

EFFECT OF SODIUM SALT STRESS ON THE GROWTH OF *ELYMUS REPENS*

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Abstract

Plants are sensitive to the external environment that may either be in form of biological stress such as any pathogenic effect or abiological stress such as salt stress. Among the salt stresses, the most prominent stress is known as sodium salt stress, because sodium is involved in a wide range of plant metabolic activities preceded by calcium. To investigate the effect of sodium ion on plant, seeds of *E. repens* are first imbibed in distilled water for 2 hours, surface sterilized with 70% ethanol and then grown in sodium nitrate, sodium bisphosphate monobasic, trisodium citrate, sodium bromide, sodium carbonate, and sodium dichromate for 7 days in the concentration of 0.2%, 0.4%, 0.6%, 0.8%, 1% of salts compared with distilled water as control for each. At 8th day of germination, root and shoot length were noted using scale and fresh weight of root and shoot was noted using digital balance while dry weight of root and shoot was noted after drying them in incubator for 72 hours. Noted data elucidated that seedlings showed high growth in sodium nitrate while low growth in trisodium citrate, sodium carbonate and sodium dichromate. Growth in sodium bisphosphate and sodium bromide was slightly higher with increasing concentration of salt but still lower when compared to control. Moreover data for each salt was also used to find out the vigour index, stem weight ratio, root weight ratio, root stem ratio and relative water content to analyse the effect of salts on different aspects of plant growth.

Key words: *E. repens*, Growth condition, Germination, Vigour index, Salt stress, and Metabolomics

Introduction

E. repens, regularly known as couch grass, is a local perennial grass. It is found mostly in Europe, Asia, the Arctic biome, and northwest Africa. It has been brought into other gentle northern atmospheres for forage or disintegration control, however is frequently viewed as a weeds (Beltagi *et al.*, 2013). Salt stress is one of main considerations in constraining harvest creation. Salinization affect more than 20% of the rural area and the phenomenon is ever increasing globally (Alzahrani *et al.*, 2019). Salinity affects seed germination of a few vegetables crops, by making an osmotic potential outside the seed limiting the ingestion of water, or by the lethal impact of Na⁺ furthermore (Sunagawa *et al.*, 2010). The immediate impacts of salt on plant development may include: (a) Reduction in the osmotic capability that lessens the available water accessible to the plant creating physiological dry spell. (b) Toxicity of extreme Na⁺ towards the cell that upsets structure of compound and different macromolecules, harms cell organelles and plasma layer, disturbs photosynthesis, breath and protein union (Ozgur *et al.*, 2013). Under salt anxiety, the photosynthetic rate of plant lessens (Maksimovic *et al.*, 2013). Most developed plants and members of grass family, are delicate to salt stress (Millar *et al.*, 2011). Notwithstanding, *Elymus* generation has spread into more minimal lands, along these lines, presenting the product to a more serious danger of salt stretch (Engels & Jensen 2010). *Elymus* is a well-known weed requiring its change to fit in the conditions with changing salinity stress. For higher yield of weed under saline condition, salinity tolerant cultivars are required to withstand soil salinization in semi and parched ranges (Agong *et al.*, 2003).

Facelli in 2008 found that plants fluctuated extraordinarily in their reaction to varying levels of salt stress. High levels of NaCl in provided supplement

unfavourably influenced tomato shoot and roots, plant hight, K⁺ focus, as well as K/Na proportion (Wang, 2011). Along these lines, the target of of the study was to inspect the impact of saline soil on plant development, chlorophyll contents, some mineral supplements of weed with an aim to identify salt-tolerant ones for semi and parched ranges.

Saud *et al.*, 2014 reviewed rice seedlings *Oryza sativa* L. vr. Fahad *et al.*, 2015 reviewed on cowpea, *Vigna unguiculata* L., lastly by Memon *et al.*, (2010) in their review on *Brassica campestris* L., where they showed that the utilization of low levels of sodium chloride stress drove to increments in plants lengths, while higher levels created deficiency. Opposite outcomes were enrolled also, counting the review done by Mustafa *et al.*, 2014 on moth bean *Vigna aconitifolia* L., Jamil *et al.*, (2007) on radish plant, *Raphanus sativus* L., by Taffouo *et al.*, (2009) on cowpea *Vigna unguiculata* by L., and Kapoor and Srivastava (2010) on *Vigna mungo* L. They found that expanding the convergences of NaCl built up a decrease in plant hight when exposed to NaCl stress.

From reported studies on impact of salinity stress on plant development, one can identify an association between reduction in plant length and sodium chloride concentration (Tavakoli *et al.*, 2016). Various reviews demonstrated negative affinity of leaf area with specific NaCl concentrations (Iqbal *et al.*, 2014). Different studies demonstrated that the fresh and dry weights of the shoot system are influenced, either partially or completely, by the degree of salt stress, sort of salt present, or kind of plant species (Hussain *et al.*, 2013).

Methods and Materials

Plant material: The experiment was conducted for available salts of sodium in incubator for 8 days using seeds of *E. repens*. The seeds were obtained from agriculture department of Peshawar University, Mardan (Table 1).

Table 7. Data of averages and standard deviation noted for *E. repens* 8 days old seedlings shows increasing vigour index, stem weight ratio, root weight ratio, relative water content (root, shoot), root stem ratio with increasing concentrations (0.2%, 0.4%, 0.6%, 0.8%, 1%) of sodium bromide as compared to control grown seedling in distilled water. ANOVA and Duncan’s test were also performed to analyse the significant difference.

Solution concentration	Seeding vigour	Stem weight ratio	Root weight ratio	Relative water content (root)	Relative water content (shoot)	Root stem ratio
Control (dil.H2O)	2239 ^b ± 638	0.5 ^c ± 0.04	0.6 ^d ± 0.2	92 ^a ± 3	92 ^a ± 1	1.6 ^a ± 1
0.2%	215 ^c ± 114	0.4 ^b ± 0.2	0.9 ^b ± 0.1	71 ^b ± 7	77 ^b ± 17	2.6 ^c ± 2.1
0.4%	110 ^a ± 32	0.4 ^b ± 0.07	1.1 ^a ± 0.4	39 ^c ± 21	62 ^{ab} ± 8	1.5 ^b ± 1
0.6%	21 ^d ± 8	0.4 ^b ± 0.08	0.1 ^b ± 0.07	97 ^d ± 4	99 ^c ± 4	0.8 ^a ± 0.3
0.8%	47 ^c ± 22	0.5 ^a ± 0	0.2 ^{ab} ± 0.05	79 ^c ± 2	78 ^d ± 5	3.1 ^c ± 0.4
1%	0.9 ^d ± 1	0.4 ^b ± 0.01	0.1 ^a ± 0	88 ^a ± 1	89 ^c ± 5	1.4 ^d ± 2
LSD _{0.05}	1.00	0.03	0.05	1.00	1.00	1.00
(P<0.05)	0.0001	0.0001	0.036	0.023	0.012	0.0001

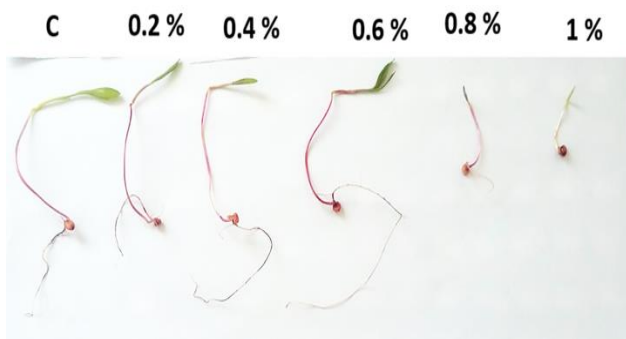


Fig. 1. 8th day seedlings of *E. repens* under sodium mono biphosphate stress.

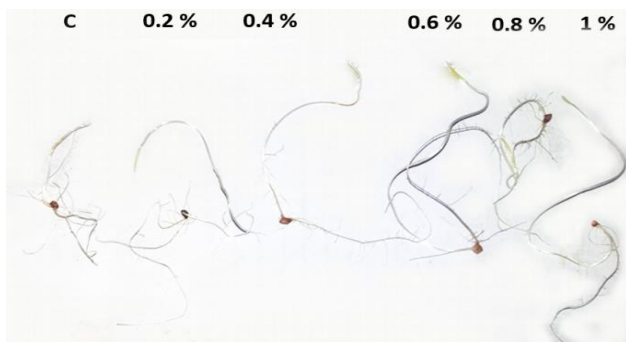


Fig. 2. 8th day seedlings of *E. repens* under sodium nitrate stress.

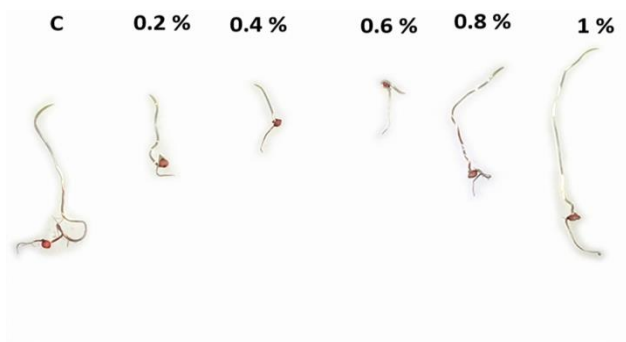


Fig. 3. 8th day seedlings of *E. repens* under sodium bromide stress.

Sodium chloride and trisodium citrate has fairly negative effect on the growth of *E. repens* as compared to sodium arsenate and sodium dichromate: Some other salts of sodium such as sodium chloride and trisodium citrate had fair inverse correlation with shoot and root length (Fig. 4), sodium chloride with their increasing concentrations as compared to sodium arsenate, sodium chloride and sodium dichromate (Figs. 5-7). Likewise, vigour index, relative water content, shoot root ratio, stem weight and root weight were also low for the above salt in the same fashion (Tables 8-15). Previous studies also suggested that arsenate and dichromate ions are regarded as nano particles which uniquely affects plants’ growth and development. These are absorbed into the cell wall of the seeds and accelerate seed germination (Okeniyi, 2015). Chloride ion is known for its negative effect on plant growth (Tavakkoli, 2011). It is involved in the opening and closing of stomata, regulating water content in plants’leaf. Chloride competes with nitrate uptake by the plant to render the plant resistant against disease development, as nitrate is an important constituent for many kinds of microbes. Beside-these advantages of chloride ions, it retards the overall growth of a plant by reducing the leaf area, and abscission of leaf margins. Similarly carbonate is also toxic for the mycorrhizae and bacteriorrhizae of the plant which are found to be associated with plant seeds and seedlings for its successive germination.

Our results also show similar results. Growth of *E. repens* seedlings was not conducive to sodium chloride and sodium carbonate in a way that with increasing concentrations 0.2, 0.4, 0.6, 0.8 and 1%, reduction in shoot and root length and fresh and dry weight as compared to control grown seedlings was observed. Sodium arsenate and sodium dichromate are the trace element, required in a nano amount for the optimal growth of plant. They also retarded the growth of shoot and root length, thus known for their negative effect on plant growth. Sodium arsenate and sodium dichromate is the trace element, required in a nano amount for the optimal growth of plant. Dichromate ions are strong oxidizing agent, certainly involved in the biosynthesis of jasmonic acid which further decides the fate of the host

cell. The accumulation of arsenic as well as macro- and micronutrients and investigated biochemical stress responses in clover shoots caused by arsenic incorporation in clover shoots. On another hand, dichromate in combination with potassium at a very low concentration such as 100 micromoles induces the growth of root hairs whereas at 200 micromoles arrest the growth of root and shoot (Domínguez-Crespo *et al.*, 2009).

Our finding also report similar data. Dichromate had negative impact on root and shoot growth of *E. repens* 8 days old seedlings. Increases in superoxide dismutase

(SOD) activity, peroxidase activity as well as decreases in chlorophyll (chl) and carotenoid concentrations correlated with increasing arsenic content in plants. The analyses of native PAGE SOD activity staining indicated one Mn-SOD and two major Cu/Zn-SOD isoenzymes in clover shoots, whose capacity increased in response to arsenate treatment (Henderson, 2015). Our findings on *E. repens* seedlings also showed similar intraction with reduced shoot and root growth in response to sodium arsenate and sodium dichromate as higher concentration was applied to the seedlings.

Table 8. Data of averages and standard deviations noted for *E. repens* 8 days old seedlings shows decreasing root and shoot length, fresh and dry weight of shoot and root with increasing concentrations (0.2%, 0.4%, 0.6%, 0.8%, 1%) of sodium chloride as compared to control grown seedling in distilled water. ANOVA and Duncan's test were also performed to analyse the significant difference.

Solution Concentration	Root length	Shoot length	Fresh weight (root)	Dry weight (root)	Fresh weight (stem)	Dry weight (stem)
Control (dil.H2O)	33 ^b ± 3	24 ^c ± 1.3	0.2 ^b ± 0.05	0.07 ^a ± 0.03	0.2 ^a ± 0.05	0.01 ^a ± 0.01
0.2%	22 ^a ± 3	12 ^d ± 2	0.1 ^a ± 0	0.03 ^{ab} ± 0.01	0.08 ^a ± 0.01	0.08 ^a ± 0.01
0.4%	19 ^a ± 0.3	10 ^c ± 0.3	0.04 ^{bc} ± 0.4	0.02 ^{ab} ± 0.01	0.06 ^{ab} ± 0.03	0.01 ^a ± 0.01
0.6%	16 ^a ± 0.3	7 ^b ± 1.4	0.03 ^{bc} ± 0.01	0.01 ^b ± 0.005	0.03 ^{ab} ± 0.02	0.01 ^a ± 0.01
0.8%	9 ^a ± 1	4 ^b ± 0.4	0.02 ^{bc} ± 0.01	0.01 ^c ± 0.005	0.01 ^b ± 0.01	0.01 ^b ± 0.01
1%	4 ^a ± 0.4	2 ^a ± 0.3	0.01 ^a ± 0	0.01 ^d ± 0.001	0.01 ^c ± 0.01	0.005 ^c ± 0.03
LSD _{0.05}	1.00	1.00	0.23	1.00	1.00	1.00
(P<)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Table 9. Data of averages and standard deviation noted for *E. repens* 8 days old seedlings shows decreasing vigour index, stem weight ratio, root weight ratio, relative water content(root, shoot), root stem ratio with increasing concentrations (0.2%, 0.4%, 0.6%, 0.8%, 1%) of sodium chloride as compared to control grown seedling in distilled water. ANOVA and Duncan's test were also performed to analyse the significant difference.

Solution concentration	Seedling vigour	Stem weight ratio	Root weight ratio	Relative water content (root)	Relative water content (shoot)	Root stem ratio
Control (dil.H2O)	530 ^b ± 68	0.2 ^a ± 0.03	1.3 ^b ± 0.5	78 ^a ± 12	94 ^c ± 3	4 ^c ± 1
0.2%	265 ^c ± 46	0.8 ^c ± 0.1	0.3 ^b ± 0.1	82 ^a ± 13	48 ^a ± 4.3	0.3 ^a ± 0.1
0.4%	183 ^c ± 15	0.4 ^{ab} ± 0.2	0.1 ^b ± 0.07	64 ^a ± 27	77 ^{bc} ± 22	2.1 ^b ± 1.2
0.6%	161 ^a ± 18	0.4 ^b ± 0.09	0.1 ^b ± 0.05	63 ^a ± 18	74 ^{bc} ± 7	1.3 ^{ab} ± 0.5
0.8%	89 ^a ± 18	0.4 ^{ab} ± 0.08	0.04 ^b ± 0.1	52 ^a ± 45	60 ^{ab} ± 10	1.5 ^{ab} ± 0.6
1%	16 ^a ± 9	0.3 ^{ab} ± 0.1	0.02 ^a ± 0.001	50 ^a ± 4	74 ^{bc} ± 20	2.5 ^b ± 1.1
LSD _{0.05}	1.00	1.00	0.19	0.175	0.27	1.58
(P<)	0.0001	0.002	0.0001	0.53	0.02	0.004

Table 10. Data of averages and standard deviations noted for *E. repens* 8 days old seedlings shows decreasing root and shoot length, fresh and dry weight of shoot and root with increasing concentrations (0.2%, 0.4%, 0.6%, 0.8%, 1%) of trisodium citrate as compared to control grown seedling in distilled water. ANOVA and Duncan's test were also performed to analyse the significant difference.

Solution concentration	Root length	Shoot length	Fresh weight (root)	Dry weight (root)	Fresh weight (stem)	Dry weight (stem)
Control (dil.H2O)	40 ^a ± 14	38 ^a ± 7	0.3 ^a ± 0.02	0.02 ^a ± 0.005	0.4 ^a ± 0.02	0.02 ^a ± 0.005
0.2%	42 ^b ± 9	55 ^d ± 10	0.16 ^b ± 0.06	0.02 ^a ± 0.01	0.2 ^a ± 0.05	0.04 ^a ± 0.03
0.4%	16 ^c ± 4	23 ^d ± 6	0.05 ^c ± 0.02	0.01 ^b ± 0.005	0.13 ^b ± 0.01	0.03 ^a ± 0.04
0.6%	9 ^d ± 4	11 ^c ± 6	0.04 ^b ± 0.03	0.01 ^b ± 0.01	0.1 ^c ± 0.01	0.06 ^b ± 0.01
0.8%	14 ^d ± 7	18 ^a ± 3	0.02 ^b ± 0.02	0.01 ^b ± 0.01	0.1 ^c ± 0.07	0.04 ^b ± 0.01
1%	2 ^c ± 1	3 ^c ± 2	0 ^c ± 0	0 ^c ± 0	0 ^d ± 0	0 ^c ± 0
LSD _{0.05}	1.00	1.00	0.23	0.76	1.00	1.00
Probability level (P<)	0.0001	0.0001	0.0001	0.56	0.0001	0.0001

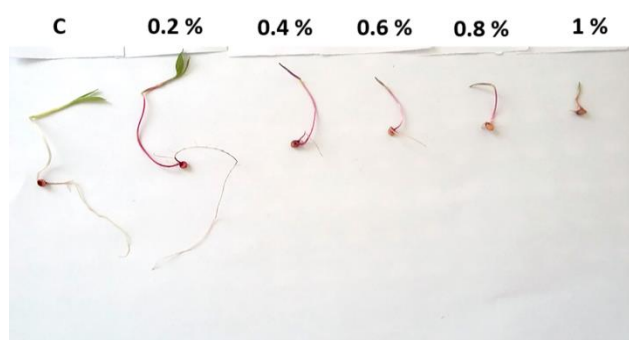


Fig. 4. 8th day seedlings of *E. repens* under trisodium citrate stress.

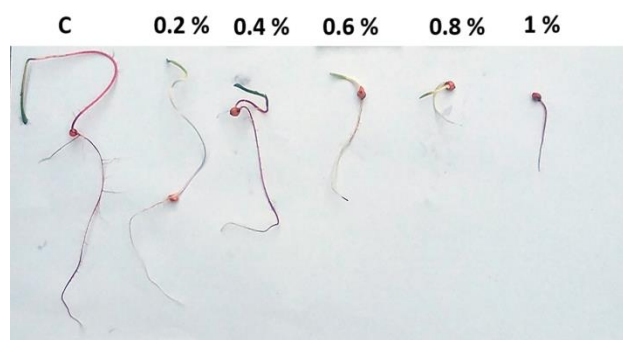


Fig. 5. 8th day seedlings of *E. repens* under sodium chloride stress.

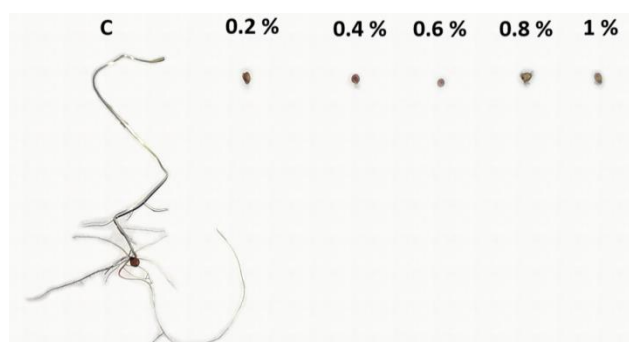


Fig. 6. 8th day seedlings of *E. repens* under sodium arsenate stress.

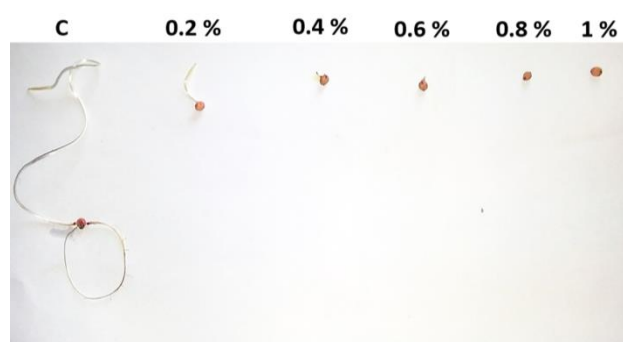


Fig. 7. 8th day seedlings of *E. repens* under sodium dichromate stress.

Table 11. Data of averages and standard deviation noted for *E. repens* 8 days old seedlings shows decreasing vigour index, stem weight ratio, root weight ratio, relative water content (root, shoot), root stem ratio with increasing concentrations (0.2%, 0.4%, 0.6%, 0.8%, 1%) of trisodium citrate as compared to control grown seedling in distilled water. ANOVA and Duncan's test were also performed to analyse the significant difference.

Solution concentration	Seeding vigour	Stem weight ratio	Root weight ratio	Relative water content (root)	Relative water content (shoot)	Root stem ratio
Control (dil.H2O)	1019 ^a ± 8	0.5 ^{ab} ± 0.02	0.5 ^c ± 0.13	91 ^c ± 3	94 ^a ± 2	1 ^b ± 0.1
0.2%	1468 ^b ± 313	0.6 ^a ± 0.2	0.3 ^b ± 0.16	70 ^d ± 28	69 ^a ± 17	0.9 ^{ab} ± 0.7
0.4%	666 ^b ± 361	0.6 ^a ± 0.2	0.1 ^a ± 0.2	64 ^b ± 13	55 ^b ± 60	1 ^a ± 0.9
0.6%	276 ^b ± 205	0.8 ^b ± 0.1	0.1 ^a ± 0.04	83 ^c ± 23	29 ^c ± 75	0.2 ^{ab} ± 0.2
0.8%	256 ^b ± 20	0.9 ^c ± 0.1	0.03 ^{ac} ± 0.06	46 ^{bc} ± 50	10 ^d ± 14	0.17 ^c ± 0.1
1%	32 ^b ± 114	0 ^d ± 0	0 ^d ± 0	0 ^a ± 0	0 ^c ± 0	0 ^d ± 0
LSD _{0.05}	1.00	0.02	1.00	1.00	1.00	1.00
Probability level (P<)	0.0001	0.023	0.045	0.0001	0.043	0.012

Table 12. Data of averages and standard deviations noted for *E. repens* 8 days old seedlings shows zero root and shoot length, fresh and dry weight of shoot and root with increasing concentrations (0.2%, 0.4%, 0.6%, 0.8%, 1%) of sodium arsenate as compared to control grown seedling in distilled water.

Solution concentration	Root length	Shoot length	Fresh weight (root)	Dry weight (root)	Fresh weight (stem)	Dry weight (stem)
Control (dil.H2O)	35 ± 7.2	32 ± 5.6	0.34 ± 0.5	0.023 ± 0.003	0.37 ± 0.02	0.05 ± 0.001
0.2%	0	0	0	0	0	0
0.4%	0	0	0	0	0	0
0.6%	0	0	0	0	0	0
0.8%	0	0	0	0	0	0
1%	0	0	0	0	0	0

Table 13. Data of averages and standard deviation noted for *E. repens* 8 days old seedlings shows zero vigour index, stem weight ratio, root weight ratio, relative water content (root, shoot), root stem ratio with increasing concentrations (0.2%, 0.4%, 0.6%, 0.8%, 1%) of sodium arsenate as compared to control grown seedling in distilled water.

Solution concentration	seedling vigour	Stem weight ratio	Root weight ratio	Relative water content (root)	Relative water content (shoot)	Root stem ratio
Control (dil.H2O)	2218 ^b ± 621	0.7 ^c ± 0.02	0.4 ^d ± 0.4	91 ^a ± 6	88 ^a ± 2	1.7 ^a ± 2
0.2%	0	0	0	0	0	0
0.4%	0	0	0	0	0	0
0.6%	0	0	0	0	0	0
0.8%	0	0	0	0	0	0
1%	0	0	0	0	0	0

Table 14. Data of averages and standard deviations noted for *E. repens* 8 days old seedlings shows zero root and shoot length, fresh and dry weight of shoot and root with increasing concentrations (0.2%, 0.4%, 0.6%, 0.8%, 1%) of sodium dichromate as compared to control grown seedling in distilled water.

Solution concentration	Root length	Shoot length	Fresh weight (root)	Dry weight (root)	Fresh weight (stem)	Dry weight (stem)
Control (dil.H2O)	31 ± 7.3	27 ± 5.1	0.31 ± 0.5	0.023 ± 0.003	0.38 ± 0.02	0.04 ± 0.001
0.2%	23 ± 3.2	13 ± 3.1	0.12 ± 0.2	0.002 ± 0.003	0.13 ± 0.0001	0.001 ± 0.002
0.4%	0	0	0	0	0	0
0.6%	0	0	0	0	0	0
0.8%	0	0	0	0	0	0
1%	0	0	0	0	0	0

Table 15. Data of averages and standard deviation noted for *E. repens* 8 days old seedlings shows zero vigour index, stem weight ratio, root weight ratio, relative water content (root, shoot), root stem ratio with increasing concentrations (0.2%, 0.4%, 0.6%, 0.8%, 1%) of sodium dichromate as compared to control grown seedling in distilled water.

Solution concentration	Seedling vigour	Stem weight ratio	Root weight ratio	Relative water content (root)	Relative water content (shoot)	Root stem ratio
Control (dil.H2O)	2315 ± 542	0.65 ± 0.03	0.5 ± 0.43	92 ± 23	95 ± 4	1.5 ± 2.1
0.2%	1313 ± 122	0.12 ± 0.02	0.2 ± 0.12	17 ± 14	13 ± 3	0.3 ± 0.001
0.4%	0	0	0	0	0	0
0.6%	0	0	0	0	0	0
0.8%	0	0	0	0	0	0
1%	0	0	0	0	0	0

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