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Research Article

In Vitro Screening of Tomato Cultivars against Cadmium Tolerance in Iraq

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Abstract: Cadmium (Cd) is a heavy metal, highly toxic and soluble in water easily taken up by the plants, produces abnormal growth, and disturbs the metabolism of plants. The present study consists of an *in vitro* study of nine tomato cultivars in comparison to the application of Cd to know their effect on different growth parameters of tomato cultivars including germination, mean generation time, shoot, and length (cm). All cultivars were scored based on a systematic procedure. Results showed that the Cd treatment influenced the germination percentage of tomato cultivars. After Cd treatment, a maximum and a minimum germination percentage of 62.67±2.89 and 25.00±5.00 were observed for T-59 and Tmt-9, respectively. However, the most susceptible cultivars were recorded for Tmt-9. The shortest mean generation time (MGT) was recorded for T-59 (4.04±2.57 days) and the longest for Tmt-9 (10.12±2.61 days). Heavy impact of Cd was recorded on shoot height; meanwhile, T-59 produced a maximum shoot height of 7.47±0.26 cm and showed the lowest Cd inhibition. The root height in Tmt-9 was 6.00±1.01 cm to 1.85±0.16 cm; thus, showed high influence with Cd application. After Cd application, for roots less inhibition of 7.10±0.62 cm was recorded. Based on the ranking score, T-59 ranked first with 16.00 points for seed germination, mean generation time (MGT), shoot length, and root length. Based on the results, it is recommended to sow the T-59 tomato variety that proved less influenced by Cd.

Keywords: Tomato Cultivars, Cadmium, Growth Evaluation, Iraq.

1. INTRODUCTION

Since cadmium (Cd) is a non-essential element that negatively affects plant growth and development. Cd is also a heavy metal toxic to all organisms [1]. Human activities giving rise to the Cd accumulation in biotic systems is becoming a major problem for environment. Cd content in the soils is increasing with the application of Cd containing fertilizers, city waste, and sewage sludge [2]. The existence of carbon based or inorganic impurities in our biospheres produce its deterioration, and which further leads to threatening issues to the global ecosystem [3]. The presence of soil with toxic heavy metals at low concentrations in the environment, for instance, chromium (Cr), arsenic (As), nickel (Ni), mercury (Hg), lead (Pb) and cadmium (Cd), which results in serious risks to life of plant and directly or indirectly also effects the human health [4, 5]. Unsustainable urbanization, boost of industrialization and less judicious ways of enhancing the agricultural practices are beings reasoned for affecting the environment. Among these, Cadmium (Cd) contamination of soil and food crops is highly addressed issues nowadays as it is a serious extent of bioaccumulation [6]. It is a silver white heavy metal, toxic in nature, quickly soluble in water thus easily translocated and taken

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by higher plants [7]. Furthermore, Cd can be swiftly absorbed by roots of many plants due to its fluidity. After accumulation in roots, Cd disturb the functional and structural properties of plants most particularly delay or inhibit the germination process and roots penetration [8]. Moreover, Cd is also recorded for its worse impact on physiological processes of many plants for instance exchange of gas, photosynthesis, respiration, and water movement. All these greatly elicit the weak metabolism of plant and consequently resulted in loss more or less [9]. The effects of Cd on plants increase by rising Cd concentration in the soil that further lead to uptake by human body through the food chain system (soil-plant-human) resulting in severe chronic diseases thus threating to the human health [10]. It is therefore essential to explore a judicious method to overcome this problem by remediate Cd-contaminated soils.

In such regards, it has been documented well to observe the effects of Cd on various crops including wheat *Triticum aestivum* L. [11], rice *Oryza sativa* L., oat *Avena sativa*, mustard *Barsica juncea*, even in commonly grown most of green leafy vegetables [12] including spinach *Spinach oleracea* L., fenugreek *Trigonella foenum-graecum* L., coriander *Corainder satium* L.) and on more importantly Tomato *Solanum lycopersicum* [13] that showed the root browning in many plants under Cd exposure. Furthermore, root length and dry mass decreased, and root diameter increased with Cd toxicity [10]. The stunting, chlorosis, necrosis, and desiccation, typical toxic symptoms of Cd stress in the foliage of plants were also noted in most of the plants. Latif *et al*. [12] further noted that Cd importantly create an impact on photosynthetic process that further leads to biochemical changes at different growth stages of plants.

Tomatoes are major source of nutrients namely iron, lycopene, vitamin C and potassium and also provide so many antioxidants that contribute essentially to human health [9, 14-16]. It is one of the unique vegetable fruits in the world for their high nutritive value, with leading producers like China, USA and Turkey. It is estimated that more than 80% of Cd contamination occurs by ingestion of vegetables and cereals [17]. In most of the vegetables, which are taken on daily basis, the accumulation of Cd in such case increases the health risk index with increasing concentration of it because of its direct toxic effect on human health. Nowadays, vegetables are one of a key food component and without this life is almost impossible but the increasing concentration of cadmium (Cd) in the food chain vegetable continuum is posing a threat to their growth as well as human life thus creating a real threat in present scenario and needs more progressive research to explore for possible solutions. Keeping all this in mind, this study has been conducted focusing the first time in Iraq showing effect of Cd on tomato cultivars as to screen the best varieties for health and productivity for local people.

2. MATERIALS AND METHODS

2.1 Experimental Design

The nine tomato seeds/cultivators (i.e., T59, T0-9, TM-1, Red-T, Chr-t, Dr-Tmto, Redone-T, Bgt-10 and Tmt-9) were purchased from local seed market at Baghdad, Iraq. The seeds were kept 25-27 °C until to be used. Pitgrow ready soil media in small plastic pots for *in vitro* experiment having all essential micro and macro nutrients was used for seed germination and seedling growth. In such manner two types of media were used for culture experiment. One is the control media that only contain Pitgrow media and other with Cd treatment media. All the seeds were sterilized well with 60% ethanol for 2 min followed washed by distilled water for various times. We inoculated three seeds of each variety into each cultured bottle which further repeated thrice. All cultures were maintained at 25-27 °C at 14:10 photoperiods at University of Misan, College of Agriculture, Iraq. Germinated seeds were counted daily according to the seedling evaluation procedure. The seeds were considered as germinated when the radical size was 2 mm. The number of germinated seeds was recorded every 24 h. In physical characteristics, 20 days after inoculating, the germination percentage using the formula (Germinated seeds number/total seeds × 100) for each replication of the treatment. After growth of seedling, the parameters included mean germination time ($MGT = \Sigma (n \times d)/N$, where n is number of seeds germinated on day d and N is the total number of germinated seeds at 10^{th} day, seedling shoot height and root length were measured in cm. In cadmium treatment for growth evaluation of tomato varieties, 50 seeds were sown in each replicate. $CdCl_2$ solutions at 15 mg/kg (based upon our literature and lab trails) concentration were applied at the time of sowing respectively. Seed germination was recorded as total number after 15 days according to whether the planetules came up obviously from soil. The comprehensive assessment of tomato cultivars expressed a total score obtained through evaluating four parameters after Cd treatment from high to low (the only exception was in MGT from low to high) with the score from the highest 5.00 points to the lowest 0.50 point. Overall, all the experiments were laid out in a Complete Randomized Design (CRD) with nine treatments (replicated thrice) for varietal comparison and two treatments (replicated thrice) for comparing tomato plants treated with cadmium and control.

2.2 Data Analysis

All the obtained data were analyzed using Student T-test for comparison of the significance of difference between the control and Cd treatment at $P < 0.05$. The data for tomato varietal studies were analyzed using Analysis of Variance (Anova) and the significance of differences were further determined using least significant difference (LSD) test at $p < 0.05$ through SAS (ver. 8.1) software. The results were presented in Mean ± S.E/S/D.

3. RESULTS AND DISCUSSION

3.1 Effect of Cd on Tomato Seed Germination

The Cd treatment influenced on germination percentage of nine tomato cultivars as presented in Table 1 and Figure 1. The analyzed results showed overall significant difference in germinations percentage of tomato cultivars ($P < 0.05$). However, in comparison to control, only three cultivars such as T-59, T0-9 and Chr-t showed more prominent difference and other cultivars were with non-significant ($P > 0.05$). After treatment of Cd, only two cultivars showed an increase in germination percentage such as Tmt-9 and Chr-t; meanwhile other seven cultivars showed decreased in germinations percentage. The maximum percentage of 81.33 ± 11.67 was recorded in T-59 ($p = 0.0238$, $t = 3.5011$) at control and the lowest germination percentage of 13.10 ± 3.48 in Chr-t at control treatment ($p = 0.0215$; $t = 4.3558$).

After Cd treatment, the maximum and the minimum percentages of 62.67 ± 2.89 and 25.00 ± 5.00 were respectively observed on T-59 and Tmt-9. These findings clearly pointed out the effect of Cd on germination percentage of tomato cultivars; however, the most susceptible cultivars were recorded for Tmt-9. It has been well reported that germination is the most vulnerable stage of higher plants and seedling development [18]. Thus, when the seed surrounding is contaminated with Cd, indiscretion in seed germination may be often noticed [19, 20]. Similar findings were noticed in the present study when in comparison to control treatment, most of the tomato cultivars germinated less. These findings are in line with those reported earlier in which it was reported that Cd stress decreased seed germination, germination index and vigour index of different crops [21].

3.2 Effect of Cd on Mean Germination Time (MGT)

After germination percentage, the effect of Cd on mean germination time (MGT) of selected tomato

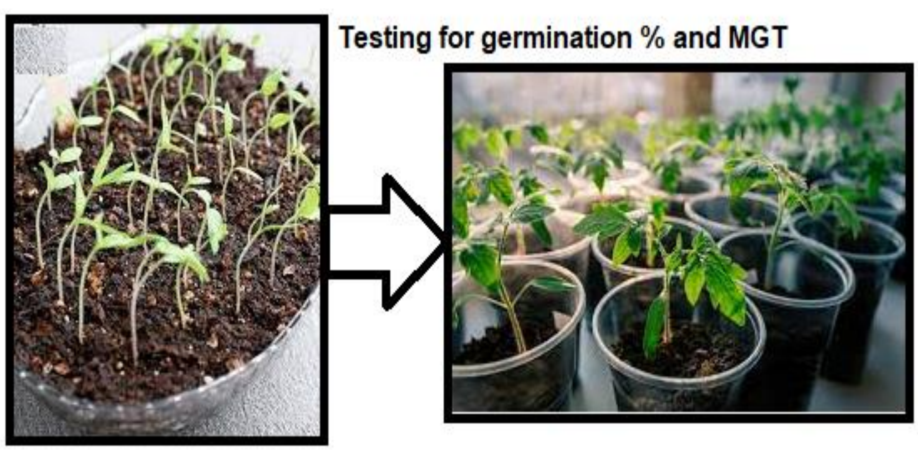

Fig. 1. Tomato seedling for testing germination percentage and calculation of Mean Generation Percentage (MGT) *in vitro* conditions.

Table 1. Effect of Cd on tomato seed germination percentage under in vitro culture.

Tomato cultivars	Control	Cd treatment	t value	p valve
T-59	81.33±11.67	62.67±2.89 a	3.5011	0.0238
T0-9	79.23±12.51	42.00±10.00 b	5.0071	0.0142
TM-1	49.47±10.72	38.00±5.08 b	1.6281	0.5055
Red-T	39.77±24.28	32.33±5.77 bc	1.2074	0.7721
Chr-t	13.10±3.48	31.33±7.64 cd	4.3558	0.0215
Dr-Tmto	36.47±23.60	29.33±7.64 cd	1.2281	0.6916
Redone-T	33.43±5.87	28.33±2.89 cd	2.4151	0.1811
Bgt-10	28.80±12.07	28.01±5.77 cde	1.2465	0.8919
Tmt-9	17.53±11.09	25.00±5.00 cde	2.1481	0.2422

cultivars was assessed. The results overall indicated a significant difference ($P < 0.05$) in MGT of tomato cultivars. Though, out of nine, six cultivars (TM-1, Red-T, Chr-t, Dr-Tmto, Redone-T and Bgt-10) took almost similar time to grow with non-significant difference ($P > 0.05$) in MGT between the control and the Cd treatment. In comparison to control treatment, Cd treatment increased the MGT in four tomato cultivars and decreased in six tomato cultivars (Table 2). However, the shortest MGT was recorded in T-59 (4.04 ± 2.57 days) than control (3.11 ± 1.01 days) and the longest in Tmt-9 (10.12 ± 2.61 days) than control (7.62 ± 4.48 days). The findings of this experiment show that T-59 had the lowest MGT than rest of other cultivars which showed less delayed in germination time meanwhile Tmt-9 found much influence with application of Cd as it delayed maximum with the highest MGT. In response to Cd treatment, the prolonged MGT may be due to inhibitory effect of Cd on germination ability of tomato seed as the Cd stress might confer reduced tolerance at the time of in vitro seed germination of tomato cultivars. Similarly, it has been observed that the higher Cd concentration in the Vigna unguiculata seeds seemed to prevent water uptake and water movement in the embryo axis which resulted in the delayed development with higher germination time of seeds [22].

3.3 Effect of Cd on Tomato Shoot Height

The results on the effect of Cd on shoot height of selected tomato cultivars under in vitro culture (Figure 2) revealed that there was a significant difference in shoot height between the control and the treatments (Table 3). The shoot height in comparison to the control decreased in response to Cd treatment in overall for all tomato cultivars. The highly significant difference between the control and the treatment ($p < 0.05$) was found in Tmt-9 as it looks heavy impact of Cd on shoot height (7.02 cm decreased to 1.61 cm). Meanwhile, T-59 produced maximum shoot height of 9.10 ± 2.03 cm in control and later after treatment of Cd remained higher 7.47 ± 0.26 cm as compared to rest of tomato cultivars and showed the lowest Cd

Table 2. Effect of Cd on Mean germination time (MGT) of tomato in vitro culture.

Tomato cultivars	Control	Cd treatment	t value	p value
T-59	3.11±1.01	4.04±2.57 d	0.4604	0.0149
T0-9	5.15±1.62	4.47±0.95 d	0.3392	0.0226
TM-1	5.32±0.51	5.37±1.42 bc	0.2392	0.5959
Red-T	7.94±2.11	6.33±2.14 bc	0.5021	0.4181
Chr-t	7.72±2.56	7.41±2.82 abc	0.2571	0.4025
Dr-Tmto	5.32±0.41	7.54±1.77 abc	2.2072	0.0623
Redone-T	7.62±1.62	8.10±0.12 abc	0.4101	0.6033
Bgt-10	8.01±3.11	10.40±5.41 abc	0.4341	0.6212
Tmt-9	7.62±4.48	10.12±2.61 a	0.6984	0.4695

Fig. 2. Measurement of root and shoot height of tomato cultivars.

inhibition in term of shoot height. Similarly, these results also found non-significant differences in shoot height in some tomato cultivars ($P > 0.05$) such as between T-59 and T0-9 and among TM-1, Red-T and Chr-t likewise. There are so many instances of Cd absorption by plant in quite smooth way by roots and then transported to shoots [23]. Such transportation of Cd, results in physiochemical changes and then badly affects the plant growth [24]. Roots are likely to be affected by heavy metals since much more metal ions are accumulated in roots than shoots [23].

3.4 Effect of Cd on Tomato Root Length

A similar trend was found in the results regarding effects of Cd on root height of selected tomato cultivars under in vitro culture (Table 4). It is observed that there was a significant difference in root height between the control and the treatments. The root height in comparison to the control decreased in response to Cd treatment in overall all tomato cultivars. The highly significant difference between the control and the treatment ($p < 0.05$) was found in Tmt-9 with 6.00 ± 1.01 cm to 1.85 ± 0.16 (maximum inhibition in overall all tomato cultivars) and showed highly influenced with Cd application. On the contrary, the maximum root height of 3.41 ± 0.11 cm in T-59 after Cd application was recorded which showed less inhibition as the root length as in control the root height for T-59 was 7.10 ± 0.62 cm. Similarly, these results also found a non-significant differences in shoot height in some tomato cultivars ($P > 0.05$) such as between T-59 and T0-9 and among TM-1 and Red-T. These results are consistent with the findings of other authors who also reported the influence of Cd on different physiological and biochemical traits of

Table 3. Effect of Cd on shoot length (cm) of tomato in vitro culture.

Tomato cultivars	Control	Cd treatment	t value	p value
T-59	9.10±2.03	7.47±0.26 a	6.9845	0.0012
T0-9	9.64±0.97	7.32±0.54 a	8.9723	0.0601
TM-1	8.21±0.31	4.64±0.71 b	6.9531	0.0011
Red-T	8.31±0.67	3.91±0.82 b	4.7421	0.0230
Chr-t	9.32±0.82	5.14±0.94 bc	5.8574	0.0014
Dr-Tmto	7.44±1.51	3.23±0.64 c	2.3741	0.0221
Redone-T	8.11±1.32	3.05±0.41 cd	6.8828	0.0011
Bgt-10	7.47±0.56	2.24±0.23 de	12.6824	0.0002
Tmt-9	7.02±0.25	1.61±0.22 f	18.1010	0.0001

plants [25, 26]. Similarly, a significant reduction in root and shoot length was recorded in Phyllanthus amarus with higher Cd stress [27].

3.5 Comprehensive Assessment of Selected Tomato Cultivars

According to the multiple comparison results (Table 5) in which tomato cultivars with the same small letters ($p < 0.05$) from ANOVA statistical analysis were thought as the same ranking and given with same score from weighted average of their deserved total scores. Based on this, T-59 displayed the highest score with 16.00 points as it performed well in seed germination, mean generation time (MGT), shoot length and root length (ranked at the first). T0-9 showed the second comprehensive score with 14.75 points and the third highest score was observed in Red-T with 10.00 points. It was obtained with an equivalent level weight coefficient of four parameters, the tomato cultivars comparatively better than others under Cd treatment particularly, T-59, T0-9 and Red-T established on growth index and index including Cd absorption, translocation, tolerance as well as some physiological and biochemical responses to Cd by tomato germinated seeds and growing seedlings. Thus, it is obvious from all our findings that Cd toxicity obviously inhibited the observed parameters of tomato cultivars and similarly previously reported for plant root growth [21]. Furthermore, root, shoot and seedling length are more crucial for last accumulation site of Cd and considered as good indicators for metal toxicity [20].

Table 4. Effect of Cd on tomato root length under in vitro culture.

Tomato cultivars	Control	Cd treatment	t value	p value
T-59	7.10±0.62	3.41±0.11 a	9.0672	0.0002
T0-9	6.91±2.15	3.01±0.08 a	4.3535	0.0624
TM-1	7.28±0.73	2.01±0.14 ab	10.2843	0.0007
Red-T	6.35±0.32	2.11±0.12 ab	19.3212	0.0003
Chr-t	6.42±0.21	2.21±0.11 bc	35.5961	0.0003
Dr-Tmto	6.62±0.52	1.84±0.04 bc	15.7413	0.0032
Redone-T	6.71±0.51	1.71±0.13 bcd	16.8314	0.0002
Bgt-10	6.11±1.14	1.09±0.15 bcd	9.7974	0.0006
Tmt-9	6.00±1.01	1.85±0.16 cde	11.8948	0.0003

Table 5. Comprehensive assessment of selected tomato cultivars.

Cultivar name	Seed germination rate			Mean germination time			Shoot height			Root length			
	MCSD	Seq.	Score	MCSD	Seq.	Score	MCSD	Seq.	Score	MCSD	Seq.	Score	Aggregate score
T-59	A	1	5.00	ab	5	2.50	ab	3	3.75	a	1	4.75	16 .00
T0-9	Bc	8	3.00	A	1	4.00	ab	3	3.75	ab	3	4.00	14.75
TM-1	Cd	5	2.50	D	11	0.00	c	7	2.00	ab	4	3.50	8.000
Red-T	D	12	0.00	C	10	0.50	a	1	4.75	a	1	4.75	10.00
Chr-t	Cd	8	0.75	Ab	5	2.50	bc	5	3.00	cd	9	1.00	7.250
Dr-Tmto	B	2	2.25	Ab	2	3.50	cd	9	1.00	bc	5	2.75	9.500
Redone-T	Cd	8	0.75	A	3	3.75	bc	6	2.50	E	12	0.00	7.000
Bgt-10	Cd	8	0.75	abc	9	1.00	d	11	0.00	de	10	0.25	2.000
Tmt-9	D	14	0.00	abc	5	1.50	d	14	0.00	e	13	0.00	1.500

MCSD: Multiple comparison of significance of difference among the average of Cd treatment Seq.: The sequence of the average of Cd treatment from high to low

Score: List the first as 5 points, the second as 4.5 points, the third as 4 points, and so on until the tenth as 0.5 point. If several cultivars have it sequence, their score is same by calculating the average of total aggregate scores deserved.

4. CONCLUSIONS

The Cd treatment greatly influenced all observed parameters of each tomato cultivars more or less. But T-59 tomato cultivar was observed less affected by Cd treatment as it showed the shortest MGT, maximum shoot and root height and with the highest score ranking thus recommend for cultivation.

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6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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