CITRIC ACID INDUCED CADMIUM STRESS TOLERANCE IN CHILI (*Capsicum annuum* L.)

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CITRIC ACID INDUCED CADMIUM STRESS TOLERANCE IN CHILI (*Capsicum annuum* L.)

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This is to certify that thesis entitled, ***"CITRIC ACID INDUCED CADMIUM STRESS TOLERANCE IN CHILI (Capsicum annuum L.)"*** *submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE in AGROFORESTRY AND ENVIRONMENTAL SCIENCE****, embodies the result of a piece of bona fide research work carried out by Suranjana Mehjabin, Registration No. 14-06146 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated:
Dhaka, Bangladesh

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Associate professor
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DEDICATED

TO

MY BELOVED PARENTS

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CITRIC ACID INDUCED CADMIUM STRESS TOLERANCE IN CHILI (*Capsicum annuum* L.)

ABSTRACT

Cadmium (Cd) is a major crop stressor posing a potential threat to food safety. Citric acid (CA) is well known to reduce Cd stress symptoms and improve plat growth. This study aims to investigate the growth and yield response to exogenous CA application under Cd stress on chili (*Capsicum annuum* L. cv. BARI Morich-2). The experiment was laid out in a completely randomized design (CRD) with three replications in pots at agroforestry farm, Sher-e-Bangla Agricultural University, Dhaka. Chili seedlings were treated with a single and combined dose of Cd and CA (2 mM and 4 mM $CdCl_2$; and 1 mM and 5 mM CA) at 15 and 30 days after transplanting. The results showed that the chili plants grown exposed to 1.0 mM and 2.0 mM $CdCl_2$ faced reduction in plant height (16 – 58%), number of leaves per plant (50 – 73%), fruit length (24 – 45%), fruit diameter (20-28%), fruit per plant (17- 49%), and yield per hectare (35- 64%) in all the growth stages. Plants only treated with 1 mM CA had maximum plant height (49 cm), number of leaves per plant (91), number of branches per plant (8), fruit length (5.44cm), fruit diameter (0.68 cm), fruit weight (1.32 gm), number of fruit per plant (50), fresh weight (115.67 gm), dry weight (1.25 gm), relative water content (91%), chlorophyll content (70.67), and yield (1.67 t/ha). 1 mM CA under 2 mM $CdCl_2$ performed best by improving plant height 5-28%, number of leaves per plant 11 – 38%, fruit length 19 – 27%, fresh weight 11 – 13%, dry weight improved 10 - 12%, and yield 41- 57% compared to the plants grown in 2 mM $CdCl_2$.

LIST OF CONTENTS

CHAPTER	TITLE		PAGE NO.
	ACKNOWLEDGEMENTS		i
	ABSTRACT		ii
	LIST OF CONTENTS		iii - vi
	LIST OF TABLES		vii
	LIST OF FIGURES		viii
	LIST OF PLATES		ix
	LIST OF APPENDICES		x
	LIST OF ABBREVIATION		xi
1	**INTRODUCTION**		1 - 3
2	**REVIEW OF LITERATURE**		4 - 18
	2.1	Heavy metal contamination and their contribution to plants	4
		2.1.1 Heavy metals	4
		2.1.2 Role of heavy metal in plants	5
		2.1.3 Heavy metals as essential plant nutrients	5
		2.1.4 Heavy metals limiting metabolism in plants	6
		2.1.5 Use of heavy metals in crop production	6
	2.2	Cadmium contamination and stress in plants	6
		2.2.1 Cadmium	6
		2.2.2 Cadmium pollution	7
		2.2.3 Cadmium accumulation in foods	7
		2.2.4 Health risk from cadmium contaminated foods	8
	2.3	Plant response to cadmium stress	8
		2.3.1 Plant height	8
		2.3.2 Foliage	9
		2.3.3 Biomass	9
		2.3.4 Fruit Quality and Yield	10
		2.3.5 Relative water content	10
		2.3.6 Chlorophyll content	11

2.4	Chili		11
	2.4.1	Chili as a major part of Bangladeshi cuisine	12
2.5	Cadmium mitigation techniques		12
	2.5.1	Different mitigation strategies	12
	2.5.2	Phytoremediation	13
	2.5.3	Organic acids for phytoremediation	13
2.6	Citric acid		14
	2.7	Plant responses to citric acid	14
	2.7.1	Plant height	14
	2.7.2	Foliage	14
	2.7.3	Biomass	15
	2.7.4	Fruit quality and yield	15
	2.7.5	Relative water content	15
	2.7.6	Chlorophyll content	15
2.8	Effect of citric acid on cadmium stressed plants		16
	2.8.1	Plant Height	16
	2.8.2	Foliage	16
	2.8.3	Biomass	16
	2.8.4	Relative water content	17
	2.8.5	Chlorophyll content	17
3	**MATERIALS AND METHODS**		19
3.1	Experimental site		19
3.2	Climate		19
3.3	Soil		19
3.4	Planting material		20
3.5	Treatments of the experiment		20
3.6	Design and layout of the experiment		20
3.7	Pot preparation		21
3.8	Cultivation technology		21
	3.8.1	Transplanting of seedlings	21
	3.8.2	Weeding	21
	3.8.3	Irrigation	21
	3.8.4	Disease and pest control	21
	3.8.5	Harvesting of fruits	22
3.9	Data collection		22
	3.9.1	Plant height	22
	3.9.2	Number of leaves and fruits per plant	23
	3.9.3	Number of branches per plant	23
	3.9.4	Length of fruit	23
	3.9.5	Diameter of fruit	23
	3.9.6	Individual fruit weight	23
	3.9.7	Fresh weight and dry weight of seedling	23
	3.9.8	Yield of fruits per hectare	24
	3.9.9	SPAD value	24
	3.9.10	Determination of leaf relative water content	24
	3.9.11	Statistical analysis	24

4	**RESULTS AND DISCUSSION**		25 - 53
4.1	Plant height		25
	4.1.1	Effect of cadmium stress on plant height	25
	4.1.2	Effect of citric acid on plant height	25
	4.1.3	Combined effect of citric acid and cadmium on plant height	26
4.2	Number of leaves per plant		29
	4.2.1	Effect of cadmium stress on number of leaf per plant	29
	4.2.2	Effect of citric acid on number of leaf per plant	29
	4.2.3	Combined effect of citric acid and cadmium on number of leaf per plant	30
4.3	Number of branches per plant		33
	4.3.1	Effect of cadmium stress on number of branches per plant	33
	4.3.2	Effect of citric acid on number of branches per plant	33
	4.3.3	Combined effect of citric acid and cadmium on number of branches per plant	34
4.4	Fruit length		35
	4.4.1	Effect of cadmium stress on fruit length	35
	4.4.2	Effect of citric acid on fruit length	36
	4.4.3	Combined effect of citric acid and cadmium on fruit length	36
4.5	Fruit diameter		38
	4.5.1	Effect of cadmium stress on fruit diameter	38
	4.5.2	Effect of citric acid on fruit diameter	38
	4.5.3	Combined effect of citric acid and cadmium on fruit diameter	39
4.6	Fruit weight		39
	4.6.1	Effect of cadmium stress on fruit weight	39
	4.6.2	Effect of citric acid on fruit weight	40
	4.6.3	Combined effect of citric acid and cadmium on fruit weight	40
4.7	Number of fruit per plant		41
	4.7.1	Effect of cadmium stress on number of fruit per plant	41
	4.7.2	Effect of citric acid on number of fruit per plant	42
	4.7.3	Combined effect of citric acid and cadmium on number of fruit per plant	42
4.8	Yield per hectare		43
	4.8.1	Effect of cadmium stress on yield per hectare	43
	4.8.2	Effect of citric acid on yield per hectare	43
	4.8.3	Combined effect of citric acid and cadmium stress on yield per hectare	44

	4.9	Fresh Weight of Plant	45
		4.9.1 Effect of cadmium stress on fresh weight of plants	45
		4.9.2 Effect of citric acid on fresh weight of plants	45
		4.9.3 Combined effect of citric acid and cadmium on fresh weight of plant	46
	4.10	Dry weight	47
		4.10.1 Effect of cadmium stress on dry weight of plant	47
		4.10.2 Effect of citric acid on dry weight of plant	47
		4.10.3 Combined effect of citric acid and cadmium on dry weight of plant	48
	4.11	Relative water content (RWC)	49
		4.11.1 Effect of cadmium stress on relative water content (RWC)	49
		4.11.2 Effect of citric acid on relative water content (RWC)	49
		4.11.3 Combined effect of citric acid and cadmium on relative water content (RWC)	50
	4.12	Chlorophyll content	51
		4.12.1 Effect of cadmium stress on chlorophyll content	51
		4.12.2 Effect of citric acid on chlorophyll content	51
		4.12.3 Combined effect of citric acid and cadmium on chlorophyll content	52
5	**SUMMARY AND CONCLUSION**		54
		Summary	54
		Conclusion	55
6	**REFERENCES**		57 - 61
	APPENDICES		62

LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
1	Effect of different levels of Citric acid and Cadmium on plant height in chili	6
2	Effect of different levels of Citric acid and Cadmium on number of leaves per plant	30
3	Effect of different levels of Citric acid and Cadmium on number of branches per plant	33
4	Effect of different levels of Citric acid and Cadmium on fruit length of chili	36
5	Effect of different levels of Citric acid and Cadmium on fruit diameter	38
6	Effect of different levels of Citric acid and Cadmium on fruit weight	40
7	Effect of different levels of Citric acid and Cadmium on number of fruit per plant	42
8	Effect of different levels of Citric acid and Cadmium on yield per hectare	44
9	Effect of different levels of Citric acid and Cadmium on fresh weight of plant	46
10	Effect of different levels of Citric acid and Cadmium on dry weight of plant	48
11	Effect of different levels of Citric acid and Cadmium on relative water content	50
12	Effect of different levels of Citric acid and Cadmium on chlorophyll content	52

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE NO.
1	Effect of Citric acid and Cadmium on plant height at different DAT	28
2	Effect of Citric acid and Cadmium on number of leaves per plant at different DAT	32
3	Effect of Citric acid and Cadmium on number of branches per plant at different DAT	35
4	Effect of Citric acid and Cadmium on fruit length of chili	37
5	Effect of Citric acid and Cadmium on fruit diameter	39
6	Effect of Citric acid and Cadmium on fruit weight	41
7	Effect of Citric acid and Cadmium on number of fruit per plant	43
8	Effect of Citric acid and Cadmium on yield per hectare	45
9	Effect of Citric acid and Cadmium on fresh weight of plant	47
10	Effect of Citric acid and Cadmium on dry weight of plant	49
11	Effect of Citric acid and Cadmium on relative water content	51
12	Effect of Citric acid and Cadmium on chlorophyll content	53

LIST OF PLATES

PLATE NO.	TITLE	PAGE NO.
1	Effect of CA and Cd stress on fruit length	37
2	Pot preparation and seed germination	69
3	Seedling stage	69
4	Flowering stage	70
5	Fruiting stage	70
6	Pesticide application	70
7	Data collection	70

LIST OF APPENDICS

APPENDIX NO.	TITLE	PAGE NO.
I	Map showing the location of experiment	62
II	Soil characteristics of experimental field as analyzed by Soil Resources Development Institute (SRDI), Khamarbari, Farmgate, Dhaka	63
III	Monthly record of air temperature, relative humidity and rainfall of the experimental site during the period from November 2019 to April 2020	63
IV	Mean table of combined effect of citric acid-cadmium on plant height	64
V	Analysis of variance (mean square) of plant height at different days after sowing	64
VI	Mean table of combined effect of citric acid-cadmium on number of leaves per plant	65
VII	Analysis of variance (mean square) of number of leaves per plant	65
VIII	Mean table of combined effect of citric acid-cadmium on number of branches	66
IX	Analysis of variance (mean square) of the data for number of branches per plant at different days after sowing	66
X	Mean table of combined effect of citric acid-cadmium on fruit length, diameter, weight, number of fruit per plant, and yield per hectare	67
XI	Analysis of variance (mean square) of fruit at length, diameter, weight, number of fruit per plant, and yield per hectare	67
XII	Mean table of combined effect of citric acid-cadmium on plant's fresh weight, dry weight, RWC, chlorophyll content	68
XIII	Analysis of variance (mean square) of plant's fresh weight, dry weight, RWC, chlorophyll content	68
XIV	Some photographs related to the study	69 - 70

LIST OF ABBREVIATION

Full form	Abbreviation
Arsenic	As
Antimony	Sb
Bangladesh Agricultural Research Institute	BARI
Bangladesh Agricultural University	BAU
Cadmium	Cd
Chromium	Cr
Citric Acid	CA
Complete Randomized Design	CRD
Copper	Cu
Days after Sowing	DAS
Days after Transplanting	DAT
et.al	Etallii (others)
Iron	Fe
Lead	Pb
Mercury	Hg
Oxygen-Evolving Complex	OEC
Parts Per Million	ppm
Reactive Oxygen Species	ROS
Relative Water Content	RWC
Root Mass Ratio	RMR
Salicylic acid	SA
Selenium	Se
Silver	Ag
Thallium	Tl
Tin	Sn
Tungsten	W
Vanadium	V
World health organization	WHO
Zinc	Zn

CHAPTER 1

INTRODUCTION

Heavy metals are abundant in nature and contribute to a broad range of functions on living beings. The earth naturally contains ninety-nine heavy metals out of the one hundred and eighteen naturally occurring elements (Duffus, 2002; Koller and Saleh, 2018), among which some nourish plants as micronutrients [zinc (Zn), nickel (Ni), copper (Cu), cobalt (Co), etc.], and some [arsenic (As), cadmium (Cd), chromium (Cr), Lead (Pb) etc.] are toxic to plants and animals (Tchounwou *et al.,* 2012; Ajah *et al*., 2015). Depending upon toxic effect on living being Cadmium (Cd), Mercury (Hg) and Lead (Pb) are marked as highly toxic metals (Appenroth, 2013; Alfaraas *et al.*, 2016). Due to their diverse properties, these heavy metals are commonly utilized in everyday life.

Heavy metals (As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Zn) frequently used in industries are toxic to organisms that have aerobic and anaerobic metabolic processes (Duffus, 2002). Widely used Cd is a noteworthy toxicant for its bio-toxicity, water solubility, and carcinogenic effect (Benavides *et al.*, 2005). A significant amount of heavy metals deposited in sediment causes hazard by eventually making its way into the food chain via water, plants, seeping into underground water supplies, or among other means (Bhuyan, 2017).

Heavy metal pollution has amplified because of diverse anthropogenic activities: industrial waste, mining operations, urban runoff, sewage treatment plant, agricultural fungicide runoff, and domestic garbage (Manea *et al.*, 2021). Since green revolution in 1960, these practices are becoming a major source of heavy metal contamination to croplands. Absorption and desorption of heavy metals or metalloids strongly influence their mobility and transportation in plants (Alfaraas *et al.*, 2016) leading to chlorosis, plasmolysis, reduction of the meristematic zone (Bini *et al.*, 2012).

Despite being a non-essential trace element Cd is readily taken up by roots, translocated (Zhou and Qiu, 2005; Liu *et al.*, 2016) and accumulated in the tissues i.e. epidermal cell wall and mesophyll cells (Bini *et al.*, 2012) and in edible parts such as leaves, fruits, and seeds (Liu *et al.*, 2016). Plants exposed to Cd, face metabolic stress (declined nutrient uptake, photosynthesis inhibition, change in nitrogen and sulfur metabolism, and alteration in some key enzymes) (Mahmud *et al.*, 2018) and physiological deviation like inhibition of cell expansion and senescence (leaf chlorosis, leaf, and root necrosis, leaf epinasty, and root growth inhibition) (Zhou and Qiu, 2005). Alfaraas *et al.* (2016) found that retarded growth, especially root growth, wilting of older leaves, stunted foliage and short brown roots are the symptoms of excessive Cd stress. The whole phenomenon is affecting not only plant growth, but also challenging soil fertility, soil microflora, even the natural ecological and evolutionary process as well as food safety (Benavides *et al.*, 2005).

Food security is adversely impacted due to high levels of heavy metal pollution in soil (Ehsan *et al.*, 2014) as it decreases the crop production, cropping area, crop diversity, and by producing unsafe food for the humans and animals. Koller and Saleh, (2018) and Manea *et al.*, (2021) found that they are carcinogenic (hexavalent Cr, As, Co, Ni, Sb, V, Hg), mutagenic (As, V), teratogenic (As), allergenic (Ni), or endocrine-disrupting (Ag, Co, Zn, Se). According to Tchounwou *et al.* (2012), the lung is the most established site of human carcinogenesis from Cd exposure, which also targets adrenals, testes, and the hemopoietic system. Bhuyan and Islam (2017) also found that Cd poses acute toxicity causing hepatic, pulmonary, and testicular injury (Baig *et al.*, 2019); and chronic toxicity in renal and bone injury (osteoporosis); carcinoma (primarily prostate and renal), and toxicity to other organs.

Heavy metal contamination in spices presents a significant threat to Bangladesh as its cuisine heavily relies on spicy dishes. Industrially processed spices have greater chances of heavy metal contamination by biogenic or chemical environmental pollutants during growing storage, transportation, and distribution (Baig *et al.,* 2019). Hot chili is an inseparable ingredient of Bangladeshi meals - a single day cannot go without using either fresh green chili or dried chili.

According to Xin *et al.* (2012) chili (*Capsicum annuum* L.) shows low to high Cd uptake depending on the degrees of exposure, duration, and cultivars. Consumption of Cd contaminated chili in the daily diet may exceed WHO (World Health Organization) permissible level 0.05 ppm (WHO, 2011). Therefore, it demands urgent research to reduce cadmium accumulation in chili plant.

Organic acids influence the availability, absorption, translocation, and detoxification of Cd (Wang *et al.*, 2017). According to Liu *et al.* (2016) plant hormones, abscisic acid, nitric oxide, gibberellic acid, salicylic acid, and citric acid play key roles in the adaptation and survival of plants under Cd stress. Exogenous citric acid (CA) has been found to reduce Cd stress via improving the antioxidant defense system, promoting some key enzymes activity (Mahmud *et al.,* 2018), and acting as a natural chelating agents to enhance phytoextraction (Wang *et al.*, 2017). Hassan *et. al* (2016) investigated Jute Mallow (*Corchorus olitorius*) with 1, 5, 10 and 20 mg/L, Cd_2^+ and 5mM CA to improving the phytoextraction. They found that Cd significantly decreased the plant growth and biomass, and increased proline contents. On the other hand, CA significantly depressed Cd_2^+ uptake and its accumulation in plant roots and shoots.

Research papers and reports are indicating serious Cd phytotoxicity, bioaccumulation, and carcinogenic effects on human life. On contrary, there are limited studies on the effect of Cd uptake by chili plants at various growth stages treated with CA in the context of Bangladesh. Most of the studies focused on the metals concentration in edible parts of vegetables. In addition, the reports describing heavy metal uptake by chili plants are scarce compared with other vegetables. Consequently, this study was proposed to investigate the function of CA in morpho-physiological attributes of chili plants under different level of Cd stress. To determine the growth and yield loss caused by Cd stress given artificially at different stages of plant development.

Objectives

1. To determine the growth and yield loss of chili plants caused by artificially created different levels of Cd stress at different developmental stages; and

2. To assess the performance of CA under Cd stressed chili plant parts at different growth stages.

CHAPTER 2

REVIEW OF LITERATURE

This chapter is a brief review of various recent researches conducted on heavy metal contamination and toxicity in plants, Cd stress in chili, and CA as a phytoremedy. It summarizes the research works done in Bangladesh as well as countries around the world.

2.1 Heavy Metal Contamination and Their Contribution to Plants

2.1.1 Heavy Metals

Metals having a density higher than 5 g/cm3, and in a broader sense atomic mass higher than 23 or an atomic number exceeding 20, are defined as heavy metals (Duffus, 2002). Out of 118 primary elements discovered until now, 99, the lion's share are classified as heavy metal as they entered 4–5 billions of years ago during earth formation from asteroids (Duffus, 2002; Appenroth, 2013; Koller and Saleh, 2018). As a natural element, heavy metals are ubiquitous, non-biodegradable, have long biological half-lives (Rahman *et al.,* 2012). They cannot be removed from water by self-purification, rather their elevation in top soil accumulates in the bottom sediment with high concentration turning into a “chemical archives” (Xu *et al.*, 2009; Bhuyan and Islam, 2018).

2.1.2 Role of Heavy Metal in Plants

Heavy metals are persistent and stable elements, which is why they cannot be degraded or destroyed, but because of their bio-accumulative characteristic they may slowly enter plants, animals, and humans (Arsenov *et al.*, 2020) through air, water, and continue progression in the food chain (Srivastava *et al.*, 2017). Many heavy metals play key role in biological processes, which are predominantly found in period 4 (Koller and Saleh, 2018), do technological wonders (Fe, Zn, Sn, Pb, Cu, W), and some can be toxic (Hg, Cd, As, Cr, Tl, Pb) even at a low concentration (Tchounwou *et al.*, 2012; Appenroth, 2013; Koller and Saleh, 2018).

2.1.3 Heavy Metals as Essential Plant Nutrients

Out of 18 essential nutrients for plant growth, seven micronutrients are heavy metals (Cu, Zn, Fe, Mn, Mo, Ni, and Co). It means in their absence, plants show deficiency symptoms (Tchounwou *et al.*, 2012; Naeem *et al.*, 2017). According to Goyal *et al.* (2020), plants need certain heavy metals for their biochemical and physiological functions involved in overall growth and development. Fe also plays a major role in respiration and chlorophyll synthesis; Cu in photosynthesis, and electrons transport system; Se in antioxidant enzymes; Co in developmental cellular metabolism; and Mn is an essential cofactor for the oxygen-evolving complex (OEC) in photosynthesis (Koller and Saleh, 2018).

2.1.4 Heavy Metals Limiting Metabolism in Plants

Tchounwou *et al.* (2012), and Hasanuzzaman *et al.* (2017), reported that heavy metal exposure can affect plants elementary cellular organelles i.e. cell membrane, mitochondria, lysosome, endoplasmic reticulum, nuclei, and some enzymes involved in metabolism, detoxification, and damage repair. It can alter nuclear proteins as well as DNA binding proteins resulting in DNA damage, cell cycle modulation, carcinogenesis or apoptosis. The study of Bhuyan and Islam (2018), revealed that forest trees are adversely affected by heavy metal toxicity causing disruption in cellular homeostasis. *Heritiera fomes*, the single dominant tree of Sundarbans, is reported to suffer from 'top-dying' because of exchangeable Sn, K, Pb, Sr, and Zn.

According to Mahmud *et al.* (2019), and Sathee *et al.* (2019), the presence of the toxic metals are capable of such cellular and metabolic alteration that can significantly hamper metabolism, and plant growth. They can directly or indirectly produce reactive oxygen species ROS putting the cell under oxidative stress. If metal-induced ROS production is not adequately countered by cellular defenses system, lipids, proteins, and nucleic acids can be damaged. However, a few toxic heavy metal (As, Cd, Cr, Pb, etc.) exposure even at low concentration, may result in damaging the photosynthetic metabolism, and electron transport system, which declines net photosynthesis (PN) leading to overproduction of ROS such as $O_2^{\bullet-}$, $^{\bullet}OH$, and H_2O_2 (Srivastava *et al.*, 2017).

2.1.5 Use of Heavy Metals in Crop Production

Since green revolution to till date, modern agricultural practices use heavy metal in agrochemical products as plant growth enhancer, i.e. inorganic fertilizers and organic fertilizers, as well as in plant protection products, i.e. insecticides, herbicides, and fungicides. With their use, crop production soared all over the globe.

According to Srivastava *et al.* (2017), phosphate fertilizers, nitrate fertilizers, potash fertilizers, and lime contains Cu, Cr, Cd, Zn, Ni, Mn, and Pb. Herbicides, insecticides, and fungicides contribute to varying concentrations of Cu, Pb, Cd, Cr, Ni, As Pb, and Zn. However, repeated use of these fertilizers and pesticides allow high levels of major toxic heavy metals to the uncontaminated croplands hampering natural resources. Studies have also found lower amount of Pb, Cd, Cr, As, Zn, Cu, Ni, and Hg from biosolids and manure i.e. livestock manures, compost sewage sludge, and fly ash. Besides, one of the most common cultural practices, land application of sewage sludge, organic waste manure, industrial byproducts, and irrigation with waste water, are also causing intrusion of Pb, Cd, Cr, As, Zn, Cu, Ni, and Hg (Ajah *et al.*, 2015).

Rahman *et al.* (2012) found that wastewater from industries or other sources carries appreciable amounts of Cd, Cu, Zn, Cr, Ni, Pb, and Mn in surface soil questioning the safe rational utilization of their use in agricultural soil. Contamination of agricultural soil with heavy metals is one of Bangladesh's most serious environmental issues because farmers frequently use untreated or inadequately treated effluent from industrial facilities and often apply chemical fertilizers and pesticides excessively.

2.2 Cadmium Contamination and Stress in Plants

2.2.1 Cadmium

Cd (atomic weight 112.41, atomic number 48) is a soft, silver-white blue tinged lustrous metallic solid, naturally occupies as Greenockite (CdS) in the earth’s crust at an average concentration of 0.1 to 0.5 ppm. Originating from geogenic processes, it is highly concentrated in sedimentary rocks, and particularly in marine phosphate, which could be as high as 15 mg Cd kg^{-1} (Tchounwou *et al.*, 2012). Cadmium (Cd) is a highly poisonous, non-essential, carcinogenic, and mutagenic heavy metal (Hassan *et. al,* 2016).

2.2.2 Cadmium Pollution

Cd is often discharged into the environment as a result of industrial operations and urban activities, as well as through the extensive use of fertilizers, manures, sewage sludge (Liu *et al.*, 2016), electroplating, burning of fossil fuels, Ni–Cd batteries, and raw city effluents (Ehsan *et al.*, 2014).

Rahman *et al.* (2012) investigated the anthropogenic heavy metal contamination on agricultural soil around Dhaka Export Processing Zone (DEPZ) using seasonal variation and indices. They found higher concentration of Cd in the wet season than the dry season, perhaps, due to more Cd containing waste water release from the industries.

Mottalib *et al.* (2016) reported that tannery effluent contaminated soil in Dhaka leather industrial area has 0.32 to 0.54 mg Cd kg^{-1} soil.

Sultana (2000) examined the soil of Bangladesh Agricultural University (BAU) campus to evaluate the effects of heavy metal contaminated sewage sludge application on crop yields and bio availability of heavy metal, and found 0.25, 14.0, 21.0 and 19.0 mg kg^{-1} Cd, Pb, Cu and Zn, respectively.

2.2.3 Cadmium Accumulation in Foods

A major route of heavy metal exposure to humans is via vegetables and spices consumption. Cadmium enters into the plants primarily through root absorption and transferred to the above-ground tissues (Hassan *et. al*, 2016). Vegetables cultivated in soils polluted with heavy metals accumulate them in their edible and non-edible parts in quantities high enough to cause clinical problems both to animals and human beings consuming these metal-rich plants. There is no good mechanism for their elimination from the human body (Rahman *et al.*, 2012; Baig *et al.*, 2019).

According to Ajah *et al.* (2015) leafy vegetables exhibit preferential uptake of Cd and Cu, leaves can accumulate large amounts of As and Cd, and Pb.

Naser (2012) studied heavy metals in soil and plant samples, and found 1.2, 0.003, 0.14, 0.080 and 0.001 ppm for Cu, Pb, Zn, Cd and Fe, respectively in Soybean.

Mottalib *et al*. (2016) studied heavy metal accumulation plant samples and found the PCF values of Cd 0.74 –0.94, Cr 0.06, Pb 0.13 – 0.43, Ni 0.15 – 0.27, As 0.13 –0.34, and Sb 0.65–0.82 in the root of spinach.

Baig *et al.* (2019) investigated As, Cd, Ni, and Pb content in the 26 common spices collected from the market of Hyderabad, Pakistan, and found that coriander seed, allspices, red chili, and black cumin seed had three to six times higher concentration than the world health organization's (WHO) permissible limit.

2.2.4 Health Risk from Cadmium Contaminated Foods

Findings from Tchounwou *et al.* (2012) suggest that human exposure to Cd may occur in a variety of ways, employment in primary metal industries, smoking cigarettes, and working in Cd-contaminated work environment, but consuming contaminated food is a critical concern. Additionally, Cd accumulated foods particularly- mushrooms, shrimp, liver, mussels, cocoa powder, and dried seaweed, may significantly raise the Cd content in human bodies. Acute ingestion can cause severe symptoms such as abdominal pain, burning sensation, nausea, vomiting, salivation, muscle cramps, gastrointestinal tract erosion, pulmonary, hepatic injury, coma vertigo, shock, loss of consciousness, and even convulsions within 15 to 30 min after consumption.

Cadmium exposure over a long period at very low concentrations can lead to anemia, anosmia, cardiovascular diseases, renal problems and hypertension (Rahman *et al.*, 2012).

2.3 Plant Response to Cadmium Stress

2.3.1 Plant Height

Wang *et al.* (2017) found that the plant height of tall fescue and Kentucky bluegrass showed significant reduction of 24% and 20% under 100 mg Cd kg^{-1} compared with the control condition.

Gill *et al.* (2011) reported that Cd reduces plant height in cotton, two wheat cultivars, C-1252 and Balcali-85, Indian mustard, rape plants, and mulberry drastically with increasing concentration. They also found that shoot growth of *Crotalaria juncea* seedlings was strongly inhibited by Cd concentrations above 0.2 mM $CdCl_2$ as well as concentrations above 3.0 mM $CdCl_2$, affected seed germination and complete cessation of seedling growth in *Sorghum bicolor*.

Mahmud *et al.* (2019) found 7.43% and 9.35% reduction in plant height at 0.5 mM $CdCl_2$, and 1.0 mM $CdCl_2$ exposure in *Brassica juncea* seedlings.

Bhuyan *et al.* (2020) reported 14% and 23% decline in the plant height of rice seedling under 1.0 mM and 2.0 mM Cd stress.

Aslam *et al.* (2014) investigated the effects of Cd on biochemical, physiological and cytological parameters of *Capsicum annuum* L. in hydroponic condition treated with five different concentrations found that that the shoot length in 20 ppm and100 ppm Cd concentrations were 42.80 cm and 27.19 cm which has decreased in dose dependent manner comparing to the controlled treatment (46.04 cm).

2.3.2 Foliage

According to Ehsan *et al.* (2014), number of leaves and leaf area significantly decreased with increasing Cd contamination. Leaf area reduced by 13.33% and 33.33%; and number of leaves dropped by 20% and 30% respectively at 10 µM and 50 µM of Cd.

Gill *et al.* (2011) reported Cd stress caused less expanded leaves with partial bleaching of green tissues and 40% shorter internode in *Elodea canadensi.* They also reported lower number of branches in cotton; significant leaf expansion inhibition in *Sedum alfredi;* lesser leaves in *Zea mays* and mulberry plants; growth of both mature and young leaf reduced in *Halianthus annus* seedlings after 7 days of exposure; and leaf chlorosis and lower leaf area in *Vigna radiata* seedlings under Cd stress.

2.3.3 Biomass

Reduced growth and biomass production occur due to altered physiological processes with increasing Cd levels in the growth medium. According to Gill *et al*. (2011), Cd exposure caused reduced dry weight of wheat; declined root growth in pea, maize, sorghum, rape seed, mustard, carrot, and radish; lower fresh and dry weight of *Phyllanthus amarus* and *Brassica napus*; altered biomass allocation pattern in *Hordeum vulgare;* and lesser root and shoot growth of *Crotalaria juncea* seedlings.

According to Arsenov *et al.* (2020), biomass production reduced with the increasing Cd concentration and over prolong time. Root mass ratio was considerably decreased by 43% and 32% respectively 3mg kg^{-1} and 6mg kg^{-1} Cd in dry soil in as compared to control after 30 days of exposure. They found that in willow trees leaf, shoot, root biomass production exhibit alteration after 30 days of Cd exposure and the highest reduction after 60 days of Cd exposure.

Mahmud *et al.* (2019) reported that fresh weight reduced by 17.46% and 20.63%, and dry weight by 12.7% and 15.87% respectively at 0.5 mM $CdCl_2$, and 1.0 mM $CdCl_2$ in *Brassica juncea* seedlings.

According to Mahmud *et al.* (2018), both fresh weight and dry weight reduced in a dose-dependent fashion. Fresh weight dropped by 19.23% and 24.35% and dry 20.22% and 25.84% respectively at 0.5 mM $CdCl_2$, and 1.0 mM $CdCl_2$ in *Brassica juncea* seedlings.

The study conducted by Bhuyan *et al.* (2020) found root length reduction by 28% and 33% of rice seedling under 1.0 mM and 2.0 mM Cd stress.

According to Mozafariyan *et al.* (2014) dry weight reduced by 20% and 31% respectively, as compared to the control treatment of *Capsicum annuum* under 0.25 mM and 0.50 mM Cd exposure.

Aslam *et al.* (2014) reported that fresh weight of plants under 20 ppm and 100 ppm Cd exposure were 21.61 g/plant and 16.22 g/plant which are respectively 9.49 % and 29.72% lesser than the control treatment (23.08 g/plant) in *Capsicum annuum.*

Results calculated by Georgieva *et al.* (1997) revealed that fresh biomass at 30 Cd mg kg^{-1} contamination decreased by 28% in pepper plants.

2.3.4 Fruit Quality and Yield

According to Mozafariyan *et al.* (2014) fruit length decreased by about 20 and 40 % along with 9% and 17% reduction in Geometric mean productivity (GP) respectively at 0.25 mM and 0.50 mM Cd contamination in *Capsicum annuum.* They also reported a slight decrease in flower number per plant, lesser days to fruit set, decrease in fruit diameter, number of fruits per plant, and flower abscission by about threefold to fourfold under 0.50 mM Cd stress as compared to the control plants.

Gill *et al.* (2011) reported that Cd stress for reduced fruiting in cotton.

Pepper plants are responsive to Cd contamination and the yield can reduce 14% when the soil has 30 Cd mg kg^{-1} (Georgieva *et al.*, 1997).

2.3.5 Relative Water Content

According to Mahmud *et al.* (2019) leaf relative water content (RWC) declines by 7.06% and 11.58% respectively at 0.5 mM $CdCl_2$, and 1.0 mM $CdCl_2$ in *Brassica juncea* seedlings.

Mahmud *et al.* (2018) reported that leaf RWC declines in dose dependent manner. Their study showed that leaf RWC decreased by 7% and 11% 0.5 mM $CdCl_2$, and 1.0 mM $CdCl_2$ in *Brassica juncea* seedlings.

2.3.6 Chlorophyll Content

Arsenov *et al.* (2020) found no significant change in chlorophyll (chl) content in *Salix viminalis* for 30 days exposure of Cd 3mg kg^{-1} dry soil. However, Cd 6mg kg^{-1} caused 17%, 14%, and 48% reduction in young leaves after 30, 60, and 90 days of exposure.

Gill *et al.* (2011) reported negative effect of Cd on *Lycopersicon esculentum*, *Triticum aestivum*, *Cucumis sativus*, *Zea mays*, *Phaeolus vulgaris*, *Hordeum vulgare*, *Oryza sativa*, *Glycine max*, *Matricaria chamomilla*, *Brassica juncea*, *Bechmeria nivea*, *Brassica campestris*, and *Vigna mungo*. They also found that chl decreased by 55% in bean plants when exposed to 50 μM Cd.

Mahmud *et al.* (2019) found that mild and severe stress decreased the chl a content by 51% and 67%, and chl b content by 47% and 66%, respectively, in contrast to the controlled seedlings of *Brassica juncea*.

Mahmud *et al.* (2018) reported that chlorophyll content (Chl a + b) declined by 50.60% and 68.69% respectively at 0.5 mM and 1.0 mM $CdCl_2$ in *Brassica juncea* seedlings.

Mozafariyan *et al.* (2014) reported *Capsicum annuum* shows a significant reduction in the concentration of chl a in the fruits of pepper subjected to 0.50 mM Cd. They also noted that chl b decreased 50% comparing to the control value in response to 0.50 mM Cd.

2.4 Chili

Chili, the hot pungent spice, is botanically a berry-fruit of plants from the genus Capsicum which are members of the nightshade family, Solanaceae. It is one of the oldest cultivated crops, as its origin of cultivation are traced to east-central Mexico some 6,000 years ago, and a part of human diets since about 7,500 BC. Now, all over the world, there are more than 400 different varieties found. *Capsicum annuum* L. is one of the five domesticated species which include *C. annuum* L., *C. baccatum* L., *C. chinense* Jacq., *and C. pubescens* (Kraft *et al.*, 2014). *Capsicum annuum* L is commonly known as chili pepper, tabasco pepper, bell pepper, cayenne pepper, mirch, paprika, hot pepper, bird pepper and so on. Both fresh and dried chili has high demand. In 2016, its global production was 34.5 million tons of green chili peppers and 3.9 million tons of dried chili peppers (FAOSTAT, 2014). It contains large amounts of vitamin C, small amounts of carotene (Pro-vitamin A), vitamin B, vitamin B6, potassium, Mg, and Fe.

2.4.3 Chili as a Major Part of Bangladeshi Cuisine

Chili plays is one of the basic ingredient in Bangladeshi cuisine and is also produced plenty of it because of having favorable soil and climatic condition. In 2014-2015 fiscal year, the average yields and production was 1.46 t/ha and more than 3 lakhs t/ha respectively in Bangladesh (BBS, 2018).

2.5 Cadmium Mitigation Techniques

2.5.1 Different Mitigation Strategies

Of late, heavy metal contaminated site restoration has become one of the most important environmental issues and a key concern (Bini *et al.,* 2012). With a view to mitigating the adverse effects of heavy metals, remediation methods are continually developed in response to rising public demand to diminish existing environmental risks for a better future.

Koller and Saleh (2018) concluded that traditional physical, thermal, chelating, and other chemical methods have significant drawbacks, including high costs and labor requirements leading to intrusive changes to soil characteristics and microflora. Modern physical and chemical methods for heavy metal pollution remediation include adsorption on new adsorbents, such as - nanocarriers, ion exchange techniques, removal through improved membrane filtration techniques, electrodialysis, and photocatalysis.

Recently, the low cost and environmental friendly technique of phytoremediation (using hyperaccumulator plants to uptake metals from contaminated soil) drew researcher's attention (Bini *et al.*, 2012).

Gao *et al.* (2010) stated that application of microorganisms, in particular fungi, could considerably lessen heavy metal toxicity to plants and promote the heavy metals accumulation in plants even when the heavy metal concentration is low in the soil.

2.5.2 Phytoremediation

Phytoextraction is the technique of utilizing high biomass producing species to uptake metal and metalloids through roots and further transportation to the areal parts (Ehsan *et al.*, 2014). It is possible to increase plants capability to absorb heavy metals by manipulation of the antioxidant system, which improves plant tolerance (Gao *et al.*, 2010).

According to Benavides *et al.* (2005), plants have homeostatic cellular mechanism as well as phytochelatin based sequestration and compartmentalization process to control metal concentration to minimize potential damage. However, this process can be boosted with application exogenous application of organic acids by increasing metal solubility as well as visual growth advantages and extensive applicability due to biodegradability (Ehsan *et al.,* 2014).

Effective phytoremediation requires high metal solubility and bioavailability. Chelating chemicals promote the release of heavy metals from the soil matrix, increase the solubility of metals in the soil, and increase metal absorption by the roots (Arsenov *et al.*, 2020). According to Gao *et al.* (2010), chelates have been found to desorb metals from the soil matrix into the soil solution, enabling metal transport into the xylem and increasing metal translocation from roots to shoots of many fast-growing and high-biomass plants. Although EDTA has been the most effective in increasing water-soluble metal concentrations, its usage is hazardous to plants and other organisms. In addition EDTA may promote metal leaching into groundwater, pollute it (Gao *et al.*, 2010; Arsenov *et al.*, 2020).

2.5.3 Organic Acids for Phytoremediation

Low molecular weight organic acids (LMWOAs), such as citric acid, oxalic acid, tartaric acid, glutamic acid, nitrilotriacetate, salicylic acid, and proponic acid etc. (Gao *et al.*, 2010, Zou *et al.*, 2019) have been proposed as an alternative to synthetic chelators for their rapid biodegradation and mineralization by soil microorganisms(Duarte *et al.*, 2011) .

According to Liu *et al.* (2016), Salicylic acid (SA), an organic acid, has been used in many plant species to alleviate Cd toxicity by regulating plant growth, reducing Cd uptake and distribution in plants, protecting membrane integrity and stability, scavenging reactive oxygen species and enhancing antioxidant defense system, improving photosynthetic capacity.

2.6 Citric acid

Citric acid (CA), a 6-carbon tricarboxylic acid synthesized by the citrate synthase. It has synergistic effects on growth, yield, and a few chemical components of many crops.

Citric acid converts Cd into more easily transported form making Cd less toxic to plants and has excellent biodegradability, therefore, it is applied to adsorb Cd from soil and sludge (Zou *et al.*, 2019).

CA is used to remove Cd from cereal grains too. Zou *et al.* (2019) developed an efficient and economical method to reduce Cd with CA treatment from Cd-contaminated rice bran. Their results showed that a significant decline of Cd was reached by 0.15 M of CA with 60 min incubation at 40 °C. In addition, the Cd removal efficiency was more than 94% with CA treatment.

2.7 Plant Responses to Citric Acid

2.7.1 Plant Height

El-Yazal (2019) reported that 0.3% foliar application CA increased plant height up to 22.39% to 22.66%, and stem diameter up to 32.38% in comparison with control treatment. However, from 0.35% and higher foliar application of CA, plant height started to decrease than the controlled condition in *Zea mays*.

According to Mahmud *et al.* (2018), plant height in plants treated with 0.5 mM CA was similar to the controlled treatment but higher dose of CA (1.0 mM CA) slightly reduced plant height than the control condition.

Anwar *et al*. (2012) found that individual CA results in gradual increase in shoot length of Sahiwal-2002 up to 1 g kg^{-1} of CA (24.41 cm) as compared to control (16.05 cm) in maize.

2.7.2 Foliage

Ehsan *et al.* (2014) reported that 2.5 mM CA slightly increases the number of leaves and leaf area in *Brassica napus* but they are statistically similar to the control condition.

El-Yazal (2019) reported that 0.3% foliar application CA increased number of leaves $plant^{-1}$ by 5.63% to 7.09% in *Zea mays* than the controlled treatment, but it started to decline from 0.35% CA to higher concentration.

2.7.3 Biomass

Mahmud *et al.* (2018) reported that the fresh weight and dry weight found in *Brassica juncea* seedlings treated with 0.5 mM and 1.0 mM CA were statistically similar to the control treatment.

Ehsan *et al.* (2014), found *Brassica napus* seedlings treated with 2.5 mM CA improved stem fresh weight, stem dry weight, leaf fresh weight, and leaf dry weight respectively by 5.70%, 7.33%, 2.48%, and 4.95% compared to the control treatment.

Gao *et al.* (2010) studied the phytoextraction of Cd and the antioxidative defense of *Solanum nigrum* L. by application of a new isolated strain (*Paecilomyces lilacinus* NH1) (PLNH1) and CA. They found that the dry biomass at maturation stage exceeded in plants treated with 20 mM CA compared to the controlled condition.

2.7.4 Fruit Quality and Yield

El-Yazal (2019) found that 0.3% foliar application CA increased all the factors contributing to the yield of *Zea mays.* It enhanced 14.04 to 15.64% ear length, 12.94% to 13.35% ear diameter, 6.92% to 6.11% number of rows ear^{-1}, 4.55% to 4.81% number of grain row^{-1}, 26.91% to 27.26% grain weight ear^{-1}, 14.44% to 13.53% weight of 100 grain, 44.32% to 43.38% ear weight plant^{-1}, and 26.89% to 27.26% grain yield fed^{-1} respectively over the control plants.

2.7.5 Relative Water Content

Mahmud *et al.* (2018) reported no significant change in relative water content after 0.5 mM and 1.0 mM CA application in *Brassica juncea* seedlings.

2.7.6 Chlorophyll Content

Mahmud *et al.* (2018) found no significant change in chlorophyll content (chl *a*, chl *b*) after 0.5 mM and 1.0 mM CA application in *Brassica juncea.*

El-Yazal (2019) stated that 0.3% foliar CA application improved chl *a* 23.72% to 23.55%, chl *b* by 17.35% to 17.17% respectively over the control plants of maize, although the rate started from 0.35% and above concentration.

Ehsan *et al.* (2014), reported that SPAD value increases significantly by 15.39% compared to the control treatment with the application 2.5 mM CA in *Brassica napus* seedlings.

2.8 Effect of Citric Acid on Cadmium Stressed Plants

2.8.1 Plant Height

According to Mahmud *et al.* (2018) plant height decreased by 18% and 24% under mild and severe Cd stress compared with the control treatment, respectively. 0.5 mM and 1.0 mM CA application considerably recovered the plant height of the stressed plants.

Wang *et al.* (2017) reported that Cd stress caused 24% and 20% decline in plant height of tall fescue and Kentucky bluegrass compared to the control treatment. Application 1 mM kg^{-1} CA resorted the detrimental effect of Cd stress by showing no additional reduction in plant height of tall fescue and Kentucky bluegrass when compared with Cd stress only.

2.8.2 Foliage

Arsenov *et al.* (2020) found no significant change in specific leaf area until 30 day exposure of Cd 3 mg kg^{-1} and 6 mg kg^{-1} in dry soil, but it decreased after 90 days by 34% and 36% in older leaves compared with control treatment of *Salix viminalis.* On the other hand, double application of CA reduced younger leaf by 19% than the plants grown in Cd concentration of 6 mg kg^{-1} soil.

Ehsan *et al.* (2014) reported that the combination of 2.5 mM CA with 10 µM and 50 µM Cd can effectively deny the inhibition caused from Cd stress and produce slightly higher number of leaves in *Brassica napus*.

2.8.3 Biomass

Arsenov *et al.* (2020) reported that in *Salix viminalis* (clone SV068) the detrimental effect of Cd 3 mg kg^{-1} and 6 mg kg^{-1} in dry soil for 30 day which caused, 32% and 43% decline on root mass ratio (RMR) as compared to control treatment. It was restored by 20 mM kg^{-1} CA of soil. However, after 60 days the combined treatment of Cd and CA caused 20% and 25%

decline on RMR. They also stated that both single or multiple application of CA at 5mM kg^{-1} soil in the Cd contaminated soil had no negative effect to shoot biomass.

According to Mahmud *et al.* (2018), in mustard plants, the combination of 0.5 mM and 1.0 mM CA with 0.5 mM and 1.0 mM $CdCl_2$ doses can improve biomass production than ones treated alone with Cd. 0.5 mM CA showed the best performance in restoration. Seedling treated with 0.5 mM CA and 0.5 mM $CdCl_2$ and 1.0 mM $CdCl_2$ had 15% and 18.64% higher fresh weight than the ones treated only with Cd. Dry weight followed the same trend.

Wang *et al.* (2017) reported that application of 1mM kg^{-1} CA restored shoot biomass of 13% in tall fescue and 10% in Kentucky bluegrass under 100 mg Cd kg^{-1} contamination.

Ehsan *et al.* (2014) found *Brassica napus* seedlings treated in combination with 2.5 mM CA and Cd concentration of 10 µM and 50 µM can overcome Cd stress and improve stem fresh weight, stem dry weight, leaf fresh weight, and leaf dry weight respectively by 21.85% and 22.92%; 38.21% and 28.67%, 19.90% and 30.84%; 19.20% and 18.26% compared to the individual 10 µM and 50 µM Cd treatments.

Gao *et al.* (2010) stated that 20mM CA kg^{-1} dry soil under 50mg Cd kg^{-1} dry soil exposure at least enhanced 30–45% of plant biomass compared to the treatment without the application of CA.

2.8.4 Relative Water Content

Mahmud *et al.* (2018) stated both 0.5 mM and 1.0 mM CA can remarkably enhance the water status and adjusted the osmotic condition by increasing leaf RWC around 4.23% to 7.01% combining with 0.5 mM and 1.0 mM $CdCl_2$ doses.

2.8.5 Chlorophyll Content

Mahmud *et al.* (2018) reported that mustard seedlings grown under the combined dose of Cd and CA improved chlorophyll content than plants exposed to Cd. Compared with Cd stress alone, applying CA with Cd can effectively restore around 30% to 70% depending upon their concentration. In addition, lower dose of CA (0.5 mM CA) was more efficient in restoration of chl content.

Ehsan *et al.* (2014) examined *Brassica napus* L. in hydroponics to improve Cd phytoextraction with the help of CA. They found that addition of 2.5 mM CA along with 10 μM and 50 μM Cd significantly increased chl *a*, chl *b*, total chl, and carotenoid contents in the leaves compared to the plants only treated with Cd.

CHAPTER 3

MATERIALS AND METHODS

The experiment was conducted to study cadmium stress and citric acid as a remedy to reduce it in a popular chili variety, BARI Morich-2, from November 2019 to April 2020 at Sher-e-Bangla Agricultural University, Dhaka-1207. The materials used and the methodology followed in the investigation are presented in this chapter.

Experimental Treatments and Sources of Plant Populations

3.1 Experimental Site

The experiment was conducted at the Agroforestry filed lab and experimental farm of Sher-e-Bangla Agricultural University, Dhaka, during November 2019 to April 2020. The experimental area was situated at 23°46′ N latitude and 90°22′ E longitude at an altitude of 8.6 meter above the sea level. The experimental site is shown in the AEZ Map of Bangladesh in Appendix I.

3.2 Climate

The experimental site is situated in a subtropical environment that has three distinct seasons: summer (March to April), monsoon (May to October), and winter season (November to February). The investigation was carried in the winter or popularly known as the Rabi season (Nov 01, 2019 – Apr 15, 2020), and the major meteorological data (air temperature, relative humidity, and rainfall) of this period were collected from Bangladesh Meteorological Department (Climate & weather division), Dhaka, which is presented in the Appendix III.

3.3 Soil

In order to avoid prior contamination, uncontaminated soil was brought from Savar, which belongs to the Madhupur Tract (AEZ-28). The soil used in the experiment had a silty loam texture, low organic matter content, and dark olive-grey color. The analytical data of the soil sample collected from the experimental area was determined in the Soil Resource

Development Institute (SRDI), Soil Testing Laboratory, Khamarbari, Dhaka and were presented in Appendix II.

3.4 Planting Material

Seeds of BARI Morich-2 were used as the test crop for this experiment. It was collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. Healthy uniform chili seeds were selected, soaked in water overnight, and sown on Nov 03, 2019.

3.5 Treatments of the Experiment

The seedlings were grown in pots exposed to low and high level of CA which is 1.0 mM and 5.0 mM, along with mild and acute cadmium which are respectively 2 mM and 4 mM of $CdCl_2$. There were 9 treatments and three replications sole or in combination with. Treatments were-

i) Control (no $CdCl_2$, Cd0 and no citric acid, CA0)
ii) 1.0 mM Citric acid (Cd0 CA1)
iii) 5.0 mM Citric acid (Cd0 CA5)
iv) 2.0 mM $CdCl_2$ (Cd2 CA0)
v) 2.0 mM $CdCl_2$ and 1.0 mM Citric acid (Cd2 CA1)
vi) 2.0 mM $CdCl_2$ and 5.0 mM Citric acid (Cd2 CA5)
vii) 4.0 mM $CdCl_2$ (Cd4 CA0)
viii) 4.0 mM $CdCl_2$ and 1.0 mM Citric acid (Cd4 CA1)
ix) 4.0 mM $CdCl_2$ and 5.0 mM Citric acid (Cd4 CA5)

Application of CA and Cd was started from 15 days after transplanting the seedlings and applied twice with 15 days intervals.

3.6 Design and Layout of the Experiment

The experiment was laid out in Completely Randomized Design (CRD) having three replications. There were 27 pots in the experiment. The size of each pot was 35.56 cm (14 inches) in diameter and 35.56 cm (10 inches) in height.

3.7 Pot Preparation

Soil was sorted to completely remove weeds and stubbles and mixed with cow dung in 1:1 proportion. Plastic pots were filled on Nov 01, 2019. In the later plant growing stages only Urea @ 5 g pot^{-1} was applied at 15 and 30 days after transplanting (DAT) to increase plant growth.

3.8 Cultivation Technology

3.8.1 Transplanting of Seedlings

Seedlings were transplanted 40 days after sowing, on Dec 02, 2019, in a way that the roots and leaves do not get torn and remain in good condition in the new pot. 27 containers accommodated 54 seedlings as 2 seedlings per pot. Plants were tagged by using laminating card.

3.8.2 Weeding

In order to keep the plants free from competition to uptake water and doses of different treatments, weeding was performed frequently.

3.8.3 Irrigation

Plant were lightly irrigated immediately after transplanting and continued throughout cultivation process until harvesting. Frequency of watering depended upon the moisture status of the soil to avoid waterlogging or drought stress to the plants.

3.8.4 Disease and Pest Control

Experimental chili plants were infected by aphids during the flowering stage. The adult insects and larvae were controlled by spraying Pyrithrin @ 1.5 ml L^{-1} twice at 7 day's interval.

3.8.5 Harvesting of Fruits

Depending upon maturity and color formation, fruits were harvested from different plants for 15 days' period. It started from April 1st 2020 to April 15th 2020.

3.9 Data Collection

Data on the following parameters were recorded from each pot during the course of experiment. Data was collected in the four stages of chili plant growth. 15 DAT = seedling stage, 30 DAT = vegetative stage, 45 DAT = flowering stage, 60 DAT = fruiting stage

a) Plant height (cm)

b) Number of leaf per plant

d) Number of branches per plant

e) Length of fruit (cm)

f) Diameter of fruit (cm)

g) Individual fruit weight (g)

h) Number of fruit per plant

i) Yield per ha (t/ha)

j) Fresh weight per plant (g)

k) Dry weight per plant (g)

l) Relative water content (%)

m) Chlorophyll content (SPAD value)

3.9.1 Plant Height

Plant height was measured from sample plants in centimeter from the ground level to the tip of the longest stem of five plants and mean value was calculated. Plant height was measured with a meter scale from three plants at 15 DAT, 30 DAT, 45 DAT, and 60 DAT.

3.9.2 Number of Leaves and Fruits Per Plant

The number of leaves, flower buds, flowers and fruits plant^{-1} were recorded by counting all the leaves, flower bud, flowers, and fruits from each plant of each pot and the mean was calculated plants at 15 DAT, 30 DAT, 45 DAT, and 60 DAT.

3.9.3 Number of Branches Per Plant

Number of Branches plant^{-1} were recorded by counting all the branches of each plants and the mean was calculated at 15 DAT, 30 DAT, 45 DAT, and 60 DAT.

3.9.4 Length of Fruit

The length of fruit was measured with a meter scale from the neck to the bottom of fruits from each plot, averaged, and expressed in cm.

3.9.5 Diameter of Fruit

Breath of fruit was measured at the middle portion of fruit from each plot with a slide calipers, average was taken, and expressed in cm.

3.9.6 Individual Fruit Weight

The weight of individual fruit was measured with a digital weighing machine from each selected plots, calculated average, and expressed in g.

3.9.7 Fresh Weight and Dry Weight of Seedling

For fresh weight and dry weight measurement, 10 seedlings from each treatment were selected. These selected seedlings were uprooted carefully, weighed in a digital balance (except the root portion); data were recorded and considered as fresh weight (FW). Dry weight (DW) was determined after drying the seedlings at 80° C for 48 h.

3.9.8 Yield of Fruits Per Hectare

Yield of fruits per hectare was measured by the following formula

Fruit yield (t/ha) = (Fruit yield per pot (kg) × 10000) ÷ (Area of pot in square meter × 1000)

3.9.9 SPAD Value

Chlorophyll (chl) content in terms of SPAD (soil plant analysis development) values was recorded using a portable SPAD 502 Plus meter (Konica-Minolta, Tokyo, Japan). In each measurement, the SPAD reading was repeated 5 times from the leaf tip to base, and the average was used for analysis.

3.9.10 Determination of Leaf Relative Water Content

Relative water content (RWC) was measured according to Barrs and Weatherly (1962). Leaf laminas from randomly chosen plants were taken. Leaves were weighed as FW and then immediately floated on distilled water in a petri plate for 8 h in the dark. Turgid weights (TW) of leaves were obtained after removing excess surface water with paper towels. Dry weights (DW) of leaves were measured after drying at 80°C for 48 h. Then, RWC was calculated using the following formula

RWC (%) = [(FW-DW)/ (TW-DW)] ×100

3.9.11 Statistical Analysis

Collected data on various characters were statistically analyzed using SPSS and Statistix 10 computer package program. The experimental data were subjected to two-way analysis of variance (ANOVA). Means were compared by Tukey's HSD test at the 5% level of significance using SPSS program.

Chapter 4

RESULTS AND DISCUSSION

This chapter represents the results of the experiment on the growth and yield of chili affected by Cd stress and the effect of CA on them. Data collected from the study on various observations were recorded, tabulated, statistically analyzed, and illustrated through figures with appropriate headings to describe the findings. The analyses of variance (ANOVA) of growth and yield parameters are presented in Appendix IV – XIII.

4.1 Plant height

4.1.1 Effect of Cadmium Stress on Plant Height

Cadmium toxicity is highly significant on plant height which is prevalent from the early growth stage until fruiting stage in a dose-dependent manner (Table 1). Acute Cd stress (Cd4) caused highest damage on plant and its toxicity amplified over time. Cd4 reduced plant height by 23.90%, 41.40%, 47.80%, and 57.56% than Cd0 (no $CdCl_2$) at 15 DAT, 30 DAT, 45 DAT, and 60 DAT respectively. The data suggests that chili plant height can be reduced to half due to acute Cd exposure during growing stage than to the plants grown in non-toxic condition. Aslam *et al.* (2014) agreed to it on his study one chili plants.

Mild Cd stress is also responsible for dropping plant height in chili plants. Cd2 application caused 16.26%, 29.81%, 23.87%, and 26.19% lower plant height than Cd0 (no $CdCl_2$) at 15 DAT, 30 DAT, 45 DAT, and 60 DAT respectively. Wang *et al.* (2017), Mahmud *et al.* (2019), and Bhuyan *et al.* (2020) reported similar results.

4.1.2 Effect of Citric Acid on plant height

From the data presented in Table 1, it is evident that CA is statistically significant that plays a positive effect on plant height through improvement over the growth stages. CA1, performed best in all the growth stages by improving plant height by 11.01%, 4.55%, 11.50%, and 12.70% than the no citric acid condition, CA0, respectively at 15 DAT, 30 DAT, 45 DAT, and 60 DAT. The result is similar to the findings of Anwar *et al*. (2012) on role of CA in stem cell elongation, cell devision.

High dose of CA application may not contribute to enhance plant height. In the experiment, CA5, high concentration of CA is statistically similar to the plants grown in CA0 treatment. However, comparing with the no citric acid condition, CA0, CA5 slightly decreased plant height by 1.17%, 4.90%, 4.67%, and 3.44% respectively at 15 DAT, 30 DAT, 45 DAT, and 60 DAT. Mahmud *et al.* (2018) reported similar results from his experiment on mustard plants.

Table 1. Effect of different levels of Citric acid and Cadmium on plant height in chili

Treatment	**Plant Height (cm)**			
	15 DAT	**30 DAT**	**45 DAT**	**60 DAT**
Levels of Cadmium				
Cd0	9.09 a	17.11 a	29.44 a	47.91 a
Cd2	7.61 b	12.01 b	22.42 b	35.36 b
Cd4	6.92 b	10.03 c	15.37 c	20.34 c
Levels of Citric Acid				
CA0	7.59 b	13.06 ab	21.81 b	33.31 b
CA1	8.53a	13.68 a	24.64 a	38.15 a
CA5	7.50 b	12.41 b	20.79 b	32.16 b
SE (±)	0.252	0.301	0.446	0.567
Significance (*P*)				
Cd	***	***	***	***
CA	**	**	***	***

Here, Cd0: 0 mM $CdCl_2$ (Control); Cd 2: 2 mM $CdCl_2$; Cd4: 4 mM $CdCl_2$;
CA0: 0 mM CA (Control); CA 1: 1 mM CA ; CA5: 5 mM CA
*** means 1% level of significance,
** means 5% level of significance,
NS means Non-Significant

4.1.3 Combined Effect of Citric Acid and Cadmium on Plant Height

The combined effect of CA and Cd indicates that the phytoremediation capacity of CA under Cd stress. Figure 1, clearly depicts that CA can effectively dismiss the detrimental effect of Cd stress and improve plant height up to a certain level of Cd toxicity. The tallest plant was found in Cd0 CA1 which is 49.00 cm at 60 DAT and the smallest in Cd4 CA5, 6.42 cm at 15 DAT (Appendix IV). Cd4 CA5 continued the trend of producing the smallest plants in all growth stages, which are. 6.42, 9.00, 13.03, and 15.57 cm at 15 DAT, 30 DAT, 45 DAT, and 60 DAT. However, Cd0 CA1 keep up trend of producing the tallest plats only in flowering and fruiting stage, which were 30.00 and 49.00 cm at 45 and 60 DAT.

At seedling stage, 15 DAT, CA performed efficiently restoring plant height with low dose and exhibited no cumulative toxic effect at high dose under severe Cd stress. Cd2 CA1 (8.50 cm), Cd2 CA5 (7.33cm), and Cd4 CA1 (7.83 cm) are statistically similar and Cd4 CA5 (6.42 cm) is not different to Cd4 CA0 (6.50 cm). It reveals that there was no detrimental effect, rather it improved plant height. Cd2 CA1 (8.50 cm) and Cd2 CA5 (7.33cm) improved plant height by 21.43% and 4.72% than Cd2 CA0 (7.00 cm), as well as, Cd4 CA1 (7.83 cm) is 8.13% greater than Cd4 CA0 (6.50 cm). The results supports Wang *et al.*, (2017)'s statement that the build-up in shoot citrate from exogenous CA application can improve plant's capacity to detoxify and Cd accumulation in the vacuoles safely.

At vegetative, flowering, and fruiting stage, 30 DAT, 45 DAT, and 60 DAT similar trend of phytoremediation was followed by Cd2 CA0, Cd2 CA1, and Cd2 CA5. However, in case of severe Cd stress, only low CA concentration could work properly and high concentration created cumulative toxicity. At 30 DAT, 45 DAT, and 60 DAT, Cd4 CA1 (11.00, 18.58, and 24.32 cm) improved plant height by 9.13%, 28.14%, and 15.09% than Cd4 CA0 (10.08, 14.50, and 21.13 cm). On contrary, at 30 DAT and 60 DAT, plant height in Cd4 CA5 (9.00 and 15.57 cm) reduced by 10.72% and 26.32% than Cd4 CA0 (10.08 and 21.13 cm). Effect of CA and Cd stress were highly significant at 60 DAT. Mahmud *et al.* (2018) reported similar findings.

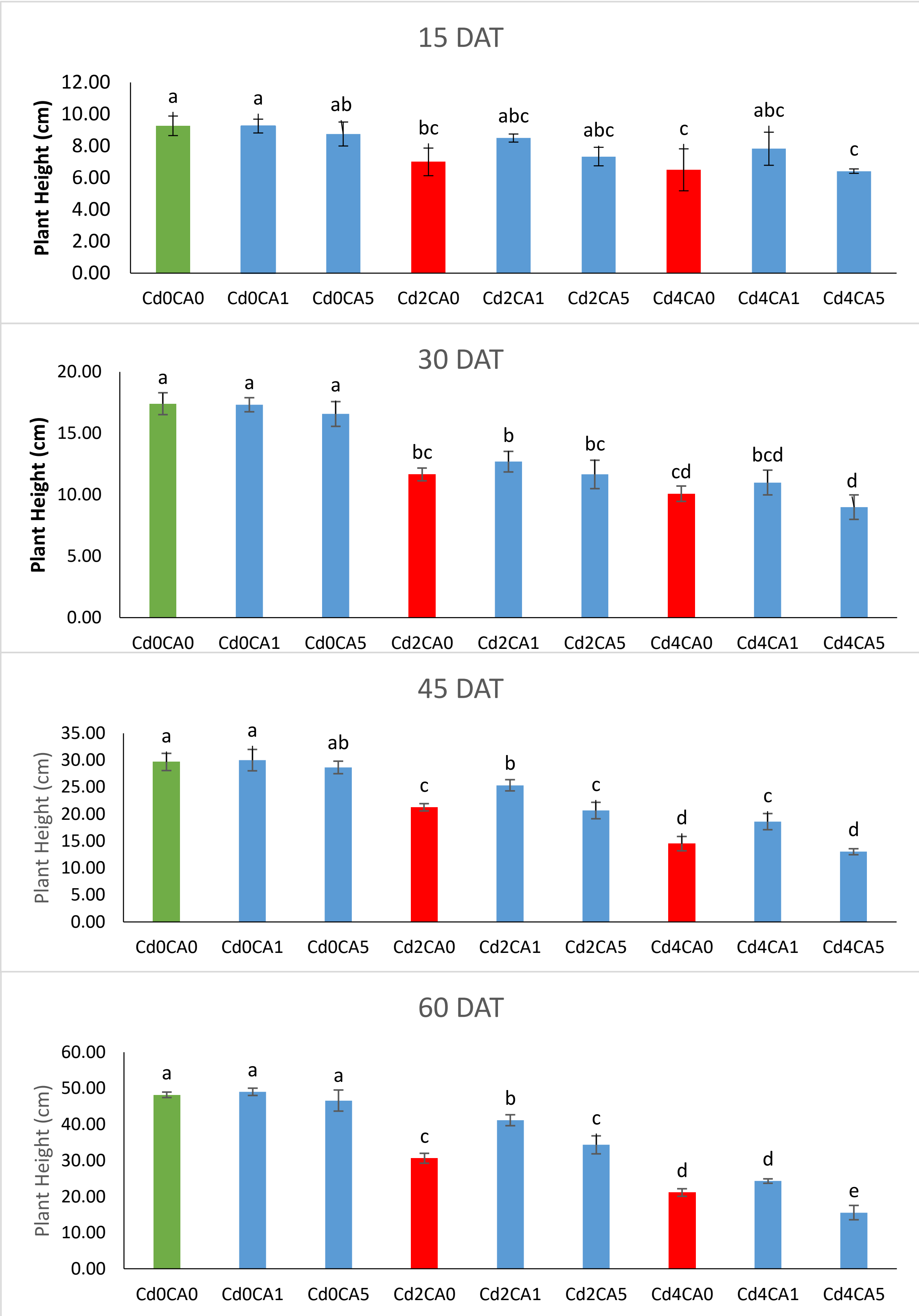

Figure 1. Effect of CA and Cd stress on plant height at different DAT ($SE \pm_{0.05}$ = 0.436, 0.521, 0.772, and 0.982 at 15DAT, 30DAT, 45DAT, and 60 DAT respectively). Cd0: 0 mM $CdCl_2$ (Control); Cd 2: 2 mM $CdCl_2$; Cd4: 4 mM $CdCl_2$; CA0: 0 mM CA (Control); CA 1: 1 mM CA ; CA5: 5 mM CA

4.2 Number of Leaves Per Plant

4.2.1 Effect of Cadmium Stress on Number of Leaf Per Plant

Leaf number per plant of chili showed statistically highly significant variation due to the diverse levels of Cd stress (Table 2). Maximum number of leaves per plant was found in Cd0 (85.67) at 60 DAT and the minimum in Cd4 (8.89) at 15 DAT. Cd2 and Cd4 are statistically different and drastically declined the number of leaves in all growth stages. Cd4 (8.89, 11.67, 17.78, 28.33), showed the highest detrimental effect of Cd toxicity by lowering leaf number by 59.39%, 73.01%, 72.93%, and 66.93% in compared to the control treatment Cd0 (21.89, 43.22, 65.67, 85.67) at 15 DAT, 30 DAT, 45 DAT, and 60 DAT respectively. Ehsan *et al.* (2014) and Gill *et al.* (2011) presented alike result stating that Cd is known to be stored in the leaves of plants grown in its contact and sharply decrease the number of leaves with higher exposure.

4.2.2 Effect of Citric Acid on Number of Leaf Per Plant

Citric acid is highly significant in terms of increasing number of leaves per plant of chili (Table 2). CA1, is statistically different compared to CA5 and the control treatment in all the growth stages of chili plant. CA1 performed best among the three doses by producing 10.07%, 15.11%, 24.49%, and 23.41% greater the number of leaves in each plant than CA0 respectively at 15 DAT, 30 DAT, 45 DAT, and 60 DAT. On the other hand, CA5 was statistically similar to CA0, although it produced slightly higher number of leaves per plant in all the growth stages. The result agrees with the findings of El-Yazal (2019) and Ehsan *et al.* (2014).

Table 2. Effect of different levels of Citric acid and Cadmium on number of leaves per plant

Treatment	**Number of Leaves Per Plant**			
	15 DAT	**30 DAT**	**45 DAT**	**60 DAT**
Levels of Cadmium				
Cd0	21.89 a	43.22 a	65.67 a	85.67 a
Cd2	11.33 b	28.22 b	57.22 b	59.89 b
Cd4	8.89 c	11.67 c	17.78 c	28.33 c
Levels of Citric Acid				
CA0	13.89 b	26.22 b	44.11 b	53.44 b
CA1	15.44 a	30.89 a	54.44 a	69.78 a
CA5	12.78 b	26.00 b	42.11 b	50.67 b
SE (±)	0.411	0.657	2.07	1.69
Significance (*P*)				
Cd	***	***	***	***
CA	***	***	***	***

Here, Cd0: 0 mM CdCl2 (Control); Cd 2: 2 mM CdCl2; Cd4: 4 mM CdCl2;
CA0: 0 mM CA (Control); CA 1: 1 mM CA; CA5: 5 mM CA;
*** means 1% level of significance,
** means 5% level of significance,
NS means Non-Significant

4.2.3 Combined Effect of Citric Acid and Cadmium on Number of Leaves Per Plant

Figure 2 illustrates that all the treatment of CA and Cd impacted on the leaf of number per plant in all stages of growth. Highest number of leaves per plant was found in Cd0 CA1 (91.33) at 60 DAT (Appendix VI). Furthermore, in each growth stages, the plants with highest number of leaves per plant were in Cd0 CA1, which are 22.23, 44.33, 73.00, and 91.33 leaf per plant at 15 DAT, 30 DAT, 45 DAT, and 60 DAT, compared to all other treatments. In addition, Cd0 CA5 is statistically similar to Cd0 CA0 at 15 DAT, 30 DAT, and 60 DAT, which implies that high dose of CA contributed to equal number of leaves as the controlled treatment. At 45 DAT, flowering stage of chili, Cd0 CA1 (73.00) had 12.88% higher leaves than Cd0 CA0 (64.67).

In terms of phytoremediation, CA is significantly effective to rule out impacts of Cd. Cd2 CA1 (12.67, 35.00, 69.00, and 79.67) produced 10.53%, 28.57%, 25.12%, and 35.98% higher leaves than Cd2 CA0 (11.33, 25.00, 51.67, 51.00) respectively at 15 DAT, 30 DAT, 45 DAT, and 60 DAT by denying Cd stress and improving growth. Likewise, Cd4 CA1 (11.00, 13.33, 21.33, and 38.33) grew 17.50% to 38.26% higher leaves than Cd4 CA0 (8.33, 11.00, 16.00, and 23.67) at 15 DAT, 30 DAT, 45 DAT, and 60 DAT. Ehsan *et al.* (2014) found similar findings in mustard plants.

However, higher dose of both CA and severe Cd stress caused cumulative toxic effect and produced the lowest number of leaves in every growth stage in Cd4 CA5, 7.333, 10.667, 16.000, 23.000 respectively at 15 DAT, 30 DAT, 45 DAT, and 60 DAT. Arsenov *et al.* (2020) reported similar result from his experiment on *Salix viminalis* with CA and Cd stress.

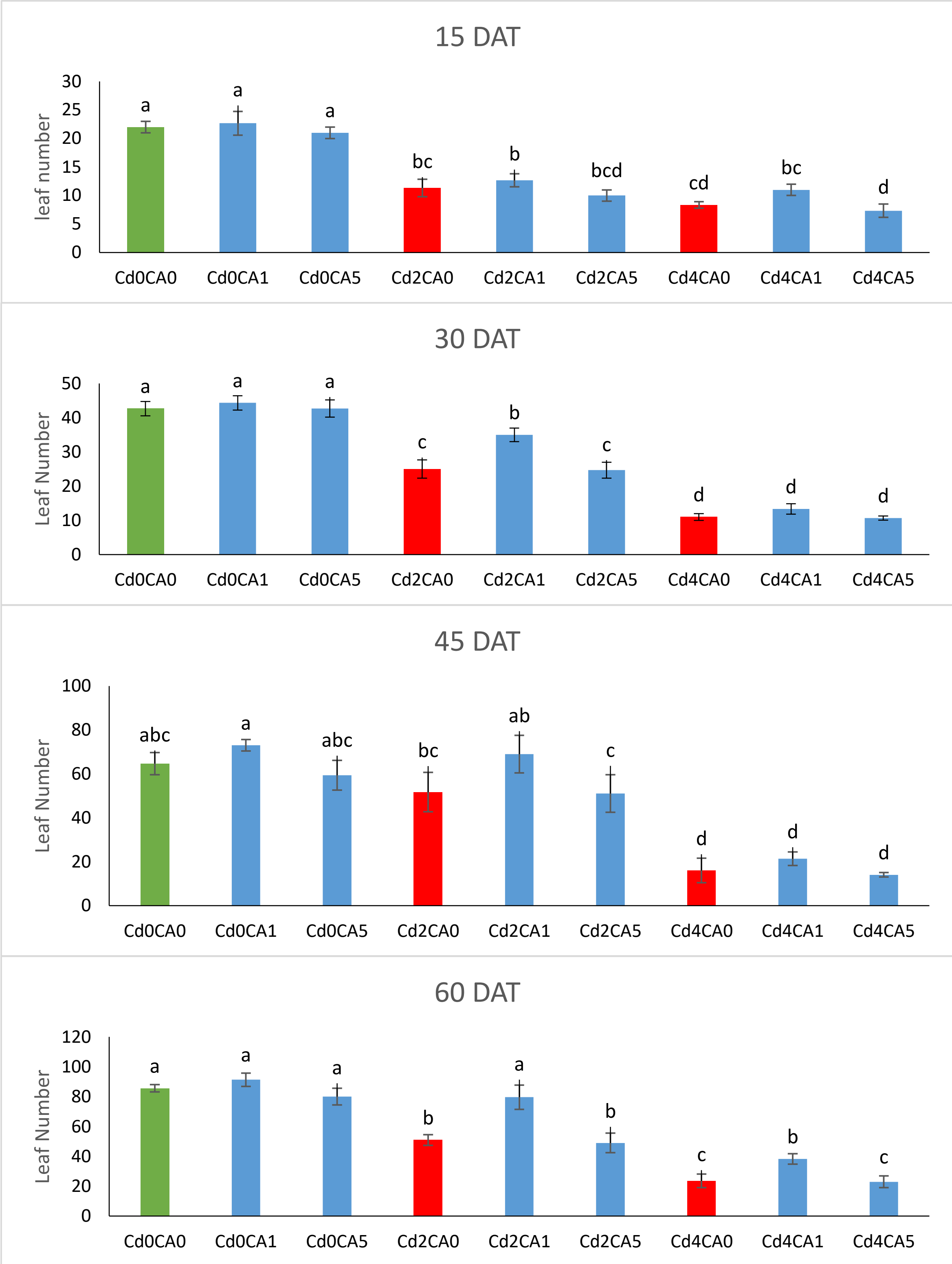

Figure 2. Effect of CA and Cd stress on number of leaves per plant at different DAT ($SE \pm_{0.05}$ = 0.711, 1.139, 3.582, and 2.910 at 15DAT, 30DAT, 45DAT, and 60 DAT respectively). Cd0: 0 mM $CdCl_2$ (Control); Cd 2: 2 mM $CdCl_2$; Cd4: 4 mM $CdCl_2$; CA0: 0 mM CA (Control); CA 1: 1 mM CA; CA5: 5 mM CA

4.3 Number of Branches Per Plant

4.3.1 Effect of Cadmium stress on Number of Branches Per Plant

Cadmium stress significantly reduced the number of branches per plant in the chili plants in dose depended manner (Table 3). The highest number of branches per plant was found in no stress condition, Cd0 (7.78) at 60 DAT. The lowest number of branches per plant was in Cd (1.44) at 45 DAT. At 45 DAT, Cd demonstrated the most detrimental effect by lowering branching around 3 fold in Cd2 (2.22), and 4.5 fold in Cd4 (1.44) than Cd0 (6.56).

4.3.2 Effect of Citric Acid on Number of Branches Per Plant

All three treatments of CA, CACA1 (4.00 and 5.89), CA5 (3.00 and 4.78), and CA0 (3.22 and 4.89) are statistically non-significant and similar to each other (Table 3). The result reveals that CA did not result in increasing the number of branches.

Table 3. Effect of different levels of Citric acid and Cadmium number of branches

Treatment	**Number of branches**	
	45 DAT	**60 DAT**
Levels of Cadmium		
Cd0	6.56 a	7.78 a
Cd2	2.22 b	5.00 b
Cd4	1.44 b	2.78 c
Levels of Citric Acid		
CA0	3.22 a	4.89 a
CA1	4.00 a	5.89 a
CA5	3.00 a	4.78 a
SE (±)	0.294	0.390
Significance (*P*)		
Cd	***	***
CA	NS	NS

Here, Cd0: 0 mM CdCl2 (Control); Cd 2: 2 mM CdCl2; Cd4: 4 mM CdCl2;

CA0: 0 mM CA (Control); CA 1: 1 mM CA; CA5: 5 mM CA;

*** means 1% level of significance, ** means 5% level of significance, NS means Non-Significant

4.3.3 Combined Effect of Citric Acid and Cadmium on Number of Branches Per Plant

There was a significant variation in the number of branches per plant due to the application of different doses of CA and Cd treatments (Figure 3). At 45 DAT, flowering stage of chili, plants treated with mild to acute Cd and/or CA and Cd had lower number of branches than the controlled ones irrespective of CA presence (Appendix VIII). Statistically, all the treatments with Cd, Cd2 CA0 (2.00), Cd2 CA1 (2.67), Cd2 CA5 (2.00), Cd4 CA0 (1.00), Cd4 CA1 (2.33), Cd4 CA5 (1.00) were similar to each other revealing that CA had no restoration effect from Cd stress in terms of number of branches per plant.

At 60 DAT, fruiting stage, Cd0 CA1 (8.00) produced the highest number of branches, and the lowest readings were in Cd4 CA0 (2.33) and Cd4 CA5 (2.33). In addition, Cd2 CA0 (4.67), Cd2 CA1 (6.00), and Cd2 CA1 (6.00); and Cd4 CA0 (2.33) and Cd4 CA5 (2.33) are statistically similar. It reveals that CA could not restore the plants under stress, however, individual CA doses slightly improved the branching number.

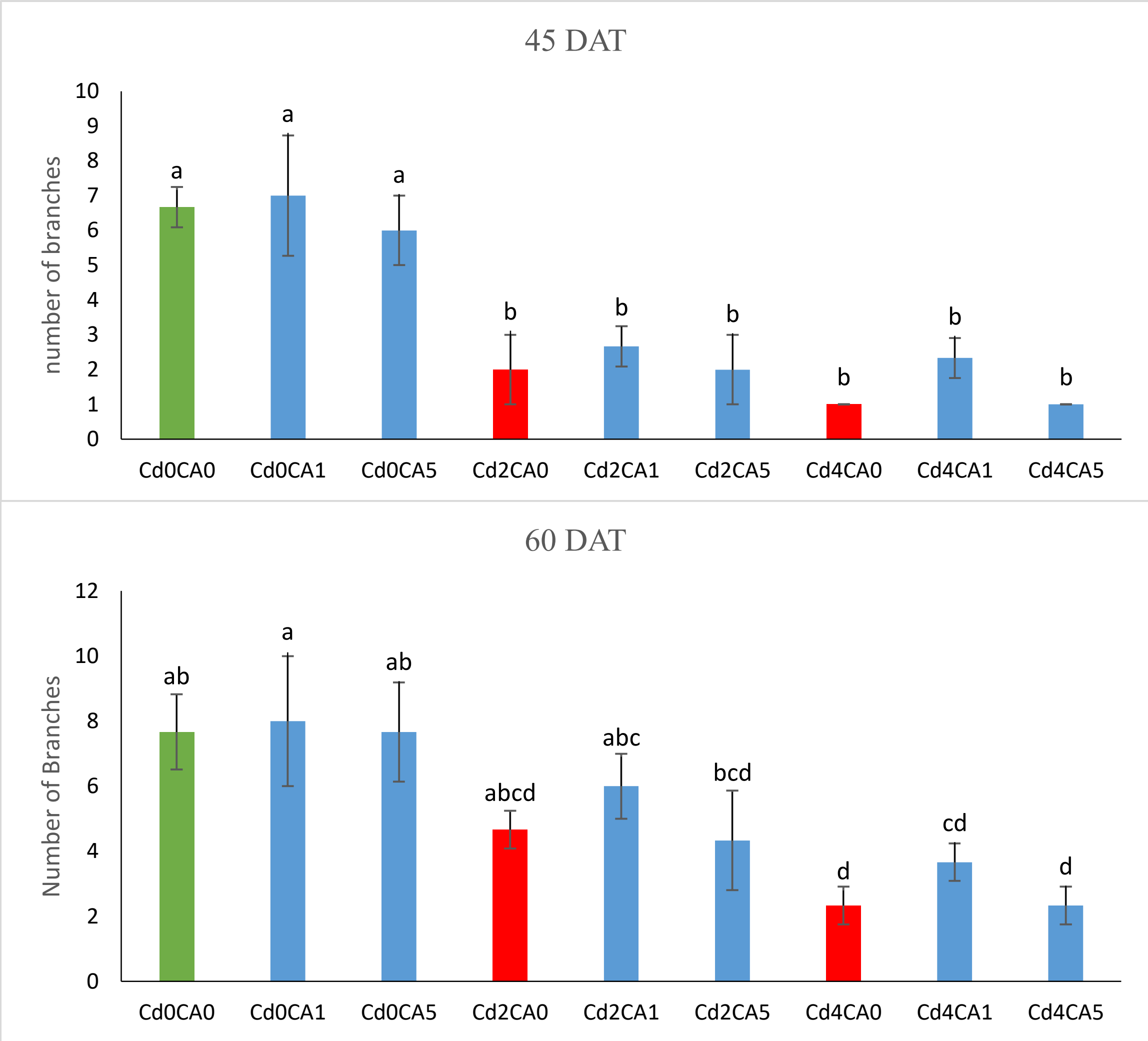

Figure 3. Effect of CA and Cd stress on number of branches per plant at different DAT (SE $\pm_{0.05}$ = 0.509 and 0.676 at 45DAT, and 60 DAT respectively). Cd0: 0 mM $CdCl_2$ (Control); Cd 2: 2 mM $CdCl_2$; Cd4: 4 mM $CdCl_2$; CA0: 0 mM CA (Control); CA 1: 1 mM CA; CA5: 5 mM CA

4.4 Fruit Length

4.4.1 Effect of Cadmium Stress on Fruit Length

Cd stress drastically reduced fruit length with increasing concentration (Table 4). Cd4 (2.98 cm) produced the smallest fruits. Fruit length declined by 23.83% and 44.51% respectively at Cd2 and Cd4 than the control treatment Cd0 (5.37 cm). Mozafariyan *et al.* (2014) and Georgieva *et al.* (1997) reported similar findings from their research on pepper plants.

4.4.2. Effect of Citric Acid on Fruit Length

It is evident from Table 4 that mild CA is highly significant in increasing fruit length at a mild level but higher dose is detrimental for chili plants in pots. CA1 (4.78 cm) performed best with 14.49% increase than the controlled treatment CA0 (4.09). On the other hand, CA5 reduced fruit length by 12.67% from CA0 (4.09). El-Yazal (2019) found similar results in *Zea mays*.

Table 4. Effect of different levels of Citric acid and Cadmium on fruit length of chili

Treatment	Fruit length (cm)
Levels of Cd	
Cd0	5.37 a
Cd2	4.09 b
Cd4	2.98 c
Levels of Citric Acid	
CA0	4.09 b
CA1	4.78 a
CA5	3.58 c
SE (±)	0.050
Significance (*P*)	
Cd	***
CA	***

Here, Cd0: 0 mM CdCl2 (Control); Cd 2: 2 mM CdCl2; Cd4: 4 mM CdCl2;
CA0: 0 mM CA (Control); CA 1: 1 mM CA; CA5: 5 mM CA;
*** means 1% level of significance,
** means 5% level of significance,
NS means Non-Significant

4.4.3 Combined Effect of Citric Acid and Cadmium on Fruit Length

Significant variation in fruit length was caused by different doses of CA and Cd treatments. Plants treated with CA alone are highly effective in increasing fruit length (Figure 4, Plate 1). Cd0 CA0 (5.36 cm) and Cd0 CA5 (5.32 cm) are statistically similar to Cd0 CA1 (5.44 cm) which had the highest fruit length (Appendix X). In addition, mild CA not only compensate

Cd stress but also improved fruit length at severe Cd stress. Cd2 CA1's (4.76 cm) fruit length was 18.70% higher than Cd2 CA0 (3.87 cm); and Cd4 CA1 (4.15 cm) is 26.75% higher than Cd4 CA0 (3.04 cm). Nevertheless, Cd4 CA5 (1.76 cm) had the lowest fruit length, perhaps, because higher CA is not effective in phytoremediation and in fact deteriorate fruit length at severe Cd stress.

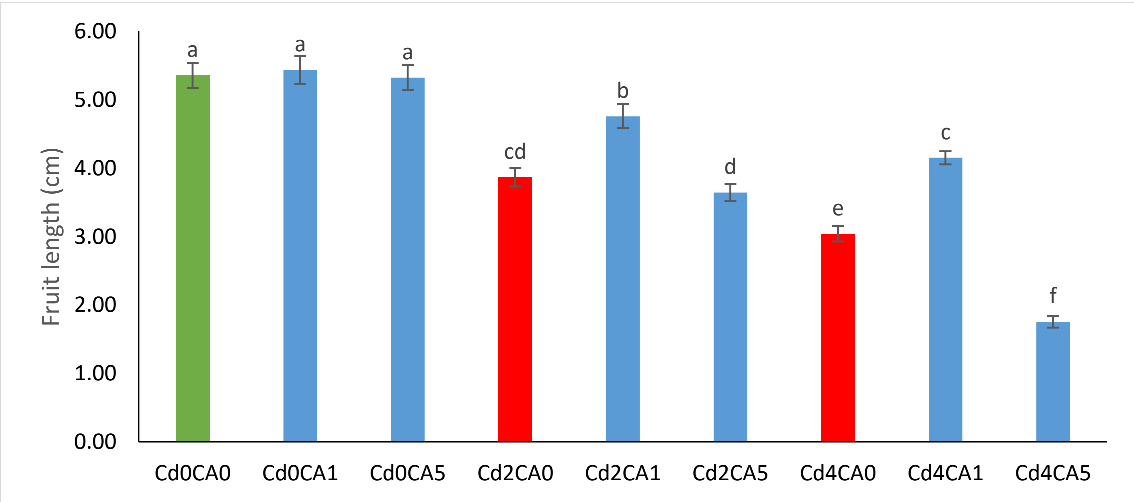

Figure 4. Effect of CA and Cd stress on fruit length (SE ± $_{0.05}$ = 0. 086); Cd0: 0 mM $CdCl_2$ (Control); Cd 2: 2 mM $CdCl_2$; Cd4: 4 mM $CdCl_2$; CA0: 0 mM CA (Control); CA 1: 1 mM CA; CA5: 5 mM CA

Plate 1. Effect of CA and Cd stress on fruit length. Cd0: 0 mM $CdCl_2$ (Control); Cd 2: 2 mM $CdCl_2$; Cd4: 4 mM $CdCl_2$; CA0: 0 mM CA (Control); CA 1: 1 mM CA; CA5: 5 mM CA

4.5 Fruit Diameter

4.5.1 Effect of Cadmium Stress on Fruit Diameter

Cd is highly significant and responsible for fruit diameter reduction with increasing concentration (Table 5). Cd2 (0.53 cm) is 19.70 % and Cd4 (0.47 cm) is 28.79% lower than Cd0 (0.66 cm). Cd4 (0.47 cm) produced the lowest fruit diameter. Mozafariyan *et al.* (2014) reported similar result from his experiment on *Capsicum annuum*.

4.5.2 Effect of Citric Acid on Fruit Diameter

CA is highly significant on fruit diameter and in the study it efficiently boosted the fruit diameter of chili plants (Table 5). CA1 has the highest fruit diameter 0.60 cm which is 11.11% higher than CA0 (0.54 cm). Furthermore, CA0 (0.54 cm) and Cd5 (0.51cm) are statistically similar. We can interpret that low concentration CA increases fruit diameter whereas higher CA dose has no toxic effect on fruit diameter of chili. El-Yazal (2019) found similar data from 0.3% foliar application CA on *Zea mays.*

Table 5. Effect of different levels of Citric acid and Cadmium number on fruit diameter

Treatment	Fruit Diameter (cm)
Levels of Cadmium	
Cd0	0.66 a
Cd2	0.53 b
Cd4	0.47 c
Levels of Citric Acid	
CA0	0.54 b
CA1	0.60 a
CA5	0.51 b
SE (±)	0.01
Significance (*P*)	
Cd	***
CA	***

Here, Cd0: 0 mM CdCl2 (Control); Cd 2: 2 mM CdCl2; Cd4: 4 mM CdCl2;

CA0: 0 mM CA (Control); CA 1: 1 mM CA; CA5: 5 mM CA; *** means 1% level of significance, ** means 5% level of significance, NS means Non-Significant

4.5.3 Combined effect of Citric Acid and Cadmium on Fruit Diameter

Significant variation in fruit diameter was caused by different doses of CA and Cd treatments (Figure 5). The highest fruit diameter was found at Cd0 CA1 (0.68 cm) and the lowest at Cd4 CA5 (0.41 cm) (Appendix X). It is also evident that every treatment where CA was applied it produced fruits with greater diameter even under severe Cd stress. Cd2 CA1 (0.59 cm) improved fruit diameter 13.56% from Cd2 CA0 (0.51 cm), as well as Cd4 CA1 (0.54 cm) is 16.67% higher than Cd4 CA0 (0.45 cm). On the other hand, Cd0 CA0 (0.65 cm) and Cd0 CA5 (0.64 cm); Cd2 CA0 (0.51 cm) and Cd2 CA5 (0.50 cm); Cd4 CA0 (0.45 cm) and Cd4 CA5 (0.41 cm) are statistically similar. It reveals that higher dose of CA alone and in combination with higher Cd has no cumulative toxic effect on the fruit diameter of chili.

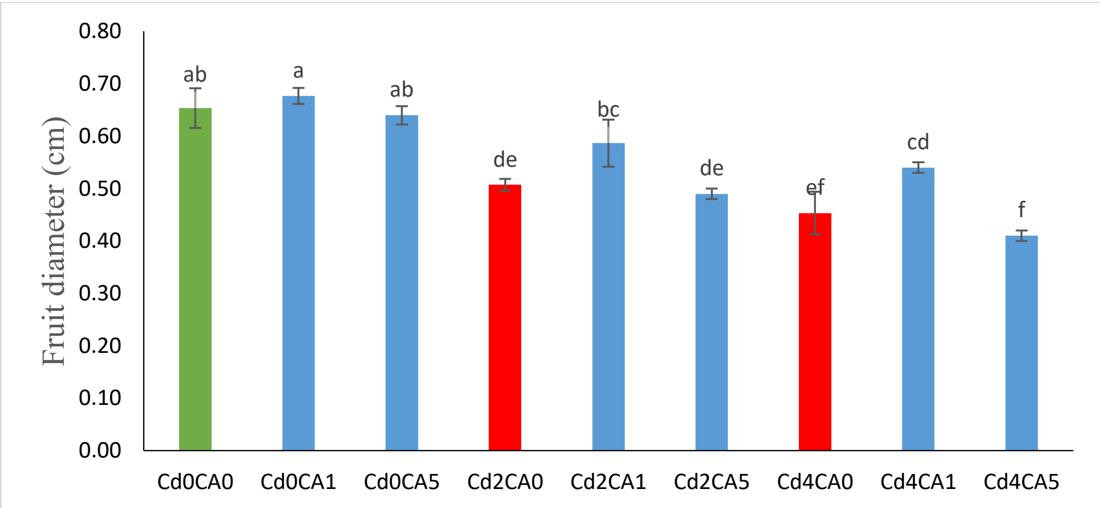

Figure 5. Effect of CA and Cd stress on fruit diameter (SE $\pm_{0.05}$ = 0.015); Cd0: 0 mM CdCl2 (Control); Cd 2: 2 mM CdCl2; Cd4: 4 mM CdCl2; CA0: 0 mM CA (Control); CA 1: 1 mM CA; CA5: 5 mM CA

4.6 Fruit Weight

4.6.1 Effect of Cadmium Stress on Fruit Weight

From Table 6, it is clear that Cd stress is highly significant on fruit weight. Cd2 (0.97 g) and Cd4 (0.67 g) are statistically different and sharply lower than Cd0 (1.21 g). We can say that Cd stress declines fruit weight with increasing concentration. Mozafariyan *et al.* (2014) and Georgieva *et al.* (1997) found similar result from their study on pepper under Cd stress.

4.6.2 Effect of Citric Acid on Fruit Weight

CA highly significant on fruit weight of chili (Table 6). Fruits produced in CA1 (1.12 g) were 13.40% heavier than the controlled treatment CA0 (0.97 g). Though fruit weight declined by 20.62% in CA5 (0.77 g) than CA0 (0.97 g). El-Yazal (2019) found alike results.

Table 6. Effect of different levels of Citric acid and Cadmium on fruit weight

Treatment	Fruit weight (g)
Levels of Cd	
Cd0	1.21 a
Cd2	0.97 b
Cd4	0.67 c
Levels of Citric Acid	
CA0	0.97 b
CA1	1.12 a
CA5	0.77 c
SE (±)	0.012
Significance (*P*)	
Cd	***
CA	***

Here, Cd0: 0 mM $CdCl_2$ (Control); Cd 2: 2 mM $CdCl_2$; Cd4: 4 mM $CdCl_2$;
CA0: 0 mM CA (Control); CA 1: 1 mM CA; CA5: 5 mM CA;
*** means 1% level of significance,
** means 5% level of significance,
NS means Non-Significant

4.6.3 Combined Effect of Citric Acid and Cadmium on Fruit Weight

There was a significant variation in single fruit weight among different doses of CA and Cd (Appendix X). From Figure 6, it is observed that the interactive effect of CA and Cd is highly significant on chili plants. Maximum fruit weight was found in Cd0 CA1 (1.32 g) and the minimum in Cd4 CA5 (0.390 g). Cd0 CA0 (1.25 g) is statistically similar to Cd0 CA1 (1.32

g), and Cd0 CA5 (1.06 g) is slightly lighter than Cd0 CA1 (1.32 g). It indicates that individual treatment of low concentration CA improves fruit weight.

In terms of phytochelation, low concentration of CA is quite effective against Cd stress. Cd2 CA1 (1.14 g) produced 18.70% heavier fruits than Cd2 CA0 (0.93 g), Cd2 CA5 (0.85 g) and Cd4 CA1 (0.84 g) are statistically similar to the control treatment. It implies that CA can protect fruits grown under Cd stress and maintain fruit weight as much as the controlled ones. On the other hand, high dose of CA induced cumulative toxicity causing fruits with lower weight under severe Cd stress. Cd4 CA5 (0.39 g) had the fruits with minimum weight.

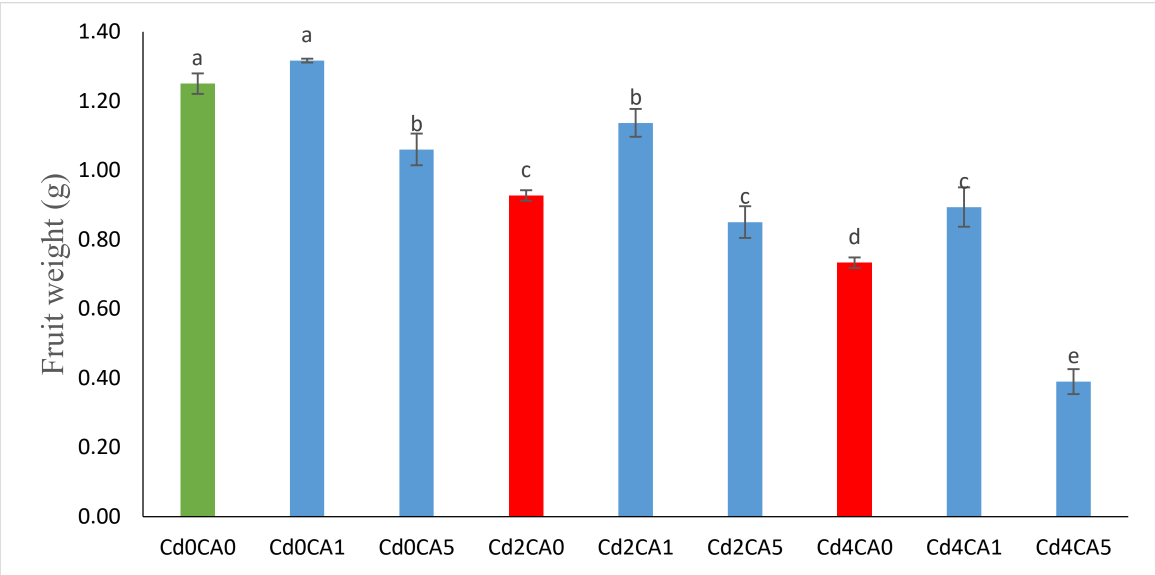

Figure 6. Effect of CA and Cd stress on fruit weight (SE $\pm_{0.05}$ = 0.021); Cd0: 0 mM CdCl2 (Control); Cd 2: 2 mM CdCl2; Cd4: 4 mM CdCl2; CA0: 0 mM CA (Control); CA 1: 1 mM CA; CA5: 5 mM CA

4.7 Number of Fruit Per Plant

4.7.1 Effect of Cadmium Stress on Number of Fruit Per Plant

Cd stress is highly significant on number of fruit per plant (Table 7). The lowest number of fruits per plant was found in Cd4 (28.22). Cd2 (38.44) and Cd4 (28.22) are statistically different and produced 16.83% and 48.94% lesser fruits than Cd0 (46.22). Georgieva *et al.* (1997) reported similar results.

4.7.2 Effect of Citric Acid on Number of Fruit Per Plant

Maximum number of fruit per plant was found in CA1 (43.44) which is 13.03% percent higher than CA0 (37.67) (Table 7). On the other hand, CA5 (31.78) is 1.64% lower than the control treatment. El-Yazal (2019) found alike data from *Zea mays*.

Table 7. Effect of different levels of Citric acid and Cadmium on number of fruit per plant

Treatment	**Number of Fruit Per Plant**
Levels of Cd	
Cd0	46.22 a
Cd2	38.44 b
Cd4	28.22 c
Levels of Citric Acid	
CA0	37.67 b
CA1	43.44 a
CA5	31.78 c
SE (±)	0.525
Significance (*P*)	
Cd	***
CA	***

Here, Cd0: 0 mM CdCl2 (Control); Cd 2: 2 mM CdCl2; Cd4: 4 mM CdCl2;
CA0: 0 mM CA (Control); CA 1: 1 mM CA; CA5: 5 mM CA;
*** means 1% level of significance,
** means 5% level of significance,
NS means Non-Significant

4.7.3 Combined Effect of Citric Acid and Cadmium on Number of Fruit Per Plant

There was a significant variation in the number of fruit per plant among different doses of CA and Cd treatments (Figure 7). Cd0 CA1 produced the highest number of fruits per plant which is 50.33. 21.65% higher number of fruits grew in Cd2 CA1 (44.67) than Cd2 CA0 (35.00); and Cd2 CA5 (35.67) is statistically similar to Cd2 CA0 (35.00)

(Appendix X). It reveals that both mild and high doses of CA is efficient in terms of phytoremediation for mild Cd stress.

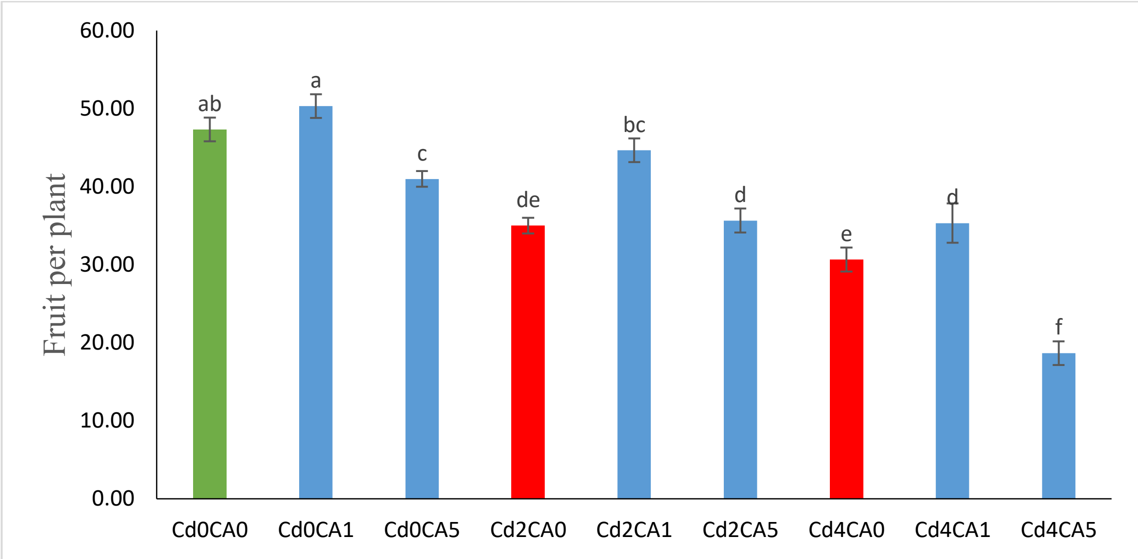

Figure 7. Effect of CA and Cd stress on fruit per plant ($SE \pm_{0.05} = 0.909$); Cd0: 0 mM CdCl2 (Control); Cd 2: 2 mM CdCl2; Cd4: 4 mM CdCl2; CA0: 0 mM CA (Control); CA 1: 1 mM CA; CA5: 5 mM CA

4.8 Yield Per hectare

4.8.1 Effect of Cadmium Stress on Yield Per Hectare

Cd is highly significant on reducing yield per ha of chili plants with increasing concentration (Table 8). Chili yield declined by 34.5% and 63.83% respectively at Cd2 (0.92 t/ ha) and Cd4 (0.51 t/ha) than the controlled treatment Cd0 (1.41 t/ha). Mozafariyan *et al.* (2014) and Georgieva *et al.* (1997) published alike findings.

4.8.2 Effect of Citric Acid on Yield Per Hectare

CA is highly significant on yield per ha of chili plants (Table 8). Maximum yield was found in CA1 (1.24 t/ha) which is 59.67% higher than CA0 (0.95 t/ha). However, high dose of CA negatively impacts on yield. CA5 (0.65 t/ha) produced the lowest yield which is 31.58% lower than the controlled treatment CA0 (0.95 t/ha). El-Yazal (2019) reported similar results.

Table 8. Effect of different levels of Citric acid and Cadmium on yield per hectare

Treatment	**Yield per ha (t/ha)**
Levels of Cd	
Cd0	1.41 a
Cd2	0.92 b
Cd4	0.51 c
Levels of Citric Acid	
CA0	0.95 b
CA1	1.24 a
CA5	0.65 c
SE (±)	0.016
Significance (*P*)	
Cd	***
CA	***

Here, Cd0: 0 mM CdCl2 (Control); Cd 2: 2 mM CdCl2; Cd4: 4 mM CdCl2;
CA0: 0 mM CA (Control); CA 1: 1 mM CA; CA5: 5 mM CA;
*** means 1% level of significance,
** means 5% level of significance,
NS means Non-Significant

4.8.3 Combined Effect of Citric Acid and Cadmium Stress on Yield Per Hectare

CA and Cd treatments caused a significant variation among the data (Figure 8). Maximum yield per hectare was found in Cd0 CA1 (1.66 t/ha) and the minimum yield was found in Cd4 CA5 (0.18 t/ha) (Appendix X). In terms as role of a phytoremedy, low concentration of CA was highly effective in mild and severe Cd stressed condition. Cd2 CA1 performed best by restoring and enhancing yield by 56.79% from Cd2CA0 (0.81 t /ha). Cd4 CA1 also improve yield per ha of chili plant by 41.07% than Cd4CA0 (0.56 t/ha). Mahmud *et al.* (2018) published alike results.

On contrary, high dose of CA application lowered yield per ha both when applied individually, and with mild and severe Cd stress condition. Cd0 CA5 (1.08 t/ha) is 27.03% lower than Cd0 CA0 (1.48 t/ha); Cd2 CA5 (0.69 t/ha) is 14.92% lower than Cd2 CA0 (0.81 t/ha); and Cd4 CA5 (0.18 t/ha) is 67.86% lower than Cd4 CA0 (0.56 t/ha).

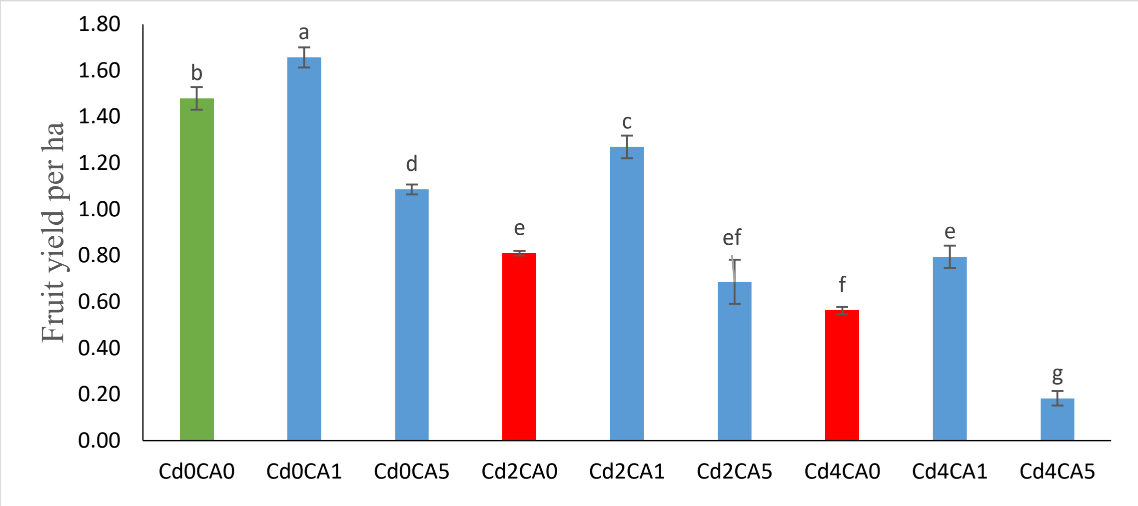

Figure 8. Effect of CA and Cd stress on yield per hectare (SE $\pm_{0.05} = 0.027$). Cd0: 0 mM $CdCl_2$ (Control); Cd 2: 2 mM $CdCl_2$; Cd4: 4 mM $CdCl_2$; CA0: 0 mM CA (Control); CA 1: 1 mM CA; CA5: 5 mM CA

4.9 Fresh Weight of Plant

4.9.1 Effect of Cadmium Stress on Fresh Weight of Plants

Cd stress is highly significant on fresh weight of plants. Cd stress reduces fresh weight of plants in dose dependent manner (Table 9). Cd2 (90.67 g) is 14.91% and Cd4 (74.33 g) is 30.25% lower than the control treatment Cd0 (106.56 g). Mahmud *et al.* (2018), Ehsan *et al.* (2014), and Gao *et al.* (2010) agreed that CA application increases fresh weight in plants.

4.9.2 Effect of Citric Acid on Fresh Weight of Plants

CA application is highly significant on fresh weight of plants and it caused variation among the data (Table 9). Mild CA is beneficial for improving fresh weight of plants but high dose causes sharp decline. CA1 (102.78 g) is 11.04% greater than CA0 (92.56 g), but CA5 (76.22 g) is 17.65% lower than CA0 (92.56 g). Arsenov *et al.* (2020), Mahmud *et al.* (2019), Mahmud *et al.* (2018), and Gill *et al*. (2011) agreed to the finding.

Table 9. Effect of different levels of Citric acid and Cadmium on fresh weight of plant

Treatment	Fresh weight of plant (g)
Levels of Cd	
Cd0	106.56 a
Cd2	90.67 b
Cd4	74.33 c
Levels of Citric Acid	
CA0	92.56 b
CA1	102.78 a
CA5	76.22 c
SE (±)	1.327
Significance (*P*)	
Cd	***
CA	***

Here, Cd0: 0 mM CdCl2 (Control); Cd 2: 2 mM CdCl2; Cd4: 4 mM CdCl2;
CA0: 0 mM CA (Control); CA 1: 1 mM CA; CA5: 5 mM CA;
*** means 1% level of significance,
** means 5% level of significance,
NS means Non-Significant

4.9.3 Combined Effect of Citric Acid and Cadmium on Fresh Weight of Plant

There was a significant variation in the number of fruit per plant among different doses of CA and Cd treatments (Table 9). Cd0CA1 produced the highest fresh weight of plant which is 115.67 g and the lowest fresh weight of plant was found in Cd4 CA5 which is 51.33 g (Appendix XII).

Mild application of CA was highly effective in recovering fresh weight of plants under mild and severe Cd stress. Cd2 CA1 (115.67 g) produced 10.51% higher fresh weight than Cd2 CA0 (92.00 g); Cd4 CA1 (91.00 g) is 12.81% greater than Cd4 CA0 (51.33 g).

On the other hand, high dose of CA treatment lowered fresh weight of plants in mild, severe Cd stress and even in individual treatment. Cd0 CA5 (99.00 g) is 5.72% lower than Cd0 CA0 (105.00 g); Cd2 CA5 (78.33 g) is 14.86% lower than Cd2 CA0 (92.00 g); and Cd4 CA5 (51.33 g) is 36.37% lower than Cd4 CA0 (80.67 g). Mahmud *et al.* (2018), Wang *et al.* (2017), and Ehsan *et al.* (2014) published similar result.

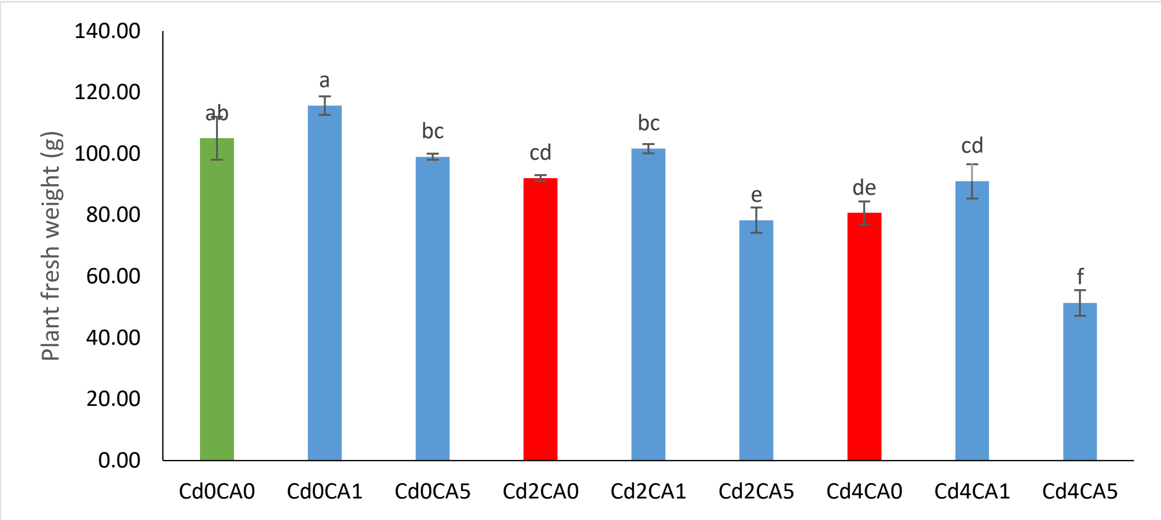

Figure 9. Effect of CA and Cd stress on fresh weight of plant (SE ± $_{0.05}$ = 2.229) Cd0: 0 mM $CdCl_2$ (Control); Cd 2: 2 mM $CdCl_2$; Cd4: 4 mM $CdCl_2$; CA0: 0 mM CA (Control); CA 1: 1 mM CA ; CA5: 5 mM CA

4.10 Dry Weight

4.10.1 Effect of Cadmium Stress on Dry Weight of Plant

Cd stress is highly significant on dry weight of plants (Table 10). Cd stress reduces dry weight of plants in dose dependent manner. Cd2 (0.98 g) is 16.95% and Cd4 (0.84 g) is 27.97% lower than the control treatment Cd0 (1.18 g). Mahmud *et al.* (2018), Ehsan *et al.* (2014), and Gao *et al.* (2010) agreed that CA application increases fresh weight in plants.

4.10.2 Effect of Citric Acid on Dry Weight of Plant

CA application is highly significant on dry weight of plants and it caused variation among the data (Table 10). Mild CA is beneficial for improving dry weight of plants but high dose causes sharp decline. CA1 (1.13 g) is 9.71% greater than CA0 (1.03 g), but CA5 (0.85 g) is 17.47%

lower than CA0 (1.03 g). Arsenov *et al.* (2020), Mahmud *et al.* (2019), Mahmud *et al.* (2018), and Gill *et al*. (2011) agreed to the findings.

Table 10. Effect of different levels of Citric acid and Cadmium on dry weight of plant

Treatment	**Dry weight of plant (g)**
Levels of Cd	
Cd0	1.18 a
Cd2	0.98 b
Cd4	0.85 c
Levels of Citric Acid	
CA0	1.03 b
CA1	1.13 a
CA5	0.85 c
SE (±)	0.014
Significance (*P*)	
Cd	***
CA	***

Here, Cd0: 0 mM $CdCl_2$ (Control); Cd 2: 2 mM $CdCl_2$; Cd4: 4 mM $CdCl_2$;
CA0: 0 mM CA (Control); CA 1: 1 mM CA ; CA5: 5 mM CA
*** means 1% level of significance,
** means 5% level of significance,
NS means Non-Significant

4.10.3 Combined Effect of Citric Acid and Cadmium on Dry Weight of Plant

There was a significant variation in the number of dry per plant among different doses of CA and Cd treatments (Figure 10). Cd0 CA1 produced the highest dry weight of plant which is 1.25 g and the lowest dry weight of plant was found in Cd4 CA5 which is 0.586 g (Appendix XII).

Mild application of CA was highly effective in recovering dry weight of plants under mild and severe Cd stress. Cd2 CA1 (1.11 g) produced 9.90% higher dry weight than Cd2 CA0 (1.01 g); Cd4 CA1 (1.03 g) is 11.96% greater than Cd4 CA0 (0.92 g).

On the other flip, high dose of CA treatment lowered dry weight of plants in mild, severe Cd stress and even in individual treatment. Cd0 CA5 (1.11 g) is 5.12% lower than Cd0 CA0 (1.17 g); Cd2 CA5 (0.87 g) is 13.86% lower than Cd2 CA0 (1.01 g); and Cd4 CA5 (0.59 g) is 36.95% lower than Cd4 CA0 (0.92 g). Mahmud *et al.* (2018), Wang *et al.* (2017), and Ehsan *et al.* (2014) published similar result.

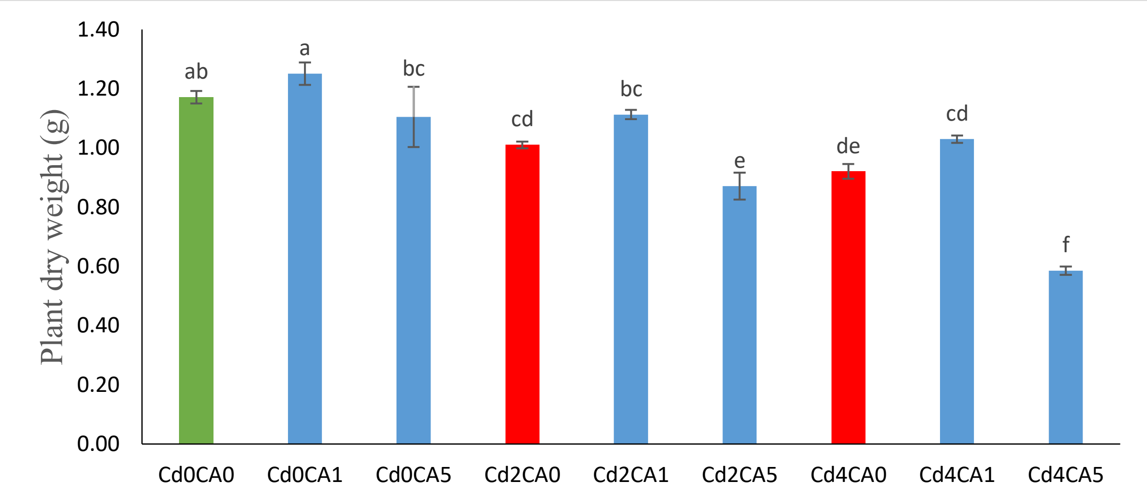

Figure 10. Effect of CA and Cd stress on dry weight of plant (SE $\pm_{0.05}$ = 0.024)
Cd0: 0 mM $CdCl_2$ (Control); Cd 2: 2 mM $CdCl_2$; Cd4: 4 mM $CdCl_2$;
CA0: 0 mM CA (Control); CA 1: 1 mM CA ; CA5: 5 mM CA

4.11 Relative Water Content (RWC)

4.11.1 Effect of Cadmium Stress on Relative Water Content (RWC)

Cd stress is highly significant on relative water content (RWC) (Table 11). Cd stress reduces dry weight of plants in dose dependent manner. Cd2 (88.73) is 2.37% and Cd4 (85.43) is 5.99% lower than the control treatment Cd0 (90.88 g). Mahmud *et al.* (2019) and Mahmud *et al.* (2018), agreed with the finding.

4.11.2 Effect of Citric Acid on Relative Water Content (RWC)

From Table 11, it is clearly evident that CA application caused variation on relative water content (RWC). CA0 (88.46) is statistically similar to CA1 (89.30) and both of them are different to Cd5 (87.29). Cd5 (87.29) is slightly lower than CA0 (88.46). Mahmud *et al.* (2018) reported alike finding.

Table 11. Effect of different levels of Citric acid and Cadmium on dry weight of plant

Treatment	Relative water content (RWC)
Levels of Cadmium	
Cd0	90.88 a
Cd2	88.73 b
Cd4	85.43 c
Levels of Citric Acid	
CA0	88.46 a
CA1	89.30 a
CA5	87.29 b
SE (±)	0.308
Significance (*P*)	
Cd	***
CA	***

Here, Cd0: 0 mM $CdCl_2$ (Control); Cd 2: 2 mM $CdCl_2$; Cd4: 4 mM $CdCl_2$;
CA0: 0 mM CA (Control); CA 1: 1 mM CA ; CA5: 5 mM CA
*** means 1% level of significance,
** means 5% level of significance,
NS means Non-Significant

4.11.3 Combined Effect of Citric Acid and Cadmium on Relative Water Content (RWC)

Application of CA and Cd caused no significant variation on RWC among the treatments. (Figure 11). Cd0CA1 (91.30) is statistically similar to the control treatment Cd0 CA0 (91.27) (Appendix XII). In combination with mild and severe Cd stress, low concentration of CA produces slightly higher RWC in the leaves. Cd2 CA1 (89.97) is 1.35% than Cd2 CA0 (88.77); and Cd4 CA1 (86.62) is 1.49% lower than Cd4 CA0 (85.35). On the other hand, Cd2 CA5 (87.47) is very close to Cd2 CA0 (88.77) and Cd4 CA5 (84.34) is also close to Cd4 CA0 (85.35). Although, Mahmud *et al.* (2018) reported significant change in RWC.

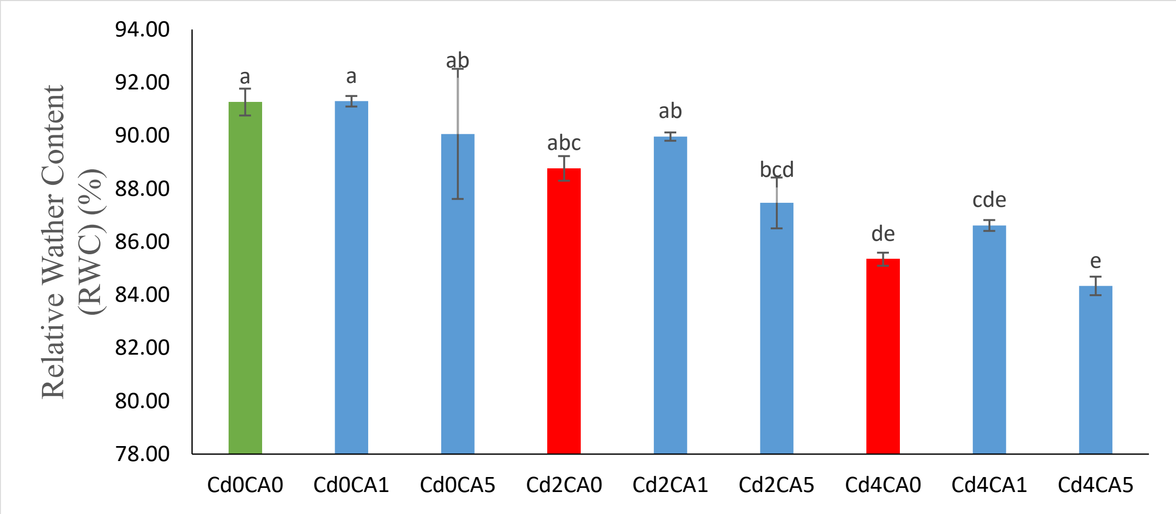

Figure 11. Effect of CA and Cd stress on relative water content (RWC) ($SE \pm_{0.05} = 0.533$);
Cd0: 0 mM $CdCl_2$ (Control); Cd 2: 2 mM $CdCl_2$; Cd4: 4 mM $CdCl_2$;
CA0: 0 mM CA (Control); CA 1: 1 mM CA ; CA5: 5 mM CA

4.12 Chlorophyll Content

4.12.1 Effect of Cadmium Stress on Chlorophyll Content

Cd is highly significant and responsible for chlorophyll content reduction with increasing concentration (Table 12). Cd2 (61.89) is 10.45 % and Cd4 (54.44) is 21.23% lower than Cd0 (69.11). Cd4 (54.44) produced the lowest chlorophyll content. Arsenov *et al.* (2020), Gill *et al.* (2011), Mozafariyan *et al.* (2014) reported similar result.

4.12.2 Effect of Citric Acid on Chlorophyll Content

CA is highly significant on chlorophyll content, and in the study it efficiently boosted the chlorophyll content of chili plants (Table 12). The chlorophyll content in CA1 (64.44) is 4.31% higher than CA0 (61.78). Furthermore, CA0 (61.78) and Cd5 (59.22) are statistically similar. We can interpret that low concentration CA increases fruit diameter whereas higher CA dose has no toxic effect on fruit diameter of chili. Mahmud *et al.* (2018) and Ehsan *et al.* (2014) reported alike findings.

Table 12. Effect of different levels of Citric acid and Cadmium on chlorophyll content

Treatment	**Chlorophyll content**
Levels of Cadmium	
Cd0	69.11 a
Cd2	61.89 b
Cd4	54.44 c
Levels of Citric Acid	
CA0	61.78 b
CA1	64.44 a
CA5	59.22 b
SE (±)	0.709
Significance (*P*)	
Cd	***
CA	***

Here, Cd0: 0 mM $CdCl_2$ (Control); Cd 2: 2 mM $CdCl_2$; Cd4: 4 mM $CdCl_2$;
CA0: 0 mM CA (Control); CA 1: 1 mM CA ; CA5: 5 mM CA
*** means 1% level of significance,
** means 5% level of significance,
NS means Non-Significant

4.12.3 Combined Effect of Citric Acid and Cadmium on Chlorophyll Content

Application of CA and Cd caused no significant variation on chlorophyll content among the treatments (Figure 12). Cd0 CA1 (70.67) contained highest amount of chlorophyll, and Cd4 CA5 (50.33) had the lowest (Appendix XII). Cd2 CA1 (64.33) is 4.92% than Cd2 CA0 (61.33); and Cd4 CA1 (58.33) is 6.69% higher than Cd4 CA0 (54.67). On the other hand, Cd2 CA5 (60.00) is very close to Cd2 CA0 (61.33) and Cd4 CA5 (50.33) is also close to Cd4 CA0 (54.67). Although, Mahmud *et al.* (2018) reported significant change in chlorophyll content.

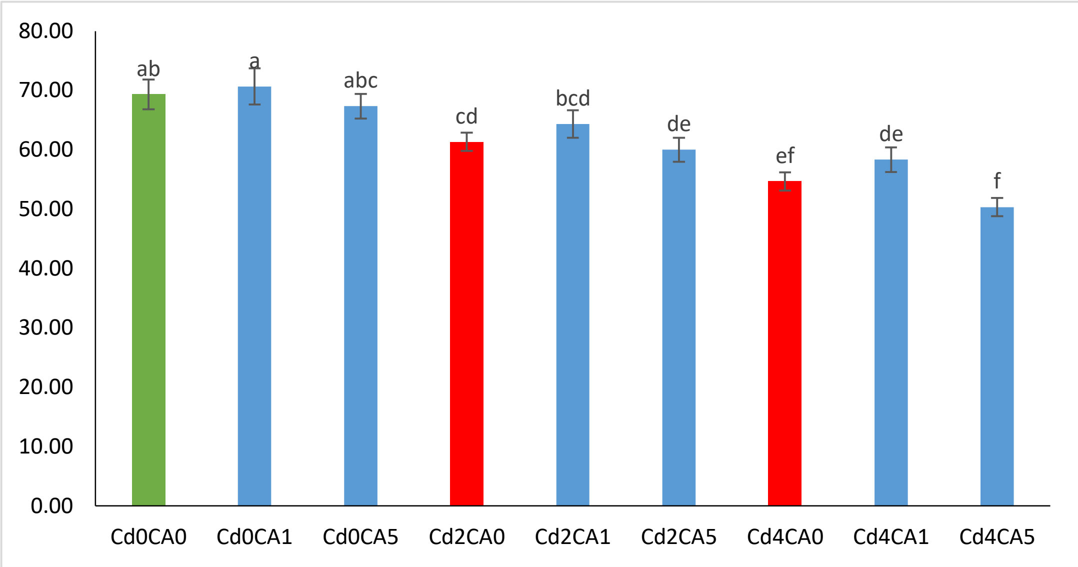

Figure 12. Effect of CA and Cd stress on chlorophyll content (SE ± $_{0.05}$ = 1.227); Cd0: 0 mM $CdCl_2$ (Control); Cd 2: 2 mM $CdCl_2$; Cd4: 4 mM $CdCl_2$;
CA0: 0 mM CA (Control); CA 1: 1 mM CA ; CA5: 5 mM CA

Chapter 5

SUMMARY AND CONCLUSION

Summary

The experiment was conducted at Agroforestry field lab and experimental farm, Sher-e-Bangla Agricultural University farm, Sher-e-Bangla Nagar, Dhaka, Bangladesh during the period from November 2019 to April 2020. The experiment was conducted to investigate the response of citric acid under cadmium stress at different growth stage in terms of growth parameters and yield attributes of chili. The study involved three treatments of cadmium viz. Cd0; no $CdCl_2$, Cd2; 2mM $CdCl_2$, Cd4; 4mM $CdCl_2$; and three concentration of citric acid viz. CA0; no citric acid; CA1; 1mM citric acid; and CA5; 5mM citric acid. Completely Randomized Design (CRD) with three replications was followed to carry out the study. Growth and yield contributing parameters were recorded at four growth stages (seedling, vegetative, flowering, and fruiting stage) which were 15, 30, 45, and 60 days after transplanting (DAT).

Cadmium stress was highly significant in reducing the vegetative characters (plant height, number of leaves per plant, and number of branches per plant) whereas low dose of citric acid successfully recovered and improved them. Results from the study showed that comparing with the control treatment, mild to severe cadmium stress can reduce 16 - 73% plant height, number of leaves per plant, and number of branches per plant in all growth stages. In order to assess the phytoremediation capability of citric acid under cadmium stress, four combined treatments viz.; Cd2 CA1, Cd2 CA5, Cd4 CA1, and Cd4 CA5 are compared with other treatments.

In case of plant height, the highest improvement (28.14%) was observed in Cd4 CA1 than Cd4 CA0 at 45 DAT. Compared to the plants under mild and or severe stress, low concentration of citric acid, Cd2 CA1 and Cd4 CA1, improved 5 – 28% plant height of chili plants in all growth stages. On the other hand, plants treated with high concentration of citric acid, Cd2 CA5 and Cd4 CA5, statistically had similar plant height as the no stress condition at seedling (15 DAT) and vegetative (30 DAT) growth stage. However, on the later phase, flowering (45 DAT) and fruiting (60 DAT) stage, plant height is reduced by 10.72 - 26.32% in Cd2 CA5 and Cd4 CA5 than Cd2 CA0 and Cd4 CA0. Perhaps, higher accumulation of Cd affected stem cell elongation. It indicates to the dual role of citric acid. CA enhances heavy metal accumulation as well as improve antioxidant defense system to develop stress tolerance in plants.

Low CA concentration is highly effective for improving number of leaves per plant of chili under both mild and severe stress. Cd2 CA1 had 11 – 36% higher number of leaves than Cd2 CA0, and Cd4 CA1 had 18 – 38% more leaves than Cd4 CA0. However, high concentration of CA under mild and severe stress caused toxic effect and drop the number of leaves. Cd4 CA5 produced the lowest number of leaves in all growth stages.

Number of branches per plant sharply dropped with increasing Cd stress. In the study, both low and high dose of CA application could not protect and restore number of branches per plant from Cd toxicity.

Fruit length of chili plants had remarkable improvement treated with low CA under mild and severe Cd stress. Cd2 CA1 had 19% longer fruits than Cd2 CA0; and fruits in Cd4 CA1 had 27% longer than Cd4 CA0. Fruit diameter, fruit weight, and number of fruit per plant maintained the same trend. However, with high CA treatment under severe Cd stress, Cd4 CA5, fruit length, fruit diameter, fruit weight, and number of fruits plants sharply declined which ultimately caused lowest yield per ha of chili.

In case of yield per ha, low dose of CA application, successfully surpassed the detrimental effects of both mild and severe Cd stress. Cd2 CA1 had 57% higher yield per ha than Cd2 CA0, and Cd4 CA1 had 41% higher yield than Cd4 CA0.

Biomass production of chili plants notably increased with low concentration of CA application. Fresh weight improved 11 – 13% and dry weight improved 10 - 12% in Cd2 CA1 than Cd2 CA0.

Relative water content and chlorophyll showed no significant change with low or high concentration of CA application.

Conclusion

Cadmium contamination in soil is a potential threat to chili at all growth stages. Cd stress drastically reduces growth, biomass production, and yield of chili plants. However, exogenous application of CA in the soil can alleviate Cd stress symptoms and induce tolerance in plants. Concentration of CA plays critical role in phytoextraction efficiency and plant's physiological and morphological characters. High concentration of CA under severe Cd stress drastically reduced growth and yield attributes. On the other hand, low concentration of CA application showed the best performance in growth (plant height, leaves, fruit length, fruit diameter, fruit weight, number of fruits per plant), yield and morphological attributes (fresh weight, dry weight, relative water content, and chlorophyll content). It significantly improved reproductive growth as the maximum number of fruit, fruit length, fruit diameter, fruit weight, and yield were found in the plants only treated with low concentration of CA. However, due to Cd stress relative water content and chlorophyll content were slightly reduced than the control treatment, but CA had no significant effect to recover them.

CHAPTER 6

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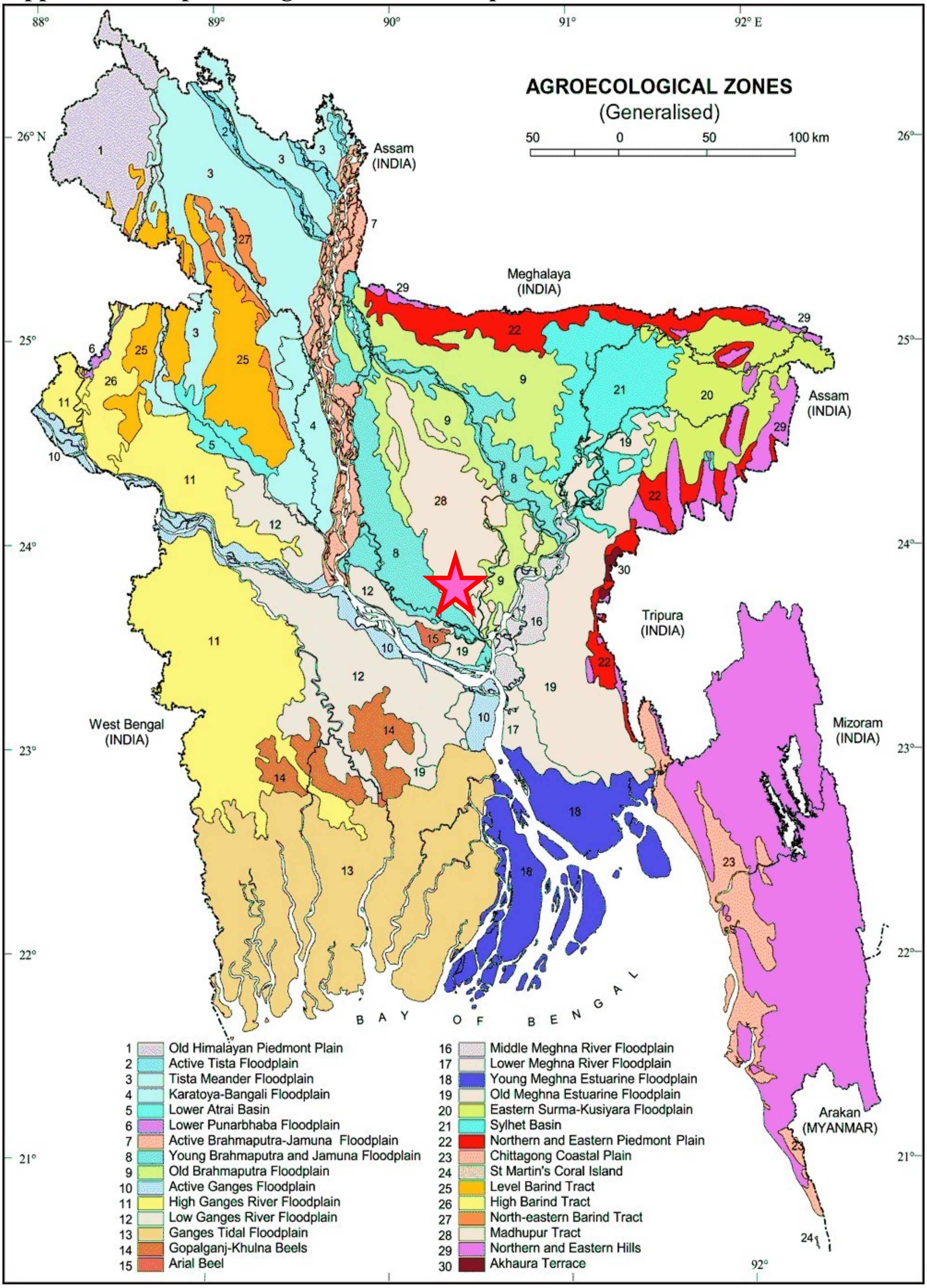
APPENDICES

Appendix I. Map showing the location of experiment



Shows the experimental site

Appendix II. Soil characteristics of experimental field as analyzed by Soil Resources Development Institute (SRDI), Khamarbari, Farmgate, Dhaka

A. Morphological properties of the soil

Morphological features	**Characteristics**
Location	Agroforestry farm , SAU, Dhaka
AEZ	Madhupur Tract (28)
General Soil Type	Shallow red brown terrace soil
Land type	High land
Soil series	Tejgaon
Topography	Fairly leveled

B. Physical properties of the soil

Particle size analysis	**Results**
Sand (%) (0.0-0.02 mm)	21.75
Silt (1%) (0.02-0.002 mm)	66.60
Clay (%) (<0.002 mm)	11.65
Soil textural class	Silty loam
Color	Dark grey
Consistency	Grounder

Source: Soil Resources Development Institute (SRDI), Dhaka.

Appendix III. Monthly record of air temperature, relative humidity and rainfall of the experimental site during the period from November 2019 to April 2020

Month	***Air temperature (${}_0$C)**	***Relative humidity (%)**	**Rainfall (mm) (total)**
November, 2019	24.9	74	37
December, 2019	19.3	74	05
January, 2020	18.5	76	21
February, 2020	21.6	59	01
March, 2020	26.4	57	30
April, 2020	27.9	72	127

* Monthly average

* Source: Bangladesh Meteorological Department (Climate & weather division), Agargoan, Dhaka.

Appendix IV. Mean table of combined effect of citric acid-cadmium on plant height

Treatment	Plant Height (cm)			
	15 DAT	**30 DAT**	**45 DAT**	**60 DAT**
Citric Acid (CA) x Cadmium (Cd)				
Cd0 CA0	9.27 a	17.42 a	29.67 a	48.17 a
Cd0 CA1	9.25 a	17.33 a	30.00 a	49.00 a
Cd0 CA5	8.75 ab	16.58 a	28.67 ab	46.58 a
Cd2 CA0	7.00 bc	11.67 bc	21.25 c	30.62 c
Cd2 CA1	8.50 abc	12.70 b	25.33 b	41.13 b
Cd2 CA5	7.33 abc	11.67 bc	20.67 c	34.33 c
Cd4 CA0	6.50 c	10.08 cd	14.50 d	21.13 d
Cd4 CA1	7.83 abc	11.00 bcd	18.58 c	24.32 d
Cd4 CA5	6.42 c	9.000 d	13.03 d	15.57 e
SE (±)	0.436	0.521	0.772	0.982
Significance(*P*)	NS	NS	NS	***

*** means 1% level of significance,

** means 5% level of significance,

NS = Non-significant

Appendix V. Analysis of variance (mean square) of plant height at different days after sowing

Source of variation	**df**	**Mean Square Values of Plant height at**			
		15 DAT	**30 DAT**	**45 DAT**	**60 DAT**
CA	2	2.9186**	3.579 **	35.826***	90.97 ***
Cd	2	11.0769***	120.176***	445.562***	1715.76***
CA* Cd	4	0.5485 NS	0.564 NS	4.914 NS	28.86***
Error	18	0.5701	0.815	1.788	2.90
CV		9.59%	6.92%	5.97%	4.93%

**Significant at 5% level,

*** Significant at 1% level,

NS = Non-significant,

CV= Coefficient of Variation

Appendix VI. Mean table of combined effect of citric acid-cadmium on number of leaves per plant

Treatment	Number of Leaves Per Plant			
	15 DAT	**30 DAT**	**45 DAT**	**60 DAT**
Citric Acid (CA) x Cadmium (Cd)				
Cd0 CA0	22.00 a	42.67 a	64.67 abc	85.67 a
Cd0 CA1	22.67 a	44.33 a	73.00 a	91.33 a
Cd0 CA5	21.00 a	42.67 a	59.33 abc	80.00 a
Cd2 CA0	11.33 bc	25.00 c	51.67 bc	51.00 b
Cd2 CA1	12.67 b	35.00 b	69.00 ab	79.67 a
Cd2 CA5	10.00 bcd	24.67 c	51.00 c	49.00 b
Cd4 CA0	8.33 cd	11.00 d	16.00 d	23.67 c
Cd4 CA1	11.00 bc	13.33 d	21.33 d	38.33 b
Cd4 CA5	7.33 d	10.67 d	16.00 d	23.00 c
SE (±)	0.711	1.139	3.582	2.910
Significance (*P*)	NS	***	NS	NS

*** means 1% level of significance,

** means 5% level of significance,

NS = Non-significant

Appendix VII. Analysis of variance (mean square) of number of leaves per plant

Source of variation	**df**	**Mean Square Values of number of leaves per plant at**			
		15 DAT	**30 DAT**	**45 DAT**	**60 DAT**
CA (A)	2	16.148***	68.59***	394.33***	959.59***
Cd (B)	2	429.593***	2242.26***	5880.78***	7421.04***
CA* Cd	4	1.037 NS	21.98**	44.44 NS	122.59***
Error	18	1.519	3.89	38.48	25.41
CV		8.78%	7.12%	13.23%	8.70%

**Significant at 5% level,

*** Significant at 1% level,

NS = Non-significant,

CV= Coefficient of Variation

Appendix VIII. Mean table of combined effect of citric acid-cadmium on number of branches

Treatment	Number of branches	
	45 DAT	**60 DAT**
Citric Acid (CA) x Cadmium (Cd)		
Cd0 CA0	6.67 a	7.67 ab
Cd0 CA1	7.00 a	8.00 a
Cd0 CA5	6.00 a	7.67 ab
Cd2 CA0	2.00 b	4.67 abcd
Cd2 CA1	2.67 b	6.00 abc
Cd2 CA5	2.00 b	4.33 bcd
Cd4 CA0	1.00 b	2.33 d
Cd4 CA1	2.33 b	3.67 cd
Cd4 CA5	1.00 b	2.33 d
SE (±)	0.509	0.676
Significance (*P*)	NS	NS

*** means 1% level of significance,

** means 5% level of significance,

NS = Non-significant

Appendix IX. Analysis of variance (mean square) of the data for number of branches per plant at different days after sowing

Source of variation	**df**	**Mean Square Values of number of branches per plant at**	
		45 DAT	**60 DAT**
CA (A)	2	2.4815 NS	3.3704 NS
Cd (B)	2	68.2593***	56.4815***
CA* Cd	4	0.2593 NS	0.4259 NS
Error	18	0.7778	1.3704
CV		25.88%	22.58%

**Significant at 5% level,

*** Significant at 1% level,

NS = Non-significant ,

CV= Coefficient of Variation

Appendix X. Mean table of combined effect of citric acid-cadmium on fruit length, diameter, weight, number of fruit per plant, and yield per hectare

Treatment	fruit length (cm)	fruit Diameter (cm)	Fruit Weight (g)	No. of Fruit per plant	Yield per ha
Cd0 CA0	5.36 a	0.65 ab	1.25 a	47.33 ab	1.48 b
Cd0 CA1	5.44 a	0.68 a	1.32 a	50.33 a	1.67 a
Cd0 CA5	5.32 a	0.64 ab	1.06 b	41.00 c	1.09 d
Cd2 CA0	3.87 cd	0.51 de	0.93 c	35.00 de	0.811 e
Cd2 CA1	4.76 b	0.59 bc	1.14 b	44.67 bc	1.27 c
Cd2 CA5	3.65 d	0.50 de	0.85 c	35.67 d	0.69 ef
Cd4 CA0	3.04 e	0.45 ef	0.73 d	30.67 e	0.56 f
Cd4 CA1	4.15 c	0.54 cd	0.89 c	35.33 d	0.79 e
Cd4 CA5	1.76 f	0.41 f	0.39 e	18.67 f	0.18 g
SE (±)	0.086	0.015	0.021	0.909	0.027
Significance (*P*)	***	NS	***	***	***

**Significant at 5% level,

*** Significant at 1% level,

NS = Non-significant

Appendix XI. Analysis of variance (mean square) of fruit at length, diameter, weight, number of fruit per plant, and yield per hectare

Source of variation	df	Mean	Square Values of number of leaves/plant at		
		fruit length (cm)	fruit Diameter (cm)	Fruit Weight (g)	No. of Fruit per plant	Yield per ha (ton/ha)
CA (A)	2	3.3061***	0.01847***	0.27638***	306.259***	0.77738***
Cd (B)	2	12.8506***	0.08384***	0.65083***	733.481***	1.79580***
CA* Cd	4	1.0303***	0.00186 NS	0.02066***	35.537***	0.02245***
Error	18	0.0221	0.00067	0.00131	2.481	0.00230
CV		3.58%	4.71%	3.80%	4.19%	5.06%

**Significant at 5% level,
*** Significant at 1% level,
NS = Non-significant,
CV= Coefficient of Variation

Appendix XII. Mean table of combined effect of citric acid-cadmium on plant's fresh weight, dry weight, relative water content, chlorophyll content

Treatment	fresh weight of plant	dry weight of plant	Relative water content	Chlorophyll content
Cd0 CA0	105.00 ab	1.17 ab	91.27 a	69.33 ab
Cd0 CA1	115.67 a	1.25 a	91.30 a	70.67 a
Cd0 CA5	99.00 bc	1.11 bc	90.07 ab	67.33 abc
Cd2 CA0	92.00 cd	1.01 cd	88.77 abc	61.33 cd
Cd2 CA1	101.67 bc	1.11 bc	89.97 ab	64.33 bcd
Cd2 CA5	78.33 e	0.871 e	87.47 bcd	60.00 de
Cd4 CA0	80.67 de	0.920 de	85.35 de	54.67 ef
Cd4 CA1	91.00 cd	1.03 cd	86.62 cde	58.33 de
Cd4 CA5	51.33 f	0.586 f	84.37 e	50.33 f
SE (±)	2.299	0.024	0.533	1.227
Significance (*P*)	***	***	NS	NS

**Significant at 5% level,

*** Significant at 1% level,

NS = Non-significant

Appendix XIII. Analysis of variance (mean square) of plant's fresh weight, dry weight, relative water content, chlorophyll content

Source of variation	df	Mean Square Values of number of leaves/plant at			
		fresh weight of plant	dry weight of plant	Relative water content	Chlorophyll content
CA (A)	2	1614.70***	0.17591***	9.1340***	61.370***
Cd (B)	2	2336.26***	0.24383***	67.6668***	484.037***
CA* Cd	4	140.87***	0.02184***	0.4823 NS	4.981 NS
Error	18	15.85	0.00181	0.8534	4.519
CV		4.40%	4.22%	1.05%	3.44%

*Significant at 5% level,
** Significant at 1% level,
NS = Non-significant,
CV= Coefficient of Variation

Appendix XIV. Some photographs related to the study

Plate 2: Pot preparation and seed germination

Plate 3: Seedling stage

Plate 4: Flowering stage

Plate 5. Fruiting stage

Plate 6: Pesticide application

Plate 7: Data collection