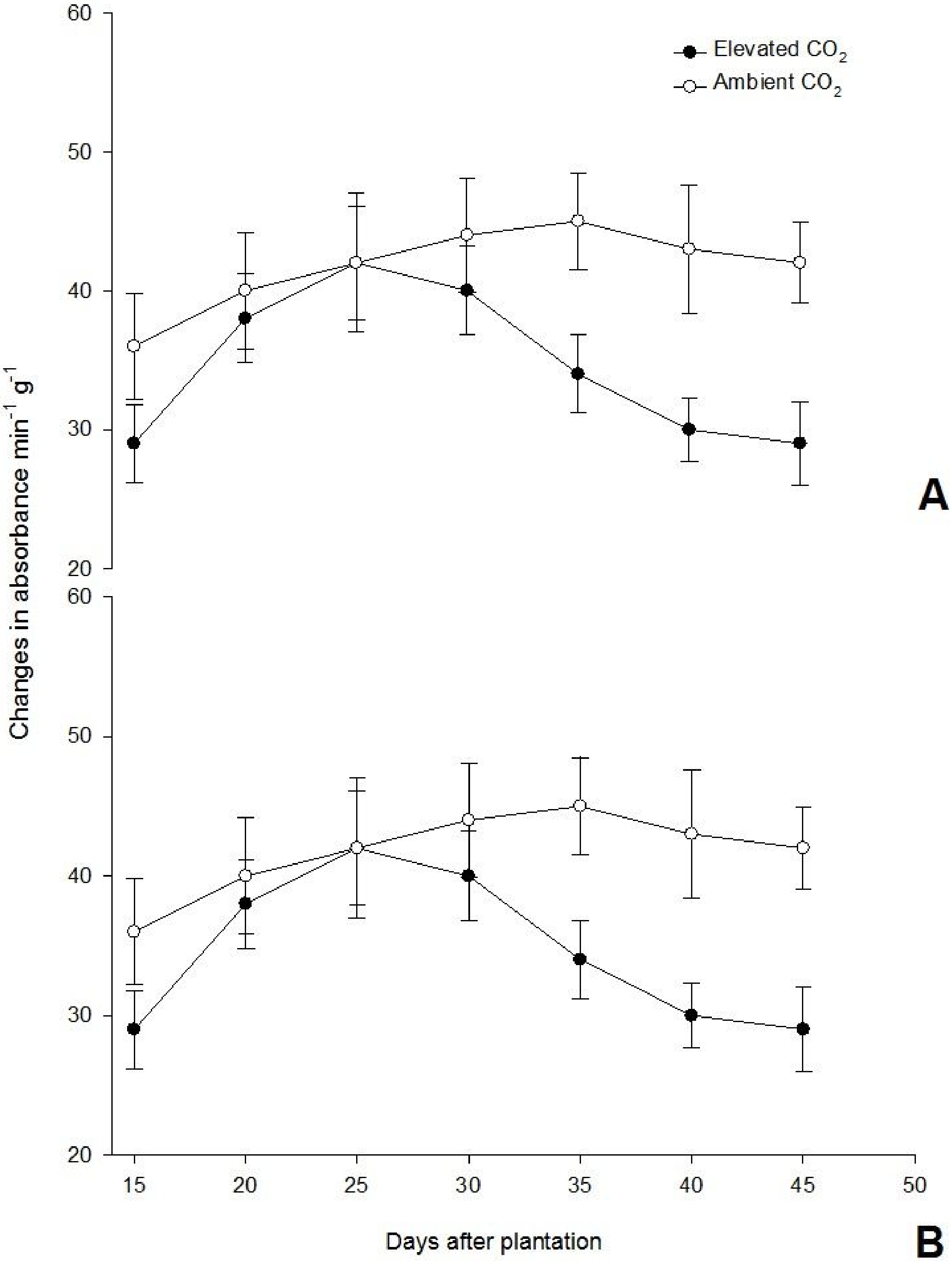

Figure 5

The Superoxide Dismutase (SOD) content in rice leaves grown in ambient and elevated CO2 (Values are means ± SEM of five replications).

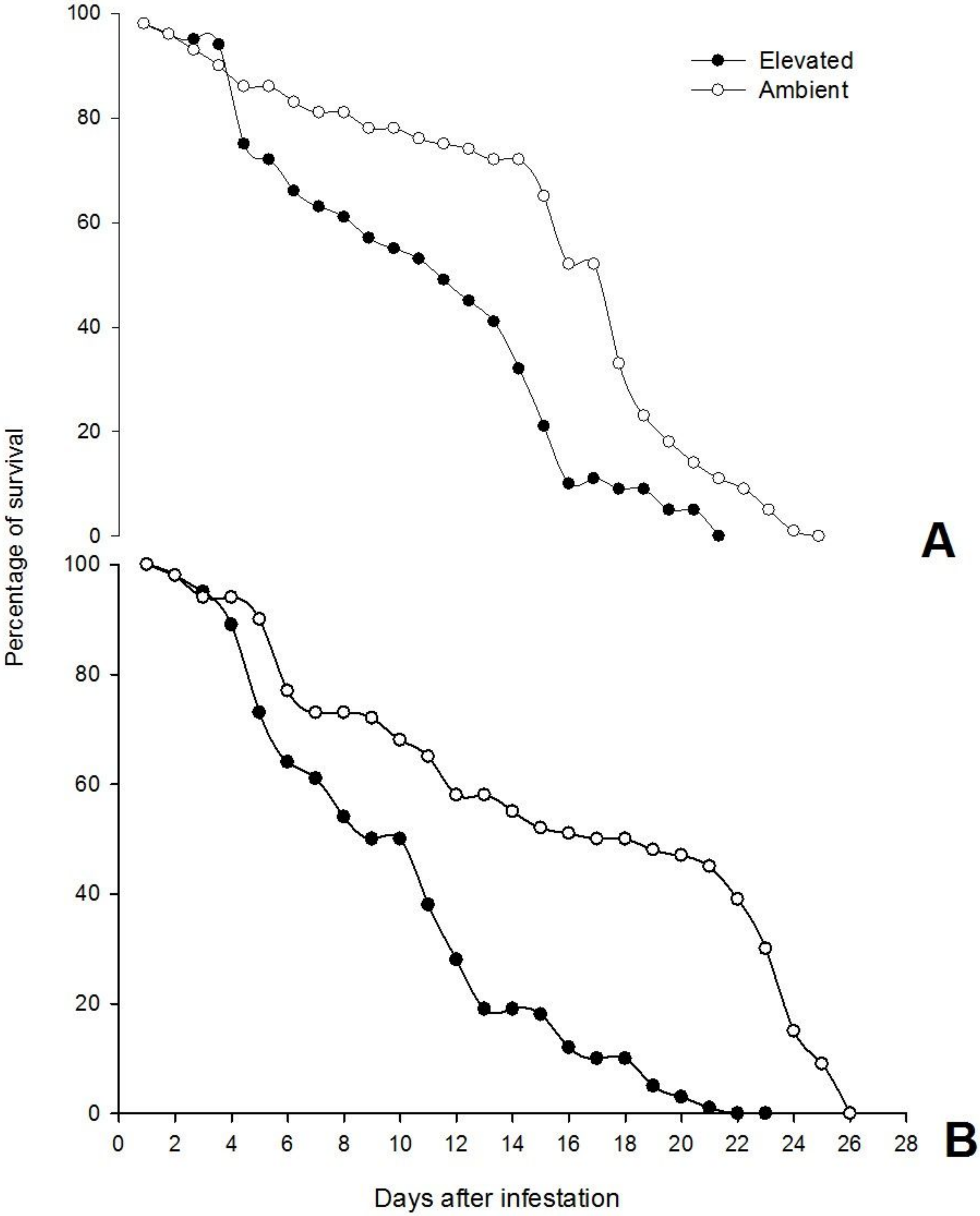

Figure 6

Survival rate of N. lugens on rice varieties (A-IR 20; B-IR 50; C-IR 64; D-ASD 16; E-ASD 19 and F-ADT 46) grown in ambient and elevated CO2 condition. Survivorship curves differ at the α= 0.05 confidence interval according to log-rank statistics

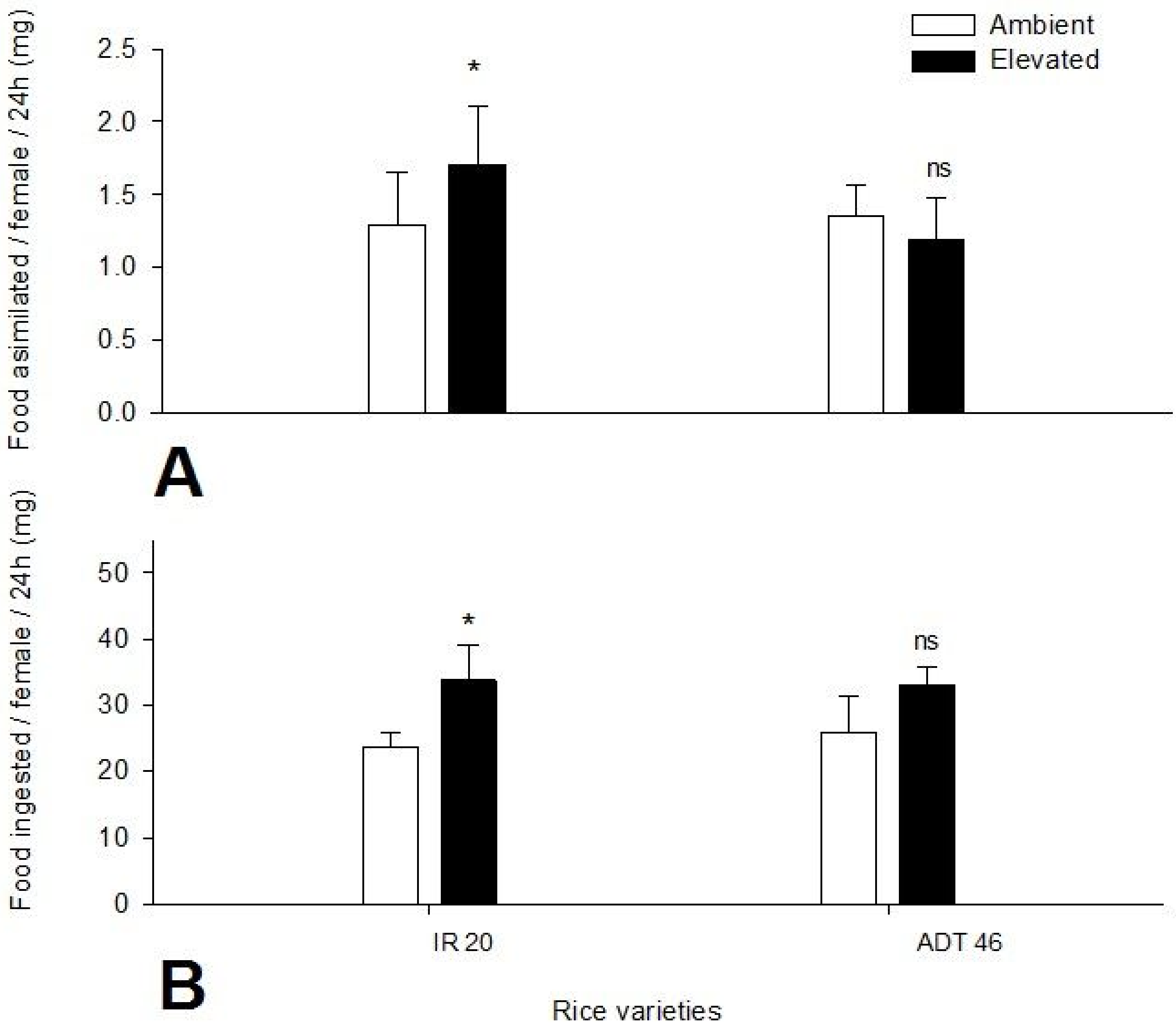

Figure 7

Food utilization of N. lugens under elevated CO2 condition (*-Significant; ns-non significant).

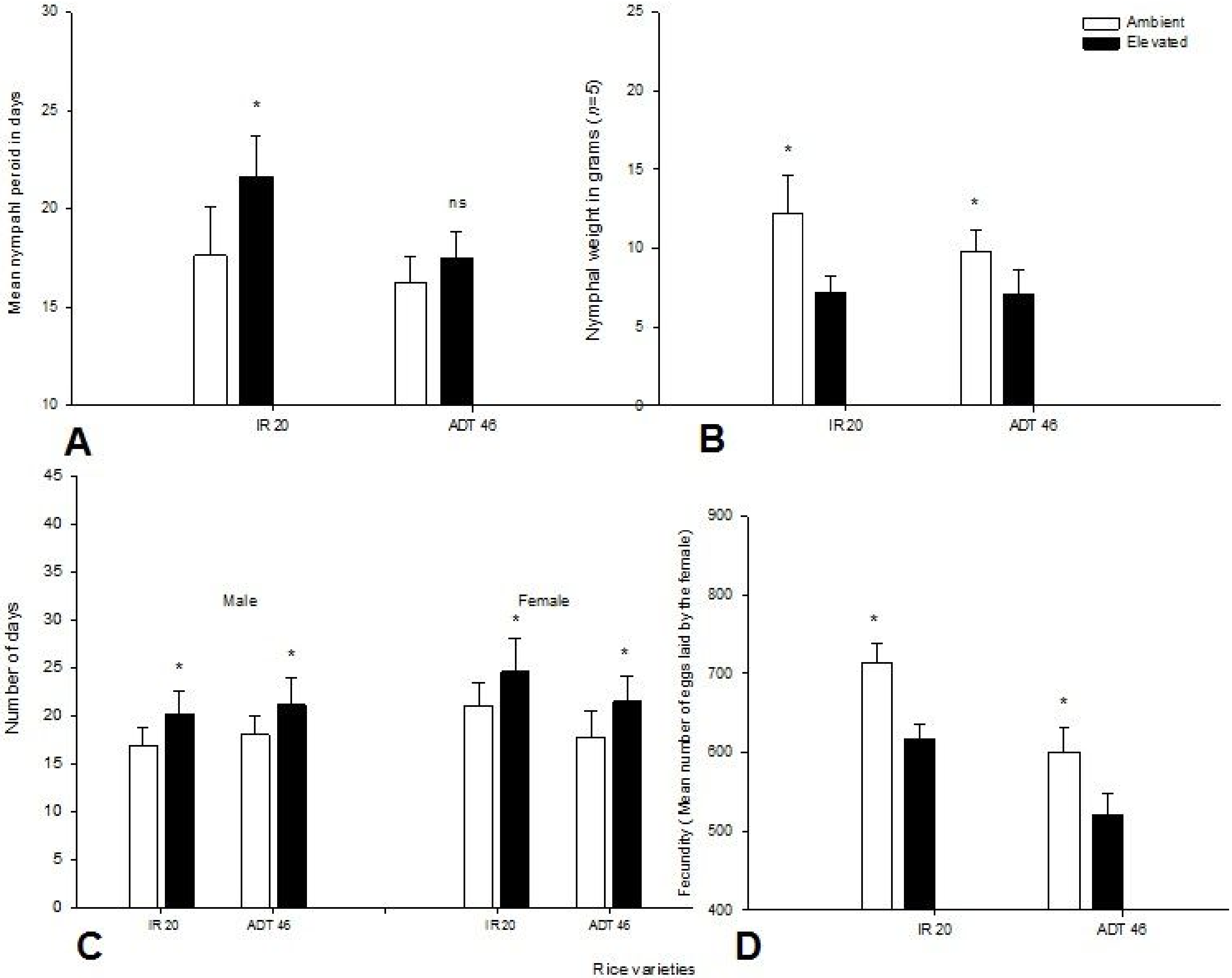

Figure 8

Mean nymphal period, nymphal weight and adult longevity of N. lugens in ambient and elevated condition (*-Significant; ns-non significant).