Interactive effect of elevated CO$_2$ and temperature on the biochemical constituents of maize, *Zea mays* (L.) and its impact on the fecundity of maize aphid, *Rhopalosiphum maidis* (F.)

Mounica D, Krishnayya PV, Srinivasa Rao M, AK Patibanda and Srinivasa Rao V

**Abstract**

The objective of this study was to estimate and analyze the biochemical constituents viz., Carbon based compounds (carbon, total soluble sugars, starch and carbohydrates), nitrogen based compounds (nitrogen, proteins, tannins, amino acids and total polyphenols) and C:N ratio of maize (*Zea mays* L.) crop foliage at 30-90 days after sowing (DAS) under elevated and ambient concentrations of CO$_2$ (550 and 380ppm ± 25 ppm, respectively) at six temperatures (20, 25, 27, 30, 33 and 35 °C) and correlate with the fecundity of maize aphid *Rhopalosiphum maidis* (F.). The results indicated that the percentage of nitrogen (4.36-7.30%), amino acids (11.54-17.40%) and proteins (5.26-11.03%) were significantly decreased while increased carbon (1.08-4.58%), carbon to nitrogen (C: N) (6.96-9.64%), total soluble sugars (2.47-7.14%), starch (3.67-7.01%), carbohydrates (3.08-6.93%), tannins (3.80-7.54%) and total polyphenols (2.70-16.95%) enhanced the nutritional quality of maize foliage under elevated CO$_2$ and temperatures.

**Key words:** Biochemical, elevated CO$_2$, temperature, fecundity, carbon compounds and nitrogen compounds

**Introduction**

The importance of cereals lies in their food value as major source of vitamins, minerals, carbohydrates, fats, oils and protein. Among the cereals, maize (*Zea mays* L.) belonging to the family of grasses (Poaceae), is one of the most important cereal crops worldwide. In India maize is grown in an area of 9.07 Mha with production of 28.72 Mt of the grains. In Andhra Pradesh maize is grown in an area of 1.18 Mha with production of 2.152 Mt of the grains. In Andhra Pradesh maize is grown in an area of 9.07 Mha with production of 28.72 Mt of the grains. In Andhra Pradesh maize is grown in an area of 1.18 Mha with production of 2.152 Mt of the grains. In Andhra Pradesh maize is grown in an area of 9.07 Mha with production of 28.72 Mt of the grains. In Andhra Pradesh maize is grown in an area of 9.07 Mha with production of 28.72 Mt of the grains.

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Materials and Methodology
Maintenance of maize crop and growth conditions (I tropical)

Seeds of maize plants (DHM-117) were sown in Open top chambers (OTCs) and growth chambers and typical representative alfisols with red soil type were maintained. The maize plants were raised under respective set conditions of elevated and ambient concentrations of CO₂ (550 and 380ppm ± 25 ppm, respectively) at six constant temperatures of 20, 25, 27, 30, 33 and 35 ± 1°C. The leaves were detached from these plants and were used for the maintenance of the R. maidis cultures for experimentation. Planting density i.e., the number of plants within a given unit of area (5 plants/m²) was maintained to avoid congestion in the area. The crop was maintained at insecticide free condition throughout the experiment to understand the impact of eCO₂ and temperature on insect pests. Foliage from 30, 45, 60, 75 and 90 days of old crop was obtained from the respective set conditions and was used for biochemical analysis.

Maintenance of R. maidis culture (II tropical)

The maize aphids, R. maidis were collected from the field and maintained at an optimum temperature of 27 ± 1°C and 75 ± 5% RH in the insectary of Entomology section, CRIDA, Hyderabad. The nymphs and adults were reared individually in petridishes (110 X 10 mm) to obtain bulk population for experiments. Light intensity of 30, 000 Lx was provided by 26 W fluorescent bulb inside the chambers during the 14 hours light period with a relative humidity of 60% (day) and 70% (night). Light illumination is provided through fluorescent lamps horizontally mounted in pairs above each shelf. Air circulation inside the chamber was maintained from a specifically designed air diffuser. The period of light, CO₂ concentrations and temperature levels were automatically monitored and controlled using Intellus Ultra Controller.

Fecundity of R. maidis

Before placing the nymph, a moist filter paper (10 cm width) was kept at the bottom of the petridish to maintain turgidity of maize leaves. The cut leaf (6 X 6 cm) from the corn seedlings of 1-2 months old was detached and placed in a petridish with a moist cotton swab on one side of the leaf margin. The cotton swabs were moistened daily to keep the leaf fresh and the leaves were changed on alternate days. The petridishes were closed and placed in the growth chamber under respective set conditions. The data on fecundity (number of off-springs produced with the life of an adult) was recorded at every 24 hours (h) interval starting from 1 day after initiation (DAI) of the experiments. The nymphs laid on maize leaves were collected daily with the help of camel-hair brush and were counted.

Estimation of the biochemical constituents in the host crop foliage at eCO₂ and temperature conditions

Leaves of maize at elevated and ambient concentrations of CO₂ (550 and 380 ppm ± 25 ppm, respectively) at six constant temperatures of 20, 25, 27, 30, 33 and 35 ± 1°C were collected and analyzed to estimate carbon, nitrogen, C:N ratio, proteins, tannins, amino acids, total soluble sugars, starch, carbohydrates and total polyphenols. The leaf samples were collected from 30, 45, 60, 75 and 90 days old plants and were shade dried for three hours and oven dried at 80°C for 72 hours. Then the leaf samples were cut into pieces, ground with a blender and passed through 1 mm sieve. The biochemical constituents of maize foliage were estimated as for the procedures detailed below Carbon - Walkkey and Black (1934) [24] method Nitrogen - Kjeldahl, 1883 [11], C:N ratio - Jackson, 1973 [10], Proteins – Lowry’s method (1951) Tannins - Folin-Denis method (Anderson and Ingram, 1993) [4], Aminoacids – Ninhydrin method (Moore and Stein, 1948) Total soluble sugars - Nelson-Somogyi (Somogyi, 1952; Dubois et al., 1956) [21, 8], Starch – Anthrone method (Yemm and Willis, 1954) [29], Carbohydrates – Anthrone method (Yemm and Willis, 1954) [29], Total polyphenols – Folin-Ciocalteau method (Malick and Singh, 1980) [16, 19].

Statistical analysis

The data pertaining to the fecundity of R. maidis was analyzed using Two way ANOVA with the effect of CO₂ and temperature levels as main factor and sub factors deployed in split plot design. Two-way analysis of variance (ANOVA) was used to quantify the nutritional quality of maize foliage at CO₂ and six levels of temperatures. Treatment means were compared and separated using LSD at p < 0.01 and p < 0.05. Pearson correlations between biochemical constituents of maize leaves (nitrogen, carbon, C: N ratio, proteins, tannins, amino acids, TSS, starch, carbohydrates and total polyphenols) with fecundity of R. maidis was estimated under the effect of two levels of CO₂ and six levels of temperatures.

Results and Discussion

Effect of elevated CO₂ and temperature on fecundity of R. maidis for three successive generations

The impact of eCO₂ at six constant temperatures on the fecundity of R. maidis was found significant in three generations. Significant differences were observed in first (F₁₁, ₂₉ = 28.75, P = <0.01), second (F₁₁, ₂₉ = 1014.3, P = <0.01) and third (F₁₁, ₂₉ = 1769.39, P = <0.01) generation under two CO₂ at six temperature conditions (Fig 1). In the first generation, the fecundity was significantly increased with temperature in the range of 20°C to 27°C (52.12 – 83.20 nymphs per female) but significantly declined at 30°C to 35°C (43.04 – 3.16 nymphs per female) under eCO₂ compared to that of aCO₂ conditions. The fecundity was significantly more with temperature in the range of 20°C to 27°C (57.44 – 87.04 nymphs per female) but significantly declined at 30°C to 35°C (41.44-1.64 nymphs per female) under eCO₂ and aCO₂ conditions compared to that of first generation. The gradual increase in the fecundity was observed under eCO₂ and aCO₂ conditions in the third generation compared to that of second generation except at the temperature of 35°C (1.28 nymphs per female) due to the highest temperature might have resulted that the reduced fecundity in R. maidis. The results indicated that significantly increase in the fecundity across the three generations under both eCO₂ and aCO₂ at six temperature conditions. Awmack et al. (1996) [5] found that eCO₂ increased the fecundity of cereal aphid, Sitobion avenae when reared on winter wheat. Lincoln et al. (1993) [14], who reported that the CO₂ induced alterations in phytochemical constituents important to insects can potentially alter their behaviours. Temperature has a direct influence on insect activity and rate of development (Zalom et al., 1983) [31]. The nutritional quality of phloem sap is the limiting resource for the growth, development and performance of aphid population (Bezemer and Jones, 1998) [6]. Singh & Painter (1964) [19] suggested that the rate of progeny production in R. maidis was based on the kind of host plant and also influenced by the effect of optimum temperature was found to be ranged from 13°C to 24°C.
**Effect of elevated CO₂ and temperature on the biochemical constituents of the maize**

The biochemical constituents viz., carbon, nitrogen, C: N ratio, proteins, tannins, amino acids, total soluble sugars (TSS), starch, carbohydrates and total polyphenols of maize foliage at 30, 45, 60, 75 and 90 days after sowing (DAS) were estimated and the results were discussed here under (Fig 2).

**Carbon content**

The carbon content was increased with temperatures ranging from 20 to 33°C but declined at 35°C at 30 DAS (33.27-32.85%), 45 DAS (34.17-33.45%), 60 DAS (34.88-33.97%), 75 DAS (35.37-34.63%) and 90 DAS (35.77-35.14%), respectively. Results indicated that the carbon per cent in maize foliage under eCO₂ conditions and temperatures increased by 1.63, 3.33, 4.58, 3.65, 3.70 and 1.08 per cent at 30 to 90 DAS compared to aCO₂ conditions, respectively.

In C₄ plants, the gas exchanging stomatal pores were kept close most of the day in order to reduce the excessive loss of moisture in dry and hot conditions. Thereby, the carbon dioxide concentration inside plant leaves was also reduced by the progression of C₃ cycle. When carbon dioxide concentration is low, photorespiration is enhanced by reducing the efficiency of photosynthesis. In order to increase the efficiency of photosynthesis, those C₄ plants conduct the C₄ cycle (Sun et al., 2009) [23].

**Nitrogen content**

The nitrogen content was increased with temperatures ranging from 20 to 33°C but declined at 35°C at 30 DAS (4.15-3.46%), 45 DAS (4.30-3.60%), 60 DAS (4.45-3.73%), 75 DAS (4.53-3.84%) and 90 DAS (4.58-3.92%), respectively. Results indicated that the nitrogen per cent in maize foliage under eCO₂ conditions and temperatures decreased by 4.97, 4.62, 4.48, 4.36, 5.17 and 7.30 per cent at 30 to 90 DAS compared to aCO₂ conditions, respectively. Crowding on nitrogen deficient plants caused a reduction in fecundity. Adams (2007) [1] reported that nutrional factors are responsible for variation in fecundity, in which an increase in nitrogen content was responsible for higher fecundity of Rhopalosiphum padi.

**C:N ratio**

The C:N was increased with temperatures ranging from 20 to 33°C but declined at 35°C at 30 DAS (8.03-9.53), 45 DAS (7.94-9.29), 60 DAS (7.84-9.10), 75 DAS (7.81-9.01) and 90 DAS (7.80-8.96), respectively. Results indicated that the C:N ratio in maize foliage under eCO₂ conditions and temperatures increased by 6.96, 8.31, 9.64, 8.31, 9.34 and 9.26 per cent at 30 to 90 DAS compared to aCO₂ conditions, respectively. Increased rate of photosynthesis, C: N ratio increases through the accumulation of non-structural carbohydrates in the plants grown under eCO₂ conditions (Wu et al., 2007 and Williams et al., 1998) [25, 26]. This, in turn, impacts the production of secondary metabolites, promoting the manufacture of carbon based secondary metabolites (e.g. phenolics) over nitrogen based ones (Bryant et al., 1983) [7].

**Proteins**

The Proteins were increased with temperatures ranging from 20 to 33°C but declined at 35°C at 30 DAS (32.67-37.43 mg g⁻¹), 45 DAS (34.63-38.04 mg g⁻¹), 60 DAS (35.65-36.24 mg g⁻¹), 75 DAS (36.02-37.06 mg g⁻¹) and 90 DAS (36.62-38.09 mg g⁻¹), respectively. Results indicated that the proteins per cent in maize foliage under eCO₂ conditions and temperatures decreased by 11.03, 8.46, 10.65, 6.99, 5.26 and 6.39 per cent at 30 to 90 DAS compared to aCO₂ conditions, respectively. The present findings are in agreement with Adishesh et al. (2017) [23] who reported that the interactive effects of aCO₂ and temperature treatment had recorded maximum soluble protein in leaves followed by eCO₂ and temperature had least soluble protein in leaves and showed a significant negative effect of eCO₂ and temperature treatments. Under eCO₂ condition plant uses more of carbon for photosynthesis resulting in lower protein content.

**Tannins**

The tannins were increased with temperatures ranging from 20 to 33°C but declined at 35°C at 30 DAS (8.32-5.44 mg g⁻¹), 45 DAS (5.46-5.51 mg g⁻¹), 60 DAS (6.54-5.62 mg g⁻¹), 75 DAS (6.68-5.83 mg g⁻¹) and 90 DAS (6.81-5.96 mg g⁻¹), respectively. Results indicated that the tannins per cent in maize foliage under eCO₂ conditions and temperatures increased by 5.19, 5.38, 4.60, 3.80, 7.54 and 6.13 per cent at 30 to 90 DAS compared to aCO₂ conditions, respectively.

Tannins are complex polyphenolic compounds with great structural diversity. They have a variable effect in decreasing digestibility of proteins. In addition, tannins also chelate the metal ions, thereby reducing their bioavailability to herbivores. When ingested, tannins reduce the digestibility of the proteins thereby decrease the nutritive value of plants and plant parts to herbivores. The present findings are in agreement with respect to higher content of tannins were found in elevated conditions favoured plant defensive chemicals as compared to ambient levels. These are carbon based compounds, along with increased carbon these compounds have also increased in the elevated climate change treatments.

**Aminoacids**

The amino acids were increased with temperatures ranging from 20 to 33°C but declined at 35°C at 30 DAS (2.18-2.12 mg g⁻¹), 45 DAS (2.22-2.15 mg g⁻¹), 60 DAS (2.24-2.18 mg g⁻¹), 75 DAS (2.60-2.24 mg g⁻¹) and 90 DAS (2.70-2.29 mg g⁻¹), respectively. Results indicated that the amino acids per cent in maize foliage under eCO₂ conditions and temperatures decreased by 14.01, 13.19, 13.82, 13.05, 11.54 and 17.40 per cent at 30 to 90 DAS compared to aCO₂ conditions, respectively. The present studies are in conformity with the findings of Sun et al. (2009) [23] who reported lower amounts of amino acids in cotton phloem under eCO₂ than aCO₂ condition. Further, in the study of Yin et al. (2010) [30] observed that the decreased N, protein content, total Amino acid of spring wheat by 13.8, 18 and 55.5 per cent, respectively when grown in an eCO₂ environment over aCO₂. More phloem sap will be ingested by cotton aphids to satisfy their nutrition requirements and to balance the break-even point of amino acids in eCO₂ (Sun et al., 2009) [23]. Likewise, this nutrient imbalance suggests that, in order to match their needs, aphids may modify their feeding and metabolism.

**Total soluble sugars**

The total soluble sugars were increased with temperatures ranging from 20 to 33°C but declined at 35°C at 30 DAS (25.98-24.22 mg g⁻¹), 45 DAS (26.36-24.57 mg g⁻¹), 60 DAS (27.69-25.61 mg g⁻¹), 75 DAS (31.55-29.30 mg g⁻¹) and 90 DAS (31.99-29.97 mg g⁻¹), respectively. Results indicated that the total soluble sugars per cent in maize foliage under eCO₂ conditions and temperatures increased by 5.72, 5.51,
The present study is in conformity with reports of Srivastava et al. (2002) who studied the effect of long-term CO2 enrichment on the foliar chemistry of mungbean (Vigna radiata L. Wilczek). Under enriched CO2 (650 µL L⁻¹), level of leaf nitrogen (protein and non-protein nitrogen) declined significantly whereas levels of starch and total soluble sugars (reducing and non-reducing) in leaves increased.

**Starch**
The starch was increased with temperatures ranging from 20 to 33°C but declined at 35°C at 30 DAS (4.77-4.56 mg g⁻¹), 45 DAS (6.99-6.50 mg g⁻¹), 60 DAS (7.70-6.73 mg g⁻¹), 75 DAS (8.11-6.91 mg g⁻¹) and 90 DAS (8.60-7.25 mg g⁻¹), respectively. Results indicated that the starch per cent in maize foliage under eCO2 conditions and temperatures increased by 3.67, 4.19, 4.25, 5.60, 6.01 and 5.63 per cent at 30 to 90 DAS compared to aCO2 conditions, respectively. The present results are in agreement with the results of Xie et al. (2015) who reported that the response of total non-structural carbohydrates (TNCs) including TSS and starch in maize leaves increased by 15.70 and 8.30 per cent, respectively under eCO2 compared with aCO2 conditions.

**Carbohydrates**
The carbohydrates were increased with temperatures ranging from 20 to 33°C but declined at 35°C at 30 DAS (31.47-29.11 mg g⁻¹), 45 DAS (33.32-31.07 mg g⁻¹), 60 DAS (35.39-32.34 mg g⁻¹), 75 DAS (39.67-36.21 mg g⁻¹) and 90 DAS (40.59-37.22 mg g⁻¹), respectively. Results indicated that the carbohydrates per cent in maize foliage under eCO2 conditions and temperatures increased by 5.56, 5.48, 5.98, 6.49, 6.93 and 3.08 per cent at 30 to 90 DAS compared to aCO2 conditions, respectively. The present results are in agreement with Xin et al. (2013) who reported that eCO2 increased carbohydrates accumulation in tomato plants. The leaf carbohydrate determinations showed that the starch, total soluble sugar and sucrose concentrations increased significantly in plants exposed to eCO2 conditions.

**Total polyphenols**
The polyphenols were increased with temperatures ranging from 20 to 33°C but declined at 35°C at 30 DAS (0.294-0.284 mg g⁻¹), 45 DAS (0.298-0.288 mg g⁻¹), 60 DAS (0.304-0.295 mg g⁻¹), 75 DAS (0.306-0.305 mg g⁻¹) and 90 DAS (0.317-0.310 mg g⁻¹), respectively. Results indicated that the total polyphenols per cent in maize foliage under eCO2 conditions and temperatures increased by 12.86, 14.67, 13.40, 11.40, 2.70 and 16.95 per cent at 30 to 90 DAS compared to aCO2 conditions, respectively. Polyphenols, non-structural compounds that constitute one of the defense mechanisms of plants and offer antifeedence to herbivores are also known to increase to a greater extent in leaves under eCO2 conditions (Bezemer and Jones, 1998) which in turn influence the growth and development of insect herbivores.

**Correlation studies between biochemical constituents of maize with fecundity of R. maidis**
The biochemical constituents of maize foliage resulted in varying a degree of correlation with fecundity of R. maidis under eCO2 conditions across the temperatures (Tab 1). A significant positive correlation at 25°C-30°C with fecundity of R. maidis with carbon (0.4717, 0.2529, 0.0999 and 0.1352), nitrogen (0.0696, 0.1148, 0.0181 and 0.0761) and C:N ratio (0.4529, 0.2565, 0.1029 and 0.1421) of maize foliage under eCO2 conditions. A significant negative correlation at 20°C and 35°C with fecundity of R. maidis with carbon (-0.015 and -0.212), nitrogen (-0.061 and -0.439) and C:N ratio (-0.039 and -0.422) of maize foliage under eCO2 conditions. A significant positive correlation at 25°C-30°C with fecundity of R. maidis with proteins (0.4455, 0.3107, 0.0829 and 0.2141) and aminoacids (0.1075, 0.3292, 0.0910 and 0.3074) content of maize foliage under eCO2 conditions. A significant negative correlation at 20°C and 35°C with fecundity of R. maidis with proteins (-0.049 and -0.2822) and aminoacids (-0.035 and -0.3971) content of maize foliage under eCO2 conditions. A significant positive correlation at 25°C-30°C with fecundity of R. maidis with TSS (0.0630, 0.3301, 0.0643 and 0.2807), starch (0.3944, 0.3499, 0.0499 and 0.2214) and carbohydrates (0.1310, 0.3333, 0.0635 and 0.2824) content of maize foliage under eCO2 conditions. A significant negative correlation at 20°C and 35°C with fecundity of R. maidis with CO2 (Tab 1). A significant negative correlation at 20°C and 35°C with fecundity of R. maidis with TSS (-0.141 and -0.1229), starch (-0.107 and -0.4229) and carbohydrates (-0.150 and -0.1550) content of maize foliage under eCO2 conditions. A significant negative correlation at 20°C to 35°C with fecundity of R. maidis with tannins (-0.111, -0.3281, -0.2463, -0.1494, -0.0882 and -0.0148) and polyphenols (-0.083, -0.3125, -0.2991, -0.0444, -0.1175 and -0.0189) content of maize foliage under eCO2 conditions.

![Image](http://www.phytojournal.com)

Fig 1: Effect of elevated CO2 and temperature on fecundity of R. maidis of maize across three generations

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**Fig 2:** Per cent change in the biochemical constituents of maize foliage under elevated CO₂ and temperature at 30-90 DAS

**Tab 1:** Correlation between biochemical constituents of maize foliage with fecundity of *R. maidis* under elevated CO₂ and temperature

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*Significant @ 5% level of significance

**References**