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Investigating the Effects of Varying Concentrations of Salinity on the Growth of Phaseolus Vulgaris

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Introduction and background information

At an initial glance, the water availability might not seem like an issue, but only a fraction of water is usable for regular agriculture. Only around 2.5% of water on earth is freshwater and most of this freshwater is stored in Glaciers and Icecaps, which decreases its accessibility. Around 30.1% of freshwater is found in underground aquifers and reservoirs. Only 1.3% of freshwater comes from sources such as lakes, rivers, and ice and snow. This can be seen in Image 1.

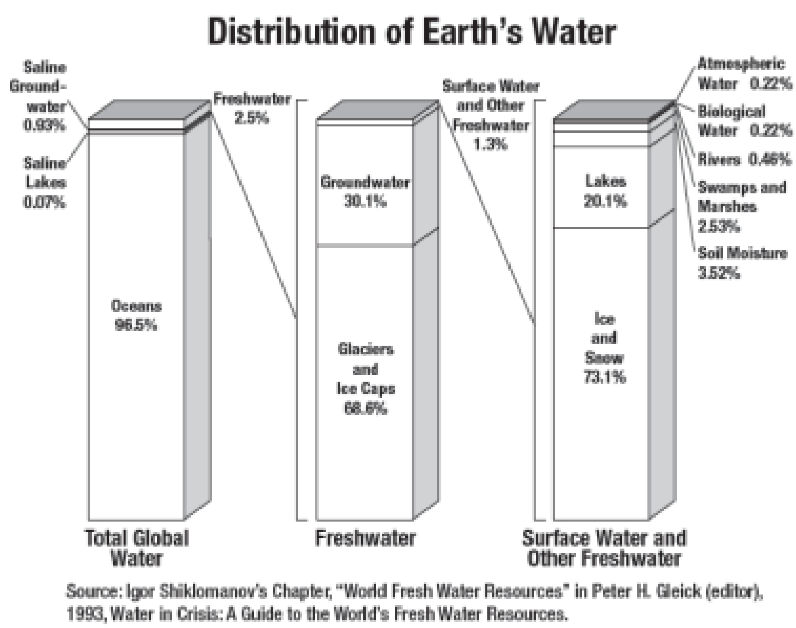

Figure 1: *shows the availability of freshwater and saltwater from various sources on earth.*

Globally, the agricultural industry consumes 70% of total freshwater available[1]. In India, 91% of available freshwater is used by the agricultural industry (Benjamin Kayatz et.al 2019). This overreliance on freshwater for irrigation is an unsustainable model for the future as the freshwater from rivers are being polluted, underground aquifers are being depleted and it is harder than ever to get access to freshwater.

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[1] https://www.worldbank.org/en/topic/water-in-agriculture#1

In India, the scarcity of freshwater is very severe. Part of this shortage is caused by the disproportionate distribution of resources to a large population, as 16% of the world's population lives in India, but it only has 4% of the world's freshwater resources[2]. This not only affects the general population of the country in a negative way, as 200,000 people die every year due to the lack of easy access to potable water[3]. The scarcity of fresh water also heavily holds back the agricultural industry on which roughly 50% of the population depends.

Not only has the water crisis had a wide impact on the farmers but it has also driven a lot of them to commit suicide as 7.4 percent of all suicides in India are committed by farmers[4]. This issue has escalated in recent years, as in Punjab 3-4 farmer suicides were reported every day in the local news in 2020. This seemingly one-dimensional problem has had devastating effects on thousands of farmers and their families. This grim situation made me wonder what could be a solution for this. I know that the majority of the water available in India is not freshwater but has some level of saline contamination. If we can use slightly saline water that can be tolerated by the plants then the dependency on freshwater will be reduced. Thus I was driven to pursue salinity studies for my extended essay.

Another reason for pursuing this study was the importance and relevance of this issue. This is an issue that is worth looking into, because it aims to aid the agricultural sector which makes up 18 percent of India's GDP[5] and 58 percent of the nation's population depends on the agriculture sector for their livelihood.

The salt used in this experiment is Sodium Chloride salt. This salt was specifically chosen for several reasons. Firstly, according to India's Central groundwater board, Sodium chloride is one of the most common contaminants in underground water reservoirs and aquifers[6]. Secondly, I come from and reside in the coastal regions of India, where the soil is naturally rich in sodium chloride, as studied by Arulmathi and Porkodi(2020).

Phaseolus vulgaris was specifically chosen to be studied for several reasons. Firstly, Phaseolus vulgaris is rich in protein and potassium, this is why it is consumed as a reliable source of protein by the vegetarian population here in India, which makes up 23% to 37% of the entire nation's population[7]. Secondly, Phaseolus vulgaris is widely consumed in my locale. While Phaseolus vulgaris is most widely consumed in Northern India, a lot of North Indians reside in my locale, hence it has always had a presence in my life and that is why I have decided to choose it as the plant for this experiment.

The review of the literature shows instances where low concentrations of NaCl promote plant growth instead of hindering it (Abdul Quados, (2010)), however contrasting results reveal that all concentrations of salt inhibit the growth. In light of the contrasting results, I wanted to confirm the level of salinity that can be tolerated by Phaseolus vulgaris. Hence, I formulated a research question.

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2 https://www.businessinsider.in/international/news/india-has-a-farmer-suicide-epidemic-and-farmers-are-protesting-new-laws-they-fear-will-make-things-worse/articleshow/80159707.cms

3 https://m.economictimes.com/news/economy/agriculture/rising-water-crisis-forces-indian-farmers-to-rethink-their-crops-selection/articleshow/77098970.cms

4 https://economictimes.indiatimes.com/news/politics-and-nation/ncrb-data-shows-42480-farmers-and-daily-wagers-committed-suicide-in-2019/articleshow/77877613.cms

5 https://www.ibef.org/industry/agriculture-india.aspx

6 http://cgwb.gov.in/wqoverview.html

7 https://www.bbc.com/news/world-asia-india-43581122

Research question

How do different concentrations of NaCl (0, 0.05, 0.10, 0.15, 0.20, 0.25 mol dm^-3) affect the growth of Phaseolus vulgaris seedlings by measuring shoot length, number of leaves, leaf surface area, and mass of plants grown in pots?

Hypothesis

The Shoot Length, Number of leaves, Surface area of the leaves, wet mass of the leaves and the dry mass of the leaves will decrease as the concentration of the salt solution given to the plants increases.

Background

Plants tend to vary in their levels of saline tolerance. The review of literature shows that much work has been done in the field of studying the effect of salt on the growth of plants. Salinity tolerance is a complex phenomenon. It results from different physiological interactions. There are many scientific reports indicating a relationship between an increase in salt concentration and decrease in plant length(Rui et al., 2009, Memon et al., 2010). Several researchers have investigated the effect of various levels of NaCl on leaf surface area and indicated that the leaf surface decreases with increase in NaCl concentration(Yilmaz and Kina, 2008, Rui et al., 2009). The Leaf number also reduces when there is an increase in the NaCl levels.(Gama et al., 2007, Ha et al., 2008)

Much research has already been done on the topic of the influence of salinity specifically in leguminous plants. Amira M.S.Abdul Qados(2011) studied the effect of salt stress on plant growth and metabolism in the Bean plant using the pot method. He reported that NaCl was responsible for an increase in the height of the plant at lower and medium levels. However, there was a decrease in the plant height at the highest level. The NaCl had little to no impact on the leaf surface area and the number of leaves at lower concentrations, whereas there was a substantial decrease in the two higher concentrations. Research done on Faba bean plants show that elevated salinity levels can cause a decrease in the growth of plants, the number of internodes and the dry and wet weight of the leaves(Fatma Bulut and Şener Akıncı,2010). A study conducted by Mena et al. (2015) on Phaseolus vulgaris reported a decrease in growth in the presence of salt similar to predictions made in this investigation.

Review of literature

As stated before, The review of literature shows several instances where low concentrations of NaCl promote plant growth instead of inhibiting it, one such study is the study by Abdul Quados, (2010), which studied the effects of 0, 60, 120 and 240 mM. It was observed that the average wet and dry mass of the plants increased when treated with 60 and 120 mM solutions. It was also observed that as the salt concentration of the treatment increased, so did the protein content of the bean. A drop in the levels of carotenoids and both chlorophyll A and B was also recorded. The higher the concentration of the salt treatment, the lower the amount of Chlorophyll A and B and Carotenoids found in the beans. However, contrasting results reveal that all concentrations of salt inhibit the growth. In light of contrasting results,I wanted to confirm the level of salinity that can be tolerated by Phaseolus vulgaris.

Materials

- The materials required for the experiment will be as follows:
- 30 plastic pots
- 15 kg soil
- Measuring Tape
- 1 digital weighing scale(1g - 5000g)
- 1 measuring cylinder (250 ml)

The Variables

Independent Variable

Independent Variable	The different values taken Purpose
Salt concentration	0.00, 0.05, 0.10, 0.15, 0.20 This independent variable and 0.25 mol dm^-3 will allow us to observe the variations in plant growth caused by salinity levels.

Dependent Variable

Dependent Variables How it is being measured
Shoot Length This parameter can be measured by measuring the length of the plant's shoot using a measuring tape.
Number of leaves This can be measured by manually counting the number of leaves on the plants.
leaf surface area A rough estimate for this value can be obtained by measuring the leaves' length and width and multiplying it.
Wet mass The wet mass of the plants can be measured by uprooting the plants and then using a digital weighing scale to check the samples' weight
Dry mass The Dry mass of the plants can be measured by uprooting samples of the plants, cutting off the roots and then packing them in envelopes and dehydrating it for 2 days. Then it can be measured using a very sensitive and precise measuring scale.

Control variables

Control Variable	The reason for why it is How is it being controlled being controlled
Type of Soil	Different types of soil can he same type of soil can have varying textures, be ordered from the mineral salt contents and supplier. The specific type varying levels of water of soil used in this retention which could have experiment is Red soil. An impact on the growth rate of the plants
Mass of soil in each pot	The mass soil could impact. The amount of soil added the growth rate of the soil. to each pot can be measured and the same amount of soil can be added to each pot. 450 grams of soil was added to each of the pots.
Number of seeds in each Pot	If a different number of The same number of seeds are sown in the pots seeds have to be sown in then the resources of the each of the pots. Five pots need to be distributed seeds were sown in each between each of the of the pots. plants, this could affect the growth rate.

Dimension of the pot	The dimension of the pot Use pots of the same could affect the packing dimensions. Every pot and density of the soil and used experiment the water retention. This has a base radius of 4.5 could also affect the cm and top radius of 7.0 growth rate. cm and a height of 12 cm.
Volume of water	The volume of water added, While preparing the salt to each of the pots will also solution, make sure that have an impact on the volume of solution growth of the plants added to every pot is the same. 100 ml of solution was added to each pot in this experiment.

Safety and risk assessment

- Gloves were worn while handling the soil.
- Every pot and row was labeled in order to avoid any confusion.
- This experiment does not have any ethical concerns.
- The experiment was set up and conducted in my house Balcony due to the COVID Pandemic health safety regulations.
- The plant matter was biodegradable, so it could be safely disposed of.

Method

Homogenous, healthy Phaseolus vulgaris seeds were obtained. 5 seeds were sown in each plastic. All the plastic pots had the same dimensions. They have a top radius of 7 cm, a bottom radius of 4.5 cm and a height of 12 cm. The big rocks and lumps were removed from the garden soil and each of the pots was filled with 450 grams of soil. The seeds were sown roughly 5 cm deep. Five replicates were set up for each of the treatments and the control group.

Five different sodium concentrations(0.05, 0.10, 0.15, 0.20 and 0.25 mol dm^-3) were used in the treatments and a control group was also set up. Similar ranges of NaCl concentration were used by the researchers in their studies (Amira M.S.Abdul Qados, 2011) (Fatma Bulut and Şener Akıncı,2010). The pots were irrigated every day with tap water for the first week. After that, the homogenous plants were divided into 6 groups and arranged in lines with each line consisting of five replicates. After the initial one week growth period, the plants were irrigated with 100 ml of sodium chloride solution,

every day for 12 more days. This experiment spanned across a total of 19 days (including the initial 7 day growth period). The growth measurements for the plants were first taken after the one week initial growth period, and once every three days after that day. These growth measurements include the number of leaves, leaf surface area and shoot length. The Wet mass of the plants was recorded on the final day of the experiment. After that the plant samples were kept in an oven for 48 hours for drying. Then the dry mass of the plant samples were also recorded and measured.

Experimental Setup

Figure 2: *Image of the experimental setup showing the five replicates for the 0.05, 0.10, 0.15, 0.20 and 0.25 mol dm^-3.*

Figure 3: *Image of the experimental setup showing the control group and the five replicates.*

Raw Data

Table 1: Raw data for growth shoot length of plant(cm) for 0.00 mol dm^-3 NaCl

Day	Length of shoot (± 1.00 cm)		
	T1	T2	T3 T4 T5
7	30.00	33.00	30.00 32.00 34.00
10	35.00	37.00	34.00 34.00 35.00
13	39.00	44.00	43.00 43.00 44.00
16	46.00	47.00	49.00 46.00 45.00

19	47.00	48.00	49.00 48.00 46.00

*T1 = Trial 1, T2 = Trial 2, T3 = Trial 3, T4 = Trial 4, T5 = Trial 5

Table 2: Raw data for number of leaves for 0.00 mol dm^-3 NaCl

Day	Number of leaves (± 1.00)		
	T1	T2	T3 T4 T5
7	2.00	2.00	2.00 2.00 2.00
10	2.00	2.00	2.00 2.00 2.00
13	5.00	5.00	4.00 5.00 3.00
16	5.00	5.00	5.00 5.00 3.00
19	7.00	5.00	5.00 6.00 5.00

Table 3: Raw data for increase of Leaf surface area for 0.00 mol dm^-3 NaCl

Day	Leaf Surface Area(±1.00 cm^2)		
	T1	T2	T3 T4 T5
10	33.80	27.04	24.24 24.00 26.23
13	34.12	28.91	26.67 26.88 28.91
16	35.00	29.73	28.20 27.29 29.08
19	35.89	31.02	28.80 28.39 30.21

Table 4: Raw data for growth shoot length of plant(cm) for 0.05 mol dm^-3 NaCl

Day	Length of shoot (± 1.00 cm)		
	T1	T2	T3 T4 T5
7	30.00	32.00	32.00 34.00 33.00
10	33.00	34.00	33.00 36.00 34.00
13	37.00	35.00	37.00 40.00 40.00
16	39.00	36.00	44.00 44.00 42.00
19	42.00	38.00	46.00 45.00 44.00

Table 5: Raw data for number of leaves for 0.05 mol dm^-3 NaCl

Day	Number of leaves(± 1.00)		
	T1	T2	T3 T4 T5
7	2.00	2.00	2.00 2.00 2.00
10	2.00	2.00	2.00 2.00 2.00
13	5.00	3.00	5.00 4.00 3.00
16	5.00	4.00	5.00 4.00 3.00
19	6.00	6.00	5.00 5.00 4.00

Table 6: Raw data for increase of Leaf surface area for 0.05 mol dm^-3 NaCl

Day	Leaf Surface Area (±1.00 cm^2)		
	T1	T2	T3 T4 T5
10	24.80	23.40	25.20 24.44 20.50
13	27.82	24.40	27.19 26.23 22.66
16	28.94	26.37	28.50 28.80 24.40
19	30.08	28.32	29.73 30.12 26.00

Table 7: Raw data for growth shoot length of plant(cm) for 0.10 mol dm^-3 NaCl

Day	Length of shoot (± 1.00 cm)		
	T1	T2	T3 T4 T5
7	30.00	26.00	27.00 30.00 30.00
10	32.00	29.00	31.00 31.00 31.00
13	35.00	36.00	35.00 32.00 35.00
16	37.00	38.00	36.00 37.00 37.00
19	37.00	39.00	37.00 37.00 38.00

Table 8: Raw data for number of leaves for 0.10 mol dm^-3

Day	Number of leaves(± 1.00)		
	T1	T2	T3 T4 T5
7	2.00	2.00	2.00 2.00 2.00
10	2.00	2.00	2.00 2.00 2.00
13	5.00	5.00	5.00 5.00 5.00
16	5.00	5.00	5.00 5.00 5.00
19	5.00	5.00	5.00 5.00 5.00

Table 9: Raw data for increase of Leaf surface area for 0.10 mol dm^-3

Day	Leaf Surface Area(±1.00 cm^2)		
	T1	T2	T3 T4 T5
10	21.84	27.60	26.51 21.32 22.40
13	24.32	28.97	27.22 23.45 23.53
16	26.34	29.81	28.98 24.65 24.64
19	27.43	30.32	30.29 26.03 25.94

Table 10: Raw data for growth shoot length of plant(cm) for 0.15 mol dm^-3

Day	Length of shoot (± 1.00 cm)		
	T1	T2	T3 T4 T5
7	29.00	30.00	28.00 28.00 30.00
10	32.00	31.00	29.00 31.00 31.00
13	34.00	33.00	30.00 33.00 34.00
16	35.00	33.00	32.00 34.00 35.00
19	36.00	34.00	33.00 35.00 35.00

Table 11: Raw data for number of leaves for 0.15 mol dm^-3 NaCl

Day	Number of leaves(± 1.00)		
	T1	T2	T3 T4 T5
7	2.00	2.00	2.00 2.00 2.00
10	2.00	3.00	2.00 2.00 2.00
13	5.00	5.00	5.00 5.00 3.00
16	5.00	5.00	5.00 5.00 3.00
19	5.00	5.00	5.00 5.00 4.00

Table 12: Raw data for increase of Leaf surface area for 0.15 mol dm^-3 NaCl

Day	Leaf Surface Area(±1.00 cm^2)		
	T1	T2	T3 T4 T5
10	19.60	18.40	22.95 20.34 23.65
13	22.54	21.89	23.92 22.04 25.90
16	23.48	23.10	25.87 23.08 27.36
19	24.93	24.03	26.38 24.66 29.84

Table 13: Raw data for growth shoot length of plant(cm) for 0.20 mol dm^-3 NaCl

Day	Length of shoot (± 1.00 cm)		
	T1	T2	T3 T4 T5
7	27.00	27.00	29.00 27.00 27.00
10	29.00	30.00	30.00 29.00 30.00
13	30.00	31.00	31.00 31.00 33.00
16	32.00	33.00	32.00 33.00 34.00
19	33.00	34.00	33.00 33.00 35.00

Table 14: Raw data for number of leaves for 0.20 mol dm^-3 NaCl

Day	Number of leaves(± 1.00)		
	T1	T2	T3 T4 T5
7	2.00	2.00	2.00 2.00 2.00
10	2.00	2.00	2.00 2.00 2.00
13	2.00	2.00	2.00 2.00 3.00
16	3.00	5.00	2.00 5.00 3.00
19	3.00	5.00	5.00 5.00 5.00

Table 15: Raw data for increase of Leaf surface area for 0.20 mol dm^-3 NaCl

Day	Leaf Surface Area(±1.00 cm^2)		
	T1	T2	T3 T4 T5
10	22.78	23.37	17.20 22.89 18.92
13	23.68	25.73	18.03 25.03 23.01
16	25.74	27.64	21.16 26.44 24.50
19	27.03	28.23	21.73 27.45 25.00

Table 16: Raw data for growth of plant(cm) for 0.25 mol dm^-3 NaCl

Day	Length of shoot (± 1.00 cm)		
	T1	T2	T3 T4 T5
7	27.00	27.00	28.00 31.00 28.00
10	28.00	29.00	29.00 33.00 29.00
13	31.00	29.00	30.00 33.00 29.00
16	32.00	30.00	31.00 34.00 30.00
19	34.00	31.00	32.00 35.00 31.00

Table 17: Raw data for number of leaves for 0.25 mol dm^-3 NaCl

Day	Number of leaves (± 1.00)		
	T1	T2	T3 T4 T5
7	2.00	2.00	2.00 2.00 2.00
10	2.00	2.00	2.00 2.00 2.00
13	3.00	2.00	2.00 2.00 2.00
16	3.00	2.00	3.00 3.00 2.00
19	5.00	4.00	5.00 3.00 3.00

Table 18: Raw data for increase of Leaf surface area for 0.25 mol dm^-3 NaCl

Day	Leaf Surface Area(±1.00 cm^2)		
	T1	T2	T3 T4 T5
10	21.20	26.46	17.64 20.40 22.36
13	22.08	27.65	18.00 22.01 24.96
16	23.47	28.96	19.32 23.83 26.00
19	24.05	29.89	21.50 24.38 27.72

Table 19: Raw data for Final wet mass of the plants

	Final Wet mass for plants at different saline levels					
	Concentration of treatment(mol dm^-3)					
	0.00	0.05	0.10	0.15	0.20	0.25
Trial						
1	3.00	3.00	5.00	4.00	2.00	2.00
2	4.00	4.00	4.00	5.00	3.00	2.00

3 5.00 4.00 3.00 5.00 2.00 2.00 4 4.00 5.00 3.00 3.00 4.00 2.00 5 5.00 5.00 3.00 3.00 3.00 1.00

Table 19: Raw data for Final dry mass of the plants

	Final dry mass for plants at different saline levels					
	Concentration of treatment(mol dm^-3)					
	0.00	0.05	0.10	0.15	0.20	0.25
Trial						
1	0.17	0.21	0.17	0.15	0.08	0.05
2	0.20	0.17	0.17	0.14	0.07	0.06
3	0.24	0.25	0.19	0.15	0.07	0.07
4	0.24	0.20	0.17	0.13	0.09	0.07
5	0.26	0.23	0.11	0.14	0.11	0.08

Processed Data

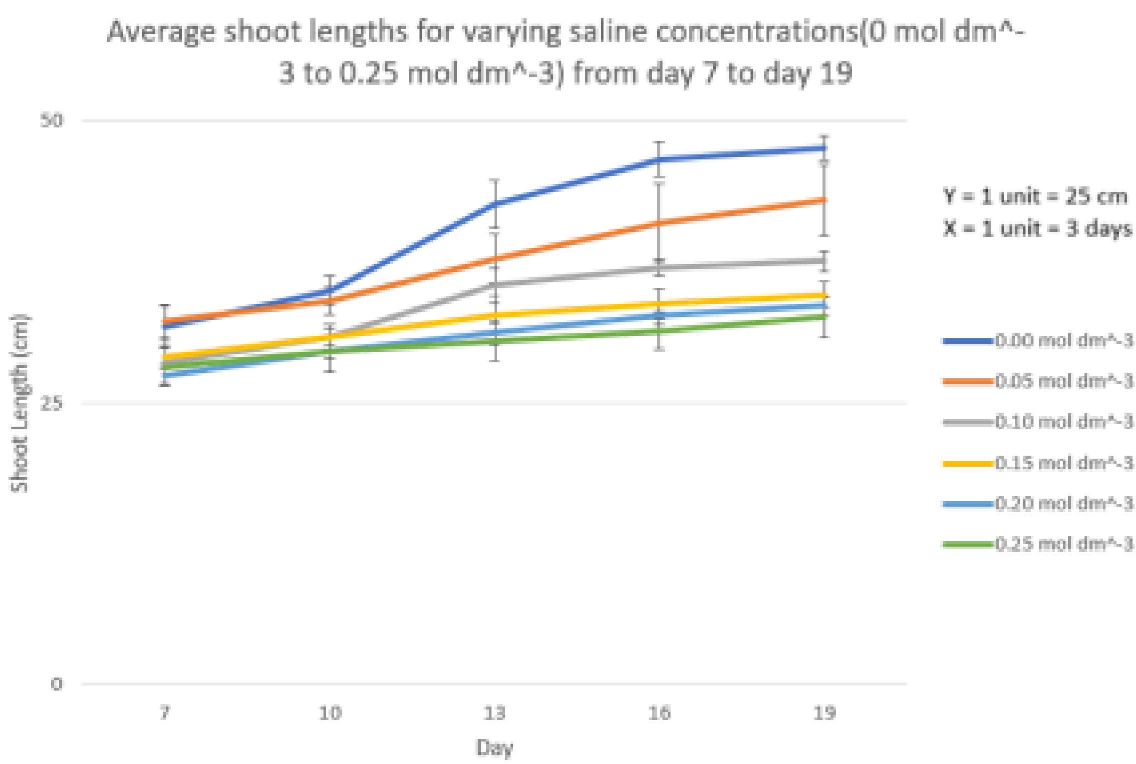
Table 19: Average Plant growth(cm) for different salinity levels(mol dm^-3)

Average Shoot Length (± 1 cm) for different saline levels(0 - 0.25mol dm^-3)								
	0.00		0.05		0.10 0.15 0.20		0.25	
Day	L	SD	L	SD	L	SD L SD L SD	L	SD
7	31.80	1.79	32.20	1.48	28.60	1.95 29.00 1.00 27.40 0.89	28.20	1.64
10	35.00	1.22	34.00	1.22	30.80	1.10 30.80 1.10 29.60 0.55	29.60	1.95
13	42.60	2.07	37.80	2.17	35.40	1.52 32.80 1.64 31.20 1.10	30.40	1.67
16	46.60	1.52	41.00	3.46	37.00	0.71 33.80 1.30 32.80 0.84	31.40	1.67

19 47.60 1.14 43.00 3.16 37.60 0.89 34.60 1.14 33.60 0.89 32.60 1.82

*SD means standard deviation

L means Length

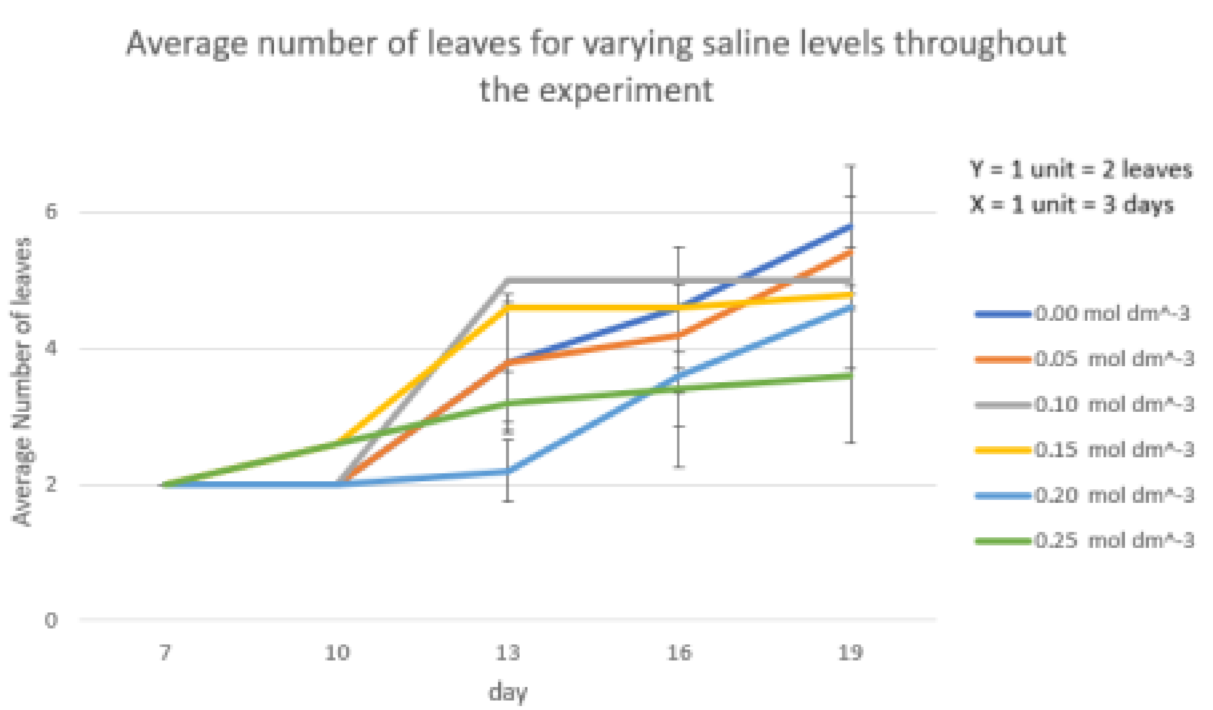


Graph 1: *Average Shoot length(cm) for different salinity levels(mol dm^-3)*

Table 20: Average number of leaves for different salinity levels(mol dm^-3)

Average number of leaves(± 1) for different saline levels(mol dm^-3)							
	0.00		0.05		0.10 0.15 0.20	0.25	
Day	L	SD	L	SD	L SD L SD L SD	L	SD
7	2.00	0.00	2.00	0.00	2.00 0.00 2.00 0.00 2.00 0.00	2.00	0.00
10	2.00	0.00	2.00	0.00	2.00 0.00 2.60 0.45 2.00 0.00	2.60	0.00
13	3.80	0.89	3.80	1.00	5.00 0.00 4.60 0.89 2.20 0.45	3.20	0.45
16	4.60	0.89	4.20	0.84	5.00 0.00 4.60 0.89 3.60 1.34	3.40	0.55
19	5.80	0.89	5.40	0.84	5.00 0.00 4.80 0.45 4.60 0.89	3.60	1.00

*SD means standard deviation, L means Length

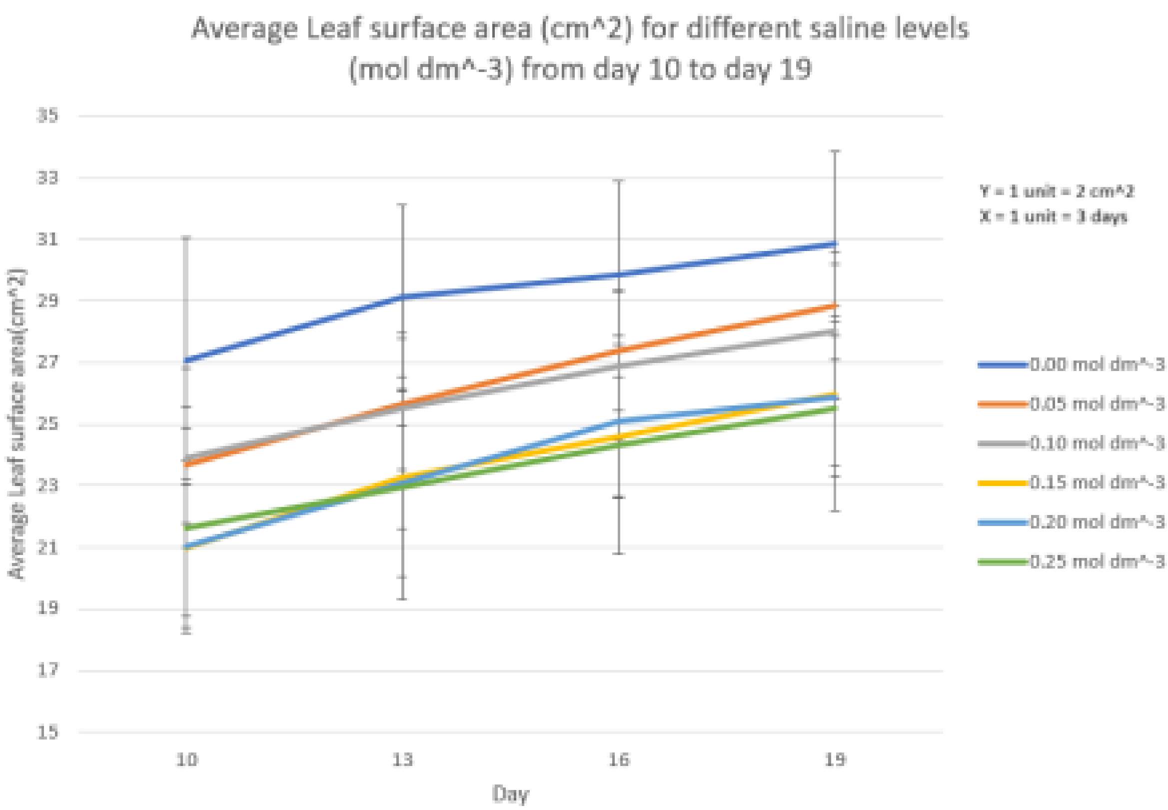


Graph 2: *Average number of leaves for different salinity levels(mol dm^-3)*

Table 21: Average leaf surface area(cm^2) for different salinity levels(mol dm^-3)

Average Leaf surface area (± 1 cm^2) for different saline levels(mol dm^-3)									
	0.00		0.05		0.10	0.15	0.20 0.25		
Day	L	SD	L	SD	L SD	L SD	L	SD L	SD
10	27.06	3.98	23.67	1.89	23.93 2.90	20.99 2.23	21.03	2.79 21.61	3.22
13	29.10	3.00	25.66	2.12	25.50 2.47	23.26 1.68	23.10	3.03 22.94	3.61
16	29.86	3.02	27.40	1.97	26.88 2.41	24.58 1.94	25.10	2.48 24.32	3.55
19	30.86	3.00	28.85	1.75	28.00 2.18	25.97 2.33	25.89	2.61 25.51	3.30

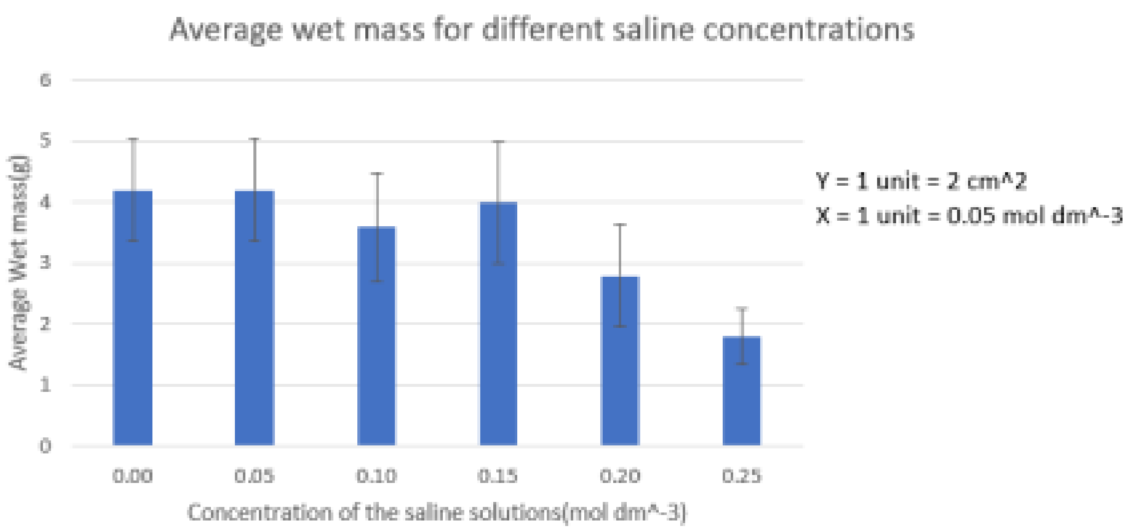
*SD means standard deviation, L means Length



Graph 3: *Average leaf surface area(cm^2) for different salinity levels(mol dm^-3)*

Table 22: Average Wet mass(g) for different salinity levels(mol dm^-3)

	Average wet mass for plants at different saline levels(± 1 g)				
	Concentration of treatment (mol dm^-3)				
	0.00	0.05 0.10	0.15	0.20	0.25
Average final wet mass fo Saline level(g)	4.20	4.20 3.60	4.00	2.80	1.80
Standard Deviation	0.84	0.84 0.89	1.00	0.84	0.45

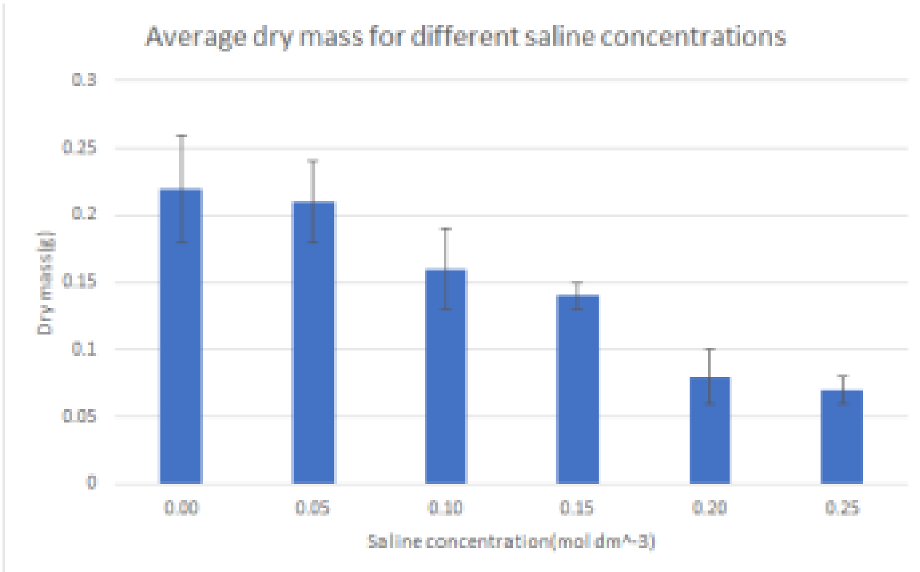


Graph 4: *Average wet mass(g) for different salinity levels(mol dm^-3)*

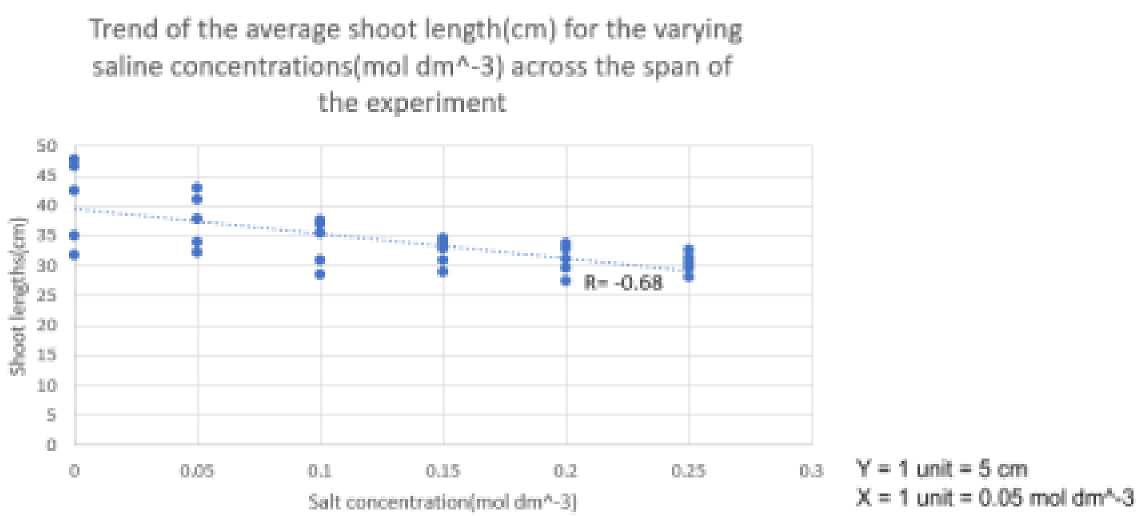
Table 23: Final Dry mass(g) for different salinity levels(mol dm^-3)

	Final Dry mass for plants at different saline level		
	0.00	0.05 0.10 0.15 0.20	0.25
Average final wet mass for each level(g)	0.22	0.21 0.16 0.14 0.08	0.07

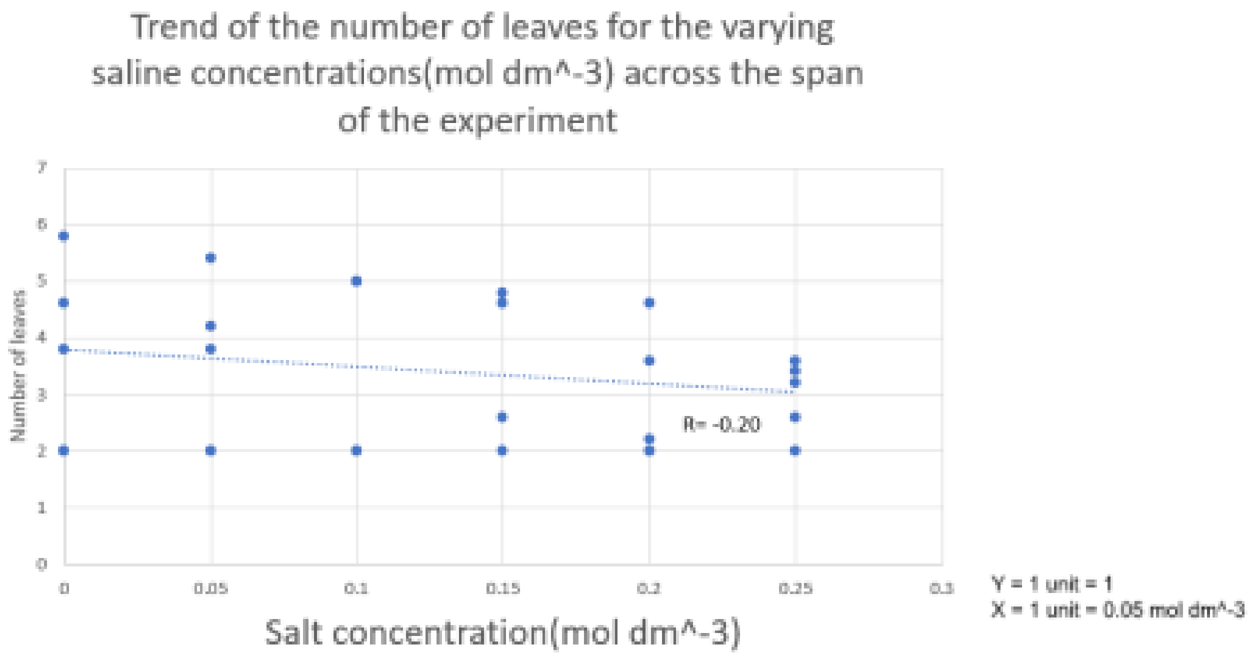
Standard Deviation	0.04	0.03 0.03 0.01 0.02	0.01



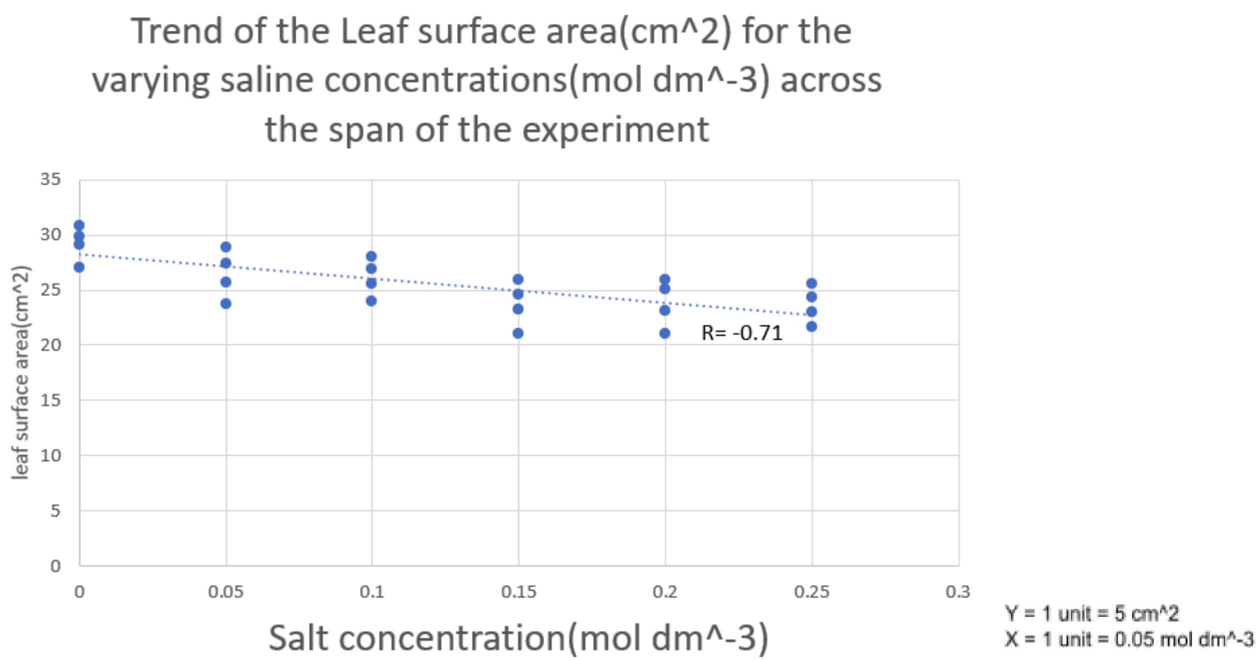
***Graph 5:** Average dry mass(g) for different salinity levels(mol dm^-3)*



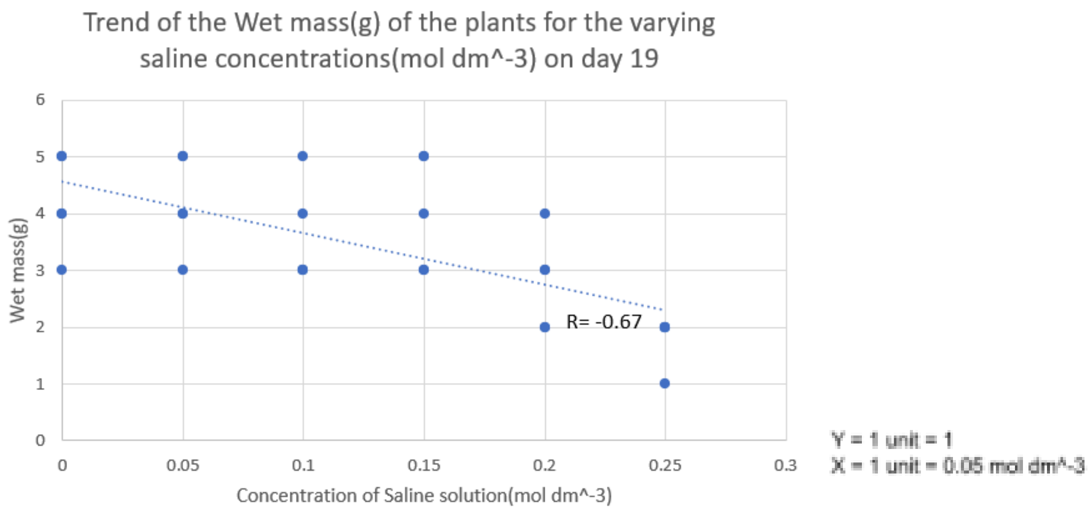
***Graph 6:** Relationship between growth in terms of shoot length(cm) and different salinity levels(mol dm^-3)*



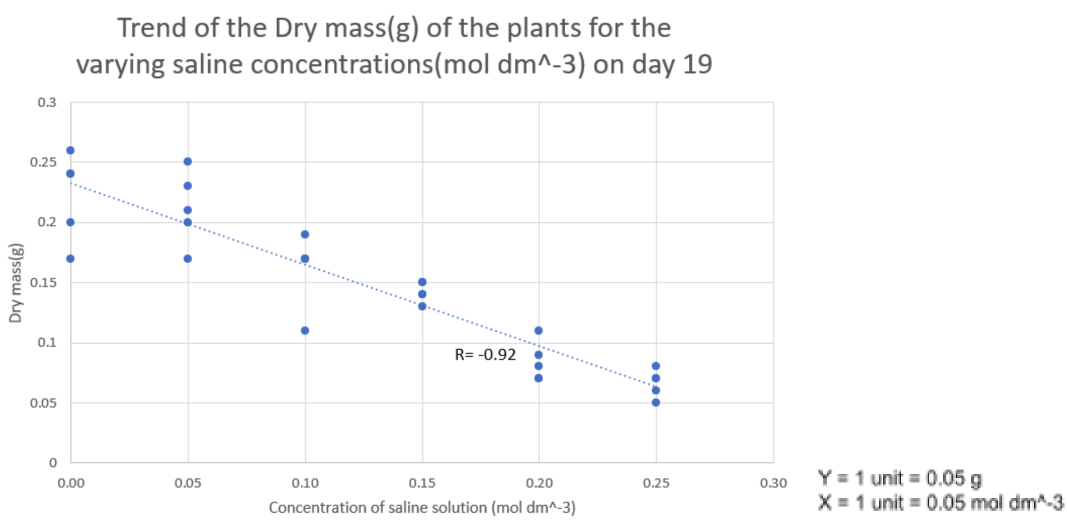
Graph 7: *Trend in the number of leaves for different salinity levels(mol dm^-3)*



Graph 8: *Trend of growth of average leaf surface area(cm^2) for different salinity levels.*



Graph 9: *Trend of growth of wet mass(g) for different salinity levels(mol dm^-3)*



Graph 10: *Trend of growth of dry mass(g) for different salinity levels(mol dm^-3)*

Sample Calculations

The first type of calculation done in this experiment was averaging, as average values were calculated for every parameter. Here is an example showing the process of calculating the average value for the 0.00 mol dm^-3 NaCl on day 7.

$$average = \frac{30.00+33.00+30.00+32.00+34.00}{5}$$

$$average = \frac{159.00}{5}$$

$$average = 31.80\ cm$$

The next calculation that was done was to find the standard deviation for the average values. The formula for standard deviation is

$$\sigma = \sqrt{\frac{\Sigma(x_i - \mu)^2}{N}}$$

Where:
σis the standard deviation value
xi is the x values *i*
μ is the population mean
And *N* is the size of the population

The next calculation is the correlation coefficient between salt treatments and growth. The Formula for the correlation coefficient is:

$$r = \frac{n(\Sigma xy) - (\Sigma x)(\Sigma y)}{\sqrt{[n\Sigma x^2 - (\Sigma x)^2][n\Sigma y^2 - (\Sigma y)^2]}}$$

Here is an example calculation showing the process of calculating the correlation coefficient of the average shoot length and the saline treatments.

$$r = \frac{30(119.46) - (1028.8)(3.75)}{\sqrt{[30*36097.04 - (1028.8)^2][30*0.6875 - (3.75)^2]}}$$

$$r = -0.684$$

Analysis

Shoot length:

The shoot length was the greatest for the control(0.00 mol dm^-3) , this is a trend that can be observed throughout the experiment. The plants which were treated with the 0.05 mol dm^-3 NaCl had the longest shoot length followed by plants with higher NaCl concentration(0.10, 0.15, 0.20 and 0.25 mol dm^-3). In Graph 1 and 6 (Table 19) , it is seen that the growth in terms of plant shoot length is inversely related to increasing saline treatments (0.05-0.25 mol dm^-3). Thus, when the saline concentration increases the average shoot length decreases.

Number of leaves

The control(0.00 mol dm^-3) had the most number of leaves on average(5.80) followed by the samples that were given 0.05, 0.10, 0.15, 0.20 mol dm^-3 NaCl(5.40-4.60) treatment. The plants that were treated with the highest NaCl(0.25 mol dm^-3) solution had the lowest number of leaves(3.60) on average. This can be observed in table 20, graph 2 and graph 7. Thus, when saline concentration increases the average number of leaves decreases.

Surface area:

The average surface area of the leaves is also highest for control(0.00 mol dm^-3 NaCl) followed by plants that were given the 0.05, 0.10, 0.15 and 0.20 mol dm^-3 salt treatments. The plants subjected to the 0.25 mol dm^-3 salt treatment had the lowest average leaf surface area as compared to the other concentrations throughout the experiment. This is evident from Table no. 21, Graph no. 3 and Graph no. 8.

Wet mass

The average wet mass was the highest for the control(0.00 mol dm^-3 NaCl),which was 4.2 g, followed by the plants that received the 0.05, 0.10, 0.15 and 0.20(mol dm^-3 NaCl), which was 4.2 - 2.8g. The plant that received the highest salt treatment(0.25 mol dm^-3 NaCl) had the lowest average wet mass(1.8g) . This trend can be clearly observed in the wet mass in Table 22, Graph 4 and Graph 9.

Dry mass

The average dry mass was the highest for the control(0.00 mol dm^-3 NaCl), which was 0.04 g, followed by the plants that received the 0.05, 0.10, 0.15, 0.20 (0.00 mol dm^-3 NaCl) which was 0.03 to 0.02g. The plant that received the highest salt treatment (0.25 mol dm^-3) had the lowest average dry mass, which was 0.01g. These findings are recorded in Table no.23, Graph no.5 and Graph no.10.

Discussion

Shoot Length:

An overall decrease in shoot length was observed as the salt concentration increased (0.00, 0.05, 0.10, 0.15, 0.20, 0.25 mol dm^-3 NaCl) (Graph 1 and 6 , Table 19). The decrease in the shoot length is due to the additional stresses exerted on the plants caused by the presence of salt in the water. There are two types of stress exerted on plants, these are osmotic pressure and ionic stress. Osmotic pressure is caused when the saline concentration of the water outside the plant is higher than the salt concentration of the root cells. This inhibits the rate of water uptake by the plant, the growth of the cells (Munns and Tester, 2008).

Similar results were obtained by Mena et al. (2015).

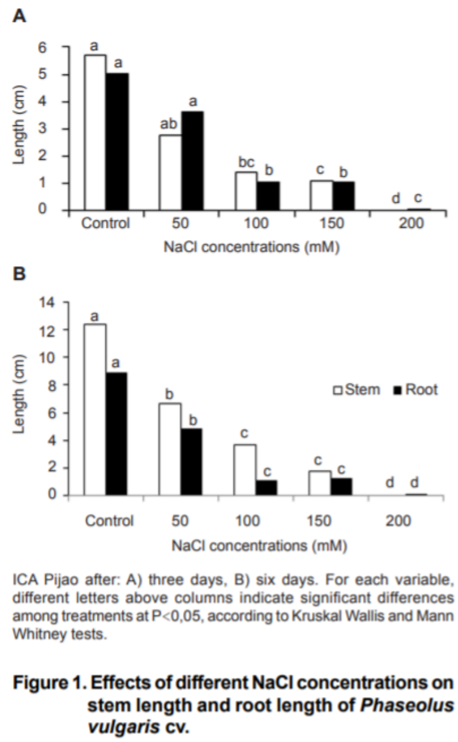


Image 4: *Results from Mena et al. (2015)*

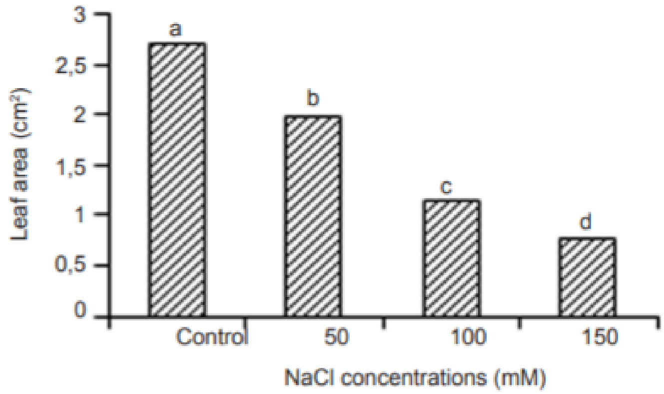
The graph indicates a decrease in length as the salt concentration increased (the range of salinity treatment studied was similar to our investigation). Contrasting results were found for Vicia faba, where the shoot length increased for lower NaCl concentrations (60 and 120 mM), however at high concentrations (240 mM) the shoot length decreased. In agreement to our findings, many scientific papers reported a decrease in shoot length as the concentration of salt increases in leguminous plants. For example Mathur et al. (2006) on moth bean (Vigna aconitifolia), Taffouo et al. (2009) on cowpea (Vigna unguiculata), and Kapoor and Srivastava, (2010) on (Vigna mungo).

Number of leaves

When plant growth was observed in terms of number of leaves, there was an overall decrease in the count as the salt concentration increased (0.00, 0.05, 0.10, 0.15, 0.20, 0.25 mol dm^-3 NaCl) (Table no. 20, Graph no.2 and Graph no.7). The reduction in the number of leaves can be attributed to the increased Na^{+}ion concentration which causes ionic stress. The ionic stress results in decreased lateral bud formation and reduced growth. Similar results were found by Abdul Quados, (2011) who reported a decrease in leaf number at higher concentrations of 120 mM and 240 mM, however, lower concentrations of 60 mM did not show a significant decrease. Similar results have also been found by Karen et al. (2002), for Cicer arietinum, Raul et al. (2003), for Phaseolus acutifolius , Vigna unguiculata , and Phaseolus filiformis.

Leaf surface area

When the leaf surface area was measured with respect to increasing salt concentrations (0.00, 0.05, 0.10, 0.15, 0.20, 0.25 mol dm^-3 NaCl), it was observed that the leaf surface area decreased (Table no. 21, Graph no.3 and Graph no.8). This decreased leaf surface area could be explained as a detrimental effect of sodium ions on photosynthesis (Netondo et al., 2004). The reduction in the leaf surface area could be an adaptive mechanism to cope with the loss of water because of the osmotic stress (Mena et al. 2015). Similar to our investigation, Mena et al. (2015) also studied the effect of salt on Phaseolus vulgaris and their findings corroborate the fact that as the salt concentration increases, the leaf surface area decreases (Image 5).



Different letters above columns indicate significant differences between treatments at P<0,05, according to Kruskal-Wallis and Mann Whitney.

Figure 2 Effects of different NaCl concentrations on leaf area of *Phaseolus vulgaris* cv. ICA Pijao after six days.

Image 5: *Results from Mena et al. (2015).*

Abdul Quados, (2010) also reported decreased leaf surface area in Vicia faba when treated with concentrations (60mM to 240mM). Similar results have also been obtained in several different plants.(Jamil et al. 2007, Zhao et al. 2007 and Yilmaz and Kina, 2008) . In this case, decrease in leaf area could be explained by the negative effect of salt on photosynthesis that leads to the reduction of plant growth and leaf growth. The lowered leaf areas per plant indicates adaptive response of P. vulgaris plants for controlling water losses under salinity conditions.

Wet mass

When the wet mass of the plants treated to the different saline treatments is compared, it is seen that the wet mass of the samples decreased(Table 22, Graph 4 and Graph 9). This can be explained by the ionic stress caused by excess Na^+, which leads to decreased photosynthesis and impaired growth of the Lateral buds. A study conducted by Abdul Quados, (2010), on Vicia Faba found contradicting results. In that study it was found that the fresh mass of the plants increased as the salt concentration also increased. They attributed this fresh weight increase to increased size of sap vacuoles to cope with salt stress. However, there are several studies that support our result of decrease in fresh mass. For example studies by Jamil et al. (2007) on Raphanus sativus , Ha et al. (2008) on Kyllinigia peruviana, Rui et al. (2009) on Bruguiera gymnorrhiza , and finally Memon et al. (2010) on Brassica campestris.

Dry mass

When Dry mass is the parameter being observed with the increase in salt concentration, it can be observed that the dry mass of the samples decreases(Table 23, Graph 5 and Graph 10). This can be explained as a result of the osmotic and ionic stress of NaCl causing reduced growth . Another study done on Vicia Faba refutes our results, as in that study, the dry mass of the samples increased as the salinity increased. However, research conducted by several different researchers on different species corroborates our findings. These include a study by Jamil et al. (2007) on Raphanus sativus , a study by Ha et al. (2008) on Kyllinigia peruviana, a study by Rui et al. (2009) on Bruguiera gymnorrhiza , and finally a study by Memon et al. (2010) on Brassica campestris.

Conclusion

This entire experiment began with the research question,' To study the effect of different concentrations of NaCl (0, 0.50, 0.10, 0.15, 0.20, 0.25 mol dm^-3) on the growth of Phaseolus vulgaris seedlings by measuring shoot length, number of leaves, surface area of leaves and change in pots'. And it is fair to say that this experiment was successful at fulfilling the objectives proposed by the research question.

Salinity has an inhibitory effect on the growth of Phaseolus vulgaris and as the salt concentration increased, the shoot length decreased. The correlation coefficient(R=-0.68) clearly indicated an inverse relationship between increasing salt concentration and decreasing shoot length. Similar result was observed in Phaseolus vulgaris by Mena et al. (2015). The leaf number decreased as salt concentration of the treatment increased with a correlation coefficient of -0.20, which is not a very strong indicator of inverse relationship between them. However, R= -0.71 was observed between leaf surface area and salt concentration which indicates a strong inverse correlation between them. Similar results were obtained by Mena et al. (2015) and Abdul Quados, (2010) and many other researchers. The fresh mass also decreased with increasing NaCl concentrations(R=-0.67). The impact of salt was very evident for the dry mass, with a R value of -0.92. Since dry mass is a more reliable measure, we can say that the impact on growth was substantial. In conclusion, it can be stated that the overall growth in Phaseolus vulgaris was impaired in the presence of even low concentrations of salt(Graph no. 1 to 9 and Table no. 19 to 23). In contrast, low concentration of NaCl increased the growth as reported by Abdul Quados, (2010).

Finally, it means that even low saline concentrations can not be used to irrigate Phaseolus vulgaris plants.

Evaluation

Hypothesis

The data obtained in this study supported the hypothesis. The results revealed that all growth parameters including shoot length, wet and dry mass, number of leaves and leaf surface area showed a decrease as the concentration of the saline treatment increased (0.05-0.25 mol dm^-3). The overall decrease in growth was a result of the osmotic and ionic stress caused by the saline treatments.

Method

The specific methodology used to conduct this experiment offers its own set of strengths and weaknesses.
strengths : The methodology of the experiment was simple as no specialized equipment was required and therefore it was conducted during the pandemic at home. The method provides a better estimate of growth as pots filled with soil are used, in comparison to any study done in petri plates to estimate growth. In this experiment the pots used had the same dimensions to obtain comparable results. The type of soil used was uniform and this reduced variation. Different saline solutions were used to irrigate the pots, however the volume of solution added to every pot was kept constant to obtain fair results.

Weaknesses

More trials could have been conducted, however five trials provided sufficient data to obtain reliable results. The results obtained for growth analysis from the pot method may vary from results obtained from field trials, however large variations may not be seen. Thus, the pot method does provide a reliable estimate of growth parameters under saline stress. Next, the methodology only studies the effect of sodium chloride on the growth of plants, but not any other types of salts abundantly found in the soil in the different regions of India like himalayan pink salt and rock salt. However, just using Sodium chloride is more relevant specifically to my locality since sodium chloride salt is more common in the coastal regions of India, hence it was not a bad choice either.

Apparatus
The apparatus used in this experiment have a certain set of strengths and weaknesses.

Strengths
All the apparatus can be used and operated from home which is important as this experiment was conducted at home.

Weaknesses
The apparatus like the weighing scale might have a limited degree of accuracy as they are not professional lab grade apparatus. However, the weighing scale used could measure mass from 1 gram to 5000 grams, which provided sufficient accuracy for the experiment.

Possible extensions
A possible extension to this experiment could be that the experiment can be conducted on a field or in farmland to obtain more accurate results. The effects of different types of salt on the growth of plants can also be studied.

Further scope of research
The experiment conducted in this essay only studies the effect that salinity has on the growth of plants, but not how it affects crop yield. A longer study could be conducted to study the effects of salinity levels on crop yields. More studies could also be conducted to compare the effects of salinity on the growth and development of varying species of glycophytic plants to see which ones are more salt tolerant and which are most salt sensitive. Biochemical chemical analysis of salt tolerant varieties could help in explaining the mechanism of salt tolerance. The pattern of sodium ion accumulation in different parts of the plant could also be investigated to explore the mechanisms.

Advanced studies for protein expression could help in identifying key proteins responsible for contributing to the attribute of salt tolerance. This study can also be conducted in a lab setting with more advanced equipment and methodology, in this case hydroponics can be used. Another possible extension to this experiment could be to study the effect of varying salt concentrations on the growth of different plant species, by using different plant species seeds, this would aid in getting a more accurate understanding and conclusion of how sodium chloride concentrations affect plant growth in different species of plants.

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