

Research Article

Effect of lithium on seed germination and plant growth of *Amaranthus viridis*

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Abstract

Lithium is one of the trace elements essential for the human body. The use of Li-based products has increased tremendously, leading to higher consumption patterns and the generation of lithium-based wastes. A higher concentration of lithium leads to the contamination of soil and water bodies. Lithium enters the food chain through the plant pathway. The food chain becomes contaminated with agricultural products produced on lithium-contaminated soil. Owing to this scenario, the present study is focused on studying the effect of lithium on the germination and growth of *Amaranthus viridis*. Germination studies were conducted in petri dishes, and the rate germination was 95% at control and 10 ppm. At higher concentrations, the rate of germination was 73% at 50 ppm, 57% at 75 ppm and 41% at 100 ppm. Pot experiments were conducted for 51 days using lithium-amended soil from 10 to 100 ppm. Pot experiments revealed that, at higher concentrations, lithium promoted the length and weight of the plant from 1.122 g/plant in the control to 2.415 g/plant at 100 ppm. The stress tolerance index was calculated for the length and dry weight of the roots and shoots, respectively. High stress tolerance at root and shoot biomass led to an increase in the biomass of the plant, which promoted the accumulation of lithium in plant parts. These results concluded that lithium stimulated plant growth at lower concentrations and increased biomass at higher concentrations, which was confirmed through the calculation of the stress tolerance index.

Keywords: *Amaranthus viridis*, Lithium, Metabolism, Pot experiments, Stress tolerance index

INTRODUCTION

The growth in the electronic industry has shown a significant impact on the economy, society, and industry. The increase in the use of electronic devices led to higher consumption and generation of e-waste. Lithium-ion batteries are an efficient energy storage technology for most portable electronic devices (Henschel *et al.*, 2020). These lithium-based batteries are often discarded, disposed or recycled to extract metals such as cobalt and nickel. The consumption of Li-based batteries has increased in the consumer and industrial sectors in recent decades. The concentration of lithium varies in different environments depending on the geochemical components, soil structure and type of water bodies.

Although lithium is considered as an essential trace element for the human body, the leachate or residue enters water bodies or soil and often leads to several health effects at elevated concentrations. Depending on the activities, higher concentrations of lithium are found in fruits, cereals, vegetables and products of animal origin (Voica *et al.*, 2021; Liaugaudaite *et al.*, 2017; Goldstein and Mascitelli 2016; Kousa *et al.*, 2013), which find its path to higher forms of the food chain (Jin *et al.*, 2021).

Crops grown and irrigated with Li-contaminated soil and water are available toxic sources of Li in humans. Agricultural lands near Li-based industries are potential sources of lithium contamination. The bioavailability of Li in soil depends on the clay content and pH of the

soil. The concentration of Li varies in different plants and indicates the amount of Li in the soil substrate (Shahzad *et al.*, 2016; Ammari *et al.*, 2011).

It is necessary to manage lithium toxicity through various remediation techniques, such as immobilization or phytoremediation, by adding chelating agents (Bolan *et al.*, 2021). The application of phytoremediation in Li has gained much importance (Tkatcheva *et al.*, 2015). Vamerli *et al.*, 2015, Maric *et al.*, 2013 and Lai *et al.*, 2008 investigated species of the *Vitaceae* family for their use in the phytoremediation and extraction of Li to aerial plant parts. Some studies have focused on detecting Li content and its accumulation rate in different plant families (Schwertfeger *et al.*, 2013; Elektorowicz and Keropian 2015; Wuana *et al.*, 2010). The translocation and immobilization of Li take place in the leaves of plants. Li acts upon plants in the following ways: disease resistance and growth are stimulated when the concentration is low. It can become toxic and inhibit development at higher concentrations (Kavanagh *et al.*, 2018). Because of the need to understand the effect of Li on plants, the present study was designed to identify the potential of *Amaranthus* subjected to its growth in pot experiments amended with Li at different concentrations under laboratory conditions and to assess its growth parameters.

MATERIALS AND METHODS

The study approach to the effect of lithium included germination studies and pot experiments, as represented in Fig. 1.

In vitro studies

The germination of seed and seedling growth is a prominent parameter for observing different amends' growth responses. Germination indices (Anjum *et al.*, 2005) included percent germination, speed of germination, speed of accumulated germination and coefficient of rate of germination. Four replicates of 25 seeds of *Amaranthus viridis* were placed at equal distances and incubated on two layers of filter paper in sterilized petri dishes (15 - 20 cm). Lithium sulfate is used extensively in lithium-ion batteries (Chen *et al.*, 2018; Zheng *et al.*, 2017), and proper disposal mechanisms are not adopted. There is a chance of lithium sulfate entering the food chain. Most of the studies were focused on lithium chloride and lithium hydroxide; thus, the objective of the present work is to study the effect of lithium (in the form of lithium sulfate) on the germination and growth of *Amaranthus viridis*. Five concentrations of lithium sulfate (10 ppm, 25 ppm, 50 ppm, 75 ppm, 100 ppm) along with a control (red loamy soil with organic manure 1:3 ratio without lithium sulfate salt) were maintained for ten days. The number of seeds germinated was counted on the 3rd, 5th and 8th days at each concentration.

Pot experiments

Pot experiments were conducted in the Department of Environmental Science greenhouse, GITAM (Deemed to be University), Visakhapatnam. Red loamy soil was collected from the uncontaminated area near GITAM. The soil was air dried and sieved and used for pot experiments.

Lithium sulfate (GR) chemical was used to prepare soil

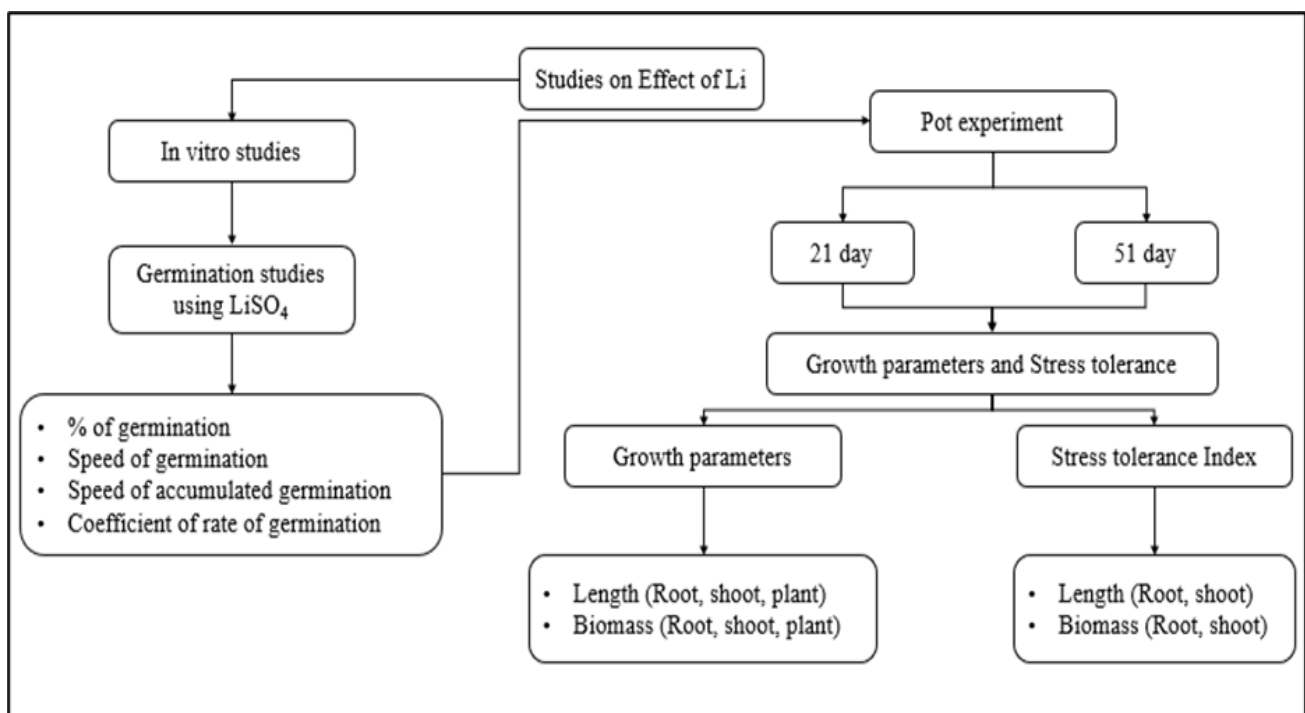


Fig. 1. Representation of the study approach

amends at different concentrations, i.e., 10 ppm, 25 ppm, 50 ppm, 75 ppm and 100 ppm. The soil without lithium was considered a control. The soil used for the pot experiment was mixed with organic manure at a ratio of 3:1. Different amounts of Li from lower to higher concentrations were prepared. Li-amended soil was taken in each pot (10 kg capacity) and watered sufficiently at regular intervals. A total of 24 pots were placed, including all four replicates at each concentration. In each pot, ten seeds were planted at an equal distance and at a depth of 10 cm. The plants' two stages of development included the vegetative phase on the 21st day and the maturation stage on the 51st day, which were considered harvest days. Plants were removed from each harvest day, and proper care was taken while removing them to keep the root system intact while washing. Growth parameters and stress tolerance were calculated on each harvest day.

Growth parameters

The growth parameters included the length and dry weights of the roots, shoots and plants, which were calculated on days 21 and 51. The length of the root was measured from the tip of the root to the base of the shoot. Shoot length was measured from the base of the shoot to the tip. These were measured in centimeters (cm). The root and shoot parts of the plants from each concentration were separated and dried in a hot air oven at 80°C for 24 hours. The average dry weights of shoots and roots were measured and calculated separately.

Stress tolerance index

The stress tolerance index was used to determine the stress tolerance potential in plants. It acts as an indicator to identify the potential genotype and high-yielding variety. Development, molecular and cellular activities help make the plants tolerant to stress, thereby helping the plant to grow without any stress-induced injury. Stress tolerance was calculated separately for the length and dry weight of roots and shoots using the following formulas (Amin et al., 2014; Gardea et al., 2004).

RLSTI = (Root length of Li-treated plant/Root length of control plant) × 100

SLSTI = (Shoot length of Li-treated plant/Shoot length of control plant) × 100

RDSTI = (Root Dry Weight of Li treated plant/Root Dry Weight of control plant) × 100

SDSTI = (Shoot Dry Weight of Li treated plant/Shoot Dry Weight of control plant) × 100

where

RLSTI – Root length stress tolerance index;

SLSTI – Shoot Length Stress Tolerance Index;

RDSTI – Root Dry Weight Stress Tolerance Index;

SDSTI – Shoot Dry Weight Stress Tolerance Index

RESULTS AND DISCUSSION

In vitro seed germination

Table 1 presents the effect of lithium sulfate, and the seeds of *Amaranthus viridis* were grown in petri dishes under laboratory conditions at different concentrations. A higher percentage (95%) of seed germination was observed at concentrations of control and 10 ppm with seeds of *Amaranthus viridis*. Lithium at lower concentrations promoted a percentage of seed germination, i.e., up to 10 ppm. Overall, in *Amaranthus*, the rate of germination decreased from the control to 100 ppm. The recovery of the percentage of germination showed a significant reduction with an increase in Li concentration, and the inhibition effect was more prominent (Table 1). A slight decrease in germination percentage was observed at a lower concentration of lithium up to 25 ppm. At medium concentrations (50 ppm and 75 ppm), the germination rate decreased to 73% and 57%, respectively. At higher concentrations, i.e., 100 ppm, there was an inhibitory effect on the percentage of germination, i.e., 41%.

Similar findings were observed in earlier studies conducted on seed germination with heavy metals such as chromium (Amin et al., 2014), cadmium, arsenic and mercury (Seneviratne et al., 2017). The percentage of germination indicates that lithium at lower concentrations had a significant stimulatory, beneficial and nutritional effect. However, an inhibitory effect was ob-

Table 1. Seed germination index of *A. viridis* exposed to different Li concentrations (*In vitro* study)

Concentrations	Average germination	Speed of germination	Speed of Accumulated Germination	Coefficient of rate of Germination	% of seed gemination
Control	9.5	12.5	22.07	0.174	95
10 ppm	9.5	12.5	22.07	0.174	95
25 ppm	7.8	5.3	16.98	0.165	78
50 ppm	7.3	6.175	17.83	0.171	73
75 ppm	5.7	2.825	12.7	0.164	57
100 ppm	4.1	2.075	7.14	0.159	41

served at higher concentrations on the percentage of seed germination. According to the study of Kalinowska et al., 2013, the effects of two lithium chemicals (lithium chloride and lithium hydroxide) on the growth of *Lactuca sativa* were studied. The study results revealed that the yield reduction of the plant is strongly correlated with increasing concentrations of lithium (≤ 20 ppm), with both salt concentrations strongly correlated with the reduction in plant growth with both lithium salts. A higher rate of inhibition was observed in lithium hydroxide than in lithium chloride. Similarly, positive effects of germination have been observed with soybean seeds exposed to a graded concentration of lithium hydroxide (Ribeiro et al., 2019).

Speed of germination

In *Amaranthus* under lower lithium concentrations, i.e., from the control to 25 ppm, there was a gradual decrease in germination speed (ontogenesis). The speed of germination was recorded as 12.5 in the control and 10 ppm and decreased to 5.3 in 25 ppm. At medium concentrations (50 ppm and 75 ppm), the speed of germination decreased from 6.18 to 2.83. At higher concentrations, i.e., 100 ppm, there was a decrease in the speed of germination to 2.08. Overall, the speed of germination decreased from the control to 100 ppm (Table 1).

A decrease in germination speed with increased concentration in *Amaranthus* seeds was significantly affected at low, medium, and higher lithium concentrations compared to the control. Except for 10 ppm, the rate of germination was 12.5, which was the same as that of the control.

Speed of accumulated germination

At lower concentrations of lithium, i.e., from the control to 25 ppm, there was a gradual decrease in the speed of the accumulated germination index. It was observed to be 22.07 in the control and 10 ppm and decreased to 16.98 at 25 ppm. At medium concentrations (50 ppm and 75 ppm), the rate of accumulated germination increased from 50 ppm to 17.83, which was more than that at 25 ppm, i.e., 16.98, and then decreased from 75 ppm to 12.70. At higher concentrations, i.e., 100 ppm, there was a decrease in the speed of accumulated germination, i.e., 7.14. The rate of accumulated germination decreased from the control to 100 ppm (Table 1).

Pot experiments:

Growth parameters

At the end of 21 days, the root length was found to be 3.6 cm/plant in the control, 5 cm/plant in 10 ppm, 5.2 cm/plant in 25 ppm and 5.5 cm/plant in 50 ppm. From the control to 50 ppm, an increase in root length was observed with an increase in concentration. At 75 ppm and 100 ppm, the root length declined from 3.9 cm/plant to 2.3 cm/plant at the end of 21 days. The root

length increased from 14 cm/plant to 15 cm/plant in control to 25 ppm, which changed from 75 ppm to 100 ppm (12.6 cm/plant to 13.9 cm/plant). In the entire treatment period of 51 days, the results showed that lithium promoted root growth with an increase in the crop growth period (Table 2).

Heavy metals induce dissimilar root growth due to excess cell division or inhibition of cell elongation, which is observed in limited soil volume. Thus, the uptake of minerals, nutrients, and water is affected, inducing mineral deficiency in plants (Jaleel et al., 2009). Higher lithium concentrations have decreased plant growth in previous studies conducted on *Brassica juncea*, *Brassica carinata*, maize and sunflower seedlings (Antonkiewicz et al. 2017; Stolarz et al. 2015; Hawrylak-Nowak et al. 2012; Li et al. 2009; Makus and Zibilske 2009). With increased concentration, the shoot length recorded a declining trend at the end of 21 days. With the increase in the treatment period, i.e., at the end of 51 days, the mean shoot length was 34.8 cm/plant at 100 ppm, 37.7 cm/plant at 75 ppm, and 40.9 cm/plant at 50 ppm. Thus, a decline in the shoot length was observed with increased concentration and time (Table 2). The study results indicated a decrease in the dry weight of plants from the control to 100 ppm (0.020 to 0.013 gm/plant) at the end of 21 days. The root weight decreased to 0.109 gms/plant in the control and 0.236 gms/plant in 100 ppm at the end of 51 days. At 25 ppm, the dry weight was recorded as 0.201 gm/plant and decreased to 0.156 at 50 ppm. The same trend was observed at 75 and 100 ppm, declining further to 0.297 and 0.236, respectively (Table 2). In the entire treatment period of 51 days, the results indicated that *Amaranthus* lithium promoted root biomass with increased crop growth. Although the essentiality of lithium and its toxic effects are not clear, previous studies on lithium toxicity mentioned that increased concentrations of lithium induced stress on plant parts such as leaves by developing necrotic and chlorotic spots (Naranjo et al. 2003). The present study revealed an increase in weight with an increased concentration of lithium in the soil. Similar results were observed in the studies conducted by Jurkowska et al., 1998, where 25 ppm lithium showed suppressed growth in oat plants, while maize and spinach growth decreased at 40 ppm. Lithium at root tips may alter root hair development and root caps and reduce root biomass (Kabata-Pendias and Mukherjee 2007; Mulkey, 2007).

As per the pot experiments conducted with lithium amends of different concentrations, it was evident that up to 51 days, i.e., harvesting day in *Amaranthus*, the shoot biomass increased with increasing concentrations from the control (0.135 gms/plant) to 100 ppm (0.204 gms/plant) at 21 days. The same trend was noticed at the end of 51 days, where the shoot biomass increased from 1.013 gms/plant in control to 2.179 gms/plant in

Table 2. Effect of Li concentration on growth parameters of *Amaranthus viridis* at 21 and 51 days (Pot experiment)

	Concentrations	Control	10ppm	25ppm	50ppm	75ppm	100ppm
21 day	Root length	3.6 ± 1.42	5 ± 1.33	5.2 ± 1.22	5.5 ± 1.35	3.9 ± 1.85	2.3 ± 0.94
	Shoot length	23.5 ± 2.12	23.1 ± 3.34	22.2 ± 1.98	20.6 ± 2.45	15.8 ± 2.39	9.6 ± 1.17
	Total Length	27.1 ± 2.76	28.1 ± 3.54	27.4 ± 2.75	26.1 ± 3.34	19.7 ± 3.52	11.9 ± 1.52
	Root biomass	0.02 ± .005	0.015 ± 0.004	0.011 ± 0.003	0.016 ± 0.004	0.011 ± 0.003	0.013 ± 0.004
	Shoot biomass	0.135 ± 0.008	0.176 ± 0.006	0.133 ± 0.005	0.149 ± 0.010	0.4 ± 0.017	0.204 ± 0.010
	Total biomass	0.155 ± 0.008	0.19 ± 0.007	0.144 ± 0.005	0.165 ± 0.011	0.411 ± 0.018	0.217 ± 0.013
51 day	Root length	14 ± 3.65	14.2 ± 4.49	15 ± 3.77	12.1 ± 1.96	12.6 ± 2.67	13.9 ± 1.37
	Shoot length	45.6 ± 6.91	54.9 ± 5.46	40.3 ± 8.96	40.9 ± 8.33	37.7 ± 7.71	34.8 ± 8.33
	Total Length	59.6 ± 6.89	69.1 ± 4.22	55.3 ± 10.45	53 ± 7.39	50.3 ± 8.92	48.7 ± 8.74
	Root biomass	0.109 ± 0.05	0.109 ± 0.05	0.201 ± 0.07	0.156 ± 0.04	0.297 ± 0.15	0.236 ± 0.09
	Shoot biomass	1.013 ± 0.22	1.013 ± 0.22	1.478 ± 0.38	0.876 ± 0.24	1.949 ± 0.85	2.179 ± 0.58
	Total biomass	1.122 ± 0.23	1.122 ± 0.23	1.679 ± 0.39	1.032 ± 0.25	2.246 ± 0.91	2.415 ± 0.62

100 ppm. A study conducted by Naranjo et al., 2003 stated that plant growth decreases at higher lithium concentrations, and leaves show chlorotic and necrotic spots, indicating that plants underwent lithium stress. However, *Amaranthus* showed dissimilarity by increasing the shoot biomass (Table 2).

The plant biomass showed a significant increase at 21 days and 51 days (harvesting day). At 21 days, the plant biomass in control was 0.155 gm/plant, and at 100 ppm, it was 0.217 gm/plant. The same trend was seen on day 51, i.e., 1.122 gms/plant in the control and 2.415 gms/plant in the 100 ppm treatment. In the entire treatment period up to 51 days, the results clearly showed that lithium promoted plant dry weight with increased lithium amendment concentrations on crop growth (Table 2). The geochemical role of plants concerning lithium is determined by biomass production rather than the structure of vegetative communities and the lithium content in the phytomass (Kashin et al., 2019).

The stress tolerance index of root length and shoot length showed a declining trend from 10 ppm to 100 ppm on days 21 and 51, respectively. On day 21, stress tolerance at root length decreased from 138.9 at 10 ppm to 63.9 at 100 ppm. Similarly, the stress tolerance index at shoot length decreased from 100.2 at 10 ppm to 41.6 at 100 ppm. On day 51, the stress tolerance index of root length decreased from 101.4 at 10 ppm to 99.2 at 100 ppm. Shoot length decreased from 120.9 at 10 ppm to 75.65 at 100 ppm.

An increasing trend in stress tolerance was not observed in either root or shoot dry weight from 10 ppm to 100 ppm on days 21 and 51, except in root dry weight at day 21. Stress tolerance decreased from 75 to 65 from 10 ppm to 100 ppm, whereas on day 51, it increased from 100 to 216.5 from 10 ppm to 100 ppm. Similarly, the shoot dry weight stress tolerance increased from 10 ppm to 100 ppm on days 21 and 51. The increase in biomass at both the root and shoot could be due to the increase in the number of cells or cell expansion. Polymers at plant cell walls may also be

Table 3. Effect of Li concentrations on the stress tolerance index of *Amaranthus viridis* at 21 and 51 days

		RLSTI	SLSTI	RDSTI	SDSTI
21 day	10ppm	138.9	100.2	75	122.58
	25ppm	144.5	94.5	55	92.9
	50ppm	152.8	89.3	80	106.45
	75ppm	108.3	68.54	55	265.16
	100ppm	63.9	41.6	65	140
	51 day	10ppm	101.4	120.9	100
25ppm		107.1	88.37	184.4	149.77
50ppm		86.4	89.69	143.11	165
75ppm		90	82.67	272.48	200.35
100ppm		99.2	75.65	216.51	215.43

responsible for increasing plant biomass (Table 3). The migration of lithium in natural environments occurs mainly in ionic forms. As the coefficient of energy is lower in lithium, a higher migration ability is observed in the shoot of the plant than in the root (Sobolev et al., 2019). Ions such as K^+ , Mg and Na^+ also influence the transport of lithium to plant parts and are thus responsible for the higher stress tolerance index at higher concentrations (Ammari et al., 2011; Sobolev et al., 2019).

Conclusion

The germination index (*in vitro*) of *Amaranthus viridis* clearly showed that all germination parameters were affected by the increased lithium concentration in the soil. The growth parameters (pot experiments), i.e., the length and dry weights of roots and shoots, on days 21 and 51 showed that lithium promoted the growth of the plant. The stress tolerance index of root and shoot length decreased from 10 ppm to 100 ppm at both 21 and 51 days. However, the stress tolerance index of root and shoot dry weight increased with an increase in concentration. This indicates that *Amaranthus* is stress-tolerant to lithium up to 100 ppm and can extract lithium from the soil to a large extent and translocate it into the aboveground parts, where it is usually stored in specific cell organelles. Plants that are tolerant to higher concentrations may accumulate higher amounts of lithium than normal plants, with no adverse effects on their growth.

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Conflict of interest

The authors declare that they have no conflict of interest.

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