Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(6): 1005-1010
Received: 09-08-2020
Accepted: 18-09-2020

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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.)

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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.

Keywords: 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 (Agricultural Statistics at a Glance, 2019) [3]. It contains 28 and 8 percent of carbohydrates and proteins, respectively and occupies prior position in cereal production.

Agriculture is one of the most vulnerable sectors to the anticipated climate change with an adverse effect on crop yields. The increased levels of atmospheric CO_2 concentrations can have a direct effect on the growth rate of crop plants. Ambient carbon dioxide (aCO_2) concentration exceeded 400 ppm and future estimations predict an increase up to 550 ppm within a few decades and temperature is predicted to increase by 1.7–4.9°C in mean global temperature by 2100 (IPCC, 2014) [9].

The increased levels of atmospheric CO_2 concentrations and temperature can have a direct effect on the growth rate of crop plants. Temperature has a direct influence on crop plants and insect activity and their rate of development. Climate change could profoundly affect the population dynamics and the status of insect pests of crops (Kun *et al*., 2014) [12]. Thus, excessively high temperatures reduce the reproductive period and fecundity (Kuo *et al*., 2006) [13]. The predicted changes in temperature and CO_2 concentration affect the population dynamics and the status of insect pests of various crops. Plants with C4 photosynthesis will respond little to rising atmospheric CO_2 because a mechanism to increase the concentration of CO_2 in leaves causes CO_2 saturation of photosynthesis at ambient conditions.

Elevated CO_2 and temperature increased the photosynthetic rate and biomass which was reduced the foliar nitrogen concentration and increased C:N ratio in leaves (Masters *et al*., 1998) [17]. This CO_2 and temperature induced changes in plant C: N ratios and nitrogen concentrations are likely to affect quality of host crop foliage as well as the feeding pattern and behaviour of herbivores. Hence, in the present study biochemical constituents were analyzed and estimated at two levels of CO_2 and six different temperature conditions.

Materials and Methodology

Maintenance of maize crop and growth conditions (I trophic)

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 CO_2 (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/m^2) was maintained to avoid congestion in the area. The crop was maintained at insecticide free condition throughout the experiment to understand the impact of *e*CO_2 and *e*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 trophic)

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 florescent 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, CO_2 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 *e*CO_2 and temperature conditions

Leaves of maize at elevated and ambient concentrations of CO_2 (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, grounded with a blender and passed through 1 mm sieve. The biochemical constituents of maize foliage were estimated as for the procedures detailed below Carbon - Walkey 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 Ingramm, 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 CO_2 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 CO_2 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 CO_2 and six levels of temperatures.

Results and Discussion

Effect of elevated CO_2 and temperature on fecundity of *R. maidis* for three successive generations

The impact of *e*CO_2 at six constant temperatures on the fecundity of *R. maidis* was found significant in three generations. Significant differences were observed in first ($F_{11, 24}$ = 28.75, P = <0.01), second ($F_{11, 24}$ = 1014.3, P = <0.01) and third ($F_{11, 24}$ = 1769.39, P = <0.01) generation under two CO_2 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 *e*CO_2 compared to that of *a*CO_2 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 *e*CO_2 and *a*CO_2 conditions compared to that of first generation. The gradual increase in the fecundity was observed under *e*CO_2 and *a*CO_2 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 *e*CO_2 and *a*CO_2 at six temperature conditions. Awmack *et al*. (1996) [5] found that *e*CO_2 increased the fecundity of cereal aphid, *Sitobion avenae* when reared on winter wheat. Lincoln *et al*. (1993) [14], who reported that the CO_2 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 CO_2 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 33oC but declined at 35oC 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 C_4 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 C_3 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 C_4 plants conduct the C_4 cycle (Sun *et al*., 2009) [23].

Nitrogen content

The nitrogen content was increased with temperatures ranging from 20 to 33ºC but declined at 35ºC 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 eCO_2 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 aCO_2 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 33ºC but declined at 35ºC 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 eCO_2 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 aCO_2 conditions, respectively.

Increased rate of photosynthesis, C: N ratio increases through the accumulation of non-structural carbohydrates in the plants grown under eCO_2 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 33ºC but declined at 35ºC 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 eCO_2 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 aCO_2 conditions, respectively.

The present findings are in agreement with Adishesha *et al.* (2017) [2] who reported that the interactive effects of aCO_2 and temperature treatment had recorded maximum soluble protein in leaves followed by eCO_2 and temperature had least soluble protein in leaves and showed a significant negative effect of eCO_2 and temperature treatments. Under eCO_2 condition plant uses more of carbon for photosynthesis resulting in lower protein content.

Tannins

The tannins were increased with temperatures ranging from 20 to 33ºC but declined at 35ºC 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 eCO_2 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 aCO_2 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 33ºC but declined at 35ºC at 30 DAS (2.18-2.12mg 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 eCO_2 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 aCO_2 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 eCO_2 than aCO_2 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 eCO_2 environment over aCO_2. 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 eCO_2 (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 33ºC but declined at 35ºC 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 eCO_2 conditions and temperatures increased by 5.72, 5.51,

6.51, 6.81, 7.14 and 2.47 per cent at 30 to 90 DAS compared to aCO_2 conditions, respectively.
The present study is in conformity with reports of Srivastava *et al*. (2002) [22] who studied the effect of long-term CO_2 enrichment on the foliar chemistry of mungbean (*Vigna radiata* L. Wilczek). Under enriched CO_2 (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 33ºC but declined at 35ºC 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 eCO_2 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 aCO_2 conditions, respectively.
The present results are in agreement with the results of Xie *et al.* (2015) [27] 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 eCO_2 compared with aCO_2 conditions.

Carbohydrates
The carbohydrates were increased with temperatures ranging from 20 to 33ºC but declined at 35ºC 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 eCO_2 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 aCO_2 conditions, respectively.
The present results are in agreement with Xin *et al*. (2013) [27] who reported that eCO_2 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 eCO_2 conditions.

Total polyphenols
The polyphenols were increased with temperatures ranging from 20 to 33ºC but declined at 35ºC 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 eCO_2 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 aCO_2 conditions, respectively.
Polyphenols, non-structural carbon compounds that constitute one of the defense mechanisms of plants and offer antefeedence to herbivores are also known to increase to a greater extent in leaves under eCO_2 conditions (Bezemer and Jones, 1998) [6] 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 eCO_2 conditions across the temperatures (Tab 1). A significant positive correlation at 25ºC-30ºC 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 eCO_2 conditions. A significant negative correlation at 20ºC and 35ºC 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 eCO_2 conditions. A significant positive correlation at 25ºC-30ºC 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 eCO_2 conditions. A significant negative correlation at 20ºC and 35ºC with fecundity of *R. maidis* with proteins (-0.049 and -0.2822) and aminoacids (-0.035 and -0.3971) content of maize foliage under eCO_2 conditions. A significant positive correlation at 25ºC-30ºC 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 eCO_2 conditions. A significant negative correlation at 20ºC and 35ºC 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 eCO_2 conditions. A significant negative correlation at 20ºC to 35ºC 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 eCO_2 conditions.

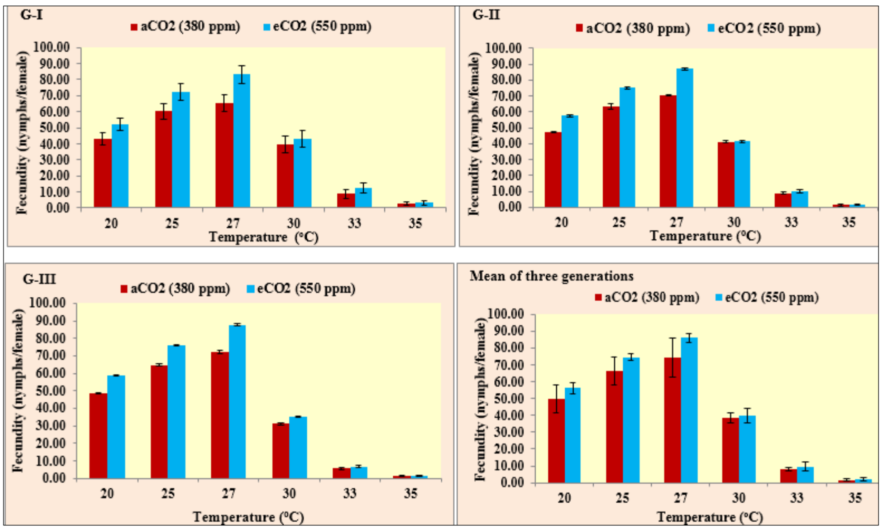

Fig 1: Effect of elevated CO_2 and temperature on fecundity of *R. maidis* of maize across three generations

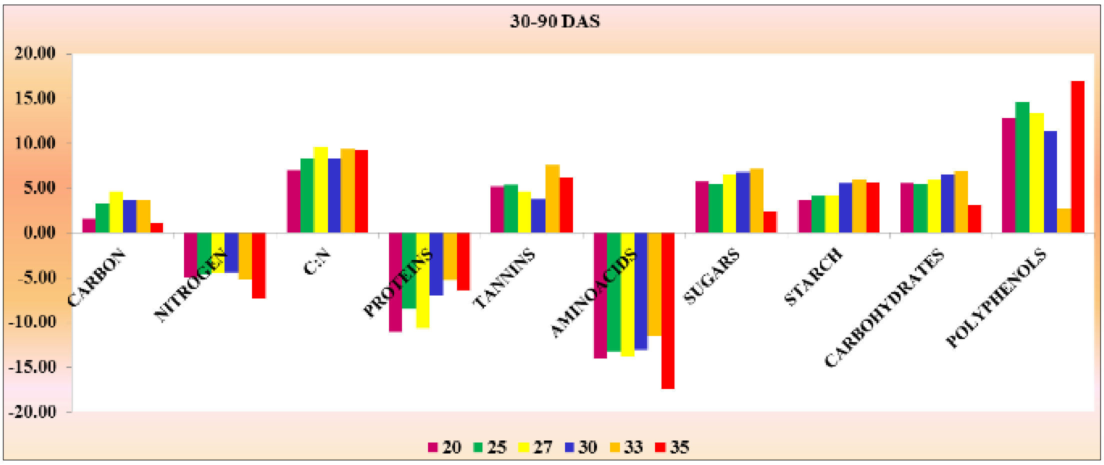

Fig 2: Per cent change in the biochemical constituents of maize foliage under elevated CO_2 and temperature at 30-90 DAS

Tab 1: Correlation between biochemical constituents of maize foliage with fecundity of *R. maidis* under elevated CO_2 and temperature

Bio Chemical constituents	Fecundity					
	T-20 ^{0}C	T-25 ^{0}C	T-27 ^{0}C	T-30 ^{0}C	T-33 ^{0}C	T-35 ^{0}C
	eCO_2	*e*CO_2	*e*CO_2	*e*CO_2	*e*CO_2	*e*CO_2
Nitrogen	-0.061*	0.0696*	0.1148*	0.0181*	0.0761*	-0.4395**
Carbon	-0.015*	0.4717**	0.2529*	0.0999*	0.1352*	-0.2123*
C: N ratio	-0.039*	0.4529**	0.2565*	0.1029*	0.1421*	-0.4228**
Proteins	-0.049*	0.4455**	0.3107*	0.0829*	0.2141*	-0.2822*
Amino acids	-0.035*	0.1075*	0.3292*	0.0910*	0.3074*	-0.3971**
Tannins	-0.111*	-0.3281*	-0.2463*	-0.1494*	-0.0882*	-0.0148*
TSS	-0.141*	0.0630*	0.3301*	0.0643*	0.2807*	-0.1229*
Starch	-0.107*	0.3944**	0.3499*	0.0499*	0.2214*	-0.4229**
Carbohydrates	-0.150*	0.1310*	0.3333*	0.0635*	0.2824*	-0.1550*
Polyphenols	-0.083*	-0.3125*	-0.2991*	-0.0444*	-0.1175*	-0.0189*

*Significant @ 5% level of significance
** Significant @ 1% level of significance

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