Original Research Article

Impact of nutrients and biofertilizers on stress tolerance in tomato under elevated CO_2induced high temperature condition

ABSTRACT:-*The goal of the study was to see how different nutrients and biofertilizers affected the stress tolerance of the tomato varietyVellayani Vijay underelevated CO_2 inducedhigh temperature condition.A pot culture study was done (mention appropriate conditions of study)with treatmentsincluded 50 ppm B + 50 ppm Zn + water spray (T1), 75 ppm B + 75 ppm Zn (T2), POP 150 ppm N + 125 ppm P+ 125 ppm K (T3), Azolla (Soil application) (T4), Azolla biofertilizerextract (20 %) (foliar application) (T5),Azolla biofertilizerextract (20%) (seed treatment) (T6), POP, KAU + PGPR1(T7), Control (water spray) (T8), Absolute control(T9). The foliar application of Azolla biofertilizerextract (20%) was found to be effective in inducing stress tolerance in tomato plants under elevated CO_2 condition by improving the relative water content, cell membrane stability index and prolinecontent. So it positively influenced the yield and quality of tomato under elevated CO_2 condition.*(how the yield and quality of tomato was positively influenced as no data is provided on growth and yield attributes)

Keywords: Biofertlizers, Climate change , Elevated CO_2 , Nutrients, Stress tolerance, Tomato

Introduction

Climate change refers to the long term variation in the average atmospheric condition by natural and artificial causes. Crop output may be severely impacted bythis and yield of the crop are negatively impacted by these meteorological factors in one way or another, which will lower food security and create financial losses to the farmers.The increase in greenhouse gases and associated higher temperature are becoming a major threat to humanity.CO_2 is an important greenhouse gas influencing crop growth and development directly.The CO_2 concentration in the earth's atmosphere is expected to reach nearly 411 ppm (Bhargava *et al*., 2021) which shows an increase of around 47% within the last 150 years. One of the most remarkable impacts of increasing CO_2 is the increase in atmospheric temperature.The rise in atmospheric temperature affectsphotosynthesis and stomatalbehaviour, which are central to plant C(full form)and water metabolism. Therefore, growth of plants under elevated CO_2 leads to a large variety of secondary effects or plant physiology resulting in modifications of performance and quality of crops. (How high temperature affects tomato? Please supplement data in the light of earlier published literature)

Agriculture,one of thelargest sectors inIndian economy. In 2020–21, the GDP of India will include an agricultural component of roughly 19.9%. In addition, 42.6% of Indians are employed in this area.Climate change can have an impact on irrigated agriculture productivity across all agro-ecological zones, because of the rise in temperature and variations in water supply.Climate change has led to a recent fall in food output, which might have a significant impact on agriculture industry earnings. Consequently, it is imperative that we adopt the climate-resilient agriculture (CRA) strategy and also execute it more rigorously in our farming practises.(Write in brief the objective and relevance of the present study.)

MATERIALS AND METHODS

The researchstudy was conductedat the Department of Plant Physiology, College of Agriculture, Vellayani, Kerala Agricultural University,Kerala, India.The tomato variety Vellayani Vijay was used in a pot culture experiment using the Open Top Chamber (OTC) facility with increased CO2 levels (500 ppm CO_2).Tomato seedlings that were one month old were placed in pots, and once they had established themselves, one set was shifted inside an OTC while the other was left outside as a control.According to the Kerala Agricultural University's package of practises, planting and aftercare were carried out (Estelitta, 2016).Real-time sensors within the OTC chamber were used to measure the microclimate inside the chamber, and the outside weather was also recorded.Three replications and nine treatments were used in the experiment's CRD design.(Provide in tabular form)The treatments comprised of T1: 50 ppm B+50 ppm Zn+water spray, T2: 75 ppm B+75 ppm Zn, T3: POP 150% N+125% P+125%K, T4: *Azolla* (soil application), T5: *Azolla* biofertilizer extract (20%) (foliar application),T6: *Azolla* bio-fertilizer extract (20%) (seed treatment), T7: POP, KAU+PGPR1, T8: Control (water spray), T9: Absolute control.(OTC construction and microclimatic conditions inside and outside OTC to be provided, Preparation of POP, KAU+PGPR1, provide full forms of the minerals with symbols in brackets and full form of POP, KAU+PGPR1)Briefly provide the planting and aftercare practices of tomato plants and how the data was collected on growth parameters at 75 days after sowing.What is the difference between control and absolute control?

Azolla biofertilizer extract (20%) preparation

1 KG of *Azolla* was boiled with 1 litre of distilled water for 30 minutes, then this mixture isfiltered using a cloth and the resultant solution gives the *Azolla* biofertilizer extract of 100% concentration (Hanafy and Gehan, 2018).About 80% distilled water was added to this filtrate to make it 20% concentration. After transplanting, foliar spraying of *Azolla* bio-fertilizer extract(20%) was carried out every 15 days.

The following list of stress tolerance -related parameterswere given below. (Grammatical error)

Relative water content (%)

Relative water content(RWC) was estimated as per Barr and Weatherly (1962) by measuring the fresh weight, turgid weight and dry weight of known number of leaf discs from the experimental plants. After measuring the fresh weight of the sample, it was submerged in distilled water for 3 hours and then the turgid weight was taken. The dry weight of the sample was measured after keeping the samples in oven at 80 ^{0}C for 3 consecutive days. The Relative water content was calculated using the following formula;

$$\text{Relative water content} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

Cell membrane stability index (%)

Cell membrane stability index was estimated as per Sairam *et al.,* (1997)by taking two sets of leaf material (100mg) in test tubes containing 10 ml double distilled water. First set was heated at 40 ^{0} C for 30 minutes in a water bath and recorded the electrical conductivity of the solution(C1). Next set was boiled at 100 ^{0} C for 10minutes on a boiling water bath and electrical conductivity (C2)was recorded.

Cell Membrane Stability Index = (1- C1/C2) ×100

Proline content (µMg $^{-1}$ tissue)(Ref)

Prolines are precipitated as a protein-sulphosalicylic acid complex during extraction of tissue with sulphosalicylic acid. The extracted proline is made to react withninhydrin under acidic conditions to form a red colour which is measuredcolorimetrically at 520nm. 0.5g of tissue was homogenized in a pestle and mortar with 10 ml of 3% aqueous sulphosalicylic acid and filtered through Whatsman No.2 filter paper. Extraction and pooling the filtrate was repeated. To 2 ml of filtrate, 2 ml each of glacial acetic acidand ninhydrin was added and mixed. It was then kept in boiling water bath for 1hr and then the reaction was terminated by placing on ice bath. 4 ml toluene was added and mixed vigorously for 20-30 sec. The chromophore (toluene) layer was aspirated and warmed to room temperature. The absorbance of red colour was measured at 520nm against a reagent blank. The amount of proline in the sample was calculated using a standard curve prepared from pure proline (range 0.1- 36 µ mole) and expressed on fresh weight basis of sample.

$$\mu M \text{ of proline / g tissue} = \frac{(\mu g \text{ proline / ml}) \times \text{ml toluene}}{115.5 \text{ g sample}} \times 5$$

All the above mentioned observations were recorded at 75 days after sowing (DAS)

RESULTS AND DISCUSSION

Provide data on how the performance of tomato plant was improved, give the growth performance of the tomato plants in terms of stem length, root length, plant height, number of leaves, fresh/dry weight for all the treatments as compared to controlunder OTC and Open conditions.

(Provide table on growth parameters of tomato 75 DAS in control and treated plants under OTC and Open conditions.)

Relative water content (%)

Relative water content of tomato leaves were recorded at 75DAS to study the effect of various nutrients and biofertilizer application and the result is presented in table 1. The treatment T5 (96.15%) showed higher value in elevated CO_2 at 75DAS compared to absolute control(92.28%). The lowest value for RWC was recordedfor T7 (88.17%). RWC was found to decrease in elevated CO_2 condition (91.19%) than opencondition (93.12%).

Cell membrane stability index (%)

The stability ofleaf cell membrane under elevated CO_2 and temperature was assessed by measuring cell membrane stability index. The effect of application of various nutrients and biofertilizers on cell membrane stability of tomato plants were recorded and presented in table 2. At 75 DAS, the highest significant value (53.83%) was observedfor T1 and lowest for T9(37.48%). A significant increase in cell membrane stability index was noticed under CO_2 enriched condition (39.10%) compared to open condition (52.50%) at 75DAS.

Proline content (µMg $^{-1}$ tissue)

Influence of application of various treatments on the proline content of tomatoleaves under elevated CO_2 condition was estimated and the result was presented in thetable 3. At 75DAS, significantly higher proline content was recorded by T1(0.15 µMg $^{-1}$tissue). Plants kept inside OTC was found to have significantly higher proline content at75 DAS(0.17 µMg $^{-1}$ tissue) compared to open condition (0.06 µMg $^{-1}$ tissue).

Relative water content is considered as the measure of plant water status, reflecting the metabolic activity in tissues. Elevated CO_2 concentration and associated high temperature is having impact on the water status of plants through stomatal mechanisms and characteristics. The water status of the leaves affects a variety of physiological characteristics such as stomatal conductance, transpiration rate, photosynthetic rate, respiration as well as the growth and development. In the present study, RWC of the plants under elevated CO_2 and ambient conditions did not show any significant difference. This was contradictory with results of Robredo *et al.* (2007) who reported that to increased CO_2 resulted in improved RWC in barley. Among treatments foliar application of 20% *Azolla* biofertilizer extract improved the relative water content of tomato under elevated CO_2 condition by 4.19%. The *Azolla* biofertilizer extract enhanced the root formation and root length and helps in better absorption of water from deeper layers of soil thus maintaining a better water status and contributing to improved RWC. (Discuss along with the results)

The stability of leaf cell membrane under elevated CO_2 and temperature was assessed by measuring cell membrane stability index. The heat stress response (Chinnusamy*et al*., 2004) is a complex phenomena that involves a variety of physiological changes that result in cell membrane damage. Heat-induced cell membranedamage results in membrane protein denaturation, enzyme inactivation, and changes in membrane permeability and integrity, resulting in reduced ion flux, electrolyte leakage, changes in relative water content, toxic compound production, and a general

disruption of homeostasis that reduces cell viability. A decreased cell membrane stability of 25.52% was observed under elevated CO_2 condition compared to open condition. Dwivedi *et al*.,2015 was measured the membrane stability index (%) to assess the stability of the cell membrane of the plants leaf inside open top chambers under elevated CO_2 and temperature condition .The mean MSI (11%) was increased under elevated CO_2 conditionacross the cultivars while there was decline in mean MSI (14%) under elevated temperature condition. Considering the effect of various treatments on cell membrane stability index, foliar application of 20% *Azolla* biofertilizer extract (23.71%) and foliar application of 50ppm B+ 50 ppm Zn+ water spray(30.37%) has showed the highestpercentage increase compared to absolute control. The application of B can increase antioxidant activities of plants and alleviating heat stress induced reactive oxygen species (ROS) damage (Eisvand*et al*., 2018) and *Azolla* biofertilizer extract influences the N uptake with a reduction in membrane oxidative damage(Maswada*et al*., 2021). (Discuss along with the results)No discussion is provided on proline content.In what way proline is helpful in improving the stress tolerance of tomato plants under high temperature.

CONCLUSION

The present study indicated the improved performance of tomato variety Vellayanivijay upon exposure to elevated CO_2. The impact of application of 50% extra N and 25% extra P and K (T3) each than therecommended doses as well as foliar application of B and Zn indicate the changingnutrient requirement of tomato under the current scenario of increasing atmospheric CO_2concentration. The response of experimental plants to foliar application of *Azolla* extract(20%) strongly suggest the effectiveness of this biofertilizer in overcoming the impacts of elevated CO_2 induced high temperature and thereby improving the plant performance. Antioxidants being economically viable secondary metabolites, the role of *Azolla* in this regard can also be further exploited in different crops. The application of extra nutrients also was found to strengthen the system by improving many of the physiological stress tolerance markers like membrane stability index, chlorophyll stability index and by enhancing non enzymatic antioxidants.How proline is helpful in improving stress tolerance?

Recommendations based on the best treatments can be developed by extending the study to field condition since the treatments were found to improve the yield and quality even under ambient condition.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

TREATMENTS	Open	OTC	Mean
T1 50 ppm B + 50 ppm Zn + water spray	89.67	92.48	91.08
T2 75 ppm B + 75 ppm Zn	89.56	92.56	91.06
T3 POP 150% N + 125 % P+ 125% K	97.16	93.72	95.44
T4 *Azolla* (Soil application)	95.32	83.61	89.47
T5 *Azolla* biofertilizer extract (20%) (Foliar application)	95.89	96.41	96.15
T6 *Azolla* biofertilizer extract (20%) (Seed treatment)	93.61	95.14	94.38
T7 POP, KAU + PGPR1	91.47	84.86	88.17
T8 Control (water spray)	96.23	86.43	91.33
T9 Absolute control	89.12	95.44	92.28
MEAN	93.12	91.19	
	T	E	T×E
SE+ -	1.90	0.95	2.67
CD	0.68	0.34	0.95

Table 1. Effect of nutrients and biofertilizers on relative water content(%) in tomato under elevated CO_2 condition

TREATMENTS	Open	OTC	Mean
T1 50 ppm B + 50 ppm Zn + water spray	61.21	46.45	53.83
T2 75 ppm B + 75 ppm Zn	53.14	44.32	48.73
T3 POP 150% N + 125 % P+ 125% K	54.01	44.25	49.13
T4 *Azolla* (Soil application)	49.21	39.32	44.27
T5 *Azolla* biofertilizer extract (20%) (Foliar application)	55.47	43.01	49.24
T6 *Azolla* biofertilizer extract (20%) (Seed treatment)	53.51	37.28	45.39
T7 POP, KAU + PGPR1	51.01	35.01	43.01
T8 Control (water spray)	49.23	33.48	41.36
T9 Absolute control	46.01	28.94	37.48
MEAN	52.50	39.10	
	T	E	T×E
SE+ -	0.69	0.25	1.19
CD	1.92	0.71	3.33

Table 2. Effect of nutrients and biofertilizer application on cell membrane stability index (%)of tomato under elevated CO_2 condition at 75 DAS

TREATMENTS	Open	OTC	Mean
T1 50 ppm B + 50 ppm Zn + water spray	0.08	0.21	0.15
T2 75 ppm B + 75 ppm Zn	0.05	0.19	0.12
T3 POP 150% N + 125 % P+ 125% K	0.05	0.20	0.13
T4 *Azolla* (Soil application)	0.06	0.15	0.11
T5 *Azolla* biofertilizer extract (20%) (Foliar application)	0.06	0.17	0.12
T6 *Azolla* biofertilizer extract (20%) (Seed treatment)	0.06	0.18	0.12
T7 POP, KAU + PGPR1	0.05	0.16	0.11
T8 Control (water spray)	0.05	0.17	0.11
T9 Absolute control	0.06	0.13	0.09
MEAN	0.06	0.17	
	T	E	T×E
SE+ -	0.01	0.01	0.02
CD	0.03	0.02	NS

Table 3. Effect of nutrients and biofertilizer application on proline content (µM g^{-1} tissue) of tomato under elevated CO_2 condition at 75 DAS

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