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Alleviation of Salinity Stress on Growth and Yield of Faba Bean (*Vicia faba* L) Plants Using Dry Yeast (*Saccharomyces cereivisiae*) Solution

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A B S T R A C T

The area of saline effected soils is increasing worldwide. Salinity significantly influences plant growth and yield. However, dry yeast (*Saccharomyces cereivisiae*) solution may play a role in alleviating salinity stress on faba bean (*Vicia faba* L) plants. Therefore, this study aimed to investigate the ability of dry yeast solution to reduce the negative effect of salinity on bean plants. In a complete randomized design with four replicates in two factors, this study was conducted. Faba bean plants were grown under saline and non-saline conditions (0 and 120mM NaCl), and treated with and without yeast solution (10g/L). The results indicated that salinity stress 120mM NaCl significantly ($p<0.001$) decreased most of studied growth and yield parameters such as plant height, number of branches, number of leaves, leaf area, number of pods plant^{-1} number of seeds pod^{-1}, weight of 10 seeds, dry weight and seed yield. On the other hand, the result shown that the application of 10g/L of yeast solution induced significant ($p<0.05$) promotive effects on most of growth and yield parameters in both saline and non-saline conditions. The study revealed that treating bean plants with a dry yeast solution could significantly improve their growth and yield traits when exposed to salinity. Overall, the study concluded that yeast could help alleviate the damage caused by salinity stress. However, combining yeast treatment with other farming practices may be necessary for optimal results.

1. Introduction

Faba bean (*Vicia faba* L.) It is considered one of the oldest legume crops in the world and Its cultivation was widespread in the Mediterranean region as a well-known food crop and as an emerging herbal medicine (Rahate et al., 2020; Mínguez and Rubiales, 2021). Worldwide, faba bean plant is considered the third most important legume crop, with about 58 countries producing this crop in large quantities (Singh et al., 2013). Faba bean seeds have a high content of digestible proteins, starch, and

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good sources of fiber, essential amino acids, and particular vitamins (Crépon et al., 2010; Sellami et al., 2021). In addition, faba beans are rich source of lysine rich proteins, carbohydrate, minerals, and numerous bioactive compounds (Dhull et al., 2022). Despite its nutritional value, bean plant cultivation faces several challenges, like other crops (Danial and Basset, 2024). Under field condition, plants are exposed during their life cycle to some biotic and abiotic factors that limit their productivity. One of the most important challenges facing bean agriculture is the problem of soil and water salinity, especially in arid and semi-arid regions. Additionally, this plant is sensitive to salinity to some degree, and therefore it is recommended to grow it in low-salt environments (Abdeen and Hefni, 2023).

Soil and water salinity are the most serious abiotic stresses that influence crop yields worldwide; salinity threatens about 6% of the world's total land area, which containing 20% of arable land and 33% of irrigated land (Safdar et al., 2019). Salinity stress significantly affects plant growth through various mechanisms, such as ionic toxicity because of excessive uptake of ions, such as sodium (Na^+) and chloride (Cl^-) (Munns and Tester, 2008; Munns et al., 2020). Salinity causes an imbalance in ionic balance, which affects some physiological processes and leads to wilting and dehydration, and thus the death of the plant (Munns, 1993 and 2002). In addition, salinity causes osmotic stress, which affects the availability and absorption of water (Sheldon et al., 2017; Safdar et al., 2019). Osmotic stress results in reducing the rate of transpiration and caused stomata closing to conserve water, which affect photosynthesis (Srivastava et al., 2019; Orzechowska et al., 2021; El-Dakak et al., 2023). Moreover, salinity reduces nutrient mobilization, affecting nutrient uptake and distribution within the plant (Ehtaiwesh, 2022a; Wekesa et al., 2022). Salinity stress induces oxidative stress and creates reactive oxygen species (ROS), which damage cellular components and contribute to plant senescence and cell death (Hasanuzzaman et al., 2021; Hualpa-Ramirez et al., 2024). Salinity stress effected seed germination and caused delay in germination, reduce germination percentage, and effect germination rate (Ehtaiwesh, 2019). Bean plants exposed to salinity stress exhibit lower total plant biomass, relative growth rateand shoot/root weight ratio of beans (Ehtaiwesh, 2022b and c). In addition, salinity adversely effected chlorophyll contents, photosynthetic, transpiration rate and stomatal conductance (Hussein et al., 2017; Safdar et al., 2019; Sofy et al, 2020).

Numerous studies aimed at the management of salinity stress, some of these studies have looked into enhancing the ability of crops to maintain good growth and productivity under salinity stress. Some of these studies suggested using plant growth promoting microorganisms (PGPMs) such as bacteria, fungi, and yeasts (Talaat et al., 2022).

Yeast *(Saccharomyces cerevisiae)* may play a role in alleviating salinity stress on plants as it contains phytohormones, vitamins and amino acids (Taha et al., 2020). Some studies found that yeast extract contains osmoprotectant compounds such as proline and betaine, which may adjust water balance within plant cells (El-Shraiy et al., 2016). This adjustment supports plants to combat water deficit caused by salinity stress (Abdelaal et al., 2021). Besides, yeast has been stated to stimulate cell division and cell expansion, enhance chlorophyll efficiency, protein and carbohydrate in plant (Dawood et al., 2013; Abdelaal et al., 2019). In addition, yeasts enhance the plant's antioxidant defense mechanisms (Abdel Latef et al., 2019). It removes the undesired free radicals and reduces oxidative damage caused by salinity stress (Abdelaal et al., 2019). Babaousmail et al., (2022) recorded that foliar application of yeast extract is a good source of nutrients through the leaves and leads to improved salt tolerance. Moreover, yeasts contain some compounds that support some metabolic processes such as enzymes activities, minerals uptake and soluble sugars production which, aiding salinity stress tolerance in plants (Hammad and Ali, 2014). A study reported that yeast improved plant growth, biochemical and elemental content in plant and improved the anatomical traits, which consequently increased the tolerance of plants to salinity stress (El-Shawa et al., 2020). Abuiflayjah et al., (2024) stated that spraying faba bean plants with yeast extract resulted in a significant increase in plant *height* and leaf area. Additionally, many studies suggest that yeast is a cost-effective bio-fertilizer that improves plant growth and productivity under saline and non- saline condition (Yousif et al., 2019; Ehtaiwesh, 2023). Recently, Abd El-Sattar and Abdelhameed, (2024) found that applying. faba bean plants with yeast extract could considerably renovate the loss of vegetative growth caused by salinity stress.

Typically, yeast (*Saccharomyces cerevisiae*) is utilized in food production, in biofuel industry, and in medical science; however, a few researchers testified that yeasts could improve plant growth under stress conditions such as salinity. Therefore, this study aimed to explore the potential effect of yeasts in improving growth and yield traits of faba bean plants.

1 Materials and Methods

Pots experiment was conducted in Jodaam farm in fall/winter of 2022. Seeds of faba bean were obtained from the local market and were used in this study.

Experimental details and growth conditions

The experiment was conducted according to completely randomized design with four replicates in two factors, two level of salinity (0 and 120mM NaCl) and two yeast treatments (with and without yeast application). Seeds of faba beans were sowed in plastic pots filed with 10 L of sandy loamy soil mixed with Peat moss (3-1) without a leaching possibility. The soil was collected from the upper surface of soil (0-10 cm deep), then the soil was air-dried and sieved through a 5-mm mesh screen and mixed with peat moss. Four healthy with equal size of faba bean seeds were sown in each pot. Before planting, 1g of urea was added to each pot. After seedling establishment seedlings were thinned and only two seedlings of each pot were kept until the end of the experiment. After planting, irrigation was applied at the appropriate times with tap water to maintain soil moisture near maximum water-holding capacity (Talaat et al, 2014). Di-Ammonium phosphates (P2O5) 46P, 18N was added 20 days of planning. 30 days of planting, pots were divided into two groups each group represent salinity treatments: 0mM NaCl (control) and 120mM NaCl. Salinity stress was induced three times 45, 65, and 85 days of sowing by irrigated plant with saline water (120 mM NaCl). At each salinity level, plants were treated either with yeast or without yeast applications. Yeast application was applied four times (40, 60. 80 and 100 days of sowing) by spraying the plants until complete covering of the plant. Other plants that were not treated with yeast were treated with water until the complete covering of the plant.

Yeast solution preparation.

10g/L of yeast solution was prepared using a commercial baker's yeast (*Saccharomyces cerevisiae*) obtained from local market. The yeast prepared by dissolving a quantity of dry yeast in water, and 1:1 ratio of sugar was added as a source of C and N. Yeast culture was grown for 48h at 2±25 ◦C before its application to the plants (Babaousmail, 2022). Yeast was sprayed on plants and untreated plants were sprayed with water. During the experiment, the pots were kept under semi-controlled environments and water was applied as needed until plants displayed physiological maturity and plants were harvested.

Data Collection

At harvest, one plant was randomly selected from each replicate for data collection. Plants were shaken slightly to remove soil particles and the lengths of their shoots were measured using a meter scale and represented as plant height (cm). The number of branches plant^{-1}, the number of leaves plant^{-1} the number of pod in plant^{-1} were counted, and then leaf area (cm^2) was measured. Leaf area (LA) was calculated using the leaf length and leaf width. Leaf length and width was measured using a ruler and leaf area was estimate following the equation of Peksen, (2007).

$$\text{Leaf area (LA)} = \text{leaf length x width of terminal leaflet.}$$

The plants were then placed in an oven at 60 °C until constant weight to record thier dry weight (g). Weights were estimated in g per plant using a balance. The number of seeds plant^{-1}, 10 seeds weight (g) and seed yield (g/plant) were calculated. The 10 seed weight was recorded in g of the weight of 10 randomly selected seeds from each replication after threshing the seeds yield was estimated in g per plant with total weight of seeds after threshing.

Statistical analysis

The experiment was conducted in four replications and obtained values were expressed as the mean ± SE. Statistical analysis was performed using SPSS for Windows software. A two-way analysis of variance test (ANOVA) was conducted to test the significance of yeast application and salinity effects on plant growth parameters.

1 Results

The P-values for growth and yield parameters of faba bean plants are presented in Table 1. The independent effect of salinity and yeast application were highly significant (P < .001) for all growth parameters that included in this study. In addition, the interaction effect of salinity and yeast application was significant (P < 0.05) for almost all growth parameters included in this study accept plant height and Number of leaves plant^{-1} parameters (Table1).

Table 1. Probability values of the effects of salinity (S), yeast (Y) and their interaction (SxY) on various growth and yield traits of bean *Phaseolus vulgaris* plants.

Traits	Salinity (S)	Yeast (Y)	S x Y
Plant height (cm)	<.001	<.001	0.120
Branches number plant^{-1}	<.001	<.001	0.033
Number of leaves plant^{-1}	<.001	<.001	0.060
Leaf area (cm^2)	<.001	<.001	0.049
Number of pods/plant	<.001	<.001	0.039
Number of seeds/plant	<.001	<.001	0.013
Weight of 10 seeds	<.001	<.001	0.049
Dry weight/plant	<.001	<.001	0.048
Seed yield/ plant	<.001	<.001	0.011

The results presented in Table 2 display the mean differences in plant growth parameters of faba bean plants due to salinity stress treatments. The results showed that bean plants that were irrigated with 0mM NaCl had higher plant growth and yield parameters, whereas irrigation of bean plants with 120mM NaCl significantly (P<.001) reduced all growth and yield parameters of bean plants.

Table 2. The main effect of salinity stress on various growth and yield traits of faba bean plants. The means were estimated using the GLM procedure in SPSS.

Traits	Salinity Level NaCl	
	0mM (NaCl)	120mM (NaCl)
Plant height (cm)	69.70	55.98 (-20)
Branches number plant^{-1}	06.50	04.63 (-29)
Number of leaves plant^{-1}	97.75	76.75 (-21)
Leaf area (cm^2)	26.38	16.58 (-37)
Number of pods plant^{-1}	11.75	07.00 (-40)
Number of seeds plant^{-1}	65.88	26.13 (-60)

Weight of 10 seeds	15.49	09.75 (-37)
Dry weight plant^{-1}	24.70	17.91 (-27)
Seed yield plant^{-1}	95.84	57.52 (-40)

Individual value is the mean of eight replicate under different salinity levels. Values in parenthesis indicate the percent decrease from the control treatment (0mM NaCl) to the (120mM NaCl)..

Table 3 presents the results of the independent effect of yeast application on growth and yield parameters of bean plants. The result indicated that yeast application significantly (P<.001) increased all growth and yield parameters of bean. The bean plants that were treated with yeast solution performed better growth as compared to plants that not treated with yeast solution.

Table 3. The main effect of yeast appiction on various growth and yield traits of faba bean plant. The means was estimated using the GLM procedure in SPSS.

Traits	Yeast Treatments	
	- yeast	+ yeast
Plant height (cm)	59.16	66.51 (+12)
Branches number plant^{-1}	05.00	06.13 (+23)
Number of leaves plant^{-1}	84.13	90.38 (+07)
Leaf area (cm^2)	19.83	23.13 (+17)
Number of pods plant^{-1}	08.50	10.25 (+21)
Number of seeds plant^{-1}	39.38	52.63 (+34)
Weight of 10 seeds	11.84	13.40 (+13)
Dry weight plant^{-1}	19.98	22.64 (+13)
Seed yield plant^{-1}	69.66	83.71 (+20)

Individual value is the mean of eight replicate under different yeast treatments. Values in parenthesis indicate the percent increase from the control treatment (-yeast) to the (+yeast).

Compared to the control plants, irrigation with saline water statistically significantly reduced plant height, which recorded 24% reduction over the control. However, plant height reduced by 9% when salinity stressed plants treated with yeast solution. Also the result indicated that when bean plants treated with yeast solution in non-saline condition, plant height trait showed an increase of 7% compared to the control Fig 1A. In addition, branches number plant^{-1} were reduced under salinity stress by 40% compared to the control, however when salinity stressed plants treated with yeast solution, the branches number plant^{-1} were reduced by 12%. The result pointed that yeast application improved plant growth under non-saline condition as indicated by an increase of 8% of branches number palnt^{-1} compared to the control Fig 1B. Leaves number plant^{-1} were also reduced by 24% under salinity stress compared to the control, and reduced by 15% under salinity stress with an application of yeast solution. The result also recorded that bean plants performed better under non-saline condition with an application of yeast solution as indicated by an increase of 4% of leaves number plant^{-1} compared to the control Fig. 1C.

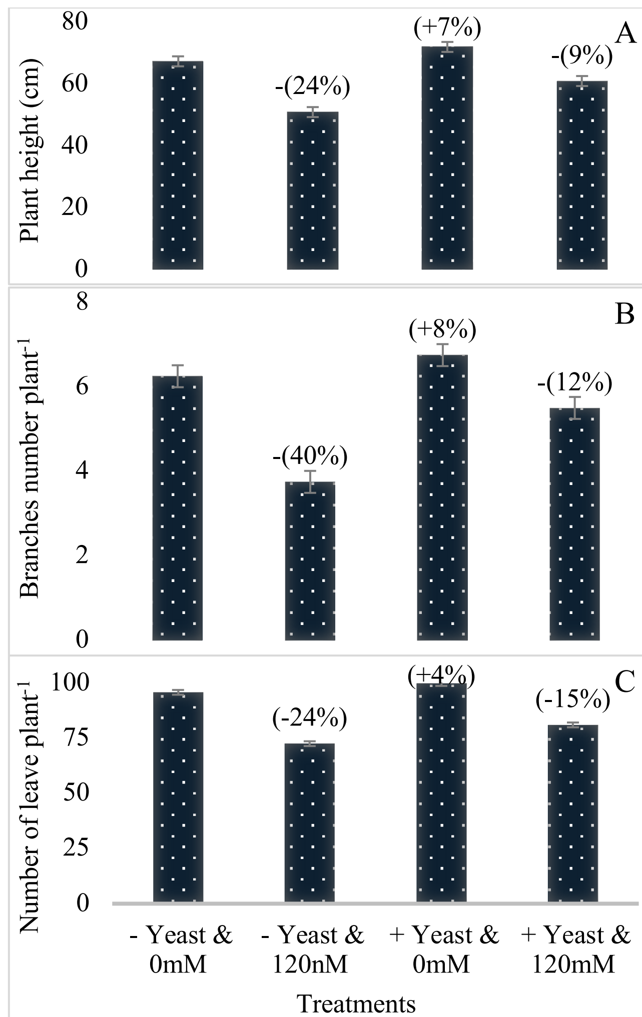

Figure 1. The interaction effect of salinity and yeast treatments on (A) plant height (cm), (B) branches number plant^{-1} and (C) leaves number plant^{-1} of faba bean plant. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicate the percent differences from the control treatment. (-) indicate percentage reduction and (+) indicates percentage increase.

Additionally, the results demonstrated that exposed of bean plants to salinity stress significantly (P<.001) diminished their growth which indicated by diminished leaf area (cm^2). The results herein indicated that 120mM NaCl salinity level reduced the values of leaf area by 38% compared to the control. However, the application of yeast to both stressed and unstressed plants significantly enhanced the values of leaf area over the respective control. The leaf area was reduced by 27% in stressed plants, and increased by 16% in unstressed plant Fig. 2A. Additionally, salinity treatment significantly reduced pods number $plant^{-1}$, which recorded 49% reduction over the control. The result showed that the application of yeast solution lessened the reduction of pods number to 27% compared to untreated plants and increased the number of pods $plant^{-1}$ under non-saline conditions by 9% Fig. 2B. The results further indicated that salinity stress significantly affected seeds number $plant^{-1}$ that indicated by significant decrease of seeds number $plant^{-1}$ by 72% compared to control. This reduction of seeds number $palnt^{-1}$ was declined to 43% reduction over the control when salinity stressed plant treated with yeast solution. Also, yeast application improved plant yield under non-saline condition which indicated by an increase of 14% of seeds number $plant^{-1}$ as compared to the control Fig. 2C.

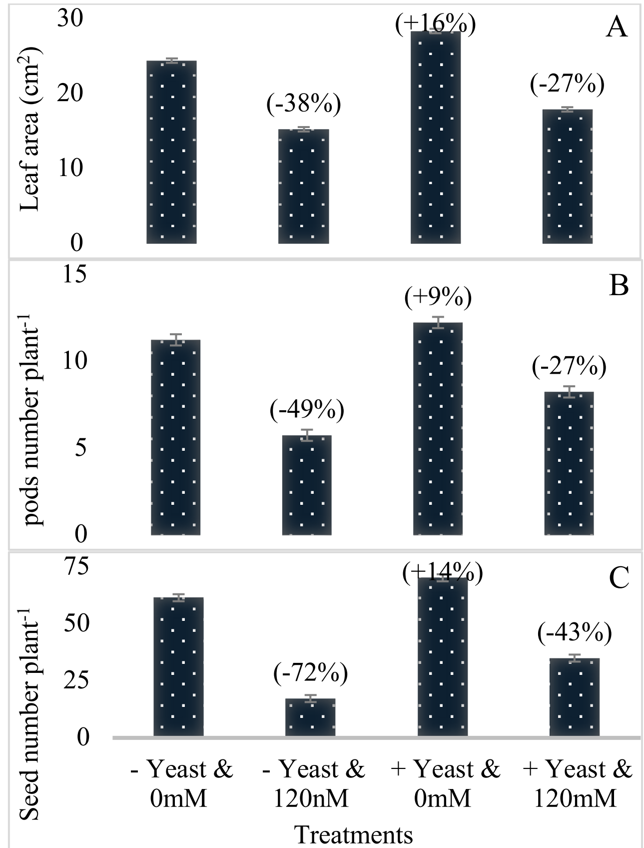

Figure 2. The interaction effect of salinity and yeast treatments on (A) leaf area (cm^2), (B) pods number $plant^{-1}$ and (C) seeds number $plant^{-1}$ of faba bean plant. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicate the percent differences from the control treatment. (-) indicate percentage reduction and (+) indicates percentage increase.

Furthermore, with the 120mM NaCl irrigation treatment, the weight of 10 seeds was reduced by 41% compared to the control treatment, however; this reduction was lessened to 28% reduction over the control when salinity stressed plants treated with yeast solution. Under non-saline condition, the application of yeast enhanced bean yield by increasing the weight of 10 seed by 8% compared to control Fig. 3A. Compared to the control, with the 120mM NaCl irrigation treatment, plant dry weight $plant^{-1}$ was reduced by 31% without yeast application and the dry weight $plant^{-1}$ was reduced by 17% with the application of yeast solution. The result also showed an increase of 8% of plant dry weight $plant^{-1}$ because of the yeast application under non-saline condition Fig. 3B. Besides, under saline irrigation 120mM NaCl seed yield $plant^{-1}$ was significantly affected. The result showed that seed yield $plant^{-1}$ was decreased by 48% and 26% without and with the application of yeast solution respectively. There was also an increase of 9% of seed yield $plant^{-1}$ in bean plants treated with yeast solution under non-saline condition Fig. 3C.

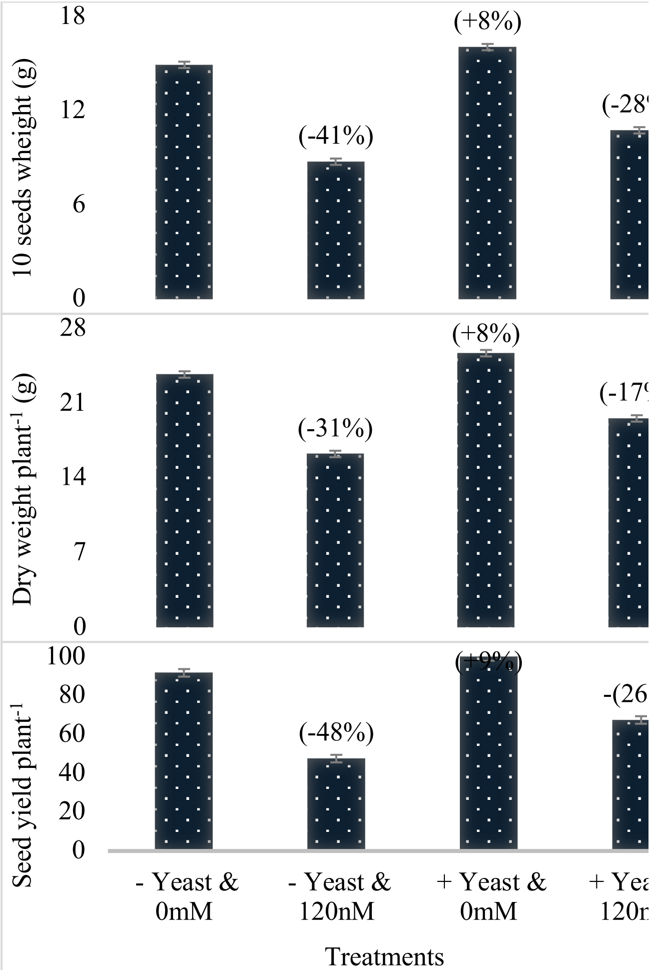

Figure 3. The interaction effect of salinity and yeast treatments on (A) 10 seeds weight (g), (B) dry weight (g) plant^{-1} and (C) seed yield plant^{-1} of faba bean plant. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicate the percent differences from the control treatment. (-) indicate percentage reduction and (+) indicates percentage increase.

1 Discussion

Soil and water salinity is a major constraint for bean production due to chloride and sodium accumulation. Increasing salinity linearly reduced plant biomass, seed yield, and leaf net carbon assimilation rate (Ehtaiwesh, 2016; Babaousmail et al., 2023). Salinity stress negatively influenced plant growth and development resulting in low crop yields (Acharya et al., 2024; Ehtaiwesh et al., 2024). The present study compared different growth and yield parameters of *Vicia faba* L plants grown at 0 and 120mM NaCl salinity levels. The results indicate consistent differences between the treatments as presented in table 2. Plant length, branches number, leaves number, leaf area, pods number plan^{-1}, number of seeds plant^{-1}, seeds weigh, dry weight and seed yield were substantially reduced under salinity stress (120mM salinity). This demonstrates that salinity stress strongly inhibited plant growth and productivity across all metrics. The extremely low p-values. (P< 0.001) provide very strong evidence that 120mM NaCl salinity impaired faba bean plants performance. This These results are similar to some early studies on different plants such as common bean *Phaseolus vulgaris* cv. (Ehtaiwesh and Abuiflayjah, 2020), pea *Pisum sativum* cv. (Popova et al., 2023), faba bean *Vicia faba* L (Afzal et al., 2022) and soybean *Glycine max* L. (Hasanuzzaman et al., 2022). This could be due to the effect of salinity on cell division, cell expansion, membrane stability, photosynthesis, ion homeostasis and generation of reactive oxygen species (Yang and Guo, 2018; Ganie et al., 2019; Hao et al., 2021).

In addition, the study compared different growth and yield parameters of faba bean plants treated with and without spraying with yeast *(Saccharomyces cerevisiae)*. The results indicated that in all parameters, the mean values were higher with yeast application compared to without yeast as presented in table 3. The results also statistically significant differences between the yeast treatment for all parameters based on the p-values (P<0.001). Plant length, number of branches, number of leaves, leaf area, number of pods, number of seeds plant^{-1}, seeds weight, plant dry weight and seed yield were substantially increased when faba bean plants treated with yeast solution. This demonstrates that yeast solution strongly superior plant growth and productivity. The extreme low p-values. (P< 0.001) provides very strong evidence that spraying plants with yeast enhances bean plant performance. These results are in line with early studies on the effect of yeast as plant growth enhancer (El-Shawa et al., 2020; Ehtaiwesh, 20.23;

Vargas et al., 2024). Many studies found that yeast has bioactive compound such as volatile organic compounds, phytohormones, enzymes, amino acids and minerals (Fawzy et al., 2012; Kowalska et al., 2022). These compounds play very significant role as plant growth enhancer. This might be due to the ability of yeast to promote plant growth and has protective role against environmental stress (Mahmoud et al., 2020).

Moreover, the study examined the interaction effects of salinity (0 and 120mM) and yeast application on faba bean plant growth and yield parameters. For all parameters, the mean values were highest under 0mM salinity with yeast application and lowest under 120mM without yeast application. Statistical analysis showed significant interactions between salinity and yeast for number of branches, leaf area, number of pods, number of seeds pod^{-1}, number of seeds plant^{-1}, seeds weight, dry weight and seed yield plant^{-1} based on p-values ($P<0.05$). However, insignificant interactions were observed for plant length and number of leaves plant^{-1}. This indicates that yeast application could overcome the injury caused by salinity stress and salinity inhibited growth more when yeast was absent. Previous studies revealed similar results (Babaousmail et al., 2022; Ehtaiwesh, 2023; Abd El-Sattar and Abdelhameed, 2024; Danial and Basset, 2024). Recent studies highlighted the potential of yeasts in sustainable agriculture, employing byproducts from the food industry and contributing to enhanced crop productivity and environmental sustainability (Raklami et al., 2024; Vargas et al., 2024).

2. Conclusions

In conclusion, the present study's results clearly show that high salinity stress (120mM NaCl) had significantly negative effects on all growth and yield measurements in faba bean plants compared to normal conditions (0mM NaCl). This demonstrates that salinity greatly hinders plant growth and development. Additionally, the findings indicate that treating plants with a yeast solution enhanced all growth and yield parameters. Yeast is a cost-effective bio-fertilizer that improves plant growth and productivity under saline and non- saline condition.

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Conflict of interest:

The authors declare that there are no conflicts of interest

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