Effect of Different Bacterial Strains on the Germination Forage Pea (*Pisum sativum* ssp. *arvense* L.) under Salt Stress

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ABSTRACT

Background: Stress factors are one of the elements that affect yield in agricultural production and salinity stress is one of the most important stress factors. Pea is an important source of nutrient for human nutrition, as well as a very important legume plant that used as animal feed.

Methods: The experiment, which was set up according to factorial arrangement in random plots, was carried out with 10 replications and a forage pea line was used. Seven different (0, 50, 100, 150, 200, 250 and 300%) salt concentrations and two different bacterial strains (*Bacillus sp*. and *Arthrobacter agilis*) were used in the study. In the study, the effects of different bacterial strains on germination percentage (%), germination rate (days), average daily germination (%), peak value (%) and germination value (%) in pea line exposed to salt were investigated.

Result: In the study, it was found that bacterial applications in salt stress were statistically significant in all parameters. It was determined that *Bacillus sp*. strain was more effective for germination percentage and germination rate. 50 mM salt + bacteria applications of the studied genotype and *Bacillus sp*. and *Arthrobacter agilis* strains were found to have positive effects on seed germination biology under salt stress.

Key words: Bacteria, Germination biology, Nutrition, Peas, Salt stress.

INTRODUCTION

Intensive and unconscious agriculture and the use of chemical fertilizers to achieve high yields cause deterioration of the physical structure of the soil, salinization and drought. Soil salinity is one of the most important problems that negatively affect productivity in crop production areas (Tiryaki, 2018). Salinization significantly decreases the growth and yield of most crop plants (Askari-Khorasgani *et al*. 2017) and accumulates in the root area (Parihar *et al*. 2015), affecting the germination biology negatively (Forni *et al*. 2017). The germination period is the period when the plant is most sensitive to salt stress in its lifetime (Ahmad *et al*. 2013). The feed pea is a one-year legume forage crop with medium sensitivity to salinity, which can be used for both grass and seed production (Senturk, 2009). As a result of many studies conducted to date, it has been revealed that salinity significantly reduces germination and sometimes even prevents germination completely, but this effect has been reported to vary depending on the plant species, variety and salt dose (Onal Asci and Uney, 2016). In the studys, it was stated that PGPR applications provide resistance against salt and drought stress (Yang *et al*. 2009). This study was carried out to determine the effect of nitrogen-fixing and phosphate-dissolving bacteria on the germination biology of forage peas at different salt concentrations.

MATERIALS AND METHODS

This study was carried out in Eastern Anatolia Agricultural Research Institute on 10.07.2020-17.07.2020 to determine the effect of different bacterial strains on the alleviation of different salt stress during the germination stage of forage pea genotype under controlled conditions. In the study, the genotype coded H_{10} [Unye 4 (18s)/09] (seed yield values of which were found to be higher than other varieties), which came forward in the research conducted by Kadioglu (2012-2014) was used as the material.

Randomized parcels of the study were set up with 10 replicates according to the factorial trial design. In the study, 7 different salt doses without bacteria application (control, 50% NaCl, 100% NaCl, 150% NaCl, 200% NaCl, 250% NaCl, 300% NaCl), nitrogen fixative (Kiziloglu, 1995) (*Bacillus sp*.) 50% NaCl + *bacillus sp*. 10^8 CFU, 100% NaCl +*bacillus sp*. 10^8 CFU, 150% NaCl +*bacillus sp*. 10^8 CFU, 200% NaCl +*bacillus sp*. 10^8 CFU, 250% NaCl +*bacillus sp*. 10^8 CFU, 300% NaCl +*bacillus sp*. 10^8 CFU and phosphorus solvent (Kiziloglu, 1995) (*Arthrobacter agilis*) 50% NaCl + *A. agilis* 10^8 CFU, 100% NaCl + *A. agilis*. 10^8 CFU, 150% NaCl + *A. agilis*. 10^8 CFU, 200% NaCl + *A. agilis*. 10^8 CFU, 250% NaCl + *A. agilis*. 10^8 CFU, 300% NaCl + *A. agilis*. 10^8 CFU) bacteria strains have been used. In the study, plant

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seeds were first subjected to surface sterilization with 10% sodium hypochlorite and then with 80% ethyl alcohol. Different doses of 10 ml NaCl solution and 10 ml bacterial strain were added to each petri dish where 25 seeds were placed. Petri dishes were allowed to germinate at 20±1°C for 7 days in a completely dark environment in a climate cabinet. During the study, the seeds were checked every day and seeds with a 2 mm root length were considered to be germinated (Demirkol *et al*. 2019).

In the study, germination percentage (%), germination speed (days), average daily germination (%), peak value (%) and germination value (%) were examined (Czabator, 1962; Ellis and Roberts, 1981; Matthews and Khajeh-Hosseini, 2007; Gairola *et al*. 2011).

$$\text{Germination percentage (GP): } n/\sum n \times 100$$

Where

n = Number of germinated seeds.

$\sum n$= Total number of seeds.

Germination speed (GS): n1/t1+ n2/t2 +……

n1, n2,… are the number of germinated seeds at times t1, t2, ….. (in days).

Mean daily germination (MDG)=

$$\frac{\text{Total number of germinated seeds}}{\text{Total number of days}}$$

$$\text{Peak value (PV)} = \frac{\text{Highest seed germinated}}{\text{Number of days}}$$

Germination value (GV)= MDG $\times$ PV

Germination percentage (%)=

$$\frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

The data obtained in the study were analyzed using the JMP 5.0.1 statistical program in the random plot factorial layout trial design. The statistical significance of the differences between the averages was carried out with the LSD comparison test.

RESULTS AND DISCUSSION

Germination percentage (GP, %)

When we examined the germination percentage, it was found that bacteria and salt application and bacteria × salt interaction were significant at 1%. It was found that *Bacillus sp*. strain was more effective, the germination percentage decreased as the salt concentration increased compared to the control, the germination percentage decreased as the salt concentration increased in the bacteria × salt interaction and the highest value was obtained from 50% salt + *Bacillus sp*. application with 83.3% (Fig 1).

Germination speed (GS, days)

When the effect of bacteria on the germination speed of the forage pea seed in salt stress was examined, it was found that the bacteria and salt were important at 1% and the bacteria × salt interaction was not significant. The order of *Bacillus sp*. (10.19) and *A. agilis* (4.67) was realized in the application of bacteria. It was found that the germination time increases as the salt concentration increases and the *Bacillus sp*. is more effective on the germination speed compared to the control (Fig 2).

Mean daily germination (MDG, %)

When the average daily germination parameter was examined, it was found that the bacterial applications were insignificant, the salt application and the bacteria × salt concentration interaction were significant at 1%. Although

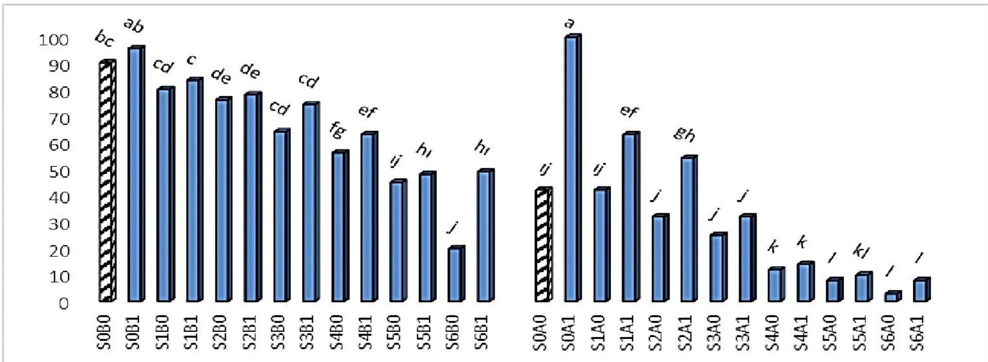

Fig 1: Effect of salt stress and bacteria application on GP in forage pea (%).

There is no difference between the mean shown on bars in the same letter (P≤0.01).

*S_0B_0= control %0 salt + %0 *Bacillus* sp. S_0B_1= %0 salt+ %10 *Bacillus* sp. S_1B_0= %50 salt+ %0 *Bacillus* sp. S_1B_1= %50 salt+ %10 *Bacillus* sp) S_2B_0= %100 salt+ %0 *Bacillus* sp. S_2B_1=%100 salt+ %10 *Bacillus* sp. S_3B_0= %150 salt+ %0 *Bacillus* sp. S_3B_1=%150 salt+ %10 *Bacillus* sp. S_4B_0= %200 salt+ %0 Bacillus sp. S_4B_1=%200 salt+ %10 *Bacillus* sp. S_5B_0= %250 salt+ %0 *Bacillus* sp. S_5B_1=%250 salt + %10 *Bacillus* sp. S_6B_0= %300 salt+ %0 Bacillus sp. S_6B_1=%300 salt+ %10 *Bacillus* sp. S_0A_0= control %0 salt+ %0 *A. agilis* S_0A_1= %0 salt+ %10 A. *agilis* S_1A_0= %50 salt+ %0 *A. agilis* S_1A_1= %50 salt+ % 10 *A. agilis* S_2A_0 = %100 salt+ %0 A. agilis S_2A_1=%100 salt+ %10 *A. agilis* S_3A_0= %150 salt+ %0 *A. agilis* S_3A_1 = %150 salt+ %10 *A. agilis* S_4A_0= %200 salt+ %0 *A. agilis* S_4A_1=%200 salt+ %10 *A. agilis* S_5A_0= %250 salt+ %0 *A. agilis* S_5A_1= %250 salt + %10 *A. agilis* S_6A_0 = %300 salt+ %0 *A. agilis* S_6A_1=%300 salt+ %10 *A. agilis*.

Bacillus sp. and *A. agilis* bacterial strains had higher values compared to salt applications in mean daily germination parameter in the study investigating bacterial applications on germination biology of pea seed with different salt concentrations, no difference was found between bacterial strains. 50% salt + bacteria application yielded the highest mean daily germination value with 5.00. In bacteria × salt interaction, the *A. agilis* strain was found to be tolerant of 250% salt + bacteria application (Fig 3).

Peak value (PV, %)

It was found that bacteria, salt + bacteria applications and bacteria x salt interaction were significant at 1% in the peak value parameter. *Bacillus sp*. strain (3.00) was found to be more effective than *A. agilis* strain (2.42). It was determined that 50% salt + bacteria application got the highest value in both bacterial applications. It was found that *A. agilis* strain was more sensitive than *Bacillus sp*. strain at 100% salt concentration in bacteria × salt interaction (Fig 4).

Germination value (GV, %)

When the germination value parameter was examined, it was found that bacteria and salt + bacteria applications were significant at 1% and bacteria × salt interaction at 5%. It was found that *A. agilis* strain was more sensitive to the salt application compared to *Bacillus sp*. strain, with the highest germination values with 50% salt + bacteria application. When the bacteria × salt interaction was examined, it was

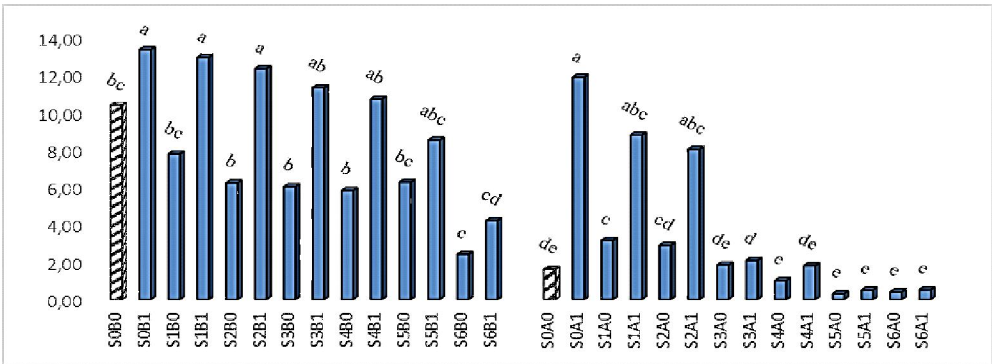

Fig 2: Effect of salt stress and bacteria application on GS in forage pea (day).

There is no difference between the mean shown on bars in the same letter (P≤0.01).

*S_0B_0= control %0 salt+ %0 *Bacillus* sp. S_0B_1= %0 salt+ %10 *Bacillus* sp. S_1B_0= %50 salt+ %0 *Bacillus* sp. S_1B_1= %50 salt+ % 10 *Bacillus* sp) S_2B_0= %100 salt+ %0 *Bacillus* sp. S_2B_1=%100 salt+ %10 *Bacillus* sp. S_3B_0= %150 salt+ %0 *Bacillus* sp. S_3B_1=%150 salt+ %10 *Bacillus* sp. S_4B_0= %200 salt+ %0 *Bacillus* sp. S_4B_1=%200 salt+ %10 Bacillus sp. S_5B_0= %250 salt+ %0 *Bacillus* sp. S_5B_1=%250 salt + %10 *Bacillus* sp. S_6B_0= %300 salt+ %0 *Bacillus* sp. S_6B_1= %300 salt+ %10 *Bacillus* sp. S_0A_0= control %0 salt+ %0 *A. agilis* S_0A_1= %0 salt+ %10 *A. agilis* S_1A_0= %50 salt+ %0 *A. agilis* S_1A_1= %50 salt+ % 10 *A. agilis* S_2A_0= %100 salt+ %0 *A. agilis* S_2A_1=%100 salt+ %10 A. agilis S_3A_0= %150 salt+ %0 A. agilis S_3A_1= %150 salt+ %10 *A. agilis* S_4A_0= %200 salt+ %0 *A. agilis* S_4A_1=%200 salt+ %10 *A. agilis* S_5A_0= %250 salt+ %0*A. agilis* S_5A_1=%250 salt + %10 *A. agilis* S_6A_0= %300 salt+ %0 *A. agilis* S_6A_1=%300 salt+ %10 *A. agilis.*

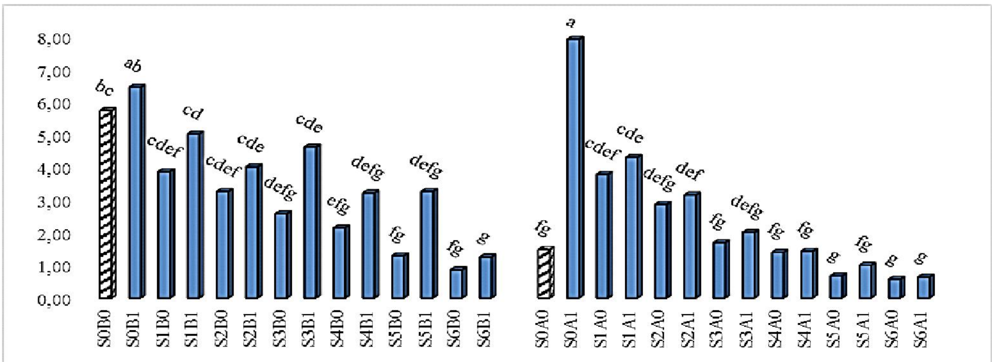

Fig 3: Effect of salt stress and bacteria application on MDG in forage pea (%).

There is no difference between the mean shown on bars in the same letter (P≤0.01).

*S_0B_0= control %0 salt+ %0 *Bacillus* sp. S_0B_1= %0 salt+ %10 *Bacillus* sp. S_1B_0= %50 salt+ %0 *Bacillus* sp. S_1B_1= %50 salt+ % 10 *Bacillus* sp) S_2B_0= %100 salt+ %0 *Bacillus* sp. S_2B_1=%100 salt+ %10 *Bacillus* sp. S_3B_0= %150 salt+ %0 *Bacillus* sp. S_3B_1=%150 salt+ %10 *Bacillus* sp. S_4B_0= %200 salt+ %0 *Bacillus* sp. S_4B_1=%200 salt+ %10 *Bacillus* sp. S_5B_0= %250 salt+ %0 *Bacillus* sp. S_5B_1=%250 salt + %10 *Bacillus* sp. $S_6B_{0=}$ %300 salt+ %0 *Bacillus* sp. S_6B_1= %300 salt+ %10 *Bacillus* sp. S_0A_0= control %0 salt+ %0 *A. agilis* S_0A_1= %0 salt+ %10 *A. agilis* S_1A_0= %50 salt+ %0 *A. agilis* S_1A_1= %50 salt+ % 10 *A. agilis* S_2A_0= %100 salt+ %0 *A. agilis* S_2A_1=%100 salt+ %10 *A. agilis* S_3A_0= %150 salt+ %0 *A. agilis* S_3A_1=%150 salt+ %10 *A. agilis* S_4A_0= %200 salt+ %0 *A. agilis* S_4A_1=%200 salt+ %10 *A. agilis* S_5A_0= %250 salt+ %0 *A. agilis* S_5A_1=%250 salt + %10 *A. agilis* S_6A_0= %300 salt+ %0 *A. agilis* S_6A_1=%300 salt+ %10 *A. agilis.*

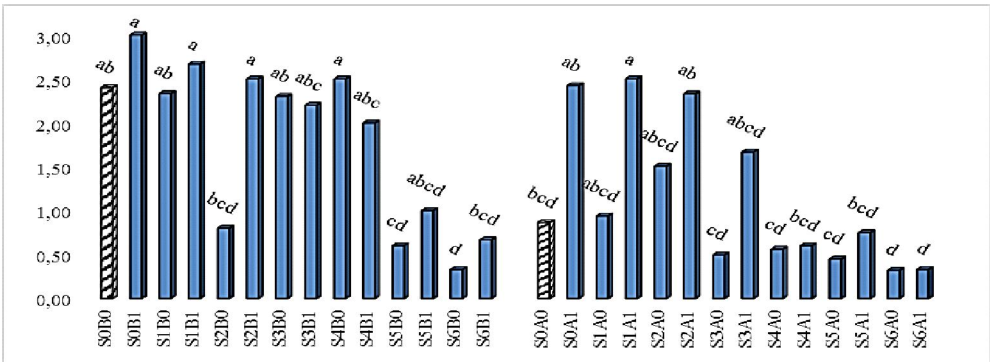

Fig 4: Effect of salt stress and bacteria application on PV in forage pea (%).

There is no difference between the mean shown on bars in the same letter (P≤0.01).

*S_0B_0= control %0 salt+ %0 *Bacillus* sp. S_0B_1= %0 salt+ %10 *Bacillus* sp. S_1B_0= %50 salt+ %0 *Bacillus* sp. S_1B_1= %50 salt+ % 10 *Bacillus* sp) S_2B_0= %100 salt+ %0 *Bacillus* sp. S_2B_1=%100 salt+ %10 *Bacillus* sp. S_3B_0= %150 salt+ %0 *Bacillus* sp. S_3B_1=%150 salt+ %10 *Bacillus* sp. S_4B_0= %200 salt+ %0 *Bacillus* sp. S_4B_1=%200 salt+ %10 *Bacillus* sp. S_5B_0= %250 salt+ %0 *Bacillus* sp. S_5B_1=%250 salt + %10 *Bacillus* sp. S_6B_0= %300 salt+ %0 *Bacillus* sp. S_6B_1=%300 salt+ %10 *Bacillus* sp. S_0A_0= control %0 salt+ %0 *A. agilis* S_0A_1= %0 salt+ %10 *A. agilis* S_1A_0= %50 salt+ %0 *A. agilis* S_1A_1= %50 salt+ %10 *A. agilis* S_2A_0= %100 salt+ %0 *A. agilis* S_2A_1=%100 salt+ %10 *A. agilis* S_3A_0= %150 salt+ %0 *A. agilis* S_3A_1=%150 salt+ %10 *A. agilis* S_4A_0= %200 salt+ %0 *A. agilis* S_4A_1=%200 salt+ %10 *A. agilis* S_5A_0= %250 salt+ %0 *A. agilis* S_5A_1=%250 salt + %10 *A. agilis* S_6A_0= %300 salt+ %0 *A. agilis* S_6A_1=%300 salt+ %10 *A. agilis.*

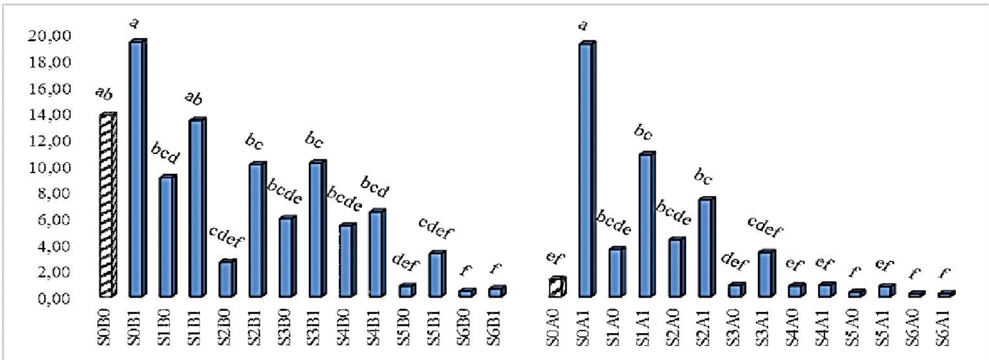

Fig 5: Effect of salt stress and bacteria application on GV in forage pea (%).

There is no difference between the mean shown on bars in the same letter (P≤0.01).

*S_0B_0= control %0 salt+ %0 *Bacillus* sp. S_0B_1= %0 salt+ %10 *Bacillus* sp. S_1B_0= %50 salt+ %0 *Bacillus* sp. S_1B_1= %50 salt+ % 10 *Bacillus* sp) S_2B_0= %100 salt+ %0 *Bacillus* sp. $S_2B_{1=}$%100 salt+ %10 *Bacillus* sp. S_3B_0= %150 salt+ %0 *Bacillus* sp. S_3B_1=%150 salt+ %10 *Bacillus* sp. S_4B_0= %200 salt+ %0 *Bacillus* sp. S_4B_1= %200 salt+ %10 *Bacillus* sp. S_5B_0= %250 salt+ %0 *Bacillus* sp. S_5B_1=%250 salt + %10 *Bacillus* sp. $S_6B_{0=}$ %300 salt+ %0 *Bacillus* sp. $S_6B_{1=}$%300 salt+ %10 *Bacillus* sp. S_0A_0= control %0 salt+ %0 *A. agilis* S_0A_1= %0 salt+ %10 *A. agilis* S_1A_0= %50 salt+ %0 *A. agilis* S_1A_1= %50 salt+ % 10 *A. agilis* S_2A_0= %100 salt+ %0 *A. agilis* S_2A_1=%100 salt+ %10 *A. agilis* S_3A_0= %150 salt+ %0 *A. agilis* S_3A_1=%150 salt+ %10 *A. agilis* S_4A_0= %200 salt+ %0 *A. agilis* S_4A_1=%200 salt+ %10 *A. agilis* S_5A_0= %250 salt+ %0 *A. agilis* S_5A_1=%250 salt + %10 *A. agilis* S_6A_0= %300 salt+ %0 *A. agilis* S_6A_1=%300 salt+ %10 *A. agilis*.

found that the *A. agilis* strain can tolerate up to 250% salt concentration (Fig 5).

In the study, it was found that salt stress has a negative effect on the germination biology of the feed pea line, bacteria reduce the effects of salt stress and *Bacillus sp*. bacteria strain gave better results on germination. Similarly; In a study investigating the effect of salt stress on germination and seedling growth in forage pea genotypes, germination rate, average germination time and plant growth were examined and it was determined that germination rate decreased as salinity increased and germination time extended (Demirkol *et al*. 2019). In a study investigating the effect of salinity stress on forage cowpea on germination and seedling growth, it was stated that as the salt concentration increased, the germination percentage (300 mM) and germination rate decreased (Okçu, 2020). In another study conducted on four different chickpea and pea varieties, control, germination percentage with 50 mM, 100 mM and 200 mM salt, mean germination speed, mean daily germination, peak value and germination value were examined. It was found that there was no germination at 200 mM salt concentration in peas and a decrease in the germination characteristics of both species with increasing salt concentration (Dadasoglu *et al*. 2020). Studies conducted with peas indicate that salt stress negatively affects plant growth (Kaya, 2021; Dadasoglu *et al*. 2021;

Prakash *et al*. 2021). Salinity stress is very important in seed germination. Salinity causes physiological and biochemical changes in seed germination and significantly affects seed germination and plant growth. Growth and germination decrease as salinity damages the plant's metabolism. Under optimum conditions, PGPRs reduce the effect of various biotic and abiotic environmental stress factors (such as salinity, drought). The bacterial strains applied in the study were found to be effective on the germination biology of pea seeds compared to the control. Nadeem *et al*. (2006), in their study examining corn under salty conditions, found that salt stress would be more harmful in a bacteria-free environment where S5, S15 and S20 strains could prevent adverse effects of salinity stress. In the study conducted with corn, *rhizobacteria* with different salt concentrations were applied and it was found that *P. fluorescens A* (N3) strain positively affected plant growth parameters in EC 9 dS m^{-1} (Kausar *et al*. 2006).

In our study on the biology of forage pea seed germination with different salt concentrations, it was determined that the biology of seed germination was negatively affected as the salt concentration increased. *Bacillus sp*. and *Arthrobacter agilis* strains minimized this negative effect. A better improvement was found at 50% salt + bacteria concentration.

CONCLUSION

In the study carried out, it was found that bacterial applications are effective in pea germination physiology under salt stress and gave better results than the results obtained under bacteria-free conditions. In terms of germination biology, it was found that the nitrogen-fixing *Bacillus sp*. strain gave a relatively better result than the phosphorus solubilizing *Arthrobacter agilis* strain. The effectiveness of different bacterial strains on the same plant may be different. At the same time, it was found that 50% salt + bacteria application had a positive effect on forage pea seed germination biology.

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