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Impact of elevated CO_2 and temperature on mean relative growth rate of maize aphid, *Rhopalosiphum maidis* fitch (Aphididae: Hemiptera) reared on maize for three successive generations

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Abstract
The objective of this study was to estimate the mean relative growth rate (MRGR) of maize aphid, *Rhopalosiphum maidis* Fitch (Aphididae: Hemiptera), reared on maize for three successive generations under elevated CO_2 (eCO_2) (550 ppm ± 25 ppm) and ambient CO_2 (aCO_2) (380 ppm ± 25 ppm) concentrations at six temperatures of 20, 25, 27, 30, 33 and 35 ±1°C in open-top chambers and CO_2 growth chambers with CO_2 and temperature regulation. Experiment was conducted by adopting agar-leaf method. The impact of eCO_2 with six constant temperatures on the MRGR of *R. maidis* was found significant in three generations. MRGR of *R. maidis* was significantly increased with temperature in the range of 20°C to 35°C (0.269 to 0.464 µg µg^{-1} day^{-1}) under eCO_2 compared to that of aCO_2 conditions across the three generations. The present results obtained in this study might provide useful information to aphid control and developing management strategies.

Keywords: elevated co$_2$; *rhopalosiphum maidis*; successive generations; temperature; mean relative growth rate

Introduction
Maize aphid *Rhopalosiphum maidis* Fitch (Aphididae: Hemiptera), a phloem feeding hemipteran insect and causes significant yield losses about 20.1 to 25 percent. It is a polyphagous species occurring worldwide on sorghum, barley and wheat besides maize [12]. It is now distributed worldwide in the tropics and warmer temperate regions [3]. In an anholocyclic life cycle female aphids produce nymphs without fertilization from the males. Aphids cause mechanical harm and malnutrition to plants by the removal of phloem sap.

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. Temperature has an indirect influence on morphological and biochemical constituents of plants. The predicted changes in temperature and CO_2 concentration affect the insect pest population dynamics of various crops. The future estimations of ambient CO_2 concentration predict an increase up to 550 ppm within a few decades [7].

Such rise in CO_2 levels affects the biological system of living organisms, including insects [5].

Temperature has a direct influence on insect activity and their rate of development. Climate change could profoundly affect the population dynamics and the status of insect pests of crops [8]. The average increase in temperature was found to be 0.87°C for the decade of 2006-2015 [7].

Hence, in the present study the mean relative growth rate of *R. maidis* for three successive generations of was examined at two levels of CO_2 and six different temperatures which would be useful in prediction of pest population.

Materials and Methods
Open top chambers
This experiment was conducted in open-top chambers (OTCs) of 4 X 4 X 4 m dimensions, located at ICAR-Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad (17° 38' N; 78° 47' E) were utilized for raising of maize crop under eCO_2 (550 ± 25 ppm) and aCO_2 (380 ± 25 ppm) concentrations. The CO_2 concentration is maintained by pumping CO_2 diluted with air by air compressor [13]. The 100 per cent CO_2 gas of commercial grade was used to elevate CO_2 levels within the chambers. CO_2 analyzer, PLC and SCADA programme with PC were used to maintain the desired level of CO_2 within the OTCs along with temperature and relative humidity sensors.

CO_2 growth chambers with CO_2 and temperature regulation
Plants and insects were maintained in growth chambers (I 36LL; Percival Scientific, Inc. Perry, USA) under 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 and 60-70 per cent relative humidity with a photoperiod ratio of 14L: 10D (14 hours of light: 10 hours of dark). 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.

Maintenance of maize crop and growth conditions
Seeds of maize plants (DHM-117) were sown in 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 ± 1ºC. The leaves were detached from these plants and were used for the maintenance of the *R. maidis* culture for experimentation. Fully grown foliage (30 days after sowing) obtained from respective set conditions was used for feeding trials and the crop was maintained at insecticide free condition throughout the experiment to understand the impact of *e*CO_2 and temperature on insect pests.

Maintenance of *R. maidis* culture

The test insect, corn leaf aphids, *R. maidis* (family: Aphididae; Order: Hemiptera) were collected from the field and maintained in the entomology laboratory of ICAR-CRIDA. The nymphs and adults were reared individually in petridishes of 110 mm diameter of 10 mm height to obtain the mass culture for experiments. The culture was maintained for a number of generations by adopting agar-leaf method [6,10] in growth chambers at 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 ± 1ºC and a photoperiod of 14L:10D. Light intensity of 30, 000 Lx was provided by 26 W fluorescent bulb inside the chambers during the 14 hours light period with relative humidity of 60 % (day) and 70 % (night).

Insect feeding method

Experiments on development of *R. maidis* for three successive generations were conducted by adopting agar-leaf method at elevated and ambient concentrations of CO_2 (550 and 380ppm ± 25 ppm, respectively) at six temperatures of 20, 25, 27, 30, 33 and 35±1ºC and a photoperiod of 14L:10D. Agar medium was prepared with 1g of agar and 100 ml distilled water. The mixture was heated to boiling point in a microwave oven and then cooled to 45ºC by stirring. The mixture was poured in petridishes and left undisturbed until it solidified. The corn leaf from the top of corn seedlings of 1-2 months old was detached and placed in agar medium. The agar medium helpful to keep the leaf fresh and the leaves were changed on every 3 days. The first instar nymphs were collected carefully from the stock culture with the help of wet camel hair brush and transferred individually into each petridish containing maize leaves obtained from respective set conditions with 25 replications per each treatment. Each nymph was examined daily and the MRGR was calculated.

Mean Relative Growth Rate (MRGR) of *R. maidis*

The MRGR of an insect was determined by using the primary data relating to first instar nymphal weight and adult aphid weight. Each first instar aphid nymph was individually weighed and recored as aphid initial weight (W_1). The adult aphid weight was recorded individually which served as final weight (W_2). All weights of aphids were recorded using AUW 220D dual range semi micro balance (0.01- 0.001 µg).

The MRGR was calculated using the formula, [1].

$$MRGR = [\ln (W_2)-\ln (W_1)]/t_2-t_1$$

Where

W_1 = Initial weight, W_2 = Final weight,

t_2-t_1 = Time taken in days between the two weighings.

Statistical analysis

The data pertaining to the first instar nymphal weight, adult aphid weight and MRGR 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. The MRGR of *R. maidis* with the effect of CO_2 (main factor), temperatures (sub factor) and generations as sub-sub factor deployed in split-split plot design.

Results and Discussion

Effect of elevated CO_2 and temperature on first instar nymphal weight *R. maidis* on maize for three successive generations

The results on impact of elevated and ambient concentrations of CO_2 (550 and 380ppm ± 25 ppm, respectively) at six temperatures of 20, 25, 27, 30, 33 and 35 ± 1ºC on first instar nymphal weight of *R. maidis* was found significant across its three successive generations (Table 1).

The impact of *e*CO_2 at six constant temperatures on the first instar nymphal weight of *R. maidis* was found significant in three generations. Significant differences were observed in first ($F_{11, 24}$ = 169.10, P = <0.01), second ($F_{11, 24}$ = 261.44, P = <0.01) and third ($F_{11, 24}$ = 183.57, P = <0.01) generation under two levels of CO_2 and six temperature conditions. In the first generation, the first instar nymphal weight was significantly increased with temperature in the range of 20ºC to 27ºC but significantly declined at 30ºC to 35ºC under *e*CO_2 compared to that of *a*CO_2 conditions. Under *e*CO_2 (550 ppm ± 25 ppm) and six constant temperatures *viz*., 20, 25, 27, 30, 33 and 35 ± 1ºC the weight was resulted that 3.67, 3.782, 3.897, 3.750, 3.165 and 2.782 µg day^{-1} whereas under *a*CO_2 (380 ppm ± 25 ppm) and six constant temperatures resulted that 3.532, 3.633, 3.742, 3.445, 3.171 and 2.363 µg day^{-1}, respectively. Likewise similar trend was observed in the second generation under *e*CO_2 (3.79, 3.869, 3.977, 3.808, 3.343 and 2.945 µg day^{-1}) compared to that of *a*CO_2 (3.585, 3.718, 3.825, 3.594, 3.265 and 2.526 µg day^{-1}) conditions across the temperatures. The first instar nymphal weight in the third generation was significantly increased under *e*CO_2 (3.85, 3.925, 4.034, 3.693, 3.026 and 2.352 µg day^{-1}) whereas under *a*CO_2 the weight was decreased and was resulted that (3.677, 3.774, 3.865, 3.384, 2.671 and 1.878 µg day^{-1}), respectively across the temperatures.

Effect of elevated CO_2 and temperature on adult aphid weight *R. maidis* on maize for three successive generations

The impact of *e*CO_2 at six constant temperatures on the adult aphid weight of *R. maidis* was found significant in three generations. Significant differences were observed in first ($F_{11, 24}$ = 3309.37, P = <0.01), second ($F_{11, 24}$ = 2770.18, P = <0.01) and third ($F_{11, 24}$ = 43.85, P = <0.01) generation under two levels of CO_2 and six temperature conditions (Table 2).

In the first generation, adult aphid weight was significantly increased with temperature in the range of 20ºC to 27ºC but significantly declined at 30ºC to 35ºC under *e*CO_2 compared to that of *a*CO_2 conditions. Under *e*CO_2 (550 ppm ± 25 ppm) and six constant temperatures *viz*., 20, 25, 27, 30, 33 and 35 ± 1ºC,

resulted that 5.43, 5.468, 5.539, 5.373, 4.772 and 4.651 µg/day whereas under *a*CO_2 (380 ppm ± 25 ppm) and six constant temperatures resulted that 5.308, 5.426, 5.487, 5.234, 4.648 and 4.138 µg day^{-1}, respectively. The adult aphid weight was significantly more in the second generation, at *e*CO_2 and temperatures resulted hat 5.49, 5.517, 5.546, 5.502, 4.823 and 4.679 µg day^{-1} whereas under *a*CO_2 and temperatures resulted that 5.335, 5.444, 5.502, 5.332, 4.789 and 4.167 µg day^{-1}, respectively compared to that of first generation. Likewise similar trend was observed in the third generation at *e*CO_2 and temperatures resulted that 5.51, 5.440, 5.568, 5.480, 4.746 and 4.568 µg day^{-1} whereas under *a*CO_2 and temperatures resulted that 5.370, 5.459, 5.517, 5.283, 4.676 and 4.001 µg day^{-1}, respectively.

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

The impact of *e*CO_2 with six constant temperatures on the mean relative growth rate of *R. maidis* was found significant in three generations. Significant differences were observed in first ($F_{11, 24}$ = 4.73, P = <0.01), second ($F_{11, 24}$ = 9.91, P = <0.01) and third ($F_{11, 24}$ = 15.23, P = <0.01) generation under two CO_2 and six temperature conditions (Table 3). In the first generation, the MRGR was significantly increased with temperature in the range of 20ºC to 35ºC under *e*CO_2 compared to that of *a*CO_2 conditions. Under *e*CO_2 (550 ppm ± 25 ppm) and six constant temperatures *viz*., 20, 25, 27, 30, 33 and 35 ± 1ºC, resulted that 0.269, 0.299, 0.335, 0.389, 0.392 and 0.464 µg µg^{-1} day^{-1} whereas under *a*CO_2 (380 ppm ± 25 ppm) and six constant temperatures resulted that 0.178, 0.227, 0.257, 0.322, 0.344 and 0.422 µg µg^{-1} day^{-1}, respectively. In the second generation, the MRGR was significantly increased with temperature in the range of 20ºC to 35ºC under *e*CO_2 compared to that of *a*CO_2 conditions. Under *e*CO_2 (550 ppm ± 25 ppm) and six constant temperatures *viz*., 20, 25, 27, 30, 33 and 35 ± 1ºC, resulted that 0.217, 0.243, 0.271, 0.352, 0.379 and 0.419 µg µg^{-1} day^{-1} whereas under *a*CO_2 (380 ppm ± 25 ppm) and six constant temperatures resulted that 0.154, 0.199, 0.235, 0.281, 0.317 and 0.374 µg µg^{-1} day^{-1}, respectively. In the third generation, the MRGR was significantly increased with temperature in the range of 20ºC to 27ºC but significantly declined at 30ºC to 35ºC under *e*CO_2 compared to that of *a*CO_2 conditions. Under *e*CO_2 (550 ppm ± 25 ppm) and six constant temperatures *viz*., 20, 25, 27, 30, 33 and 35 ± 1ºC, resulted that 0.232, 0.243, 0.296, 0.411, 0.416 and 0.540 µg µg^{-1} day^{-1} whereas under *a*CO_2 (380 ppm ± 25 ppm) and six constant temperatures resulted that 0.197, 0.218, 0.253, 0.365, 0.468 and 0.514 µg µg^{-1} day^{-1}, respectively. The results are in agreement with [11], who found that increased weight and relative growth rate of *R. padi* at *e*CO_2 conditions. The adult weight of aphids, *viz*., pea aphid, *Acyrthosiphon pisum* Harris (Aphididae: Hemiptera) on peas [9], potato aphid *Macrosiphum euphorbiae* Thomas (Aphididae: Hemiptera) on blue bind weed, *Solanum dulcamara* [4] decreased with increase in temperatures. In case of peach potato aphid, *Myzus persicae* Sulzer (Aphididae: Hemiptera) had a close linear correlation with temperature, decrease in body size at higher temperature. In contrast, green peach aphid, *Myzus antirrhinii* Lichtenstein (Aphididae: Hemiptera) the temperature relations were reversed [2]. The present results obtained in this study might provide useful information to aphid control and developing management strategies.

Effect of elevated CO2, temperature and generations on first instar nymphal weight, adult aphid weight and MRGR of *R. maidis* on maize

The effect of *e*CO_2, temperature and generations on first nymphal instar weight, adult aphid weight and MRGR of *R. maidis* on maize was furnished in Table 4. The first nymphal instar weight of *R. maidis* showed non-significant difference by CO_2 conditions ($F_{1, 24}$ = 0.30^{NS}, P =<0.01). The first nymphal instar weight of *R. maidis* was significantly varied by temperature conditions ($F_{11, 24}$ = 97.81, P =<0.01). The first nymphal instar weight of *R. maidis* was significantly varied by generations ($F_{3, 24}$ = 128.56, P =<0.01). The interaction between CO_2, temperature and generations was found significant ($F_{14, 24}$ = 92.12, P =<0.01). The adult aphid weight of *R. maidis* showed non-significant difference by CO_2 conditions ($F_{1, 24}$ = 0.91^{NS}, P =<0.01). The adult aphid weight of *R. maidis* was significantly varied by temperature conditions ($F_{11, 24}$ = 30.61, P =<0.01). The adult aphid weight of *R. maidis* was significantly varied by generations ($F_{3, 24}$ = 67.49, P =<0.01). The interaction between CO_2, temperature and generations was found significant ($F_{14, 24}$ = 40.56, P =<0.01). The MRGR of *R. maidis* showed non-significant difference by CO_2 conditions ($F_{1, 24}$ = 2.68^{NS}, P =<0.01). The MRGR of *R. maidis* was significantly varied by temperature conditions ($F_{11, 24}$ = 58.47, P =<0.01). The MRGR of *R. maidis* was significantly varied by generations ($F_{3, 24}$ = 4.52, P =<0.01). The interaction between CO_2, temperature and generations was found significant ($F_{14, 24}$ = 165.39, P =<0.01).

Table 1: Effect of elevated CO_2 and temperature on first instar nymphal weight, adult aphid weight and mean relative growth rate of *R. maidis* on maize in first generation

Temperatures (ºC)	First instar nymphal weight (µg day^{-1})		Adult aphid weight (µg day^{-1})		Mean relative growth rate (µg µg^{-1} day^{-1})	
	aCO_2 (380 ppm)	*e*CO_2 (550 ppm)	*a*CO_2 (380 ppm)	*e*CO_2 (550 ppm)	*a*CO_2 (380 ppm)	*e*CO_2 (550 ppm)
20	3.532±0.022	3.67±0.019	5.308±0.006	5.43±0.004	0.178±0.011	0.269±0.021
25	3.633±0.018	3.782±0.018	5.426±0.004	5.468±0.008	0.227±0.021	0.299±0.026
27	3.742±0.015	3.897±0.020	5.487±0.009	5.539±0.010	0.257±0.023	0.335±0.049
30	3.445±0.027	3.750±0.011	5.234±0.005	5.373±0.005	0.322±0.033	0.389±0.033
33	3.171±0.029	3.165±0.033	4.648±0.009	4.772±0.012	0.344±0.039	0.392±0.027
35	2.363±0.053	2.782±0.104	4.138±0.013	4.651±0.098	0.422±0.041	0.464±0.028
F test	169.10**		3309.37**		4.73**	
S.Em ±	0.011		0.003		0.009	
LSD (p =0.05)	0.023		0.006		0.018	
LSD (p =0.01)	0.030		0.008		0.023	
CV (%)	1.18		0.21		9.50	

Factor 1 (CO_2)			
aCO_2 (380 ppm)	3.314	5.041	0.292
eCO_2 (550 ppm)	3.508	5.207	0.358
F test	1817.18**	17403.25**	280.24**
S.Em ±	0.005	0.001	0.004
LSD(p = 0.05)	0.009	0.003	0.008
LSD (p = 0.01)	0.013	0.004	0.011
CV (%)	1.15	0.21	10.48
Factor 2 (Temperature (ºC))			
20	3.600	5.374	0.224
25	3.708	5.447	0.262
27	3.820	5.514	0.296
30	3.598	5.304	0.358
33	3.168	4.711	0.367
35	2.573	4.395	0.443
F. test	6711.48**	90797.95**	331.86**
S.Em±	0.008	0.002	0.006
LSD(p = 0.05)	0.016	0.004	0.012
LSD (p = 0.01)	0.021	0.006	0.016

All values are mean ± standard deviation; ** Significant @ 1% level of significance NS = Not-significant

Table 2: Effect of elevated CO_2 and temperature on first instar nymphal weight, adult aphid weight and mean relative growth rate of *R. maidis* on maize in second generation

Temperatures (ºC)	**First instar nymphal weight (µg day^{-1})**		**Adult aphid weight (µg day^{-1})**		**Mean relative growth rate (µg µg^{-1} day^{-1})**	
	***a*CO$_2$ (380 ppm)**	***e*CO$_2$ (550 ppm)**	***a*CO$_2$ (380 ppm)**	***e*CO$_2$ (550 ppm)**	***a*CO$_2$ (380 ppm)**	***e*CO$_2$ (550 ppm)**
20	3.585±0.029	3.790±0.023	5.335±0.015	5.490±0.010	0.154±0.006	0.217±0.017
25	3.718±0.012	3.869±0.022	5.444±0.003	5.517±0.007	0.199±0.011	0.243±0.016
27	3.825±0.015	3.977±0.009	5.502±0.007	5.546±0.004	0.235±0.015	0.271±0.027
30	3.594±0.013	3.808±0.014	5.332±0.004	5.502±0.003	0.281±0.016	0.352±0.043
33	3.265±0.022	3.343±0.016	4.789±0.009	4.823±0.005	0.317±0.028	0.379±0.032
35	2.526±0.041	2.945±0.053	4.167±0.030	4.679±0.037	0.374±0.042	0.419±0.031
F test	261.44**		2770.18**		9.91**	
S.Em ±	0.007		0.003		0.008	
LSD (p =0.05)	0.015		0.007		0.015	
LSD (p =0.01)	0.019		0.009		0.020	
CV (%)	0.74		0.23		9.33	
Factor 1 (CO_2)						
aCO_2 (380 ppm)	3.419		5.095		0.260	
eCO_2 (550 ppm)	3.622		5.261		0.314	
F test	4992.25**		17487.07**		311.67**	
S.Em ±	0.003		0.21		0.003	
LSD(p = 0.05)	0.006		0.001		0.006	
LSD (p = 0.01)	0.008		0.003		0.009	
CV (%)	0.71		0.003		9.20	
Factor 2 (Temperature (ºC))						
20	3.686		5.415		0.185	
25	3.794		5.480		0.221	
27	3.901		5.524		0.253	
30	3.701		5.418		0.331	
33	3.304		4.807		0.335	
35	2.736		4.423		0.394	
F. test	13883.40**		70116.76**		440.61**	
S.Em±	0.005		0.002		0.005	
LSD(p = 0.05)	0.010		0.005		0.011	
LSD (p = 0.01)	0.014		0.006		0.014	

All values are mean ± standard deviation; ** Significant @ 1% level of significance NS = Not-significant

Table 3: Effect of elevated CO_2 and temperature on first instar nymphal weight, adult aphid weight and mean relative growth rate of *R. maidis* on maize in third generation

Temperatures (ºC)	**First instar nymphal weight (µg day^{-1})**		**Adult aphid weight (µg day^{-1})**		**Mean relative growth rate (µg µg^{-1} day^{-1})**	
	***a*CO$_2$ (380 ppm)**	***e*CO$_2$ (550 ppm)**	***a*CO$_2$ (380 ppm)**	***e*CO$_2$ (550 ppm)**	***a*CO$_2$ (380 ppm)**	***e*CO$_2$ (550 ppm)**
20	3.677±0.013	3.85±0.019	5.370±0.005	5.51±0.004	0.197±0.012	0.232±0.011

25	3.774±0.019	3.925±0.009	5.459±0.006	5.440±0.387	0.218±0.014	0.243±0.064
27	3.865±0.017	4.034±0.014	5.517±0.004	5.568±0.006	0.253±0.020	0.296±0.021
30	3.384±0.017	3.693±0.025	5.283±0.006	5.480±0.003	0.365±0.036	0.411±0.045
33	2.671±0.044	3.026±0.023	4.676±0.007	4.746±0.007	0.468±0.046	0.416±0.032
35	1.878±0.078	2.352±0.048	4.001±0.013	4.568±0.041	0.514±0.045	0.540±0.039
F test	183.57**		43.85**		15.23**	
S.Em ±	0.009		0.032		0.010	
LSD (p =0.05)	0.019		0.063		0.019	
LSD (p =0.01)	0.025		0.083		0.026	
CV (%)	1.01		2.18		9.36	
Factor 1 (CO_2)						
aCO_2 (380 ppm)	3.208		5.051		0.336	
eCO_2 (550 ppm)	3.481		5.219		0.358	
F test	5304.69**		173.71**		20.25**	
S.Em ±	0.004		0.013		0.005	
LSD(p = 0.05)	0.008		0.026		0.010	
LSD (p = 0.01)	0.010		0.036		0.014	
CV (%)	0.97		2.14		12.12	
Factor 2 (Temperature (ºC))						
20	3.764		5.440		0.214	
25	3.850		5.450		0.233	
27	3.950		5.543		0.275	
30	3.539		5.382		0.388	
33	2.849		4.825		0.444	
35	2.115		4.711		0.527	
F. test	22779.71**		1055.47		752.88**	
S.Em±	0.007		0.022		0.006	
LSD(p = 0.05)	0.013		0.044		0.013	
LSD (p = 0.01)	0.018		0.058		0.017	

All values are mean ± standard deviation; ** Significant @ 1% level of significance NS=Not-significant

Table 4: Effect of elevated CO_2, temperature and generations on first instar nymphal weight, adult aphid weight and mean relative growth rate (MRGR) of *R. maidis* on maize

Temp (ºC) X Generations		**First instar nymphal weight (µg day^{-1})**		**Adult aphid weight (µg day^{-1})**		**Mean relative growth rate (µg µg^{-1} day^{-1})**	
		***a*CO$_2$ (380ppm)**	***e*CO$_2$ (550 ppm)**	***a*CO$_2$ (380ppm)**	***e*CO$_2$ (550ppm)**	***a*CO$_2$ (380ppm)**	***e*CO$_2$ (550ppm)**
F1	20	3.532±0.022	3.67±0.019	5.308±0.006	5.43±0.004	0.178±0.011	0.269±0.021
	25	3.633±0.018	3.782±0.018	5.426±0.004	5.468±0.008	0.227±0.021	0.299±0.026
	27	3.742±0.015	3.897±0.020	5.487±0.009	5.539±0.010	0.257±0.023	0.335±0.049
	30	3.445±0.027	3.750±0.011	5.234±0.005	5.373±0.005	0.322±0.033	0.389±0.033
	33	3.171±0.029	3.165±0.033	4.648±0.009	4.772±0.012	0.344±0.039	0.392±0.027
	35	2.363±0.053	2.782±0.104	4.138±0.013	4.651±0.098	0.422±0.041	0.464±0.028
F2	20	3.585±0.029	3.79±0.023	5.335±0.015	5.49±0.010	0.154±0.006	0.217±0.017
	25	3.718±0.012	3.869±0.022	5.444±0.003	5.517±0.007	0.199±0.011	0.243±0.016
	27	3.825±0.015	3.977±0.009	5.502±0.007	5.546±0.004	0.235±0.015	0.271±0.027
	30	3.594±0.013	3.808±0.014	5.332±0.004	5.502±0.003	0.281±0.016	0.379±0.043
	33	3.265±0.022	3.343±0.016	4.789±0.009	4.823±0.005	0.317±0.028	0.352±0.032
	35	2.526±0.041	2.945±0.053	4.167±0.030	4.679±0.037	0.374±0.042	0.419±0.031
F3	20	3.677±0.013	3.85±0.019	5.370±0.005	5.51±0.004	0.197±0.012	0.232±0.011
	25	3.774±0.019	3.925±0.009	5.459±0.006	5.440±0.387	0.218±0.014	0.243±0.064
	27	3.865±0.017	4.034±0.014	5.517±0.004	5.568±0.006	0.253±0.020	0.296±0.021
	30	3.384±0.017	3.693±0.025	5.283±0.006	5.480±0.003	0.365±0.036	0.411±0.045
	33	2.671±0.044	3.026±0.023	4.676±0.007	4.746±0.007	0.468±0.046	0.416±0.032
	35	1.878±0.078	2.352±0.048	4.001±0.013	4.568±0.041	0.514±0.045	0.540±0.039
F test		92.12**		40.56**		165.39**	
S.Em ±		0.042		0.030		0.011	
LSD (p = 0.05)		0.088		0.063		0.022	
LSD (p = 0.01)		0.119		0.085		0.030	
CV (%)		4.68		1.50		12.87	
Factor 1 (CO_2)							
aCO_2 (380 ppm)		3.411		5.130		0.323	
eCO_2 (550 ppm)		3.440		5.161		0.316	

F test	2.17^{NS}	0.91^{NS}	2.68^{NS}
S.Em ±	0.024	0.019	0.006
LSD (p = 0.05)	0.050	0.040	0.012
LSD (p = 0.01)	0.068	0.054	0.016
CV (%)	4.37	3.90	12.74
Factor 2 (Temperature (ºC))			
20	3.324	5.073	0.291
25	3.545	5.229	0.341
27	3.322	5.066	0.298
30	3.532	5.228	0.342
33	3.301	5.052	0.300
35	3.529	5.227	0.345
F test	97.81**	30.61**	58.47**
S.Em ±	0.024	0.033	0.007
LSD (p = 0.05)	0.048	0.065	0.013
LSD (p = 0.01)	0.064	0.085	0.017
CV (%)	8.36	5.24	12.53
Factor 3 (Generations)			
F_1	3.411	5.124	0.325
F_2	3.521	5.178	0.287
F_3	3.345	5.135	0.347
F test	128.56**	67.49**	4.52*
S.Em ±	0.031	0.026	0.008
LSD (p = 0.05)	0.065	0.054	0.017
LSD (p = 0.01)	0.088	0.074	0.023

All values are mean ± standard deviation; ** Significant @ 1% level of significance NS = Not-significant

Conclusion

The results concluded that the MRGR of *R. maidis* was significantly increased with temperature in the range of 20ºC to 35ºC (0.269 to 0.464 µg µg^{-1}day^{-1}) under *e*CO_2 compared to that of *a*CO_2 conditions across the three generations.

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