



Original article

Effects of CO₂ enrichment by fermentation of CRAM on growth, yield and physiological traits of cherry tomato

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ABSTRACT

Carbon dioxide (CO₂) concentration in greenhouses is sub-optimal for vegetable production. Many techniques have been used to increase CO₂ concentration in greenhouses but most of them are expensive with certain limitations and drawbacks. We adopted a new strategy to elevate CO₂ concentration in the greenhouse throughout the day via crop residues and animal manure composting (CRAM). During the whole cultivation period, CRAM-treated greenhouse had doubled CO₂ concentration which significantly increased the yield of cherry tomatoes (*Lycopersicon esculentum* L.) i.e. up to 38%. The influence of CRAM procedure on cherry tomato quality was also investigated and the concentrations of total soluble solids (TSS) and soluble sugar were found to be significantly higher in cherry tomatoes grown under composting greenhouse than that of non-composting greenhouse. Additionally, CRAM-CO₂ enrichment also resulted in increased concentrations of ascorbic acid (Vitamin C) and titrate acid as compared with the control. In contrast, the concentration of nitrate was considerably decreased in cherry tomato grown under CO₂ enriched condition than that of control. The increase in active oxygen metabolisms such as POD, CAT and SOD while a decrease in MDA, as well as APX was observed for cherry tomatoes grown under CO₂ enriched condition. Hence, CO₂ fertilization by using CRAM in greenhouse significantly improved quality and increased the yield of cherry tomatoes.

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1. Introduction

Tomato is one of the most commonly used vegetables for cooking and food industry condiment around the globe. Tomato enriches a lot of nutrients and provides our body antioxidants, fibre and some vitamins (Petronia et al., 2019). Red colour of tomato is due to the accumulation of beta carotene that contains ascorbic acid and a great amount of phenolic contents (Stewart et al., 2000). Cherry tomato (*Solanum Lycopersicum*) is an ancestor of the domesticated form of the cultivated tomato and generally contains higher bio-active compound as compared to large ones (Ranc et al., 2008; Choi et al., 2011). Greenhouses are being used for the

production of cherry tomato, as it has many advantages over field production including higher quality, enhanced yield and extended growing season for vegetable production. In China alone, the land utilized for the production of vegetables is almost 17.8 million hectares out of which 14.6% is consumed by greenhouse (National Bureau of Statistics of China, 2005). However, tomato's poor quality complaints have been reported in the past few years in China and the better flavour is demanded by the consumers (Baldwin et al., 2000, Causse et al., 2003). One of the major factor for the low quality of vegetables was probably due to the low concentration of CO₂ in the greenhouse (Chongwei et al., 2009). Furthermore many studies revealed, that the ideal concentration of CO₂ for the production of vegetables in the greenhouse is 800–1000 μL L⁻¹ (Kläring et al., 2007). However, the recorded concentration of CO₂ in the greenhouse during day time, due to hermetic conditions is about 110–260 μL L⁻¹ (Kläring et al., 2007). Additionally, photosynthetic activity, water usability and the yield of vegetable depend upon the elevation of CO₂ in the greenhouse (Kauret et al., 2013). There are many well-known benefits of CO₂ enrichment in greenhouses such as an increase in biomass and size of the canopy,

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photosynthetic rate etc. (Ziska, 2008) which ultimately increase the growth and development of the plant. Moreover, many studies revealed that CO₂ enrichment in greenhouse improves water use efficiency and product yield (Arena et al., 2011; Klaiber et al., 2013; Brestic et al., 2018). Other reported that by applying a high concentration of CO₂ and the presence of enough heat in the greenhouse will make the greenhouse environment optimum for plant growth (Halmann and Steinberg, 1998).

Mostly, commercial growers prefer to use supplemental CO₂ to improve crop yield and quality (Li and Wang, 2013). In greenhouses, various techniques have been developed to elevate CO₂ concentrations like the direct injection of gas inside the greenhouse, ventilation as well as chemical production by combining ammonium bicarbonate with sulfuric acid (Klaringet al., 2007; Linker et al., 1999). But they are all very expensive and are difficult to operate. Hence, CO₂ insufficiency in the greenhouse is still a limiting factor which leads to an undesirable taste of vegetables and ultimately gives lower profit to the farmers.

Since the pre industrial times, with the involvement of human activities the atmospheric (CO₂) has been elevated about 40% and the current (CO₂) of 400 ppm is continuously increasing and will reach to 650 ppm by the year 2050 (IPCC, 2007). Day by day increase in CO₂ in an open environment is also due to mismanagement of wastages (crops and animal residues) and anti-ecosystem activities of the human being. According to the (National Bureau of Statistics of China, 2005), about 23% of entire Chinese agriculture waste was burnt in the field which results in Carbon (C) emissions of 5.5×10^7 t y⁻¹ (Cao et al., 2011). This C emission in the atmosphere can be significantly reduced by using composting. Consequently, in China alone, about 2.75 billion tons of farmyard manure (FYD) is produced every year and about 220 (million ton) animal waste (Ding et al., 2006) is released in water bodies. The massive discharge of animal manures produced by high-density livestock results in P and N to flow into waterbodies which ultimately results in eutrophication (Zvomuya et al., 2006). Composting of crop residues is a way to handle waste efficiently and reducing the CO₂ concentration in the environment. In contrast, the mismanagement of agricultural wastages (such as crop-residues and animal-manure) and anti-ecosystem activities of human beings are increasing CO₂ in the environment. Composting encourages our farmers to utilize presently unused manure properly and this practice also diminishes dangerous releases of manures into the waterbodies. Furthermore, optimum fermentation conditions for composting to increase CO₂ concentration in the greenhouse were reported by (Chongweijin et al., 2009). According to which the C:N ratio which is most crucial factor must be 40:1. The pH, water content, and temperature for rice straw and pig manure biodegradation should be 6.5–7.0, 71% (w/w) and 50C, respectively. In the present study, we adopted the most simple and efficient unit of composting CRAM (crop residues and animal manure) to elevate CO₂ concentration in greenhouses by placing CRAM unit directly in the greenhouse. This strategy is very economical, the increase in yield and higher quality of cherry tomato will encourage farmers to adopt this technique.

2. Materials and methods

2.1. Plant materials and experimental design

The cherry tomato variety used in this experiment was “Huangfei”, treated with two treatments: Control i.e normal condition (local conventional cultivation), and CO₂ enrichment, CRAM, by fermentation of organic wastes. In order to conduct the experiment, two neighbouring greenhouses were used (only 40 cm apart) in Huzhou, China. Each greenhouse had the same dimensions

(42 m × 6 m × 3 m) and had same soil condition. There were 5 composting units placed in the greenhouse for CRAM treatment. The composting units were made of timber with 1.2 m height and 0.6 diameters. To maintain sufficient aeration in a unit, bars of 6 cm (width) at the bottom with 2 cm intervals were used. The 25 kg of composting material (equivalent to a dry weight of wheat straw) and moist manure (8 kg) were added. At the initial stage, about 90 L of water was added to maintain the moisture level at 70% throughout the experiment (Du et al., 2004). Three fungal species *Panusconclmtw* zj3, *Trichoderma viride* zj2, and *Aspergillus niger* zj1 were inoculated with the CRAM mixture to enhance the production of CO₂ via fermentation. The optimum pH (6.5–7) of CRAM mixture was maintained by using pickled vegetable juice (after every 15 days) (Jin et al., 2009). Before sowing, fertilizers were applied as: organic fertilizer 2000 kg, manure fertilizer 25 kg, potassium fertilizer was added in two splits 20 kg and 75 kg, respectively. Each greenhouse had 3 ridges, the distance between the plants was 45 cm, the distance between ridges was 75 cm, the width of the ridge was 40 cm, and there were about 1300 plants in the greenhouse. During the experiment, the water and fertilizer applications were kept the same in all greenhouses.

2.2. CO₂ determination in greenhouse

To estimate the CO₂ concentration, CO₂/ temperature monitor (Telaire 7001, USA) was installed in the greenhouse.

2.3. Determination of plant growth parameters and yield characteristics

From each treatment plant growth parameters were examined by taking five plants. Plant height (at the peak flowering stage), leaf area (using portable leaf area meter; LI-3000C, LI-COR, USA), number of flowers per plant, the total fruit mass per plant and no. of fruits per plant were measured. Physico-chemical parameters of the soil used for this experiment were calculated according to (Bao, 2000) (Table 1). Table 2.

2.4. Evaluation of quality traits

By using anthrone method, total soluble sugars concentration was determined from spectrophotometric determination (Fales, 1951) following by (Buysse and Merckx, 1993). By using the titration method (2, 6-dichlorophenol indophenol sodium salt dehydrate) contents of ascorbic acid were measured (Horwitz, 1980). Total soluble solids (TSS) were obtained according to Mamatha et al. (2014). Nitrate concentration in cherry tomato fruits was analyzed by following the similar procedure of (Cataldo et al., 1975). Nitrate concentration in fruits was calculated as mg kg⁻¹. Acidity was calculated by following AOAC method (942.15) (AOAC 2000).

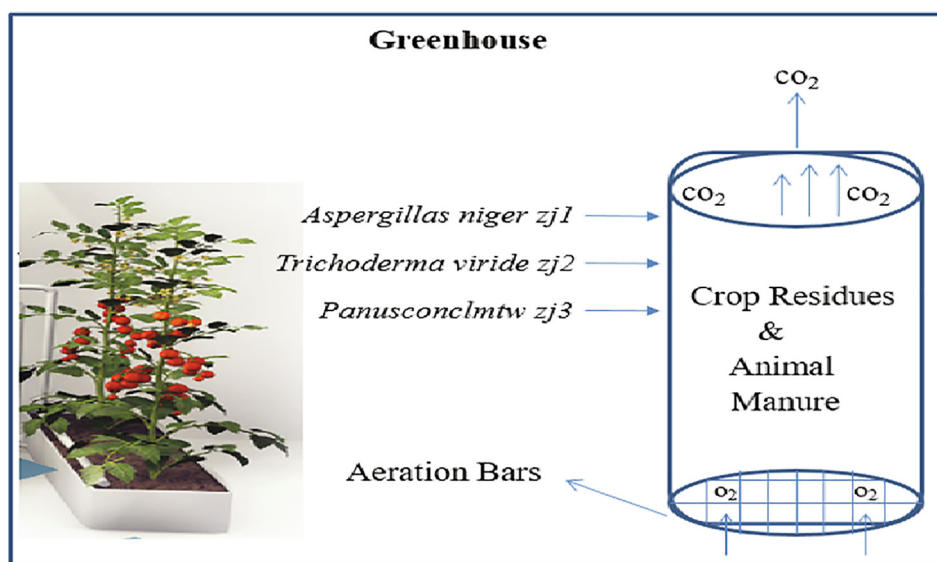
Table 1

Basic Physico-chemical parameters of experimental soil.

Soil physiological index	Control	+CO ₂ (CRAM)
OM (%)	5.2	7.04
Total N (gkg ⁻¹)	0.26	0.35
NH ₄ ⁺ -N (mgkg ⁻¹)	4.36	5.91
NO ₃ ⁻ -N (mgkg ⁻¹)	3.54	4.8
Available P (mgkg ⁻¹)	2.3	3.12
Available K (mgkg ⁻¹)	42.26	55.06
TOC (%)	3.01	4.08
PH	6.86	6.98
EC (mgL ⁻¹)	6.15	6.54

Table 2Effects of CO₂ enrichment by fermentation of CRAM on growth of cherry tomato.

Treat.	Seedling	Transplant	CRAM	First flower	First harvest	End harvest
Year. Month. Day						
Control	2016.11.05	2016.11.20	2017.01.15	2017.03.02	2017.03.20	2017.06.20
+CO ₂	2016.11.05	2016.11.20	2017.01.15	2017.02.25	2017.03.12	2017.06.15

Control and +CO₂ correspond to normal condition and CO₂ enrichment by fermentation of CRAM.**Fig. 1.** Graphical abstract of CRAM procedure in the greenhouse for the production of CO₂.

2.5. Measurement of photosynthetic parameters

The photosynthetic parameters e.g. intercellular concentration, transpiration and stomatal conductance in cherry tomato plants were measured using portable photosynthesis system LI-6400 (Li-COR, Lincoln, NE, USA) and Chlorophyll content was measured by using SPAD.

2.6. Measurements of antioxidant enzyme activities and MDA content

The antioxidant enzyme activities were determined by following the methodology of (Chen et al., 2010; Wu et al., 2003). Briefly, 0.5 g of plant leaf sample was supplemented with 8 ml of 50 mM PBS buffer (pH 7.8) and homogenized in pestle and mortar. Then centrifugation of homogenate was followed for 20 min (10000 rpm, 4 °C). The resulted supernatant was used for the measurement of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) as well as malondialdehyde (MDA) content.

2.7. Statistical analysis

Mean values of each treatment were the presented data. The measurements were done with three replicates. DPS (Data processing system) software package was used for all Statistical analysis

by using ANOVA followed by the Duncan's Multiple Range Test (DMRT) to evaluate the effects of different treatments ($P < 0.05, 0.01$).

3. Results and analysis

3.1. Effects of CO₂ enrichment by fermentation of CRAM on growth and yield of cherry tomato

While evaluating the difference in CO₂ concentration in both greenhouses, the results indicated that CO₂ level in control was recorded between 480 and 570 $\mu\text{L L}^{-1}$, while in the CRAM-CO₂ greenhouse the recorded concentration of CO₂ ranged between 1000 and 1500 $\mu\text{L L}^{-1}$ during the entire cultivation period. CRAM fermentation resulted in elevated CO₂ level more than 100% as compared to the control greenhouse. Graphical abstract for the production of CO₂ in the greenhouse is shown in (Fig. 1).

3.2. Growth morphological traits

CO₂ enrichment via using CRAM procedure in the greenhouse had a significant impact on cherry tomato growth and morphology i.e. plant height (37%), stem diameter (17%), leaf width (14%) and chlorophyll content increased by (11.4%) than that of the control

Table3Effects of CO₂ enrichment by fermentation of CRAM on morphological traits of cherry tomato.

Treat.	Plant height (cm)	Stem diameter (cm)	Leaf width (cm)	Chlorophyll
Control	76.7	1.12	49.7	42.1
+CO ₂	105.0**	1.31**	56.7**	46.9**

Control and +CO₂ correspond to normal condition and CO₂ enrichment by fermentation of CRAM. *and **, indicate significant differences between +CO₂ and control at significant levels of 0.05 and 0.01, respectively.

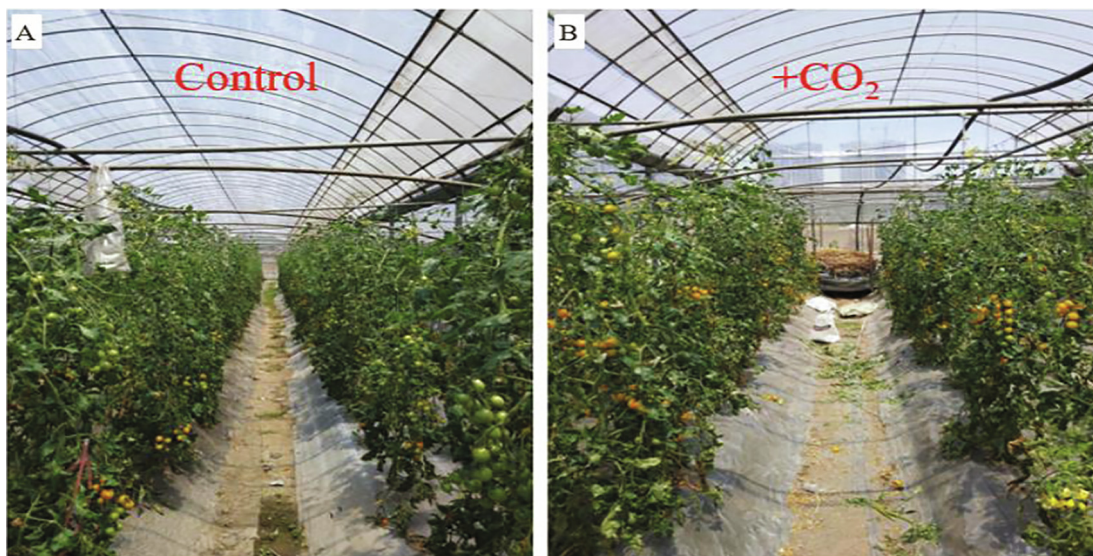


Fig. 2. Morphology of cherry tomato plants at the flowering stage under control (A) and CO₂ enrichment (B) condition. Control and +CO₂ correspond to normal condition and CO₂ enrichment by fermentation of CRAM.

Table 4
Effects of CO₂ enrichment by fermentation of CRAM on yield traits of cherry tomato.

Treatment	Single fruit weight (g)	Fruit diameter (cm)	Fruit number	Yield (kg/hm ²)
Control	13.8	3	12.2	1300
+CO ₂	19.0*	3.6*	27.4**	1800*

Control and +CO₂ correspond to normal condition and CO₂ enrichment by fermentation of CRAM. *and **, indicate significant differences between +CO₂ and control at significant levels of 0.05 and 0.01, respectively.

greenhouse (Table 3). Moreover, the result of the conducted study revealed significant alteration in size, weight, number of fruits and duration of ripening in cherry tomatoes grown under CO₂ enriched conditions as compared to control. Furthermore, it can be seen from (Fig. 2) that under the CO₂ enriched greenhouse, almost all the cherry tomato fruits were turned yellow, while in control con-

dition, only the fruit at the bottom of the plant changed to yellow. The cherry tomatoes grown in CRAM-treatment greenhouse changed colour 10d earlier, as compared to the control greenhouse.

In addition, yields of cherry tomato grown in control greenhouse and CRAM-CO₂ enriched greenhouse was 1300 (kg/hm²) and 1800 (kg/hm²) respectively, indicating a massive increase in yield of CO₂ treated cherry tomato plants by 500 kg/hm² (Table 4). Influences in physiology and morphology directly associated with the yield and quality related traits. A visible phenotypic difference was observed between mature cherry tomato fruit grown in CRAM-CO₂ treated greenhouse and control greenhouse (Fig. 3).

3.3. Effect of CO₂ enrichment by CRAM on the quality of cherry tomato

CO₂ enrichment via CRAM procedure significantly influenced soluble sugar (6%), soluble solids (7.2%), titrate acid (0.4%) and Vitamin C (Ascorbic Acid) up to (5%), while nitrate concentration decreased by (2%) in cherry tomato as compared to control (Fig. 4). The fruit diameter and individual fruit weight for CRAM-CO₂ enriched cherry tomato showed significant difference of 25% and 37.7%, respectively. Overall, cherry tomato grown under CRAM-CO₂ enriched condition exhibited a significant increase in the quality trait, as compared to the control greenhouse.

3.4. Effects of CO₂ enrichment by fermentation of CRAM on physiological traits of cherry tomato

3.4.1. Photosynthetic characteristics

Significant enhancement of photosynthetic rate (*Pn*) (c.f. by 20.22% higher than the control), stomatal conductance (*Gs*) (75%), intercellular CO₂ (*Ci*) (9%), and transpiration rate of (*Tr*) (43%) were



Fig. 3. Difference of mature cherry tomato fruit in control greenhouse and CRAM-CO₂ treatment greenhouse. (Scale bar is 1 cm).

observed in cherry tomato under CRAM-CO₂ enriched greenhouse as compared to the control (Table 5).

4. Active oxygen metabolism

Activities of ascorbate peroxide (APX), peroxidase (POD), catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) contents were determined in leaves of cherry tomato Fig. 5. The SOD and APX activities were observed as almost similar between control (CK) and +CO₂ enriched cherry tomato. The POD and CAT activities in leaves of cherry tomato under CRAM-CO₂ treatment were increased by 26.66% and 14.70% respectively, MDA contents were decreased by 18.03% in CRAM-CO₂ enrichment as compared to the control. (Fig. 5).

5. Discussion

Cherry tomatoes are used in many ornamental dishes and are mostly grown in greenhouses (Kauret al., 2013). However,

Table 5

Effects of CO₂ enrichment by fermentation of CRAM on photosynthetic characteristics of cherry tomato.

Treatment	<i>Pn</i>	<i>Gs</i>	<i>Ci</i>	<i>Tr</i>
Control	17.8	0.4	313.8	3.5
+CO ₂	21.4*	0.7*	341.2*	5.0*

Control and +CO₂ correspond to normal condition and CO₂ enrichment by fermentation of CRAM. *, indicate significant differences between +CO₂ and control at 0.05.

low concentration of CO₂ in the greenhouse is the main factor that deteriorates the quality of cherry tomatoes (Jin et al., 2009). So, fertigation of CO₂ is essential in the greenhouse in order to obtain massive yield and quality. To fulfill the required amount of CO₂ concentration in the greenhouse, CRAM procedure (crop residues as well animal manures) is the most economical and efficient procedure as compared to others strategies such as direct gas injection, ventilation and chemical production (Leakey et al., 2009). It can maintain optimum concentration of CO₂ (for the

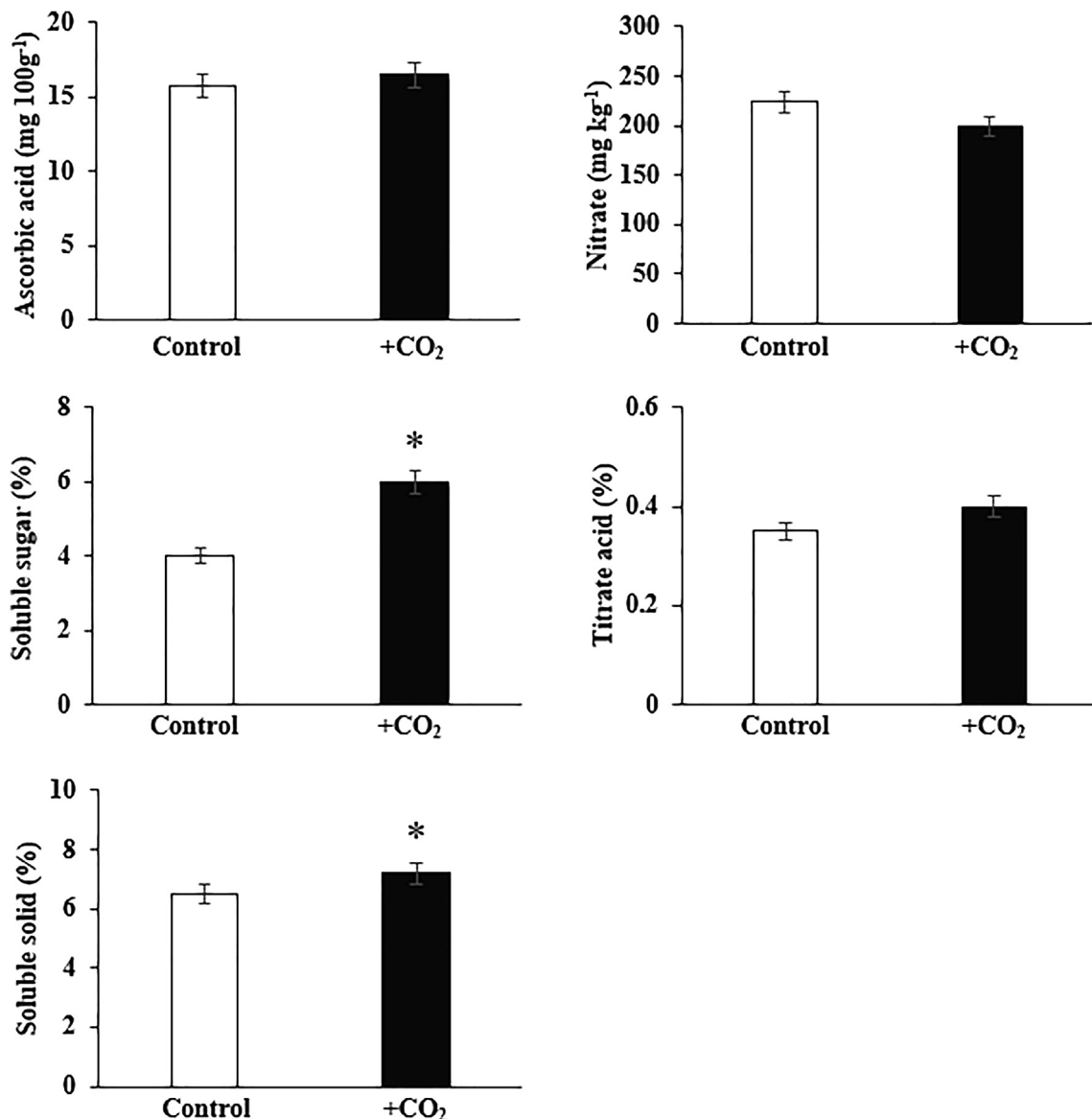


Fig. 4. Effects of CO₂ enrichment on quality of mature cherry tomato fruits. Control and +CO₂ correspond to normal condition and CO₂ enrichment by fermentation of CRAM. *, indicates significant differences between +CO₂ and control at the level of 0.05.

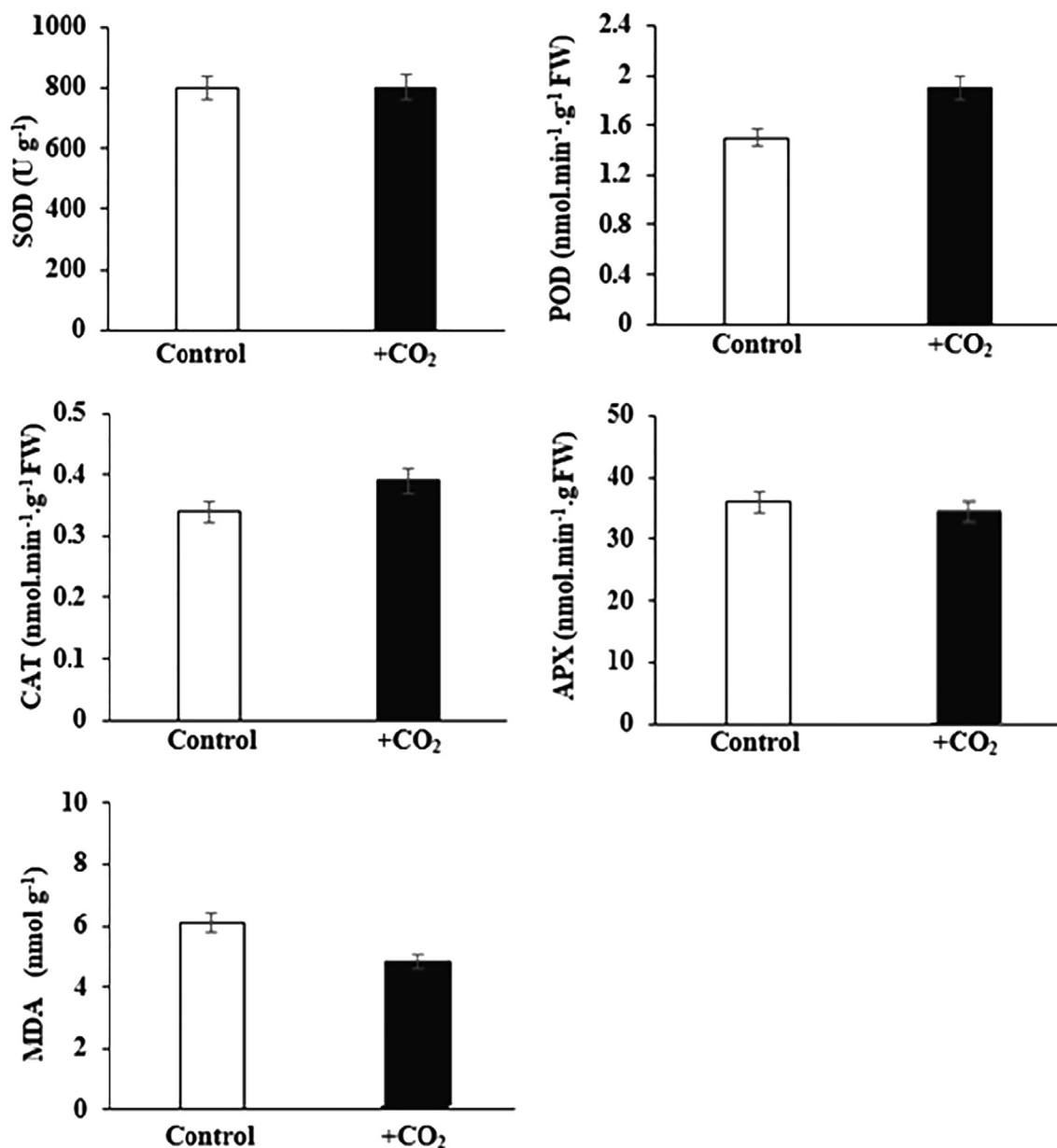


Fig. 5. Effect of CO₂ enrichment on anti-oxidant enzymes activities of cherry tomato. Control and +CO₂ correspond to normal condition and CO₂ enrichment by fermentation of CRAM. *, indicates significant differences between +CO₂ and control at 0.05.

greater time period) required for the successful growth of plants in the greenhouse, as compared to other procedure like chemical production (1–2 h). In addition, the CRAM procedure is easy and simple to operate in the greenhouse. It can be made from common material like bamboo, plastic and timber etc. Therefore, the cost of this technique is very low and economical for farmers. This study revealed that the CRAM-CO₂ enrichment increased the production of cherry tomatoes in greenhouse notably. However, it has also been studied that too high concentration of CO₂ may have adverse effects on plants (Yuming et al., 2015). This possible negative effect can be controlled in the greenhouse, by altering the number or the size of the composting unit (CRAM). For 100 m³ greenhouse, almost 4.5 kg of moist manure and approximately 14 kg of agriculture wastes are required for optimal production of CO₂ in the greenhouse (Yu, 2005).

Many studies reported that optimum CO₂ concentrations are important in order to obtain massive yield and higher quality of

vegetables in the greenhouse (Buddendorf and woltering, 1994). In general, elevated CO₂ can enhance the production of vegetables (Long et al., 2006; Parajuli et al., 2019). It has been frequently observed that elevated CO₂ increased the fruit set percentage of tomato 226 (Mamatha et al., 2014). Current study reveals significant alteration in size, weight, number of fruits, quality, yield and duration of ripening under CO₂ enriched cherry tomato as compared to control. For instance, in CO₂ enriched greenhouse, almost all fruits of cherry tomato had changed to yellow colour 10 days earlier than that in control greenhouse. In the present study, plant growth parameters such as leaf width, plant height and plant biomass were found significantly higher for cherry tomatoes grown under CRAM-CO₂ enriched condition. Consequently, under enriched CO₂ condition (Kadamet et al., 2012; Conroy, 2012) increased leaf area was observed as well as plant height was increased for gladiolus plants and *Pinus radiata*, respectively. The concentration of CO₂ was significantly higher in composting

greenhouse as compared to the non-composting greenhouse which results in increased biomass of cherry tomato. In previous literature, it was reported that higher level of CO₂ in the greenhouse, significantly enhanced the growth of the plant, this was probably due to the increased photosynthetic rate of the plant (Heineke et al., 1999; Porter, 1984). Besides morphological traits, beneficial substances i.e. ascorbic acid and soluble sugars were also significantly increased in cherry tomato under elevated CO₂. (Islam et al., 1996) also reported higher ascorbic acid and sugar contents for tomatoes grown under CO₂ enriched condition as compared to ambient concentration. (Idso et al., 2002) also found that the vitamin C content of sour orange (*Citrus aurantium*L.) increased when grown under elevated CO₂ condition and the same scenario was observed for strawberry by (Wang et al., 2003). Many other studies revealed that increased plant growth by increased CO₂ was often correlated with the increased concentration of ascorbic acid and soluble sugars (Springer et al., 2008). In the present study, the concentrations of titrate and soluble solid in cherry tomato were increased in CO₂ enriched greenhouse than that of control and cherry tomatoes quality increases in response to CO₂ enrichment; these results are in consistence with (Mamatha et al., 2014). Furthermore, the concentration of nitrate decreased considerably in cherry tomatoes grown under CO₂ enriched condition as compared to the control. This might be due to increase in nitrate reductase activity under increased CO₂ condition (Fonseca et al., 1997) or due to the dilution effect of vigorous growth (Vornee et al., 2002). As we know, nitrate is non-toxic itself but its conversion into metabolites like nitrite and N-nitroso compounds (NOCs) has an adverse effect on human health, among metabolites NOCs are highly carcinogenic (Hill, 1999; L'HirondelandL'Hirondel, 2002). The enhanced (Pn) at higher CO₂ concentration increased plant growth as well as yield in both C3 and C4 crops (Reddy et al., 2010). In the present study, we found that the Photosynthetic parameters such as Pn, Gs, Ci, Tr increased significantly for cherry tomato plants grown under elevated CO₂ enriched condition as compared to the control. (Kimball et al., 2002) also observed a significant increase in Pn along with Gs, Ci and Tr for tomatoes which were exposed to CO₂ enriched condition. Moreover higher chlorophyll content was observed for cherry tomatoes grown under high CO₂. In previous studies, it was reported that the constraint of plants/crops for resourceful photosynthesis was satisfied by generated CO₂ during the growing period (Springer et al., 2008). In contrast, the MDA concentration was substantially higher in cherry tomato plants growth in control greenhouse as compared to cherry tomatoes grown under CO₂ enriched condition. This suggests that cherry tomato plants grown under CO₂ enriched condition developed an efficient antioxidant defensive system for ROS. Moreover, under CO₂ enriched condition POD and CAT remarkably increased for cherry tomatoes growth under CO₂ enriched condition but SOD remains unchanged in both treatments. These results indicated that the CRAM-CO₂ enrichment in greenhouse triggered an increase in POD and CAT antioxidant enzymes allowing cherry tomato plant to perform better under CO₂ enriched circumstances. These results are inconsistency with the previous finding, reporting increased antioxidative-enzymes activities in response to CO₂ enrichment for tomato plants (Wangjun et al., 2004; Lenthéric et al., 2003). Further, the higher ascorbate levels observed in the CO₂ treated fruits were probably related to the decrease in APX activity. These specific changes in activity may explain the increase in the overall production of cherry tomato grown under CO₂ enriched condition.

Overall, the increase in fruit set percentage along with a number of fruits per plant resulted in higher fruit yield and quality per plant at CRAM-CO₂ enriched greenhouse (Table 4) was probably because of increase in sink strength as compared to source strength in cherry tomatoes. Furthermore, more carbohydrate might be partitioned to the tomato fruit, during the fruit develop-

ment which leads to higher yields. Our findings are consistent with the previous reports (Yelleet al., 1990; Reinert et al., 1997) which also observed an increase in yield for tomato plants grown under CO₂ enriched condition. Similarly (Islam et al., 2006) found significantly developed fruits for tomato plants grown under CO₂ enriched condition. Thus control on climate in the greenhouse could lead to the significantly higher yield and quality of cherry tomato.

6. Conclusion

In the current study, our findings suggest that CO₂ enrichment in greenhouse via using CRAM technique is an efficient approach to increase CO₂ concentration. More importantly, this technique is an economical and easily adaptable management tool for farmers to use in the greenhouse for cherry tomato production. Consequently, the implementation of this technique would readily be accepted by the farmers as it is economical as well as increases the quality and quantity of cherry tomato production. Furthermore, by using this strategy the environmental pollution problems caused by improper disposal of agricultural bi-products and by burning of crop residues can be reduced significantly.

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