The Libyan Journal of Science
University of Tripoli Vol. 26, No. 02
https://uot.ed.ly/journals/index.php/ljs

The Effect of Yeast (*Saccharomyces cereivisiae*) on Growth of Common Beans (*Phaseolus vulgaris* L.) Plants under Salinity Stress

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ARTICLE INFO

Article history:

Received 16/06/2023

Received in revised form 12/11/2023

Accepted 02/12/2023

ABSTRACT

Plant growth-promoting microorganisms (PGPMs) such as bacteria and fungi have some beneficial effects on plants growth and their application in agriculture has increased in recent years. Yeasts are mainly known for their application in the food industry and medical industry. However, the use of yeasts as PGPMs has not been widely investigated; therefore, this study was aimed to evaluate yeast potential role in reducing the injury induced by salt stress. Common beans (*Phaseolus vulgaris* L.) plants were grown under saline and non-saline conditions (0 and 100mM NaCl), with and without yeast (10g/L) foliar application. The result found that salinity at 100mM NaCl significantly ($p<.001$) reduced all plant growth traits compared to plants treated with 0mM NaCl. At the same time, the results showed remarkable efficiency of yeast application ($P<.001$) in promoting beans seedlings growth, increasing plant height, number of branches, number of leave, increasing plant fresh and dry weight, relative growth rate and relative water content. Interestingly, the results indicated that the application of yeast solution alleviate the injury caused by salinity. The result showed significant ($p< 0.05$) increase in all growth traits of beans. Overall the study conclude that yeast foliar application as environmental friendly, sustainable and efficient strategy can improve plant responses to mitigate the adverse effects of salinity stress.

Keywords: Plant growth promotion; salinity stress; *Saccharomyces cereivisiae*; *Phaseolus vulgaris* L.; growth traits.

1. Introduction

Common beans plants (*Phaseolus vulgaris* L.) Is the world's most highly consumed vegetable and is one of the most important legumes worldwide because of their high nutritional value rich in (protein phosphorus, iron), and also due to their functional properties, which include carbohydrates, vitamins, lectins, soluble fiber, phenolic acids, flavonoids, and proanthocyanidins. [1] As a food, legumes could be used as dried beans or in the fresh state as green beans and widely used as a vegetable when harvested with young tender pods before mature. [2] Common beans is grown worldwide, including the regions suffering from water and soil salinity, such as the North Africa region, in such regions, beans crop production is restricted by a many biotic and abiotic stress factors like pathogenic disease, drought, salinity, and temperature.

Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate, as well as changes in plant metabolic processes. [3, 4] Salinity reduces bean seed germination and lengthens the time needed for germination. [5] In addition, salinity had adverse effects on the total plant biomass and fresh pod yield, relative growth rate, other morphological parameters such as plant height, number of leaves, root length and shoot/root weight ratio of common beans. [6-8] Also salinity adversely effected chlorophyll contents, photosynthesis, transpiration rate and stomatal conductance in common bean plants. [9-12] Recently, there are some new approaches to alleviate the deleterious effects of salinity stress. One of these approaches its using plant growth promoting microorganisms (PGPMs) such as yeasts.

Plant growth promoting microorganisms (PGPMs) are well defined as microorganisms include: fungi, bacteria, actinomycetes and yeasts, that can improve plant growth and productivity, increase plant nutrient uptake and defend plants from various biotic and abiotic stresses. [13-15] (PGPMs) has the ability to promote plant growth through many mechanisms such as nitrogen fixation, solubilization of insoluble minerals, increase polysaccharide secretion, biocontrol activity and production and regulation of plant. [16,17] PGPMs inoculation can also increase plant uptake of several other nutrients such as calcium, iron, magnesium, sulfur and zinc because of acidification of the soil rhizosphere through organic acid production. [18] One direct mechanisms that may adopted by the endophytic yeasts in improving the plant growth and productivity is synthesis of plant growth promoting compounds such as auxins and cytokinins. [19] Another mechanisms that may adopted by some fungi and yeasts is enhancing nitrogen fixation process. [20] Also some yeasts may synthesis some enzymes that enhance nutrient availability and uptake such as phosphatases, siderophores amylase, cellulase and protease. [19] In addition, some strains of yeast may synthesis bioactive secondary metabolites. [21,22] Yeast typically used in several fields include fermentation industry such as in bread making and in beverage and food industry in general, biofuel production industries, as well as its use in medical science. [23] Recently, there are some researchers suggest that yeast has the potential to be used as plant growth promoting factor. [24] A study suggested that *Saccharomyces cerevisiae* could act as a plant growth promoting and a biocontrol agent for sustainable agriculture in unadorned conditions as well as its use as biocontrol as it has the potential to inhibit plant pathogens. [23,25] Mostly yeast and their products were used in food industry, biofuel industry and medical science; however, there are only very few literatures reported that yeasts have the ability to be used as plant growth promoting agents. Therefore, this study aimed to investigate the effect of yeasts as plant growth agents on growth of common beans plants (*Phaseolus vulgaris* L.).

2. Material and methods

A pot experiment was conducted in Jodaam farm, 30km from Tripoli, Libya in spring of 2022. The experiment was conducted according to completely randomized design with four replicates in two factors, two level of salinity (0 and 100mM NaCl) (Ehtaiwesh and Abuiflayjah, 2020) and two yeast treatments (with and without yeast foliar application). Seeds of common beans were sown in plastic pots filed with 10 kg of sandy loamy soil mixed with Peat moss (1-3) without a leaching possibility. The soil was obtained from the upper soil surface (0-10 cm deep), the soil then was air-dried, sieved through a 5-mm mesh screen and mixed with peat moss. The electrical conductivity (EC) of the soil solution was about 2.4 dSm^{-1}. Five healthy equal size common bean seeds were selected and sown in each pot. However, 1g of urea was added to each pot before planting. After seedling, establishment they were thinned and only two seedlings per pot were kept until the end of the experiment. After planting, they were irrigated at the appropriate times with 0.8 dSm^{-1} EC of tap water to maintain soil moisture near maximum water-holding capacity. [26] Di-Ammonium phosphates (P2O5) 46P, 18N was added 15 days after planning. 25 days after planting, pots were divided into four groups, each group represent one treatment with four replications each. Two groups used for salinity treatments: 0mM NaCl (control) and 100mM NaCl. At each salinity level, plants were treated either with yeast or without yeast applications. Plants were irrigated with 250 mL of the corresponding treatment three times a week during the growth period.

2.1. Yeast solution preparation

Fallowing the modified mothed of [27] a 10% yeast extract 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 ±25 ◦C before its application to the plants. [27] Yeast was sprayed on plants and soil surface at the time of irrigation. Untreated plants were sprayed with water.

2.2. Data Collection

After 7 weeks of planting, four replications (4plants) of each treatment were collected for data collection. Plants were shaken slightly to remove soil particles and the lengths of their shoots and roots (cm) were measured using a meter scale and represent as plant height. The number of branches $plant^{-1}$, the number of leaves $plant^{-1}$ 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 estimated according to the equation of [28] .

Leaf area (LA) = leaf length x width of terminal leaflet.

The relative growth rate (RGR) was calculated during two periods of plant growth at 25 and 45 days after planting using the equation of [10].

$$RGR = (W2 – W1/t2\text{-}t1) x 100 \quad \text{……(1)}$$

Where, W plant dry weight (g), t the time (days), and the subscripts 1 and 2 are initial and the second sampling of plant weight.

Relative water content (RWC) was calculated according to Rady et al., (2016), [29]. where 2 cm leaf disks were weighed immediately after collection to obtain fresh weight (FW), and then disks were placed in a Petri dish containing fresh water and kept in the dark. After 24 h, the turgid weight (TW) was obtained. For the dry weight (DW), the disks were oven-dried for 24 h at 75°C until constant weight, and then the RWC was then calculated following the formula:

$$RWC\ \% = (FM - DM)/(TM - DM) \times 100 \quad \text{….(2)}$$

After fresh weight (g) of plants were obtained, the plants were then placed in an oven at 60 °C until constant weight to record plant dry weight (g).

2.3. Statistical analysis

The experimental traits were conducted in four replications and obtained values were expressed as mean ± SE. Statistical analysis was performed with SPSS for Windows Software v. 28. A two-way analysis of variance test (ANOVA) was conducted in order to test the significance of yeast application and salinity effects on plant growth variables. The means were compared by Duncan's multiple range test at $p \leq 0.05$

3. Results

The P-values for growth traits of common bean plants are presented (Table 1). The independent effect of salinity was highly significant ($P < .001$) for all growth traits that included in this study. Also, the independent effect of yeast application was highly significant ($P < .001$) for all growth traits. In addition, the interaction effect of salinity x yeast application was significant ($P < 0.05$) for all growth traits included in this study (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	0.049
Number of branches plant^{-1}	<.001	<.001	0.033
Number of leave plant^{-1}	<.001	<.001	0.045
Leaf area (cm^2)	<.001	<.001	0.040
Fresh weight plant^{-1} (g)	<.001	<.001	0.047
Dry weight plant^{-1} (g)	<.001	<.001	0.017
Relative growth rate	<.001	<.001	0.038
Relative water content %	<.001	<.001	<.001

The results presented in table 2 shows the marked differences in plant growth traits of common bean plants because of salinity treatments, plants that irrigated with 0mM NaCl had higher plant growth traits. Irrigation of common bean plants with 100mM NaCl significantly (P<.001) reduced all growth traits of common bean compared to the 0mM NaCl treatment.

Table 2. The main effect of salinity level on various growth and yield traits of bean Phaseolus vulgaris plant plant. The means was estimated using the GLM procedure in SPSS.

Traits	Salinity Level	
	(control) 0mM NaCl	100mM NaCl
Plant height (cm)	36.94^a	22.75^b
Number of branches plant^{-1}	10.75^a	6.38^b
Number of leave plant^{-1}	30.25^a	19^b
Leaf area (cm^2)	31.27^a	17.97^b
Fresh weight plant^{-1} (g)	27.12^a	20.63^b
Dry weight plant^{-1} (g)	8.13^a	5.38^b
Relative growth rate	24.38^a	14.5^b
Relative water content %	83.45^a	58.71^b

[*] Individual value is the mean of four replicate under different salinity levels. Values followed by different letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

The independent effect of yeast application on growth traits of common bean plants indicated that yeast application significantly (P<.001) increased all growth traits of common bean (Table 3). The common bean plants that were treated with yeast performed better growth as compared to plants that not treated with yeast application.

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

Traits	Yeast Treatments	
	With yeast	Without yeast (control)
Plant height (cm)	31.29^a	28.4^b
Number of branches plant^{-1}	9.38^a	7.75^b
Number of leave plant^{-1}	26.38^a	22.88^b
Leaf area (cm^2)	27.46^a	21.78^b
Fresh weight plant^{-1} (g)	25.63^a	22.13^b
Dry weight plant^{-1} (g)	7.5^a	6^b
Relative growth rate	20.75^a	18.13^b
Relative water content %	76.05^a	66.11^b

[*]Individual value is the mean of four replicate under different yeast treatments. Values followed by different letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

The result showed that salinity negatively affect plant growth (Fig 1), which indicated by reducing in plant height. The average value of plant height of common bean was ranged from 20.75 cm under salinity level of 100mM NaCl to 37.8 cm under yeast treatment without salinity. Whereas the salinity of 100mM NaCl with yeast treatment combination had a significantly higher ($p < 0.05$) plant height whit the average of 24.75cm compared to salinity treatment alone (Fig. 1A). Also, salinity harmfully affected plant biomass production which indicated by the number of branches plant^1. The result revealed that salinity stress significantly (P<.001) decreased the branches number plant-1. Under non-saline condition, the average number of branches was 10.25branch plant-1, which reduced to 5.25branch plant-1 under saline condition. However, yeast application significantly (P<0.033) improved their values in both non-stressed as well as salinity stressed plants. The number of branches plant-1 was 11.25branch plant-1 and 7.5branch plant-1 in yeast treated plants under non-saline, and saline condition respectively (Fig. 1B).

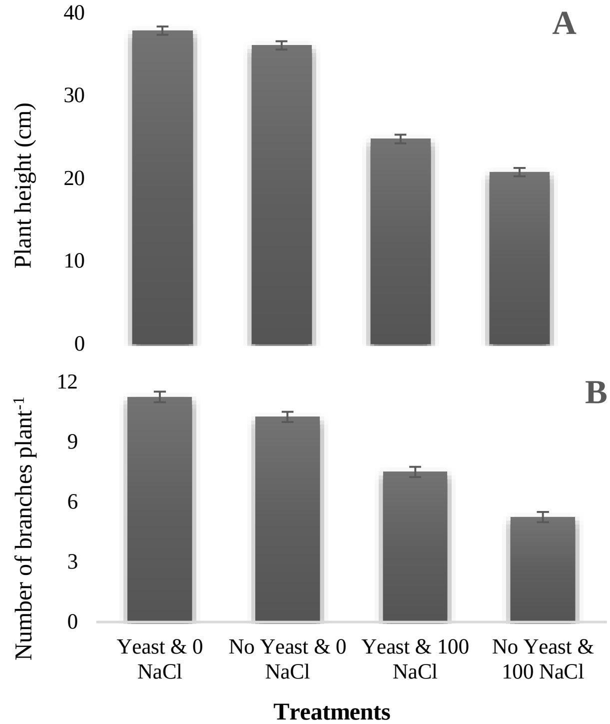

Fig. 1. The effect of salinity and yeast treatments on (A) plant height (cm) and (B) number of branches plant^{-1} of common bean plant. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4)

In addition, the results indicated that yeast foliar application resulted in higher number of leaves per plant of common bean plants, regardless of the level of salinity (Fig 2). The results showed that treating common bean plants with salinity (100mM NaCl) resulted in significant (P<.001) reduction in leave number plant-1 as compared to non-saline condition. However, treating salinity stressed plants with yeast resulted in significant (p<0.045) increase in leave number plant-1 compared with salinity stressed plants without yeast application (Fig. 2A). Moreover, the effect of salinity stress on leaf area (cm^2) was also observed in these results. The exposure of bean plants to salinity stress resulted in significant (P<.001) reduction of leaf area. In fact, yeast foliar application had significant (P<.001) role in enhancing leaf growth in non-saline as well as in saline conditions. Under non-saline condition, the leaf area of common bean plant was recorded to be about 29.13 cm^2, whereas the leaf area was recorded to be 14.44cm^2 in salinity stressed plants. Yet when salinity stressed bean plants treated with yeast foliar application the leaf area of bean increased to 21.5cm^2 (Fig. 2B).

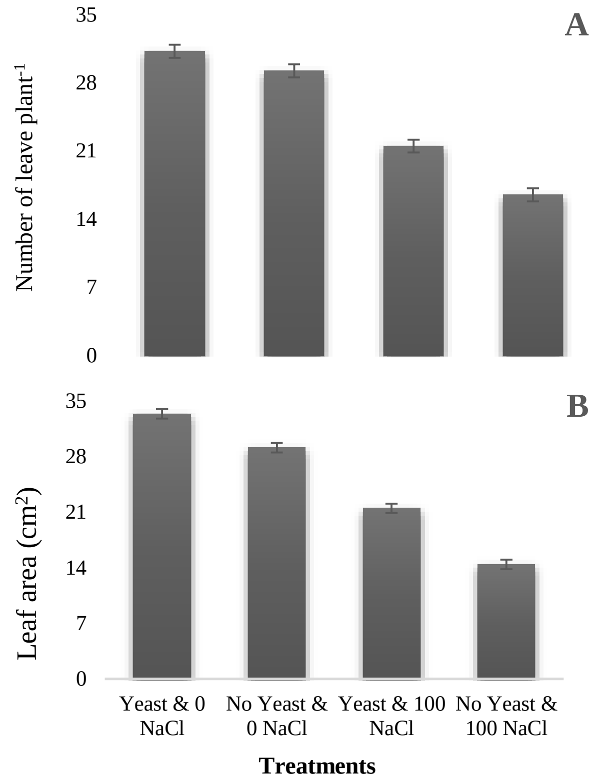

Fig.2. The effect of salinity and yeast treatments on (A) number of leave plant^{-1} and (B) leaf area (cm^2) of common bean plant. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4).

Additionally, the result illustrated that exposed common bean plants to salinity stress significantly (P<.001) decreased their growth (Fig3) which indicated by decreased plant fresh weight (g). The result herein indicated that 100mM NaCl level of salinity reduced the values of plant fresh weight from 25.75g to 18.5g plant^{-1}. However, the subsequent application of yeast to both non-stressed as well as stressed plants significantly (P<.047) enhanced the values of plant fresh weight, over the respective controls (Fig. 3A). Generally, mean plant dry weight values varied between treatments, however, salinity treatment significantly (P<.001) reduced plant dry weight. The application of yeast increased plant dry weight under non-saline conditions, which gave 8.5g of plant dry weight, compared to untreated plants, which gave 7.75g of dry weight plant^{-1}. The results further indicate that yeast application affect plant dry weight trait of common bean, which indicated by significant (p< .017) increase of plant dry weight when salinity stressed plants treated with yeast. The dry weight of salinity stressed plants was 4g; whereas salinity stressed plants that treated with yeast was 6.5g (Fig. 3B).

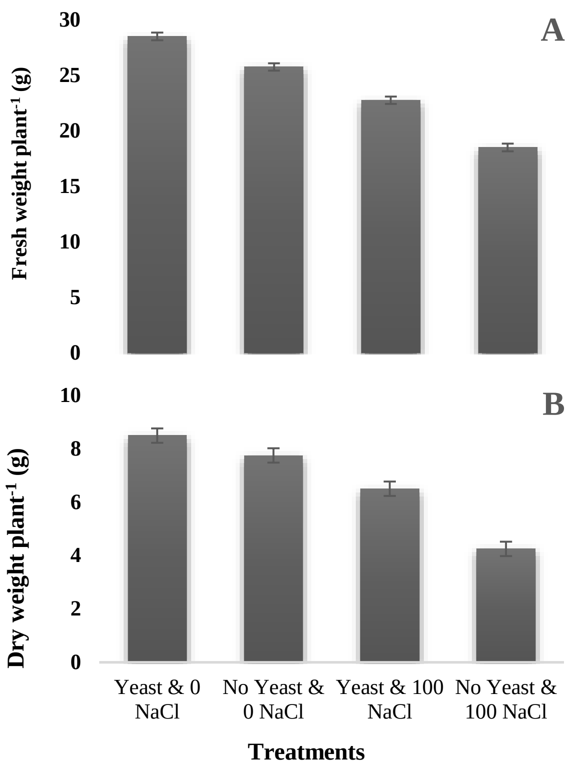

Fig.3. The effect of salinity and yeast treatments on (A) plant fresh weight (g) and (B) plant dry weight (g) of common bean plant. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4).

decrease in the relative growth rate of common bean plants (Fig4). The result shown that the minimum values of relative growth rate was recorded in plants exposed to the 100mM NaCl salinity level (Figure 4A). Nevertheless, a different response was detected when this trait was studied in yeast treated plants, in both conditions (0mM and100mM NaCl). As the result shown, the application of yeast increased the relative growth rate from 12.5 under salinity stress without yeast application, to 16.5 under the same salinity stress with the application of yeast (Fig. 4A). Similarly, common bean plants grown under saline conditions exhibited reduced values of relative water content compared to values of common bean plants grown under non- saline conditions. However, yeast application to salt-stressed and non- stressed plants significantly (P<.001) proved effective in improving this trait. The result demonstrated that yeast treatment to bean plants subjected to 100mMm NaCl salinity level improved relative water content as shown in Fig. 4B. The relative water content increased from 50% under salinity stress without yeast application, to 68% under the same salinity stress with the application of yeast.

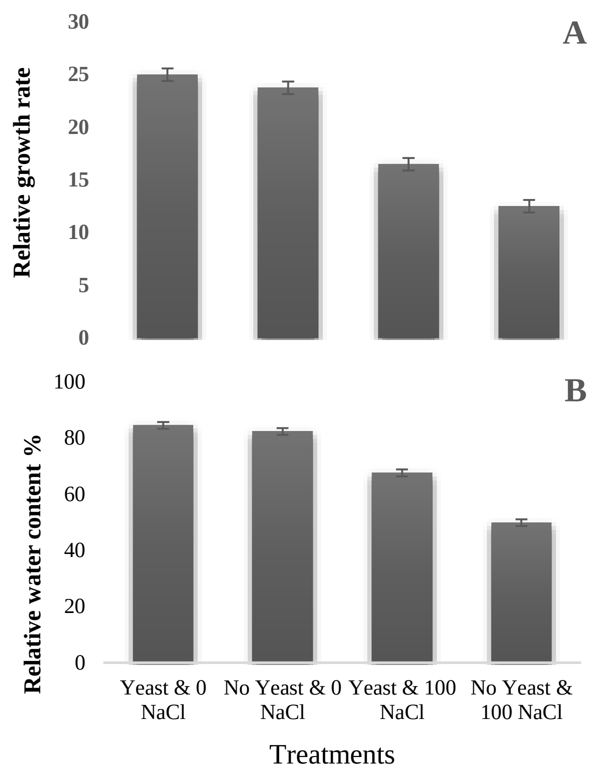

Fig.4. The effect of salinity and yeast treatments on (A) relative growth rate and (B) relative water content (%) of common bean plant. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4).

4. Discussion

Raised salt concentrations in agriculture areas represent one of the most important risk to environmental quality and sustainable crop production. Therefore, improving plant salinity tolerance become an emergent task for many researchers. The current study's unique achievement is the evaluating an environmentally friendly approach which may lessens the negative effects of salinity on plant growth and development. Salinity stress interrupts plant growth and limited plant yield (Ehtaiwesh, 2016; Shahid et al., 2020). Recently, many reports have focused on developing bio-stimulants substances in crop management mainly under stressful conditions (Rady et al., 2016;

Abdelmoteleb et al., 2022; Talaat et al., 2022). Therefore, applying bio-stimulants substances in lessening salt stress toxicity can be the best alternative option ecologically. Fungi such as *(Saccharomyces cerevisiae)* is considered a plant growth promoting yeast for different crops as it plays an important role in cell division and cell enlargement (Farrag et al., 2016). The result of this study indicated that salinity harmfully affected plant growth traits of common bean, which could be because salinity decreases soil water potentials and decreases water uptake by plant roots, resulting in a physiological drought in the plant and decreased cell division and cell elongation. This finding was in agreement of early findings in different crops such as common bean, wheat, pea, beans and barley (Gamaet al., 2007; Ehtaiwesh, 2019; Ehtaiwesh and Abuiflayjah, 2020; Ehtaiwesh and Emsahel, 2020; Talaat et al., 2022). However, the application of yeast as plant growth promoting microorganisms (PGPMs) reduced the negative effect of salinity on almost all growth parameters included in this study. Tables 1 and 2 showed that even under salinity stress (100mM NaCl), yeast application plays a favorable role in increasing plant height, number of branches and number of leave, and consequently increasing plant fresh and dry weights. In a similar study, on the effect of some yeast strain on *Lactuca sativa* plant growth concluded that yeast strain could be used as a bio-fertilizer for healthy and safe crop production under drought, salinity and Al toxicity stresses (Silambarasan et al., 2019b). The result of this study revealed that the application of yeast increased plant biomass production. This outcome agree with previous studies on different plants such as rice (Verma et al., 2019), cucumbe (Kanga et al., 2015), tobacco (Fernandez-San Millan et al., 2020), and beans (Bilek et al., 2022). Also, early study found that field application of *S. cerevisiae* improved tomato growth and yield (Karajeh, 2014). In addition, a study on Lettuce plants indicated that spraying plants with *S. cerevisiae* showed significant increases in vegetative growth, yield and its components (Farrag et al., 2016). Other studies showed that the yeast had an indirect beneficial effect on some plants by improving symbiotic parameters such as nodulation and mycorrhization (Bilek et al., 2022). The effect of yeast application in enhancing plant growth in both saline as well as non-saline condition could be due to the effect of yeast in promoting the production of plant hormones which needed for cell division and cell elongation. In addition, recent study indicated that PGPA regulated the antioxidant machinery, glyoxalase system, and photosynthetic capacity, and plays a critical role in salt stress mitigation of barley plants (Talaat et al., 2022). Therefore, yeast application showed promise as alternatives to chemical fertilization, especially in salinity-affected areas.

5. Conclusion

The development of new environmentally safer technologies is an international trend and urgent for crops production, where chemicals are usually used extensively. Among these technologies, the use of products from microorganisms as bio-fertilizers. This study conducted to examine the potential of yeast foliar application in alleviating the deleterious effects of NaCl-salinity on some growth traits of common bean (*Phaseolus vulgaris*) plants. In this study, yeast application showed promise as alternatives to chemical fertilization, especially in salinity-affected areas. The findings of this study suggest that yeast is a cost-effective bio-fertilizer that improves plant growth and productivity under saline and non- saline condition.

6. Acknowledgment

The autors is grateful to Plant Science Department, University Az Zawia for providing the study equipments.

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