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The impact of Salt Stress on The Growth and Photosynthetic Pigments of *Vicia faba* (L).

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ABSTRACT

Water used for crop irrigation is often of insufficient quality in the Mediterranean region, where sea water invades the porous karst matrix and salinizes Sources of soil and water. Measuring the productivity of horticultural crops under saline conditions helps decide if and when crops should be irrigated if water is saline, thus balancing crop water and salt stress. To examine the effects of saline irrigation water on (*Vicia faba* L.) biomass and yield parameters, a greenhouse pot experiment was set up. NaCl salinity was applied as follows: After 15 days from irrigation, plants were subjected to graded levels of salinity: 0, 50, 100, 200 and 300 mM NaCl, applied in stepwise daily increments to avoid induction of salt shock to the seedlings. Height of Plant (cm), The number of lateral branches, number of (leaves, pods, seeds) per plant, shoot weight (g), is correlated with an increase in stressed plant photosynthetic pigments. five weeks after salinity therapy, the weight of the pod (g) and the weight of the seed (g) were determined. Increased irrigation water salinity statistically significantly decreased the calculated parameters ($P < 0.05$) relative to regulation, except for the number of branches and pods. Salinity is associated with an increase in photosynthetic pigments in stressed plants. The productivity of *Vicia faba* has decreased in proportion to the degree of salinity of irrigation water.

1 Introduction

The worldwide cultivated land of Faba bean (*Vicia faba* L.) was 3.7 million ha by 1980 and decreased to 2.1 million ha by 2014. In their significance to humans, legumes are second only to cereals (Graham & Vance, 2003). They account for 27% of the world's primary crop production, with grain legumes alone accounting for 30 % - 60 % of the nitrogen requirements for human dietary protein (Vance *et al.*, 2000). Seeds are expected to increase in line with the growing demand for high-protein, economical and nutritious foods, Particularly given the growing concern about the dangers of eating animal products. The use of faba bean in human and animal diets has health benefits because it presents a high protein

content and is a source of several nutrients including Fe, Mg, Zn, K and Ca, amino acids, carbohydrates, vitamins and essential nutraceuticals (Koivunen *et al.*, 2016).

In rotation with cereals, the addition of pulse crops such as faba beans improves soil: physical, chemical and biological properties, soil fertility, interferes with cycles of pests, diseases, and decreases the use of 63 inorganic nitrogen fertilizers through the use of The Biological Fixation of Nitrogen (BNF) (Jensen *et al.*, 2012). This capacity of fixation, ranges from 90 to 200 kg N ha⁻¹ (Herridge *et al.*, 2008; Neugschwandtner *et al.*, 2015) With a size of up to 300 kg N ha⁻¹ (Singh *et al.*, 2013). The variations in nitrogen fixation rely on the variety, agronomic practices, soil properties,

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particularly the presence of compatible symbiotic rhizobacteria in the soil (Argaw & Mnalku, 2017).

Large soluble salt concentrations in the rhizosphere cause stress on plant salts. Salt stress induces imbalances of water and nutrients as well as activates unspecific processes of oxidation in plant cells. Growing in a saline setting results in morphological, physiological, biochemical and molecular changes in plant cells that have a negative effect on crop growth and productivity. (Wang *et al.*, 2003).

Plant salt stress is thus characterized by osmotic and ionic stress, along with oxidative stress. Does high soil salinity cause signals? Can alterations in the capacity of soil water, allowing the plant to take water from the soil, causing osmotic stress, which can disrupt cellular activities and lead to cell death. Ionic stress causes changes in nutrient uptake as well as transport and partitioning within the plant, as well as changes in plant physiological processes, molecular disruption, and growth retardation are further caused by Wang *et al.*, (2008). The increased development of reactive oxygen species is characterized by oxidative stress, leading to unspecific oxidation of proteins and membrane lipids, causing DNA injuries (Schutzendubel & Polle, 2002).

Plant salt stress has been frequently studied, typically concentrating on those plant physiological aspects affected by the salinity; However, from an agronomical standpoint, research is especially important in addressing the issue of salinity's impact on crop productivity, i.e. growth, development, and yield. (Sen & Mandal, 2016). It is necessary to determine how plants react to high salinity in order to improve crop productivity under saline conditions. When studying plant salinity responses, biomass production and yield are considered useful parameters. (Radwan *et al.*, 2000). Measuring the productivity parameters of horticultural crops under saline conditions in this context is a valuable instrument for modifying the management practices of saline farming.

The purpose of this research was to study the effects of saline irrigation water on biomass and yield parameters of the faba. The focus was on determining the response of the plant with regard to the degree of salinity to which the plants were exposed. The ultimate objective was to establish an optimum agricultural management strategy for *V. faba* under saline conditions, i.e. whether and when to irrigate saline water crops, thus balancing crop water and salt stress, taking into account potential losses in yields.

2 Materials and Methods

2.1 Germination conditions

Preparing and sowing of seed

Intact seeds of *V faba*, which were homogeneous and identical in size and colour, and free from wrinkles, washed thoroughly with tap water and planted in plastic pots of 15 cm diameter, full of one kg of water-washed sand, 5 seeds per pot. Seeds were watered with 0.2 mM of CaSO₄ for 15 days. After 15 days from sowing the seedlings (about 10 cm long) were successively thinned to one per pot.

2.2 . Treatments.

Plants were subjected to graded levels of salinity after 15 days of irrigation: 0, 50, 100, 200, and 300 mM NaCl, applied in stepwise daily increments to avoid inducing salt shock in the seedlings in the following manner: On the first day, one-fifth of the pots were left untreated as a control, while the remaining four-fifths were treated with 50 mM NaCl. On the second day, one-fourth of the treated pots were kept at 50 mM NaCl, while the other three-fourths were kept at 100 mM NaCl. On the third day, one-third of the pots receiving 100 mM NaCl remained so, while the other two-thirds received 200 mM NaCl. The fourth day began with pots receiving 200 mM NaCl were divided into two halves, one half received 200 mM NaCl and the other half received 300 mM NaCl.

The experiment was set up under greenhouse conditions at the Faculty of Science, Zintan University during the period from November to December 2020. Irradiance ranged from 1500 to 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ from natural sunlight, with an average temperature of 27/20 °C in a 14/10 h light/dark cycle and a relative humidity of about 70%.

2.3 Measurement of biomass and yield parameters.

Five weeks after the NaCl salinity treatment started, *V. faba* biomass and yield parameters were measured. Shoots were separated from the roots by carefully cutting the plant above the soil. Lateral branches were counted, and the plant was then placed on a flat surface for height measurement (cm) The number of leaves was determined. Then there's the weight of the shoots (g) was measured using the technical balance. Pods were removed from plants, counted, and their weight was measured (g). After, seeds were separated from the pods, counted, and seed

weight was measured separately from the pods (g). An aliquot of fresh leaves was kept frozen at -10°C in

order to estimate of photosynthetic pigments. Dry weights were recorded after drying of fresh plant material at 80 °C for 48 h and were corrected for the leaf portion kept frozen.

2.4 Estimation of photosynthetic pigments

Photosynthetic pigments were determined according to the method described by (Wellburn & Lichtenthaler, 1984). Frozen leaf discs were macerated in 80% acetone using a cold mortar and pestle in dim light with a pinch of magnesium carbonate to neutralize the plant acids. The slurry was centrifuged and 80 percent acetone was used to dilute the clear extract and absorbance was read at 646 and 663 nm for chlorophyll (a and b) estimation and at 470 nm for carotenoids. The concentrations of chlorophyll a, chlorophyll b and carotenoids were expressed ($\mu\text{g ml}^{-1}$) using the following equations:

$$\text{Chlorophyll a} = 12.21 E_{663} - 2.81 E_{646}$$

$$\text{Chlorophyll b} = 20.13 E_{646} - 5.03 E_{663}$$

$$\text{Carotenoids} = \frac{(1000 E_{470} - 3.27 \text{Chla} - 104 \text{Chl b})}{229}$$

Statistical analysis

The results were analyzed by comparing (F) values obtained from a one-way ANOVA using the SPSS statistical package (Anderson et al., 1997). The lowest significant (LSD) between the means, at the (5%) level, were determined for various treatments, following the method of (Steel et al., 1960).

3 Results.

3.1 Effect of salt stress on plant growth.

3.1.1 Plant height.

Analysis of variance result was showed that there were significant ($p \leq 0.05$) differences on seedling height due to concentration of NaCl (Table 1). The maximum value (55.92cm) of Shoot height was recorded from *V. faba* in non-salinized plants, while the lowest value was recorded from the seedling (40.78cm) in 300 mM NaCl treated plants (Fig 1a). The findings of this study indicated that the concentration of NaCl has an effect on the growth of *V. faba*. Shoot growth consistently decreased as NaCl concentration increased. Generally, salinity might be affect *V. faba* seedling growth by disturbing transport system and metabolic processes.

3.1.2 Number of leaves and Number of pods per plant

The number of leaves and number of pods per plant in terms was sharply reduced from 51.8 and 4.8 , respectively in non-salinized plants to 28.8 and 1.8 , respectively in seeds treated with 300 mM NaCl . (Fig 1a).

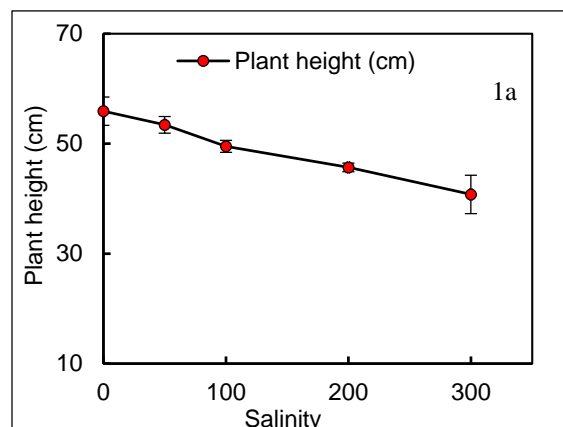


Fig 1a Plant height of *V. faba* was treated to graded levels of water stress on a sand culture, for 15 days. Each statistic represents the average of five replicates \pm SE.

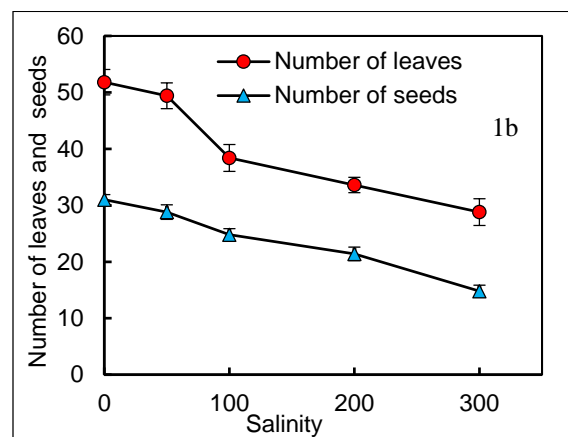


Fig 1b Number of leaves and seeds of *V. faba* subjected to graded levels of water stress on a sand culture for 15 days. Each value is the mean of 5 replicates \pm SE.

Similarly, salt concentration had a significant impact. Salinity drastically reduced the number of branches and pods per plant, dropping from (3.60-4.80) in control plants to (1.60-1.80) in plants subjected to 300 mM NaCl (Fig 1c).

3.1.3 Fresh and dry weight.

Such that fresh weight of the shoots, pods and seeds decreased by 140.9, 136.2 and 66.08 g, respectively, which is 0.7, 13.2 and 22.9%,

respectively compared to the control, with the 300mM NaCl irrigation treatment (Fig 1d).

Salt stress has a negative influence on the fresh weight and pods weight per plant of the *V. faba*, according to the findings in Table 2., which decreased from (140.9g,136.24g) to (119.4 g.,117.6 g) (Fi 1d), and the total weight of seeds decreased from (66.07g) to (37.32 g) in plants exposed to 300 mM NaCl (Fig 1e)

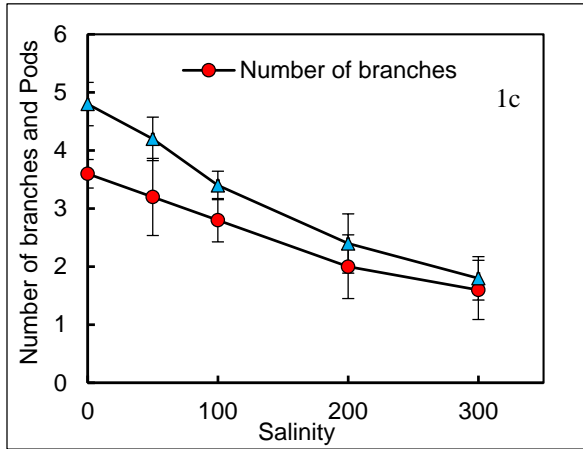


Fig. 1c Number of branches and Pods of *V. faba* subjected to graded levels of water stress on a sand culture for 15 days. Each value is the mean of 5 replicates ± SE..

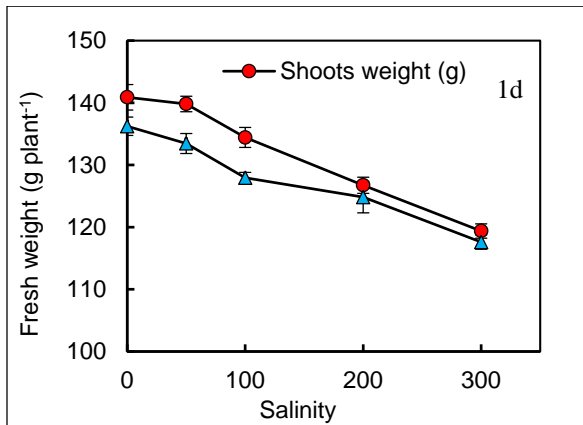


Fig. 1d Fresh weight shoots and pods of *Vicia faba* subjected to graded levels of water stress on a sand culture for 15 days. Each value is the mean of 5 replicates ± SE.

3.1.4 Effect of salt stress on chemical content

3.1.5 Photosynthetic pigments

The effect of salt stress on the chlorophyll content of the bean plants under study, including the chlorophyll 'a', 'b', and carotin ratio, is demonstrated using different concentrations of sodium chloride. The

results show that salt concentration and chl. 'a' content have an inverse relationship. Chlorophyll 'a' content decreased as concentration increased, reaching its lowest level, in terms of was reduced from 0.65 in non-salinized plants to 0.61 in plants treated with 300 mM NaCl.

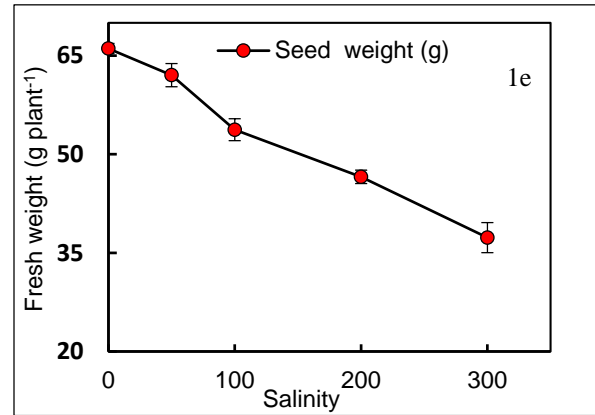


Fig 1e Fresh weight of seeds of *V. faba* subjected to graded levels of water stress on a sand culture for 15 days. Each value is the mean of 5 replicates ± SE

The results show that salinity had little effect on chlorophyll content up to 200 mM salt concentration, but that it changed dramatically at 300 mM NaCl concentration. In the *V. faba*, it is obvious that the maximum salt concentration (300 mM) has a deleterious impact on the chl. 'a':'b' ratio. However, it appears that chl. 'a' is more sensitive to salinity than chl. 'b.' The amount of chlorophyll in plants is proportional to the health of the plant. (Rodriguez & Miller, 2000): (Zhang et al., 2005) when compared to non-salinized plants, (Fig 1f).

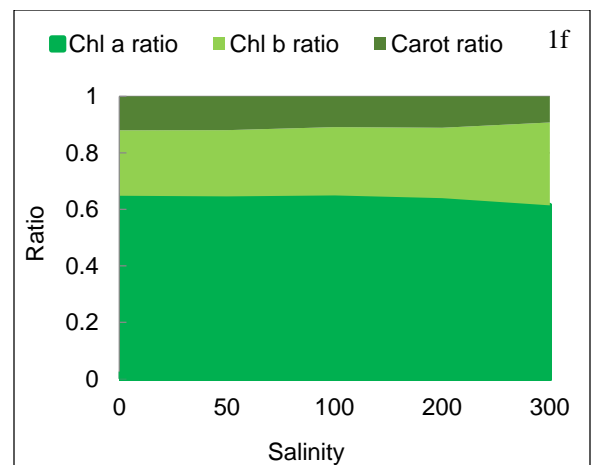


Figure 1. f) Pigment ratio of *Vicia faba* subjected to graded levels of water stress on a sand culture for 15 days. Each value is the mean of 5 replicates ± SE.

Table 1. Plant height (cm) ,Number of leaves, Number of seeds per plant , Number of branches per plant and Number of pods per plant of *V. faba* subjected to graded levels of water stress on a sand culture for 15 days. Each value is the mean of 5 replicates \pm SE.

NaCl Treatment	Plant height (cm)	Number of leaves	Number of seeds per plant	Number of branches per plant	Number of pods per plant
NaCl (0)	55.92 \pm 2.57 ^a	51.80 \pm 2.24 ^a	31.00 \pm 0.24 ^a	3.60 \pm 0.24 ^a	4.80 \pm 0.37 ^a
NaCl (50)	53.44 \pm 1.51 ^{ab}	49.40 \pm 2.27 ^{ab}	28.80 \pm 0.66 ^{ab}	3.20 \pm 0.66 ^a	4.20 \pm 0.37 ^a
NaCl (100)	49.52 \pm 1.09 ^{abc}	38.40 \pm 2.37 ^c	24.80 \pm 0.37 ^{bc}	2.80 \pm 0.37 ^{ab}	3.40 \pm 0.24 ^{ab}
NaCl (200)	45.70 \pm 0.78 ^{bcd}	33.60 \pm 1.36 ^{cd}	21.40 \pm 0.55 ^{cd}	2.00 \pm 0.55 ^{ab}	2.40 \pm 0.50 ^{bc}
NaCl (300)	40.78 \pm 3.48 ^{cde}	28.80 \pm 2.35 ^{de}	14.80 \pm 0.50 ^e	1.60 \pm 0.50 ^{abc}	1.80 \pm 0.37 ^{cd}

Means having the same letters in a column were not significantly different at $p < 0.05$

Table 2. Shoots weight (g) , Pod weight (g), Number of seeds per plant , and Seed weight (g), of *V. faba* subjected to graded levels of water stress on a sand culture for 15 days. Each value is the mean of 5 replicates \pm SE

.NaCl Treatment	Shoots weight (g)	Pod weight (g)	Seed weight (g)
NaCl (0)	140.9 \pm 1.05 ^a	136.24 \pm 1.47 ^a	66.08 \pm 0.17 ^a
NaCl (50)	139.8 \pm 1.25 ^{ab}	133.46 \pm 1.60 ^{ab}	62.03 \pm 0.36 ^{ab}
NaCl (100)	134.4 \pm 1.60 ^{abc}	127.95 \pm 0.87 ^{bc}	53.73 \pm 0.52 ^c
NaCl (200)	126.8 \pm 1.30 ^d	124.84 \pm 2.53 ^{cd}	46.56 \pm 0.25 ^d
NaCl (300)	119.4 \pm 1.15 ^e	117.60 \pm 1.15 ^{de}	37.32 \pm 0.28 ^e

Means having the same letters in a column were not significantly different at $p < 0.05$

4 Discussion

4.1 Effect of salt stress on plant growth.

Salt stress had a significant impact on the growth of *V. faba*, particularly the aboveground parts. When studying plant responses to salinity, biomass production and yield are important parameters to consider. (Radwan et al., 2000), especially from the perspective of saline agriculture management strategies. a wide range of salinity has been examined in this experiment (0-300 mM NaCl). Saline water irrigation statistically significantly reduced plant height by increasing the level of salinity from 0 to 300 mM NaCl. (Hamada, 1995) with his study of maize *Zea mays* L., (Misra et al., 1997), are other results that support what has been shown here (1997). *Oryza sativa* L. with their study of rice seedlings., (Dantas et al., 2005) in their study of cowpea, *Vigna unguiculata* L., and finally in their study of *brassica campestris* L. by (Memon et al., 2010). They suggested that the use of low sodium chloride concentrations contributed to an increase in the length of plants, while higher concentrations resulted in a shortage. In comparison,

the research conducted by Mathur et al., 2006) on moth bean *Vigna aconitifolia* L. (Jamil et al., 2007) on radish plants, *Raphanus sativus* L., (Victor Desire Taffouo et al., 2009) on cowpea *Vigna unguiculata* L., and (Kavita et al., 2010) on *Vigna mungo* L.

They found that increasing NaCl concentrations caused a decrease in plant lengths. In general, we can conclude that when plants are treated with low salt concentrations, the elongation of the stem can induce osmotic adjustment activity in the plants, which can boost growth. On the other hand, the noted decrease in the length of the stem, also due to sodium chloride solution treatment this could be due to the salt's negative effect on photosynthesis rate, the changes in the activity of the enzyme (which subsequently affects the synthesis of proteins), in addition the decrease in carbohydrate and growth hormone levels, all of which could lead to growth hormone inhibition. (Mazher et al., 2007).

Water stress affects *V. faba* shoot growth, and among the various aspects of shoot growth, fresh weight appears to be more affected than stem dimensions., number of leaves and number of pods per plant, in terms was sharply reduced. According to

(Sultana *et al.*, 1999) reduced mean pod weight by 15% and pods per plant by 48%. Higher saline stress reduced seed output by 67 percent due to seed weight and quantity reductions, as well as affecting broad bean product quality. It was discovered that the reduction in photosynthesis in salinized plants resulted in low concentrations of assimilates in the leaves, resulting in low levels and poor translocation of assimilates from the source, resulting in reduced grain dry matter. (Miransari & Smith, 2007). These results have been confirmed by (Welfare *et al.*, 2002), with their study on *Cicer arietinum* L. and (López-Aguilar *et al.*, 2003), with their study on the leaves of the tepary bean (*Phaseolus acutifolius* L.), cowpea (*Vigna unguiculata* L.), and wild bean (*Phaseolus filiformis* L.).

They point out that, compared with control plants, sodium chloride treatment decreased the number of leaves. They note that when treated with 50 and 100 mM of sodium chloride, there is a decrease in the number of leaves. The decrease in leaf number can be attributed to sodium chloride accumulation in cell walls and the cytoplasm of older leaves. At the same time, their vacuole sap is unable to absorb more salt and therefore reduces the salt concentration within the cells, contributing eventually to their rapid death and reduction in salt concentrations (Munns, 2002). Generally speaking, there is a rise in the new weights of the plants. In this analysis, these results for fresh and dry weights for the shoot system agree with the findings provided by (Andriolo *et al.*, 2005) in their lettuce study (*Lactuca sativa* L.), where they stated that the salt treatment increased the fresh weight by about 28%, and also agree with the results of (Dantas *et al.*, 2005) study Cowpea (*Vigna unguiculata* L.), where they report that 10 mM of sodium chloride is used to increase the fresh and dry weight of their seedlings' shooting systems. Other supporting findings include those of (Niazi *et al.*, 2005), who stated that the treatment of 200 mM of sodium chloride with fodder beet (*Beta vulgaris* L.) and sea beet (*Beta maritima* L.) increases the fresh weight of the shooting device, which was essential for both forms.

The research by Orak *et al.*, 2005 on common vetch (*Vicia sativa* L.) and (Nedjimi *et al.*, 2006) on (*Atriplex halimus* L.) where they report an increase in the fresh and dry weight for root and shoot systems of the plants with concentrations of NaCl.

4.2 Photosynthetic pigments

salinity decreased the chlorophyll index (CI) and total chlorophyll of salt-sensitive cultivars of soybean (Miransari & Smith, 2007) and tomato (Florina *et al.*, 2013). Tantawy *et al.*, 2009 discovered that high levels of salinization cause a significant drop in the concentration of pigment fractions and, as a result, the total chlorophyll content when compared to non-

salinized plants. Our findings on a decrease in chlorophyll 'a', 'b', and total chl. agree with Tort & Turkyilmaz, 2004) who stated that barley exposure (*Hordeum vulgare* L.) to nil, 120, and 240 mM of sodium chloride resulted in a decrease in chlorophyll 'a', chlorophyll 'b', and total chlorophyll content in their study on *Centaureum erythraea* (L.), (Šiler *et al.*, 2007)(Lee *et al.*, 2004) stated that as salt concentrations increased, chlorophyll 'a', 'b', and total chlorophyll decreased.

The increasing photosynthetic pigment concentration (particularly Chl a and carotenoids) in *V. faba* leaves under water stress is likely the result of greater reduction in leaf area than in pigment synthesis, rather than of salinity-induced stimulation of pigment synthesis. This greening of the foliage was, however, associated with lower proportions of Chl b in the total pigment content in favor of Chl a and carotenoids. Similar greening of foliage is a well-known symptom of P deficiency and was also suggested to arise as a result of greater reduction in leaf area than in pigment synthesis (Hawkesford *et al.*, 2012). Furthermore, salinity stress alters the ultrastructure of chloroplasts (Locy *et al.*, 1996) (Keiper *et al.*, 1998).

What also includes supporting outcomes include what(Turan *et al.*, 2007) on bean plant *Phaseolus vulgaris* (L.),(Jaleel *et al.*, 2008), on *Catharanthus roseus* (L.), (Victor Desire Taffouo *et al.*, 2009), on cowpea (*Vigna unguiculata* L.) (Taffouo *et al.*, 2010), on *Vigna subterranean* (L.). Under saline conditions, the rate of photosynthesis also decreases (Sixto *et al.*, 2005). In terms of salt stress, photosystem II is a relatively sensitive component of the photosynthetic system (Allakhverdiev *et al.*, 2000).

Demonstrate that sodium chloride salt stress reduced the total chlorophyll content. The results of the "increase" in chlorophyll content with increasing salt concentrations agree with the findings of Misra *et al.*, 1997). They demonstrated stressed rice seedlings of *Oryza sativa* L. The chlorophyll content of 15-day-old seedlings increased dramatically when treated with sodium chloride. Furthermore,, Jamil *et al.*, 2007) reported that increasing the sodium chloride concentrations (0, 50, 150 mM) increased the total chlorophyll content of sugar cane leaves (*Beta vulgaris* L.), and the increase was significant. (Khan *et al.*, 2009) found that three tolerant wheat genotypes, Lu-26s, Sarsabz, and KTDH-22, had less chlorophyll degradation when exposed to NaCl. (Tantawy *et al.*, 2009). There was also a decrease in total chlorophyll content in tomato with increasing salinity. (Mane *et al.*, 2010) discovered a decrease in total chlorophyll content with increasing NaCl concentration in *Phaseolus vulgaris*, indicating a decrease in both chlorophyll 'a' and 'b'. The decrease in chlorophyll content under stress is a commonly reported

phenomenon, and according to various studies, this could be due to a variety of factors, one of which is membrane deterioration. (Mane *et al.*, 2010)

5 Conclusion

The results of this study revealed that different NaCl concentrations had a significant influence on the majority of the parameters tested. *V. faba* can be considered salt-tolerant during growth, as the shoots can withstand up to 200 mM NaCl with limited damage. Saline water system water significantly decreased faba bean stature, number of seeds per plant, shoots weight, case weight, just as the seed weight. The salinity treatments had no effect on the number of lateral branches or pods per plant. According to the findings of this study, the total chlorophyll content in the leaves of all *V. faba* species increases at lower salinity levels. Furthermore, *V. faba* productivity decreased proportionally to irrigation water salinity level, implying that the optimization of agricultural management strategy for growing *V. faba* in areas where irrigation water is affected by salinity is possible in such a way to allow for certain yield loss due to use of irrigation water of certain salinity level, in order to avoid a greater one due to use of irrigation water of higher salinity level. As a result, the best saline agriculture management strategy may be to accept acceptable yield loss in order to avoid plant water stress.

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Conflict of interest: The authors declare that there are no conflicts of interest.

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