

Assessment of seedling growth parameters and disease-like symptoms of leafy *Pisum sativum* under salt stress in high water capacity peat

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Abstract

Irrigation water salinization is a serious problem affecting seedling growth at the planting circle's first step. Sown seeds of leafy form peas (*Pisum sativum* L.) were exposed to saline water once at first watering via irrigation with (0; 0.5; 1; 2; 4 and 8 g/L NaCl) salt solutions. Seedling growth parameters as; the seedling emergence rate, mean emergence day, root length, fresh root weight, dry root weight, shoot length, fresh shoot weight, dry shoot weight, fresh seedling weight, dry seedling weight, and root/shoot ratio (in terms of; length/length, fresh weight/fresh weight, and dry weight/dry weight) were calculated and evaluated in Tukey Test after analysis of variance. At the end of the two weeks, the evaluated data indicated that the used plant type negatively affected in most parameters related to length, fresh weight, and root/shoot ratio only in the highest dose (8 g/L) of the NaCl treatment. Although there were no differences between salt treatments in seedling emergence rate, the data were between 16.7-50%. In addition, the plants treated with a certain amount of salt (1, and 2 g/L NaCl) showed the best growth visually. Beside the parameters, disease-like symptoms were evaluated, and the data showed that although no statistical differences were found, the certain amount of (0.5; 1, and 2 g/L NaCl) salt stress treated seeds showed lesser damages and disorders.

Keywords: Salt stress, *Pisum sativum*, seedling, disorder, seedling health

Introduction

High salt levels in plant growth steps are known to affect most of the plant's physiological and biochemical processes as well as growth and yield. The abiotic plant stress makes plants predispose to another biotic effect. In most cases, the harmful effects of salt stress are tried to be revealed through biochemical, analytical, and visual measurements. In addition, adaptation of different plant genus, species, and even cultivars to the stress factor may be different than supposed.

Pisum sativum L. is one of the valuable legumes that contains valuable nutritional and bioactive ingredients as carbohydrates, protein, lipids, minerals, and vitamins. Among these, starch, globulin, and polyunsaturated fatty acids can be described as the mostly found carbohydrate, protein, and lipid groups respectively. Nitrogen, potassium, phosphorus, manganese, copper, zinc, and a minor amount of selenium in addition to vitamins especially tocopherols are the main minerals and vitamin groups (Wu *et al.*, 2023)^[7]. The pea has different morphotypes or varieties (Cousin, 1997; Pekşen *et al.*, 2004; Juozaitytė *et al.*, 2007)^[1, 6, 4] including leafy peas (snow peas, sugar peas) with edible pods and green peas (vining peas, shelling peas) which is consumed as fresh, canned, frozen, or dried seeds without pods. Besides human consumption, some pea varieties are mostly for animal feeding. This plant, which contains such important nutrients and has various uses, is the most well-known plant around the world and is grown in many countries (FAOSTAT, 2022)^[3].

Peas have large cultivation areas and may need to be grown in a land that is becoming increasingly saline. For this reason, it is important to know the salt tolerance point of peas and to observe the first symptoms that may be encountered in salty soils or salty irrigation water to avoid misdiagnoses.

In this study, a leafy pea cultivar that treated with salty irrigation water at the seedling stage to obtain the effect on some important growth parameters of seedlings and visual disease-like symptoms for reaching healthy plants.

Materials and Methods

The study was conducted at Kocaeli University, Agricultural Faculty, in November, 29- December, 13, 2023 under a controlled environment. The seeds of leafy form peas (*Pisum sativum* L.) that were examined for salt stress, harvested last growing season, and purchased from a seed company in pesticide-treated form (Figure 1.a). The Randomly selected thousand-seed weight is 253 g.

In the experiment, the seeds were placed in plastic trays with thirty-two cells, and each cell consisted of approximately 60 g of first-use peat, which had a water retention capacity of 400%. The seeds were irrigated with (0; 0.5; 1; 2; 4 and 8 g/L NaCl) salt solutions in tap water of 40 ml with only one seed hole. No other water application was required along with the study. In a controlled environment the air temperature was at 23±1°C, the air humidity was at 60±7%, and the peat medium temperature

was at $22 \pm 1^{\circ}\text{C}$. The experiment was held in non-sterile conditions.

Along 14 days, seedling emergence was observed daily to calculate the seedling emergence rate and mean emergence day. At the end of the 14th day, shoot length, fresh shoot weight, dry shoot weight, root length, fresh root weight, dry root weight, fresh seedling weight, dry seedling weight and root/shoot ratio (in terms of length/length, fresh weight/fresh weight, and dry weight/dry weight) were calculated (Figure 1.b. Figure 1.c). At the same time disease-like symptoms were observed diagnostically. If a plant was not emergence to the surface or not capable in giving true leaves healthily, the seedling was assumed as diseased whatever the reason.

The study was conducted in a Completely Randomized Design (CRD) with a total of 6 treatments (5 salinity and control) each comprising three replicates. Each replicate consisted of 8 seeds that were placed in seedling hole and a totally of 144 seeds were sown, calculated, and evaluated. The recorded data were analyzed through statistical software (SPSS Statistics 16.0). A two-way analysis of variance (ANOVA) was used to test for differences among salt treatments. Comparisons were made using the Tukey Test at least $P \leq 0,05$ degree. Results were shown as mean values and standard deviation (\pm) in the tables.

Results and Discussion

Analysis of variance revealed that significant differences among the salt treatment for seedling emergence day; length of root, shoot, and seedling; weight of fresh root, fresh shoot, and fresh seedling; and ratio of root/shoot in terms of fresh weight and length.

The earliest mean emergence days were found in 2 and 1 g/L NaCl concentrations as 5.6 and 6.13rd days respectively while the latest was recorded in 8 g/L of NaCl concentration as 8th day (Figure 2.a). Although there were no differences between salt treatments in seedling emergence rate (Figure 2.i), the data were between 16.7-50% (Table 1). In a study (Dadaşoğlu *et al.*, 2020) [2] conducted for observations the salt stress effect on seed germination of *Cicer arietinum* and *Pisum sativum* in Petri trials under 0, 50, 100, 150 and 200 mM NaCl concentrations, among used four pea cultivars, germination rate changed between 0-97% in cultivars and under salt treatments. The study is strongly parallel to this study and indicated that germination was not only affected by salt treatment it was also strongly affected by cultivars. In another study (Ouerghi *et al.*, 2016) [5], which was conducted to find out the physiological and morphological characteristics of *Pisum sativum* under 0, 25, 50, 75, and 150 mM of NaCl salt levels in Petri trial, germination percentages were found between 58-100% and the highest dose indicated the least results. In the study mean germination time was highest in 75 mM salt treatment that the result is in agreement with this study because of the effect of similar medium dose activation effect on the emergence time.

Root length (13.26-15.46 cm/plant) and seedling length (20.91-24.48 cm/plant) remained statistically unchanged up to 4 g/L NaCl, and 8 g/L NaCl significantly in decreasing in root length (5.95 cm) and seedling length (10.70 cm). The parameter showed that certain salinity concentrations up to 4 g/L had no negative effects (Figure 2.b, Figure 2.d). But shoot lengths are higher in 1 and 2 g/L NaCl concentrations (9.83 and 9.02 cm/plant) than in lesser and higher salt concentrations (Figure 2.c). The results indicated that certain doses have a positive effect in the parameter (Table 2). In a study (Yousef *et al.*, 2020) [8] conducted to obtain salt stress on the growth of *Cicer arietinum* and *Pisum sativum* seedlings in sand culture with NaCl of 0; 0.25 and 0.50 Molar. The study indicated that *P. sativum* seedlings root length, and fresh and dry plant weight were in decreasing with increasing concentrations. In addition, shoot length was found the highest in 0.25 Molar concentration. Their results are conformable with this study because of obtaining the highest shoot length in the certain concentrations.

The weight of fresh root, fresh shoot, and fresh seedling were also only negatively affected by 8 g/L NaCl concentration. Fresh root, fresh shoot, and fresh seedling changed between 0.3972-0.4974; 0.6028-0.7532, and 1.0188-1.2465 g/plant respectively in 0-4 g/L concentrations, but in 8 g/L NaCl concentration, the fresh root weight was 0.0745 g; fresh shoot weight was 0.2294 g and the fresh seedling rate was 0.3039 g/plant. The results indicated that the parameters were sharply in decreasing and affected by only the highest dose 8 g/L NaCl concentration (Table 3; Figure 2.e; Figure 2.f; Figure 2.g).

The root/shoot ratio in terms of fresh weights (f.w./f.w.) was higher in 0-4 g/L (0.5263-0.7361) than in 8 g/L NaCl (0.2921). The root/shoot ratio in terms of length (l./l.) was the highest in the control seedling (Figure 2.h) than the other applications while the dry weight root/shoot ratio (d.w./d.w.) gave no statistical results (Table 5). Among all measured and calculated growth parameters, seedling emergence rate (Table 1), dried root, shoot and seedling weight (Table 4), the dry root/shoot ratio (Table 5) also showed no statistically significant results. Disease-like symptoms including no germination capacity, no healthy seedling growth as shoot tip burning, and shoot and root rot were also observed and found between 58.3-87.5%. Although the results did not show statistical differences, certain amounts of salt-treated seeds (0.5; 1; 2 g/L) showed lesser damages numerically (Table 6; Figure 2.j).

Conclusion

Salinity stress and tolerance to this stress factor involve many growth parameters. The present study revealed that the used leafy *Pisum sativum* cultivar is partially tolerant to salt stress up to 4 g/L with a lower germination capacity than being the other studies and expected. Under certain NaCl concentrations (0.5 g/L) emergence rate can be increased (Figure 2.i), and diseases-like symptoms (Figure 2.j) can be suppressed. This promising study revealed that the need to continue studies with many pea varieties, under different substrates and environmental conditions to support these results.

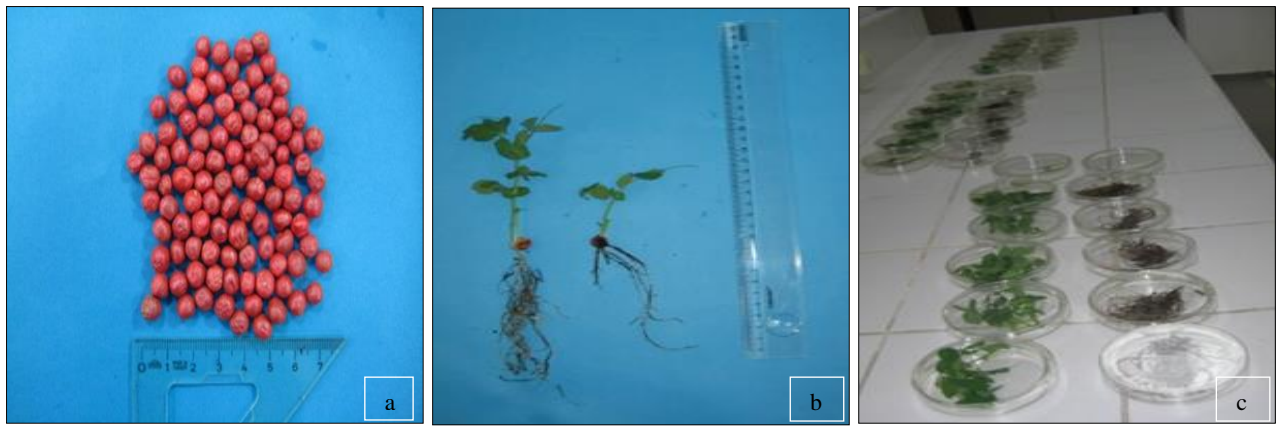


Fig 1: The leafy *Pisum sativum* L.; a) The pesticide-treated seed samples before sowing, b) Left seedling from 2 g/L NaCl treated seed, and right seedling from 8 g/L NaCl treatment, c) Preparation of shoot and root parts for fresh and dry weight calculation for all treatment of *Pisum sativum* L.

Table 1: Seedling emergence rate and mean emergence day of *Pisum sativum* under salt stress

Salt Treatment (NaCl)	Seedling Emergence Rate*		Emergence Day**	
	(%)	±S.D.	(day)	±S.D.
0 g/L	25,0	12,5	6,60 ab	0,36
0,5 g/L	50,0	21,7	6,63 ab	0,32
1 g/L	37,5	12,5	6,13 a	0,51
2 g/L	41,7	19,1	5,60 a	0,53
4 g/L	41,7	19,1	6,23 ab	0,93
8 g/L	16,7	7,2	8,00 b	1,00
Mean	35,42±17,8 P<0,2		6,53±0,94 P<0,05	

*No significant at least $P < 0,05$ probability level at ANOVA, **Significant at $P < 0,05$ probability level at Tukey Test

Table 2: Root, shoot and plant length of *Pisum sativum* under salt stress

Salt Treatment (NaCl)	Root Length*		Shoot Length**		Seedling Length*	
	(cm)	±S.D.	(cm)	±S.D.	(cm)	±S.D.
0 g/L	14,72 a	2,73	7,37 ab	0,95	22,08 a	3,26
0,5 g/L	13,26 a	2,34	7,65 ab	1,39	20,91 a	3,72
1 g/L	13,36 a	1,09	9,83 a	1,03	23,19 a	2,08
2 g/L	15,46 a	3,65	9,02 a	2,73	24,48 a	6,33
4 g/L	13,59 a	0,75	8,22 ab	0,82	21,81 a	1,57
8 g/L	5,95 b	0,55	4,75 b	0,55	10,70 b	0,00
Mean	12,72±3,70 P<0,01		7,81±2,04 P<0,05		20,53±5,50 P<0,01	

*Significant at $P < 0,01$ probability level, **Significant at $P < 0,05$ probability level at Tukey Test

Table 3: Fresh root, fresh shoot, and fresh seedling weight of *Pisum sativum* under salt stress

Salt Treatment (NaCl)	Fresh Root Weight*		Fresh Shoot Weight*		Fresh Seedling Weight*	
	(g/plant)	±S.D.	(g/plant)	±S.D.	(g/plant)	±S.D.
0 g/L	0,4626 a	0,1589	0,6174 ab	0,1238	1,0801 a	0,2826
0,5 g/L	0,4060 a	0,1051	0,6028 ab	0,1628	1,0188 a	0,2091
1 g/L	0,3972 a	0,0551	0,7532 a	0,0613	1,1504 a	0,1106
2 g/L	0,4974 a	0,1123	0,7492 a	0,2473	1,2465 a	0,3589
4 g/L	0,4502 a	0,0343	0,7144 a	0,0958	1,1646 a	0,1207
8 g/L	0,0745 b	0,0557	0,2294 b	0,0713	0,3039 b	0,1270
Mean	0,3813±0,1662 P<0,01		0,6111±0,2209 P<0,01		0,9940±0,3754 P<0,01	

*Significant at $P < 0,01$ probability level at Tukey Test

Table 4: Dry root, dry shoot, and dry seedling weight of *Pisum sativum* under salt stress

Salt Treatment (NaCl)	Dry Root Weight*		Dry Shoot Weight*		Dry Seedling Weight*	
	(g/plant)	±S.D.	(g/plant)	±S.D.	(g/plant)	±S.D.
0 g/L	0,1185	0,0824	0,1293	0,0870	0,2478	0,1690
0,5 g/L	0,1943	0,0823	0,2317	0,0802	0,4250	0,1546
1 g/L	0,1546	0,6101	0,2272	0,0584	0,3818	0,1189
2 g/L	0,1992	0,1316	0,2462	0,1343	0,4455	0,2628
4 g/L	0,1856	0,0882	0,2485	0,1128	0,4341	0,2007
8 g/L	0,0163	0,0101	0,0375	0,0286	0,0538	0,0387
Mean	0,1448±0,0964 P<0,14		0,1871±0,1107 P<0,07		0,3313±0,2046 P<0,09	

*No significant at least $P < 0,05$ probability level at ANOVA

Table 5: Root/Shoot Ratios of *Pisum sativum* under Salt Treatments

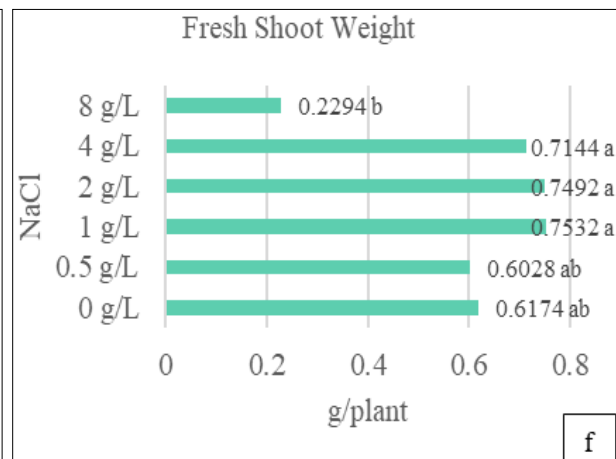
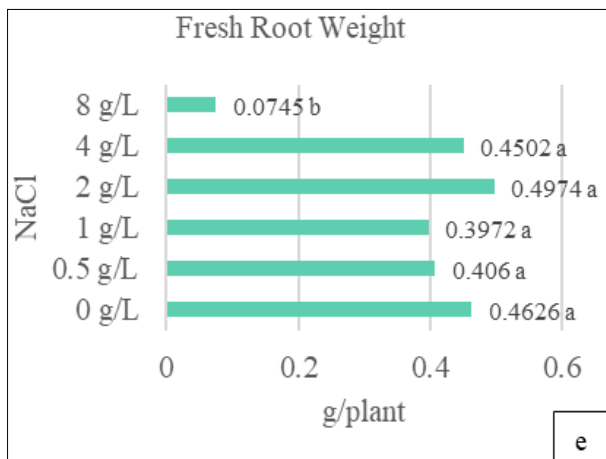
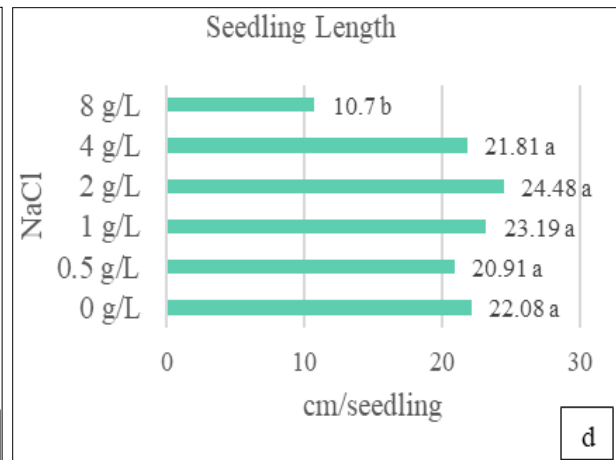
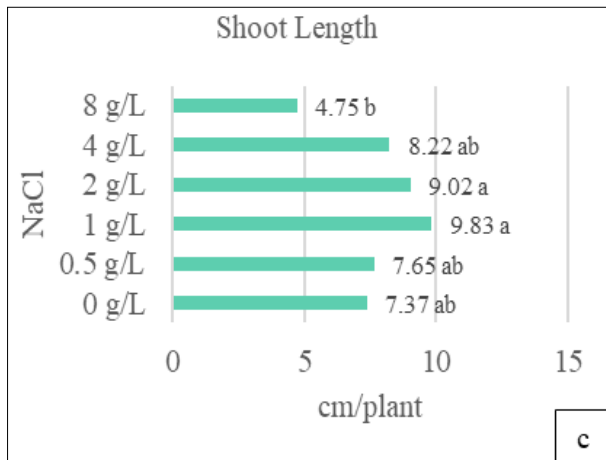
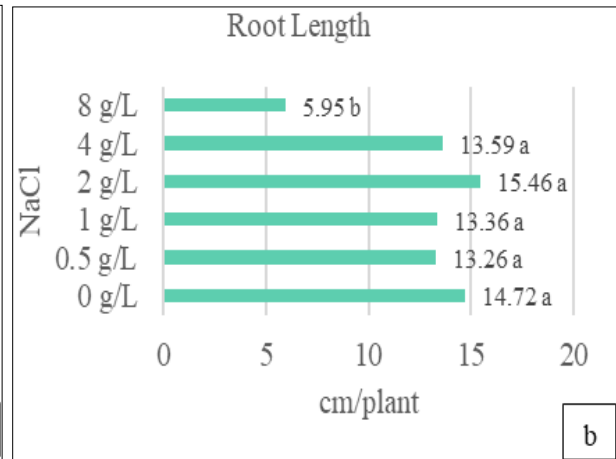
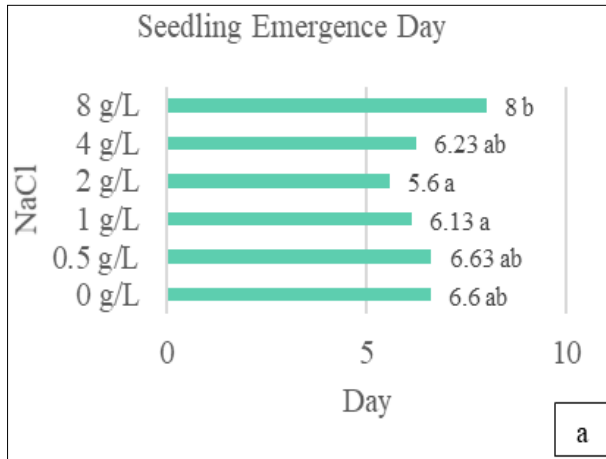
Salt Treatment (NaCl)	Root/Shoot Ratio*		Root/Shoot Ratio**		Root/Shoot Ratio***	
	(f.w./f.w.)	±S.D.	(l./l.)	±S.D.	(d.w./d.w.)	±S.D.
0 g/L	0,7361 a	0,0996	2,01 a	0,35	0,8860	0,1504
0,5 g/L	0,6982 a	0,2447	1,74 ab	0,01	0,8292	0,1882
1 g/L	0,5263 ab	0,0483	1,36 b	0,05	0,6624	0,1250
2 g/L	0,6826 a	0,0852	1,74 ab	0,15	0,7922	0,2295
4 g/L	0,6354 ab	0,0711	1,66 ab	0,07	0,7423	0,0529
8 g/L	0,2921 b	0,1594	1,27 b	0,27	0,5473	0,1494
Mean	0,5951±0,1923 <i>P</i> <0,05		1,63±0,30 <i>P</i> <0,01		0,7432±0,1759 <i>P</i> <0,1880	

*Significant at *P*<0,05 probability level, **Significant at *P*<0,01 probability level at Tukey Test ***No significant at least *P*<0,05 probability level at ANOVA

Table 6: Disease-like symptom Ratios of *Pisum sativum* under Salt Treatments

Salt Treatment (NaCl)	Disease-like Symptom Ratios *	
	(%)	±S.D.
0 g/L	79,2	14,4
0,5 g/L	58,3	14,4
1 g/L	62,5	12,5
2 g/L	62,5	21,7
4 g/L	66,7	14,4
8 g/L	87,5	0,0
Mean	69,44±16,2 <i>P</i> <0,1	

*No significant at least *P*<0,05 probability level at ANOVA



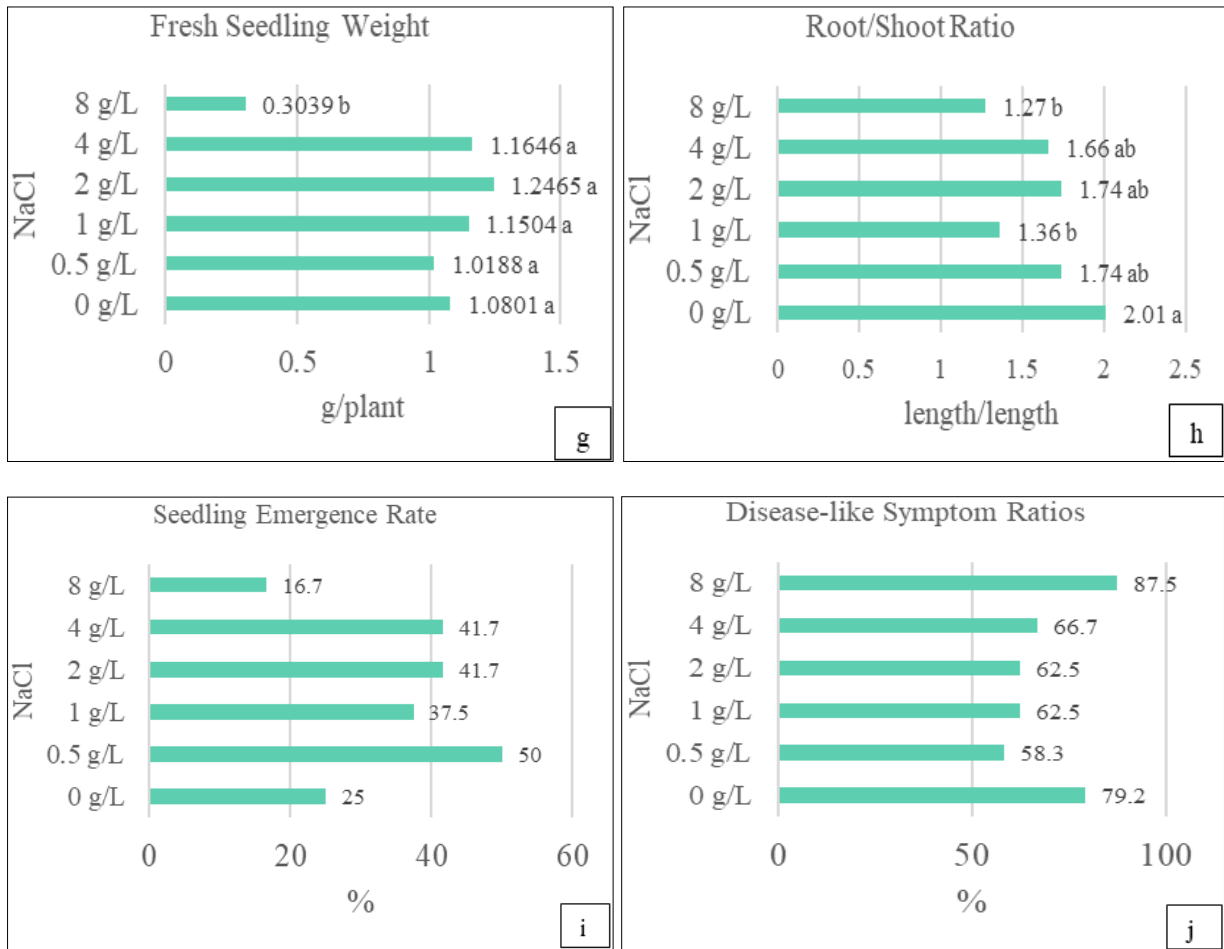


Fig 2: Seedling of *Pisum sativum* growth and health parameters under salt stress; a) Seedling emergence day, b) Root length, c) Shoot length, d) Seedling length, e) Fresh root weight, f) Fresh shoot weight, g) Fresh seedling weight, h) Root/Shoot ratio, i) Seedling emergence rate, j) Disease-like symptom ratios

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