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Original Research Article

AMELIORATION OF CADMIUM STRESS ON TOMATO (*LYCOPERSICON ESCULENTUM*) BY TRIACONTANOL APPLICATION

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Abstract *Lead (Pb), copper (Cu), zinc (Zn), and cadmium (Cd) tend to build up in the roots of tomato plants and are then taken up by the leaves and fruits, resulting in significant removal from the soil. Plant growth and development are at risk due to the soil's buildup and toxicity of heavy metals (HMs). Cadmium, a hazardous element, inhibits the development and yield of tomatoes. Triacontanol (TRIA) enhances plant development when exposed to low concentrations of Cd, hence mitigating its harmful effects. This work used the exogenous triacontanol (TRIA) application to mitigate the adverse physiological impacts of cadmium (Cd) on tomato plants. The current study investigated tomatoes' physical characteristics, functions, and chemical composition to get a deeper understanding of how Triacontanol responds to stress caused by cadmium. Two experiments were laid out under a Completely Randomized Design (CRD), and three replications under a hydroponic system. In the first experiment, the effects of Cd on tomato were grown hydroponically and exposed to cadmium chloride at five concentration levels (control, 1.5, 03, 06 and 12µmol/L). While in the second experiment tomato grown hydroponically, and cadmium used in the form of CdCl2 at five concentration levels (control, 1.5, 03, 06 and 12µmol/L) with one level of TRIA 10µM/L was be applied to facilitate the Cadmium stress on plant. The Hoagland's solution was used to irrigate the plants after the initial 72 hours of pretreatment in both experiments at 35±1℃; 70-75%RH. Data for tomato plants' morphological, physiological, and biochemical characteristics was recorded. Results revealed that mitigation through triacontanol (TRIA) @ 10µmol/L best affects cadmium stress. It was noted that triacontanol (TRIA) @ 10µmol/L significantly affects tomato plants' morphological, enzymatic, and physiological characteristics at 1.5µmol/L of cadmium stress.*

Keywords: *Tomato; Cadmium Stress; Triacontanol (TRIA); Heavy Metal Toxicity; Plant Physiology; Hydroponic System; Morphological Characteristics, Enzymatic Response, Biochemical Analysis; Mitigation Strategies*

Introduction

The tomato, scientifically known as Lycopersicon esculentum, belongs to the Solanaceae family, and other family members include eggplant, chilli peppers, potato, and tobacco. (Svobodová and Kuban, 2018). The tomato, a widely grown vegetable crop, is widely consumed in many culinary forms. As the fourth most popular fresh-market vegetable after potato, lettuce, and onion, it is a nutrient-rich food that contains water, proteins, fibres, carbs, calories, a significant amount of vitamins, and a wide range of thirty-seven minerals (Tanveer *et al.*, 2019). The tomato plant is a perennial, meaning it grows back every year in mild climates where it is commonly planted outside. A weak, woody stem often develops in a vine-like fashion around neighbouring plants, and the plant typically reaches a height of 1-3 m (3 to 10 ft). Each five to nine leaflets on an otherwise pinnately structured leaf may grow eight centimetres long. The leaves range in length from 10 to 25 centimeters. A profusion of glandular trichomes adorns the stem and leaves, making them stand out. This small flower has five pointed lobes on its corolla and is golden. Its diameter is around 1-2 cm. A cluster of three to twelve flowers is arranged in a cymose fashion (Smith, 2001; Bionity, 2022). The tomato is native to Central, South, and the southern portions of North America, ranging from Mexico to Peru (Bionity, 2022). Tomatoes are widely grown in warm temperate and tropical locations (Basyal *et al.*, 2019). In the fiscal year 2011-2012, Pakistan's agricultural landscape saw a total tomato production of 577,900 metric tonnes. This yield was obtained from cultivating 57,400 hectares of land devoted to growing tomatoes. Khyber Pakhtunkhwa province contributed by dedicating 13,700 hectares of agricultural land,

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resulting in an annual output of 130,150 metric tonnes. During the last twenty years, Pakistan's area of land dedicated to tomato cultivation has seen a significant increase of 54% (Imran, 2022). Tomato fruits are composed predominantly of water, comprising approximately 94% of their overall composition (Klunklin and Savage, 2017), These fruits contain measured quantities of soluble carbohydrates, a diverse array of organic acids (Li *et al.*, 2022), many organic acids such as citric, malic acids, aminobutyric acid, cis-aconitic acid, and formic acid, in addition to some histidine, lysine. Additionally, (Agius *et al.*, 2022) have identified the presence of histidine and lysine, while (Fuentes *et al.*, 2022) have reported the occurrence of minerals and mineral salts. Moreover, tomato fruits exhibit elevated concentrations of essential vitamins, including but not limited to vitamins A, B1, B2, and C, as articulated by (Mengistie and Awlachew, 2022). Tomatoes are versatile beyond fresh consumption via culinary changes. These precise operations produce soups, sauces, liquid extracts, and powders. The versatile tomato fruit is utilized in sauces, soups, protein-packed foods, and salads. Additionally, tomatoes provide the basis for purées, sauces, and refreshing liquids. Canned and dried tomato derivatives are economically significant. Tomatoes are high in carbs, sugars, fibre, proteins, and lipids (Eidelman, 2013). Lycopene is advantageous for human health due to its antioxidative properties, which may counteract free radicals and help prevent or repair cellular damage. Tomato fruits that have been cooked are more efficient. Tomato also protects blood constituents against the adverse impacts of lead (Mallick PK, 2021).

Metal (HM) stress is a major environmental barrier to plant growth, development, and production (Anjum *et al.*, 2021;Singh *et al.*, 2016; Dhalaria *et al.*, 2020). Over 5 g/cm3 elements are classified as HMs. Hence 53 of the 90 naturally occurring elements are HMs (Khalef *et al.*, 2022). Heavy metals, including cadmium (Cd), zinc (Zn), copper (Cu), chromium (Cr), mercury (Hg), and lead (Pb), may impair human health, agriculture, and natural ecosystems (Adefemi and Awokunmi, 2013). Heavy metals are absorbed by plants when they are naturally soluble in soil or when root exudates make them soluble (Mao *et al.*, 2022). Plant growth and upkeep need heavy metals. However, high amounts of these elements may harm plants. Plants may also obtain non-essential elements by gathering important metals (Aliu *et al.*, 2022). Non-degradable metals harm plants at high amounts. High metal concentrations block cytoplasmic enzymes and destroy cell structures via oxidative stress (Goyal *et al.*, 2020). The displacement of essential nutrients at plant cation exchange sites causes indirect damage (Upadhyay, 2022). Heavy metals harm soil microbes, which affects plant growth indirectly. High metal concentration may reduce soil microorganisms, reducing organic matter decomposition and soil fertility. Heavy metals and soil microorganisms may impede enzyme activity, essential to plant metabolism. Damage, whether direct or indirect, slows and kills plant growth (Chibuike and Obiora, 2014). Heavy metal toxicity affects plant growth and development differently depending on the metal. Metals, including Pb, Cd, Hg, and As, which do not benefit plant development, are detrimental even at low-growth medium concentrations (Tangahu *et al.*, 2011). High levels of readily absorbed heavy metals generate free radicals and reactive oxygen species in plant cells. Unregulated oxidation starts a chain reaction involving nucleic acids, proteins, and lipids. Cell damage and oxidative stress come from this mechanism. Thus, sensitive plants grown in heavy metal-exposed locations have altered metabolic processes and reduced growth, biomass production, and yield (Goyal *et al.*, 2020). The worldwide problems of heavy metal toxicity and bioaccumulation harm plants, animals, and humans. Industrialization, urbanization, and agriculture have degraded the soil and ecology in most industrialized and growing nations (Anjum *et al.*, 2021; Sahab *et al.*, 2021). Cadmium is toxic to all living things and does not seem involved in metabolic activities. Cadmium in the soil is easily transferred through the plant and distributed to all organs (Dutta *et al.*, 2020). According to the WHO, plant Cd concentrations should be between 0.2 and 0.8 mg kg-1 (Grochowska-Niedworok *et al.*, 2020). Cadmium poisoning causes oxidative stress, which weakens the immune system and generates reactive oxygen species (ROS). ROS, or reactive oxygen species, targets carbohydrates, enzymes, and proteins, increasing cell membrane lipid peroxidation (Suhani *et al.*, 2021). Cd content classifies plants as Cd accumulators or avoiders. Cadmium (Cd) may alter plant absorption, transport, and use of potassium, calcium, magnesium, and phosphorus. It also affects plant water control (Mourato *et al.*, 2018). High Cd levels cause chlorosis, growth inhibition, root browning, and plant mortality (Haider *et al.*, 2021a).

Cadmium affects root hair development, cell wall properties, and transporter function (Migocka and Klobus, 2007; Loix *et al.*, 2017; Bahmani *et al.*, 2016). Plants absorb water and nutrients inconsistently (Perfus-Barbeoch *et al.*, 2002; Souza *et al.*, 2018). Cadmium toxicity causes too much ROS, destroying plant membranes, cellular macromolecules, and organelles (Abbas *et al.*, 2018). (Xu *et al.*, 2017) cadmium inhibits plant iron and zinc absorption, producing leaf chlorosis. Plants lose iron and photosynthesis due to Cd's reduction of root

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Fe (III) reductase and other enzymes. It reduces aerial plant nitrate uptake and transport by inhibiting nitrate reductase. Cadmium (Cd) also impacts carbon dioxide-fixing enzymes (Piršelová and Ondrušková, 2021). (Hossain and Komatsu, 2013; Jamla *et al.*, 2021; Emamverdian *et al.*, 2015) thoroughly investigated plant heavy metal stress in plants. This research has helped us understand plants' structural, functional, chemical, cellular, and molecular reactions to high heavy metal levels (Sofo *et al.*, 2022; Muratova *et al.*, 2022; Thakur *et al.*, 2022). Plants compartmentalize, structurally change sections, manage osmotic pressure, activate antioxidant defense systems, maintain ion balance, and increase metal ion transporter development under heavy metal (HM) stress (Shariatipour *et al.*, 2022; Waqeel and Khan, 2022; Khan *et al.*, 2022). Chemical and biological methods may lessen heavy metal toxicity's impact on polluted land and water. Chemical precipitation, filtration, electrochemical treatments, coagulation/flocculation, phytoremediation, soil remediation, stabilization or in situ immobilization, excavation, and ion exchange remove heavy metals (Sarwar *et al.*, 2021, 2022; Parveen *et al.*, 2022; Rajendran *et al.*, 2022; Liu *et al.*, 2018) examined methods. (Feng *et al.*, 2022; Wilson *et al.*, 2022; (Xiong *et al.*, 2022); (Sharma and Bhateria, 2022) found that biochar, compost, and nanoparticles boost plant growth and minimize heavy metal toxicity. Significant efforts have been undertaken to develop heavy metal-resistant agricultural plant kinds. These methods include genomics, genetic engineering, and plant breeding (Saraswat *et al.*, 2022). Phytohormones may increase heavy metal stress tolerance in the environment (Ezeh *et al.*, 2022). Despite low quantity, phytohormones and chemical signals influence plant development and growth (EL Sabagh *et al.*, 2022). They also stimulate non-living signal transduction pathways (Meena *et al.*, 2022).

Prior research suggests that applying external phytohormones has effectively improved the plant's capacity to withstand stress when subjected to heavy metals (Kaur *et al.*, 2022). Furthermore, when exposed to HM, the natural levels of phytohormones are changed, affecting several plant stress adaptation characteristics (Sytar *et al.*, 2019). Expression profiling, mutation testing, microarray analysis, proteomics, system biology, and bioinformatics have established a robust framework for identifying and characterising phytohormone-mediated molecular pathways that enable plants to withstand heavy metal stress (Singh *et al.*, 2016). However, phytohormones' particular involvement in regulating heavy metal stress signaling components remains unclear. Plant growth regulators modulate several physiological and biochemical processes to increase stress tolerance (Kumar and Verma, 2018). Their pivotal functions promote exploring novel phytohormones and clarifying their involvement in governing various plant physiological functions (Sarwar *et al.*, 2021, 2022;Sabagh *et al.*, 2021). Triacontanol is a newly synthesized phytohormone that, when applied topically to several plant species, has shown the capacity to promote plant growth at very low doses. Across many crops, TRIA has improved photosynthesis, protein synthesis, water absorption, mineral nutrient uptake, nitrogen fixation, enzyme activity regulation, and the accumulation of different organic compounds in leaf tissues (Sarwar *et al.*, 2021, 2022; Verma *et al.*, 2022). TRIA has attracted substantial attention in recent years as a plant growth regulator. It has the potential to greatly affect the capacity of some plants to tolerate abiotic stresses such as salt, cold, and arsenic toxicity (karam *et al.*, 2017).

Objectives

The primary goals of my research endeavour are:

1. The aim of this investigation is to ascertain if Triacontanol can be used as a treatment to reduce the negative impact of cadmium stress on tomato plants.
2. To assess the changes in the morphology, physiology and biochemical characteristics generated by exogenously administered Triacontanol on Tomato under cadmium stress.

MATERIALS AND METHODS

A triacontanol dosage optimization experiment was done to ascertain the most effective amount for tomato plants in mitigating the effects of cadmium-induced stress.

Plant growth and treatment

Located at 29◦22’36" N and 71◦45’ 42" E, the Department of Horticulture experimental site at IUB was used to seed the tomato crop. We chose a tomato genotype that does well in stressful environments because it is well-adapted. The seeds were first sanitised with 95% ethanol, distilled water was used for three rinses., and then cleaned with a 70% sodium hypochlorite solution. The seeds of a single tomato genotype were planted in plastic containers containing fine sand. After seed germination, seedlings were irrigated with half strength Hoagland solution as a growth regulator. After the germination, they were transplanted in hydroponic tubs at the four-leaf stage. Before transplanting, the roots of plants are washed completely with distal water to prevent contamination. After the transplanting, half the strength of Hoagland solution was applied to the distilled water solution with the same ratio for all tubs. The treatments (two sets of tubs) were as follows: (i) Control, treated with a solution of cadmium in the form of $CdCl_2$ 1.5 μmol/L, $CdCl_2$ 6 μmol/L, $CdCl_2$ 9 μmol/L, $CdCl_2$ 12 μmol/L in absence of Triacontanol (ii) Control, treated with cadmium in the form of $CdCl_2$ 1.5 μmol/L, $CdCl_2$ 6 μmol/L, $CdCl_2$ 9 μmol/L, $CdCl_2$ 12 μmol/L and

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application of Triacontanol @10 µmol/L for each tub. The solution was in three different intervals after 24 hours of the last application. Each treatment had three replications. In the tubs of the hydroponic system, 6 air pumps were installed for the aeration.

Harvesting and Measurement of Growth Attributes

After 45 days of planting, the crops were harvested. After the plants were harvested, quick measurements were performed at the research site to determine their fresh weights (FWs), root and shoot lengths, and overall plant size. Fresh samples were kept in a biomedical refrigerator at a temperature of -30 degrees Celsius to conduct fresh analysis. To get the dry weights (DWs) of the samples and perform an ionic content analysis using the microwave digestion method, each treatment had three samples that were oven-dried at a temperature of 65 degrees Celsius for three days.

Finding the lengths of roots

After forty days of development, we removed the seedlings and rinsed them with distilled water to eliminate any sand or other debris. Root length, measured in centimetres (cm) from the base of the hypocotyls to the root tip, was taken from each set of seedlings in triplicate using a meter rod. We found the mean of all the replicates.

Determination of the fresh biomass of plants

After taking precise measurements of the roots' and shoots' lengths, we delicately separated them and wrapped them in filter paper to remove any remaining moisture. This was then reflected in the revised digital balance.

After the stems and roots of each plant were measured, they were dried in a Schawabach drying oven (Memmert-110) at 70 °C for one week after the fresh weights were taken. After measuring the root and shoot dry weights using a digital scale, we averaged the two measurements to get the final weight of each duplicate.

Criteria for root morphology Using the WinRHIZO image analyzing system (Regent Instruments, Quebec, Indonesia), the morphology of the roots and associated data (root length, root average diameter, volume, and root surface area) were examined. An Epson Perfection V800 scanner was used to scan the roots after they were gently dispersed across a plastic box. The scanned root pictures were processed to digitalize the data. We examined the root's surface area and diameter (Naciri *et al.*, 2021).

Leaf morphology parameters

A method developed by Regent Instruments in Quebec, Indonesia, called WinFOLIA, was used to analyse the total leaf area. The Epson Perfection V800 scanner was used to scan the meticulously distributed leaves on a plastic box. Data was digitalized. Analyzed was the total area of the leaves (Naciri *et al.*, 2021).

Measurements of photosynthesis and chlorophyll fluorescence

The approach indicated by (Setal *et al.*, 2016) was followed to assess the chlorophyll fluorescence and photosynthesis in intact broccoli plant leaves using a portable PhotosynQ (Mutispeq VII, USA) equipment between 9:00 and 11:00 am, just before harvesting. To recap, each plant's completely expanded 4th and 5th leaves were utilized to quantify Chl, Fv/Fm, qP, and qL. To evaluate the photosynthetic machinery, we employed linear electron flow (LEF), heat dissipation (NPQt), and assessment of Photosystem-II efficiency.

Assay for Protein

The protein was determined by using enzyme extract and Bradford in 1:4 ratio. Take 400µl enzymes extract+ 1600µl Bradford Reagent and check Absorbance at 595nm on a spectrophotometer.

Finding enzymes that neutralise free radicals

Fresh tomato leaves were analyzed for antioxidant enzyme activity. To achieve this, the leaves used for measuring photosynthesis were sliced, washed with deionized water, and promptly placed in a container suitable for freezing. Next, 1.5 g of leaf material was extracted using a potassium phosphate buffer with a pH of 7.6 (50 mM), Na2-EDTA (0.1 mM), Triton X-100 (0.3% v/v), and polyvinyl pyrrolidone (PVP) (1% w/v). The antioxidant enzyme activities were assessed by centrifuging the samples at 4°C for 12 minutes at 12,500 g. The SOD activity was analyzed following the methodology described by (Giannopolitis and Ries, 1977), while the activities of CAT and POD were determined using the procedures outlined by (Maehly and Chance, 1954).

Experimental Design and Statistical Analysis

Two variable factors: Cadmium (5 in number) and TRIA application. All the numerical data obtained were subjected to statistical analysis using a complete randomized design with two factors factorial at $\alpha = 0.05$ by statistix 8.1 software (Version 8; Analytical software, USA). Analysis of variance (ANOVA) will be obtained for every parameter to find out the significant differences between the variables. Significance among the means was concluded by using Least Tukey HSD application ($p \leq 0.05$ level) application (Steel *et al.*, 1997).

Results

Plant biomass and parameters of root and shoot

Table 1 shows that cadmium stress significantly affected many plant growth indices, including shoot length, shoot fresh weight, root fresh weight, and root dry weight. Linearly, the observed plant development parameters decreased with increasing concentrations of CdCl2 heavy metal. The most notable decrease in plant growth parameters was

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when the greatest concentration (12 µmol/L CdCl2) of heavy metal stress was administered. Compared to the control, the experimental group showed significant differences in the following parameters: root length (-47.72%), root fresh weight (-53.63%), root dry weight (-24.08%), shoot length (-50.20%), shoot fresh weight (-66.27%), shoot dry weight (-49.91%), and root length (-47.72%). As the amount of CdCl2 stress grew, this suggests that plants' ability to accumulate biomass dropped significantly. Triacontanol significantly mitigated the detrimental impacts of CdCl2 on tomato plants when administered at doses of 10 µmol/L TRIA. When exposed to heavy metal stress at a concentration of 1.5 µmol/L CdCl2, TRIA showed its best effects, reducing shoot length by -7.94%, shoot fresh weight by -14.98%, shoot dry weight by -9.65%, root length by -9.70%, root fresh weight by -9.41%, and root dry weight by -7.85% when compared to the control. Additionally, the growth metrics such as root volume, root average area, root surface area, and leaf average area were noticeably reduced when exposed to cadmium chloride at a dosage of 12 µmol/L. Nevertheless, according to Table 2, TRIA reduced heavy metal stress by 34.55% in root volume, 14.19% in root average diameter, 12.67% in root surface area, and 14.70% in leaf average area.

Assessment of antioxidant enzymes

An increase in superoxide dismutase activity was seen in both cadmium stress and cadmium treatments supplemented with TRIA. Cd stress resulted in a 148.42% drop in POD activity relative to the control group. Pretreatment with TRIA may restore CAT activity, as seen in Figure 1. When exposed to 12 µML-1 of CdCl2, the protein activity decreased by 61% compared to the control. Fig. 1 shows that when seedlings were treated to 10 µML-1 of TRIA and 1.5 µML-1 of CdCl2, protein activity was 17.78% lower in the treatment group than in the control group. Stress with CdCl2 reduced CAT activity and carotenoid levels compared to the control group. The exogenous pretreatment enhanced TRIA activity under CdCl2 stress, with a concentration of 1.5 µML-1, resulting in a -24.89% and -6.96% drop, respectively, as shown in Figure 1.

Table 1: **Effect of TRIA on the growth rate of Tomato after six days of CdCl$_2$ application**

TRIA Levels	CdCd$_2$ Levels	Shoot Length (cm)	Shoot Fresh Weight (g)	Shoot Dry Weight(g)	Root length (cm)	Root Fresh Weight (g)	Root Dry Weight (g)
TRIA (0µmol/L)	**Control**	61.25 ab	6.72 ab	1.96 ab	15.37 ab	1.83 ab	0.81 b
	1.5	52.33 bc	5.57 bc	1.76 abcd	11.73 bcde	1.64 bc	0.76 bc
	3	44.33 cd	4.8 cd	1.36 cde	11.33 cde	1.36 cd	0.72 cde
	6	39 de	3.82 de	1.19 de	9.5 de	1.24 d	0.67 def
	12	30.5 e	2.27 e	0.98 e	8.03 e	0.85 e	0.61 f
TRIA (10µmol/L)	**Control**	66.98 a	7.59 a	2.08 a	16.83 a	2.02 a	0.88 a
	1.5	61.67 ab	6.45 abc	1.88 abc	15.2 abc	1.83 ab	0.81 ab
	3	49.83 bcd	5.83 bc	1.45 bcde	12.4 bcd	1.59 bc	0.78 bc
	6	39.83 de	4.77 cd	1.26 de	10.33 de	1.36 cd	0.73 cd
	12	32.17 e	3.71 de	1.04 e	8.3 e	1.12 de	0.65 ef

Table 2: Effect of TRIA on the growth rate of Tomato after six days of CdCl$_2$ application

TRIA Levels	CdCd$_2$ Levels	Root. Vol(cm^3)	Root. avg. Diameter (mm)	Root Surface Area (cm^2)	Leaf avg, Area (cm^2)
TRIA (0µmol/L)	**Control**	1.12 b	2.25 ab	22.5 ab	2.97 ab
	1.5	0.78 bcd	1.94 bc	18.39 bc	2.58 ab
	3	0.63 cd	1.75 bcd	14.16 cd	2.05 ab
	6	0.45 d	1.61 cd	11.73 cd	1.49 ab
	12	0.4 d	1.29 d	11.03 d	1.24 b
TRIA (10µmol/L)	**Control**	1.56 a	2.57 a	25.76 a	3.34 a
	1.5	1.02 bc	2.2 ab	22.5 ab	2.85 ab
	3	0.79 bcd	1.9 bc	17.15 bcd	2.63 ab
	6	0.5 d	1.81 bcd	13.03 cd	1.69 ab
	12	0.49 d	1.4 cd	11.37 d	1.52 ab

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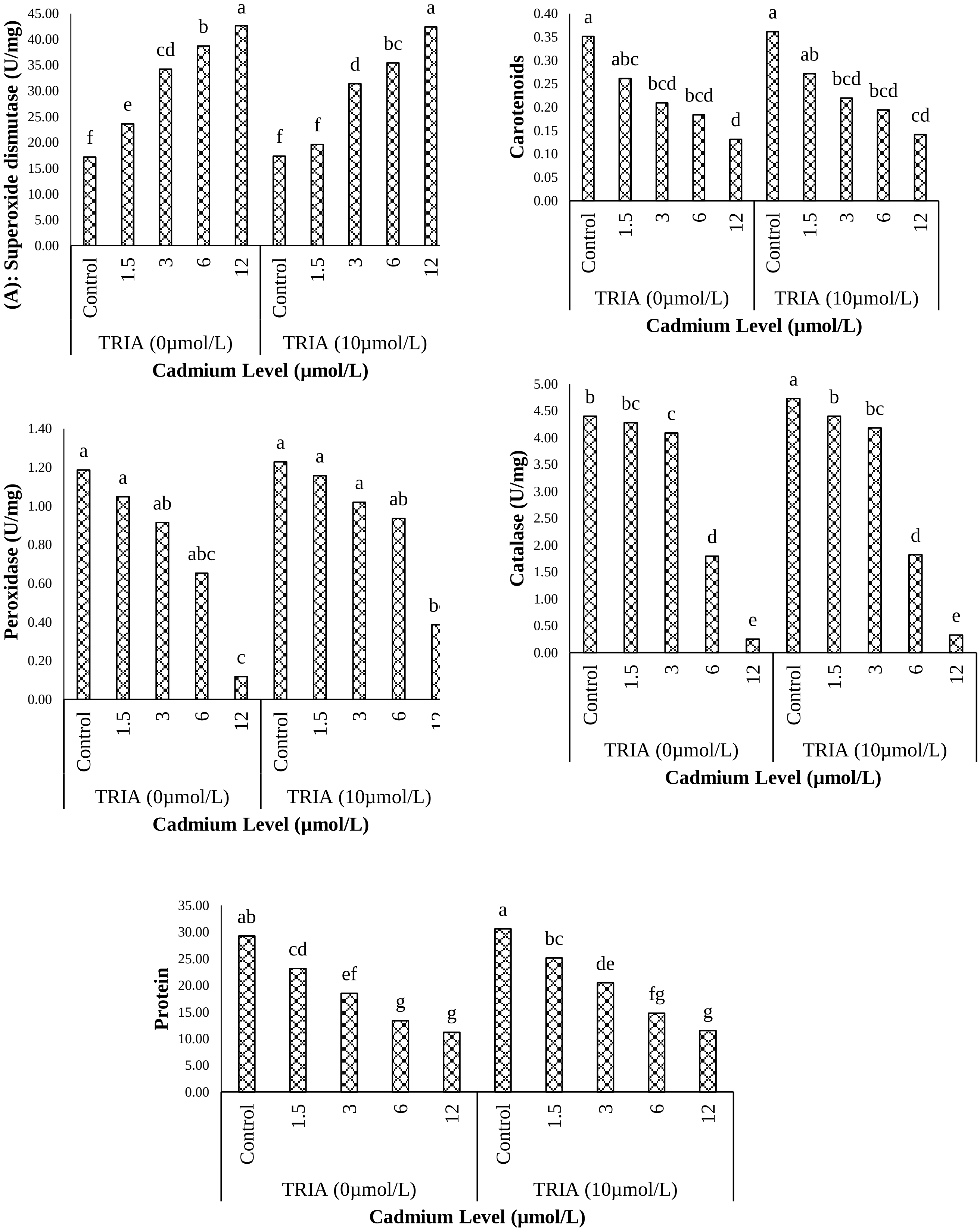

Figure 1 illustrates the impact of cadmium and triacontanol on many aspects of tomato leaves, including the levels of superoxide dismutase (SOD), peroxidase (POD), protein content, carotenoids, and catalase (CAT) content

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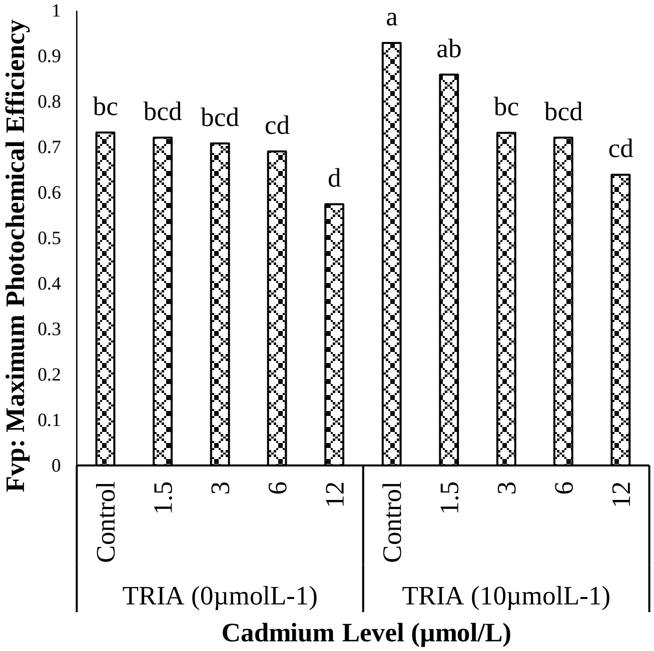
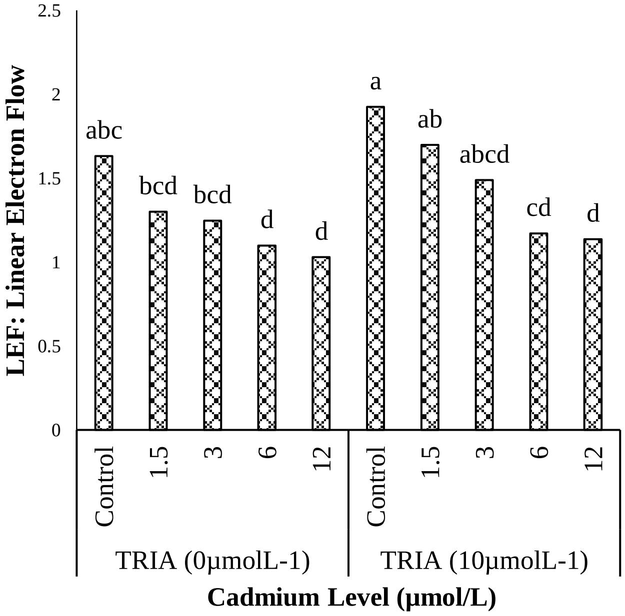
Leaf chlorophyll photosynthesis and fluorescence

The stress caused by $CdCl_2$ resulted in a progressive decrease in the primary light energy conversion rate (Fv/Fm), light energy flux (LEF), and quantum yield of primary photochemistry (qp). However, the application of TRIA increased these parameters, as shown in Figure 2. Nevertheless, the highest value of qL was achieved at a concentration of 1.5 mmol L-1 of $CdCl_2$ in the presence of TRIA. The Fv/Fm ratio declined by 21%, the LEF decreased by 36%, and the qP decreased by 23% when exposed to the maximum Cd level of 12 mmolL-1 $CdCl_2$. On the other hand, TRIA enhanced the Fv/Fm ratio by 8%, the LEF by 11.82%, and the qP by 13.43% at a lower Cd level of 1.5 mmolL-1 CdCl2, compared to the control (Fig. 2). Typically, the control plants had the highest levels of chlorophyll fluorescence. It can be inferred that the excessive accumulation of CdCl2 in tomato cells led to toxicity, which significantly disrupted the light energy conversion process known as LEF. As a result, the rate of converting light energy was negatively impacted. However, when a lower dose of $CdCl_2$ stress (1.5 mmol $CdCl_2$ L-1) was combined with TRIA, there was a significant improvement in the coefficients of photochemical quenching, particularly in the case of qp. The photosynthetic efficiency of tomato leaves was quantified by assessing the chlorophyll content, the efficiency of photosystem II (PSII), non-photochemical quenching (NPQt), and the quantum yield of non-photochemical quenching (PhiNo) (Fig. 3). The CdCl2 somewhat reduced the PSII and Ch (SPAD) levels, while increasing NPQt and PhiNO. The efficient effect of TRIA at a concentration of 1.5 mmolL-1 $CdCl_2$ is shown in Figure 3.

Discussion

Cadmium toxicity is responsible for the reduction of nutrient uptake, inhibition of cell division and elongation, and damaging photosynthetic pigments and net photosynthesis, which ultimately results in strong inhibition of plant growth (Haider *et al.*, 2021a ; Dias *et al.*, 2013). In our study on tomato seedlings, shoot fresh weight decreased in Cd-treated samples; TRIA treatment reduced Cd-induced growth inhibition. The decrease in plant growth was proportional to the uptake of Cd. Our data showed that CdCl2 supply, significantly increasing Cd accumulation in tomato plants, is related to tomato growth reduction. That is following other studies on *Lepidium sativum*, *Brassica juncea*, and *Lycopersicon esculentum* (Kapoor *et al.*, 2014; Alotaibi *et al.*, 2021; Carvalho Bertoli *et al.*, 2012). (karam *et al.*, 2017). TRIA mitigated Cd's inhibitory effects by decreasing Cd accumulation in brasica plants. Prior research has reported on the capacity of TRIA to regulate plant physiology and biochemistry to enhance development in the presence of metal-induced stress (Karam *et al.*, 2016).





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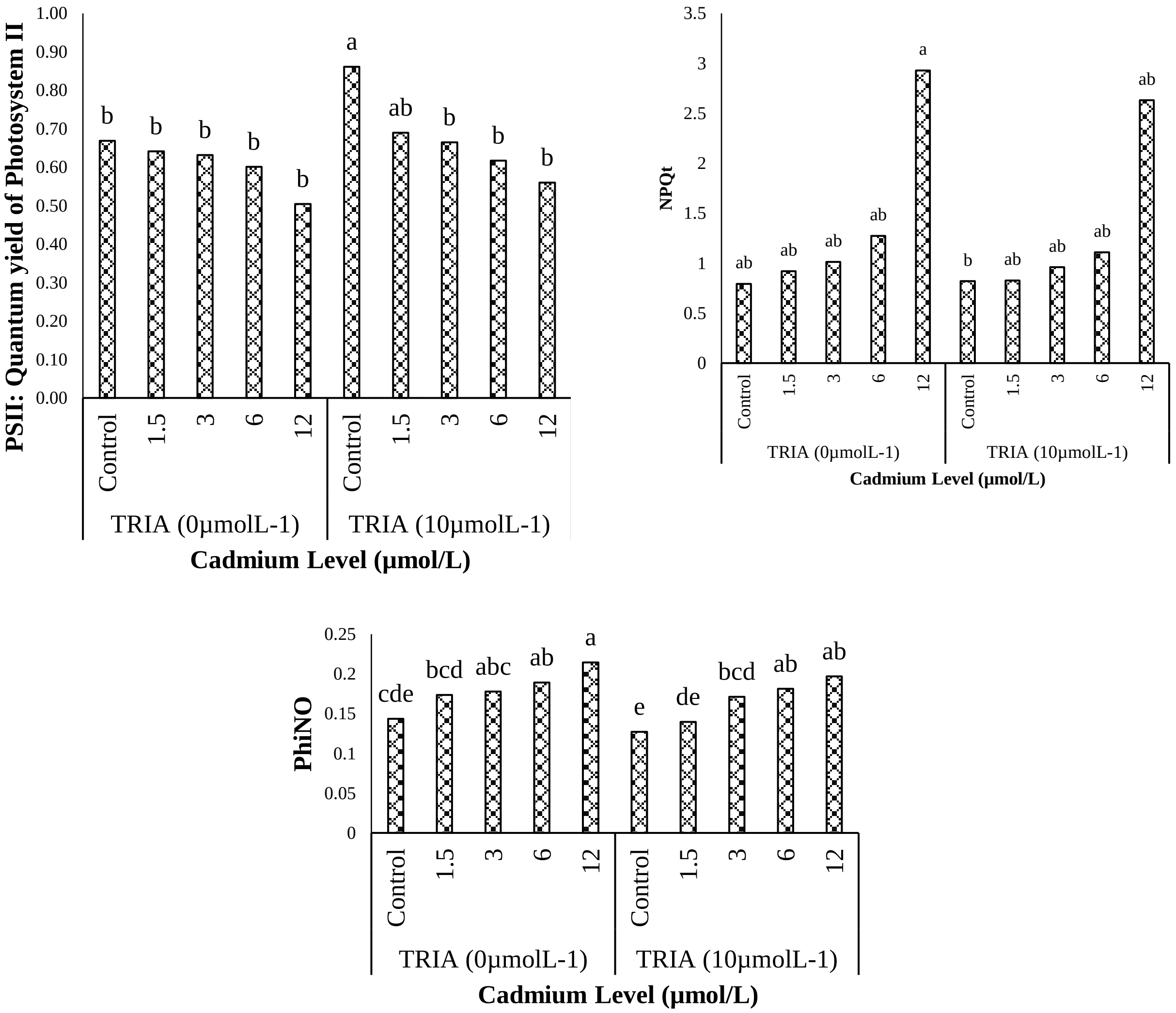

Fig 2. Chlorophyll fluorescence and photosynthesis in tomato leaves as influenced by CdCl2 and TRIA

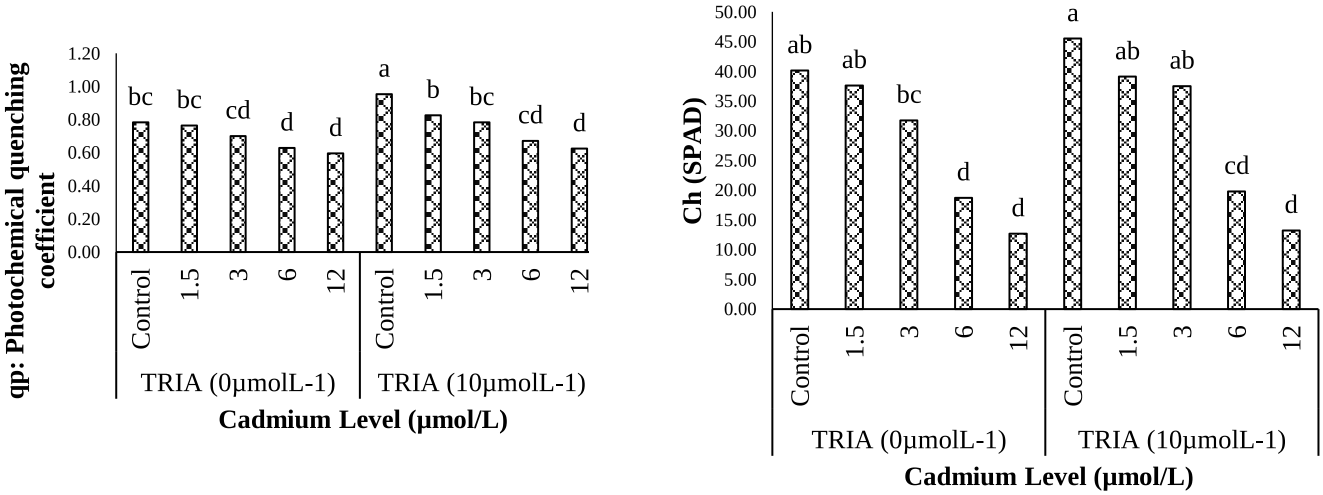

Figure 3. Non-photochemical quenching (NPQt), Photosystem-II, PhiNO, and leaf chlorophyll (SPAD value) in tomato leaves as influenced by CdCl2 heavy metal and TRIA

Particular attention has been paid to TRIA's capacity to stimulate agricultural plants' development via its synergistic interaction with phytohormones and production of 9-b-L (+) adenosine, which has structural similarities with cytokinin. (Naeem et al., 2012). Among the many crops that are negatively affected by cadmium stress are tomatoes. However, according to (Haider *et al.*, 2021b), tomatoes are particularly vulnerable to low yields, poor quality, and reduced biomass accumulation when exposed to high quantities of cadmium. In this study, higher doses of Cadmium stress, such $CdCl_2$ 12µmol/L , significantly inhibited photosynthetic and enzymatic activities, resulting in stunted plant growth and development. As shown in Table 1, most of the effects of CdCl2 stress are felt by the roots. This, in turn, causes a decrease in root and shoot growth, leading to shorter roots and shoots, smaller leaves, and lower chlorophyll concentrations. Cd (cadmium) toxicity in eggplant (*Solanum melongena L.*) seedlings, Cd declined growth, pigment contents and photosynthesis, and increased the rate of dark respiratory oxygen uptake. These effects were accompanied by Cd accumulation in tissues (Chourasia *et al.*, 2021). Because of the ionic toxicity and osmotic imbalance brought on by the high levels of Cd, the plants cannot absorb sufficient quantities of water and nutrients (Haider *et al.*, 2021b). Cd stress responses in tomato plants. plants undergo osmotic stress when subjected to Cd stress Findings from a study by (Chen *et al.*, 2023) suggest that an excess of CdCl2 in the cytosol may interfere with the biochemical and physiological functions of plants. This is because Cadmium can damage the proteins in the cell membrane and lead to electrolyte leakage in Sorghum Cultivars (Hassan *et al.*, 2020). Several studies have documented TRIA-mediated improvements in growth, yield, photosynthesis, protein synthesis, water and nutrient intake, nitrogen fixation, and enzyme activity in diverse crops (Verma *et al.*, 2022). In this study, Triacontanol applied exogeniously to the tomato plant not only hindered the Cd stress at maximum level but showed high results against the 1.5mmol/L CdCl2 mitigation with least reduction in (61.67 ab Shoot length), (6.45 abc Shoot Fresh Weight g), (1.88 abc Shoot Dry Weight g), (15.2 abc Root length cm), (1.83 ab Root Fresh Weight g), (0.81 ab Root Dry Weight g) of tomato plants as compared to those plants where Triacontanol is not applied as shown in table 1. The amount of chlorophyll in a plant's leaves indicates its resistance to abiotic stress (Talebzadeh and Valeo, 2022) . A growing body of evidence suggests that abiotic variables such cold, heat, salt, and drought diminish leaf chlorophyll content and, thus, limit plant growth (Mandal *et al.*, 2022). Previous research has shown that triacontanol may improve the resistance of slonace crops to abiotic stress, lending credence to our current discovery (Islam and Mohammad, 2020). Measuring leaf chlorophyll using Fv/Fm, qP, and NPQt values is common practice since they are sensitive markers of photosynthetic machinery functioning. In this study, increasing cd levels significantly reduced the FvP/FmP, qP, and PS-II efficiency values (p 0.05). Supporting this idea, the results demonstrated a strong correlation between LEF and chlorophyll fluorescence, and LEF declined as CdCl2 stress levels rose. A decrease in photosystem-II efficiency and leaf chlorophyll content is often associated with reduced electron transport to the photosystem-II electron acceptor QB (secondary acceptor plastoquinone), according to (Ali *et al.*, 2022). For Solanum melongena (Liaqat *et al.*, 2019) and Ricinus communis (Li *et al.*, 2010), the heat stress response decreases the efficacy of photosystem-II. For salt stress, it is an increase in non-photochemical quenching (NPQt). These results support the findings of (Tsai *et al.*, 2019), who discovered that triacontanol protected the PS-II apparatus from cd stress. Given its statistically favorable correlation with LEF and chlorophyll concentrations, PS-II will likely play a pivotal role in photochemical reactions during photosynthesis. Based on its strong positive correlation with chlorophyll and LEF concentrations, PS-II is expected to impact the photochemical reactions during photosynthesis significantly. The capacity of photosystem-II to generate energy and facilitate growth is contingent upon its efficacy. The impact of CdCl2 concentrations was significantly mitigated by the tomato TRIA concentration in this investigation. TRIA treatment substantially increased the expression of genes associated with photosynthesis, including ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and rbcS. By increasing rbcS expression, the researchers identified a more efficient photosynthetic pathway (Islam and Mohammad, 2020). This study revealed that Triacontanol at a concentration of 10µmol/L exhibited the greatest positive impact on FvP/FmP, PS-II, qP, NPQt, LEF, PhiNO, and Ch (SPAD) when applied against CdCl2 at a concentration of 1.5µmol/L. In addition, RuBisCO activity and concentration were increased, and the condition of the photosystem was enhanced. As studied (Sachdev *et al.*, 2021), alterations in ion transport and the generation of reactive oxygen species (ROS) transpire in response to abiotic stress. (Alharbi *et al.*, 2021) report that evidence suggests reactive oxygen species (ROS) signaling regulates cadmium (Cd) accumulation in the cytosol of plants, and that TRIA also regulates such oxidative responses. The capacity of a plant to resist the production of reactive oxygen species (ROS) is directly related to its stress tolerance. Enzymatic and non-enzymatic antioxidants, such as catalases (CAT), peroxidases

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(POD), and superoxide dismutase (SOD), are used by plants in response to reactive oxygen species (ROS). The present study shown that 10 mmolL-1 TRIA increased the synthesis of SOD, POD, and CAT activities in comparison to the (or control). TRIA found to be resistant to Cd stress, as shown by increased catalase (CAT) and peroxidase (POD) activity. Previous studies support our work that TRIA enable plants to increased levels of SOD, POD, and CAT which help them resist Cd stress. For instance, SOD plays a part in the process of cellular O2 removal (Sarwar *et al.*, 2021, 2022; karam *et al.*, 2017) as explained in above image. Tomato plants were shown to enhance their ability to cope with Cd stress by increasing levels of antioxidant enzymes (APX, SOD, and CAT) and photosynthetic activity (LEF, PS-II, NPQt, and chlorophyll contents) when exposed to 10μmol/L TRIA best against 1.5μmol/L CdCl2. Plants subjected to high levels of Cd stress (6 and 12μmol/L CdCl2), on the other hand, showed markedly diminished levels of these physiological traits, which in turn inhibited plant development and biomass accumulation.

Conclusion

According to the results, exogenous TRIA treatment reduced the growth-inhibiting effects of Cd stress on tomato plants and increased photosynthetic pigment and parameter levels when Cd stress was present. Overall, the crop's performance was improved after spraying TRIA on the leaves, which raised the values of all the studied attributes. In its role as a potent plant growth regulator (PGR), TRIA effectively controls several plant physiological and biochemical processes, including photosynthesis of chlorophyll, enzymatic activities like catalases, peroxidases, and superoxide dismutase, and chlorophyll photosynthesis (FEF/FmP, PS-II, qP, NPQt, LEF, PhiNO, and SPAD). TRIA improves photosynthesis and other physiological traits while increasing crop growth and yield. It boosts the amount of active substances and their content as well. The present study found that a TRIA concentration of 10μmol/L might lessen the negative impacts of cadmium stress and improve tomato yield and quality. However, studies on TRIA biosynthesis, action mechanism, and signaling receptors are required to control several metabolic and developmental processes in crop plants.

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Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Ethical approval was given from Ethical Review committee of department.

Consent for publication

The consent form was approved from Ethical Review committee of department.

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Conflict of Interest

Regarding conflicts of interest, the authors state that their research was carried out independently without any affiliations or financial ties that could raise concerns about biases.

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Author Contribution

MAS conducted research and wrote up initial draft of manuscript. MRS, RH, AS, and TH provided resources. RR and MBET made final editing in the manuscript. All authors approved final version of manuscript.

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