Seed priming with bio-priming improves stand establishment, seed germination and salinity tolerance in canola cultivar (Hayola 401)

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Abstract

This research has been done to examine the effect of bio-priming on germination parameters related to seeds of canola cultivar (Hayola 401) under high salinity as factorial in seed laboratory of Shahed University based on a Completely Randomized Design (CRD) in three replications. Experimental factors included bio-priming with bacteria strains inoculation at three levels: seed inoculation with water as a control, inoculation of seed with *Bacillus subtilis* bacteria, inoculation of seed with *Macrophomina phaseolina* fungus, and combination of *Bacillus subtilis* bacteria and *Macrophomina phaseolina* fungus. Salinity treatment was applied at four levels (0, 2.5 $dS.m^{-1}$, 5 $dS.m^{-1}$, and 7.5 $dS.m^{-1}$). Results of variance analysis showed that the effect of priming and high salinity was meaningful for all characteristics. The highest length measurement for the radicle and relative content of leaf water were obtained in salinity 2.5 $dS.m^{-1}$. The maximum root and seedling length and length matrix of seedling vigor were related to fungus priming in bacteria and high salinity as 7.5 $dS.m^{-1}$. Seeds primed with fungi under high salinity 2.5 $dS.m^{-1}$ produced the highest mean dry weight of seedlings, germination percentage, and the mean germination. Also, the results included the highest mean germination period, germination speed factor, and germination rate for bacteria priming in the salinity control. Generally, according to the results, using *Bacillus subtilis* bacteria and *Macrophomina phaseolina* fungus could improve germination index and modify the effects of salinity.

Keywords: Hayola 401; salinity; germination; *Bacillus subtilis* bacteria; priming

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Introduction

Canola seed (*Brassica napus* L.) is the third major world source of edible seed oil after soybeans and palm (Singh and Sharma, 2016). Abiotic stresses such as salinity always limit growth, distribution, and production of crops, especially in arid and semi-arid regions. According to a recent estimate, 1128 million ha of global land is affected by salinity and sodicity (Akhtar et al., 2015). Salinity is one of the most limiting factors for agricultural productivity worldwide causing the decrease in crop average yields by more than 50% (Singh et al., 2015). In addition to the toxic effects of the sodium and chloride ions, salinity disturbs

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plants' water relations due to the decreased availability of water from soil solution as a result of lowered osmotic potential. Different plant species have developed various mechanisms to cope with these effects (Hameed et al., 2012). Seed germination as the most important stage of plant growth is also affected by salinity. Increased Na^+ uptake in plants under salinity disturbs those metabolic processes that require low Na^+ and high K^+, Ca^{2+} or both for normal functioning (Akhtar et al., 2015). Physiological changes including stomatal conductance lowered water potential and osmotic adjustment in plants grown under salt conditions have been considered as effective responses to saline conditions (Alvarez and Sanchez-Blanco, 2014). Seed priming, as a low-cost method, is a technique commonly used to increase germination percentage especially under unfavorable conditions (Ataei Somagh et al., 2016). Seed pretreatment has been considered to be a common economic strategy with low risk for increasing the germination percentage, speed, uniformity, seed growth, and also improving the quality and quantity of crops under the unfavorable environmental situation which can increase plant resistance against salt stress. (Riazi, 2008). This research has been done to examine the effect of bio-priming on germination parameters related to canola seed (Hayola 401) under salt stress.

Material and Methods

Experimental design and seed priming

The experiment was conducted at Seed Biology Laboratory, Department of Environmental Science, Shahed University, Iran to examine the effects of bio-priming on the germination index of canola seeds (Hayola, 401) under salt stress in 2015.

Pretreatment and the composition of bacteria and fungus

The two factors of the study included four levels of bio priming solution and four levels of natural salinity. The experimental design was randomized blocks with a factorial design of 4 × 4, including four bio priming or microbial priming (non-inoculated seeds that were only hydro primed with distilled water, an absolute control, an inoculation with *Bacillus subtilis*bacteria, an inoculation with *Macrophomina phaseolina* fungus, and co-inoculations with bacteria and fungus); also, there were four salinity levels in the study (0, 2.5 $dS.m^{-1}$, 5 $dS.m^{-1}$, and 7.5 $dS.m^{-1}$) (Table 1). Different concentrations of salt were used during the experiment to get the electrical conductivities (EC) 0, 2.5, 5, and 7.5 $dS\ m^{-1}$ as shown in Table 1.

Table 1
Chemical analysis of water sample of Lake Houzz Soltan

EC (ds/m)	Cl^- gr l^{-1}	$SO4^{2-}$ gr l^{-1}	Ca^{2+} gr l^{-1}	M g^{2+} gr l^{-1}	Na^+ gr l^{-1}	K^+ gr l^{-1}
690	218.7	48.8	0.086	19.5	128	1.23

Sterilizing of petri dish and seeds

Canola seeds (*Brassica. napus* L.) were purchased from Pakan Bazr Company, Isfahan, Iran. Prior to seed priming, the seeds were surface sterilized. Seeds were then rinsed with water and distilled water, and surface sterilized in 40% sodium hypochlorite (v/v) for 10 min, followed by 70% alcohol (v/v) for 10 s, and again rinsed thoroughly with sterile deionized water before they were primed. One hundred seeds per replication were put in each Petri dish. Seeds were taken into a glass beaker and soaked in priming solution.

Microorganisms and preparation of the inoculant

Caryopses were stripped from the spikes and those with seeds were selected and stored in the dark for 4 months at 4 °C until the beginning of the experiment. Bacteria used in this work were isolated from the rhizosphere of the Taleghan area (Karaj, Iran). *Bacillus subtilis* bacteria. (BS 04-542 strain) were obtained from pasture soil samples collected in Taleghan. Bacillus *subtilis* was purified and multiplied in dextrose yeast glucose sucrose (DYGS) medium at pH 6.0 (Rodrigues Neto et al., 1986). One bacterial strain was cultivated separately in 100 ml of Tryptic Soy Broth (TSB) medium at 28° C and was continuously shaken (200 rpm) for 24-48 h depending on the bacterium. Cultures were centrifuged at 8000 g

and pellets were suspended in sterile distilled water for the inoculation process. The absorbance of the cultures at 600 nm was determined and adjusted to 1.5 for all cultures with sterile distilled water. Moreover, in order to guarantee that there were no antagonistic effects between the strains in the consortium, an antagonist test was done as follows: cultures of the three strains with equal absorbance at 600 nm were mixed. The strain of entomopathogenic fungi was obtained from the fungal collection of ''Karaj Institute" (IKs), Karaj, Iran and preserved by freeze-drying (lyophilization) technique. To confirm the identity of strains already characterized on the basis of morphological characters according to the taxonomic keys of Humber (2012), molecular techniques were used (Humber, 2012). Seeds were inoculated with *Macrophomina phaseolina* fungi and maintained in a greenhouse for two days before germination test and thus, seed inoculation was checked for colonization according to Russo et al. (2015).

Non-inoculated seeds were used as absolute control; co-inoculation was accomplished with 1.0 ml of a bacterial suspension containing *Bacillus subtilis* and 1.0 ml of a *Macrophomina phaseolina* suspension containing a strain (10^7 $UFCml^{-1}$).

The beakers were covered with a clean paper and then kept for 24 h in the absence of light (25 ± 1° C). After 24 h the seeds were withdrawn from the solution and dried by keeping between two layers of some filter paper followed by keeping under bright light till they were completely dry i.e. ± 10% of initial weight. The dried seeds were then kept in an airtight plastic zipper pouch at 25 ± 1° C for two days (Basra et al., 2005). For each treatment, one hundred seeds were kept between two layers of filter papers in a glass Petri dish 90 mm in diameter for 5 and 10 days (d).

The Petri dishes were kept inside an incubator at 26 ± 0.5° C under aphotic conditions. Seeds were considered as germinated only when radical lengths were found to be ≥ 2 mm. after 5 d and 10 d, the seedlings were washed in distilled water and used for measurements of germination parameters and seedlings growth.

Germination parameters and seedlings growth

The germinated seeds were counted daily at a determined time. Seeds with rootlet lengths of 2 mm were considered as germinated (ISTA, 2010). After the period of seed germination, the length of seedlings was measured with a cm ruler. In order to measure the dry weight, the germinated seeds were first kept in an oven (70° C) and then weighed (mg).

Germination percentage (GP) was calculated as follows: at the end of the experiment period (day 10), the number of germinated seeds were reported based on a percentage :

$$GP = (N \times 100) / M$$

In this equation N, M, and G are the total germinated seeds at the end of the experiment, the total seeds planted, and the germination percentage, respectively.

Mean germination time (MTG), i.e. the mean time needed for germination, is a matrix for the speed of germination measured as below (Ellis and Roberts, 1981):

$$\mathrm{MTG} = \frac{\sum(\mathrm{nd})}{\sum \mathrm{n}}$$

where n is the number of germinated seeds per day and d is the number of days since the start of the experiment.

Mean daily germination (MDG) is a matrix for daily germination speed which was measured as follows (Scott et al, 1984):

$$MDG = \frac{FGP}{d}$$

In this equation, FGP is the index for final germination percentage and d is the index for the days needed for the final germination (during the experiment period).

Germination coefficient (GC) which is the exact point of the Mean Germination Time was calculated as the following equation (Ghasemi et al., 2012):

$$\mathrm{GC} = \frac{1}{MTG} \times 100$$

where GC is the germination coefficient and MTG is the time needed for germination.

Coefficient velocity (CV), which includes the inverse multiplication of mean germination time by germination percentage, was calculated

using the following equation (Ghasemi et al., 2012):

$$CV = \frac{1}{MGT} \times \mathrm{GP}$$

where CV is the germination coefficient, MTG is the mean time for germination, and PG is the germination percentage.

Uniformity germination (UG) was calculated as follows (Omidiet al., 2015):

$$UG = \frac{1}{V} \times 100$$

where UG is the mean time for germination and V stands for germination variance.

To measure the length index of seedling vigor, 5 seedlings were randomly selected and then the lengths of radicles and rootlets were measured at the end of the experiment. In the next step, the length index of seedling vigor was identified by the following equation (Omidiet al., 2015):

Germination percentage × (average root length (RL) of primary root + the average length of the primary stem (SL)) = the length index of seedling vigor (SVI).

$$\mathrm{SVI} = (\mathrm{RL} + \mathrm{SL}) \times \mathrm{GP}$$

Germination rate (RG) which includes the inverse of mean germination time was calculated using this equation :

$$\mathrm{RG} = \frac{1}{MTG}$$

Germination variance (VG) was calculated using the following equation (Omidi et al., 2015):

$$\mathrm{VG} = \frac{\sum(\mathrm{Di} - \overline{\mathrm{D}})2ni}{\sum \mathrm{N}}$$

where Di is the number of days after planting, N is the number of germinated seeds, and VG is the germinated variance.

In order to measure the relative water content (RWC) or the relative moisture of leaf, plant leaves were put in a test tube with a cap and distilled water content and, after 6 hours, the weight loss of leaves were determined.

Finally, the relative leaf humidity was obtained by the following equation (Singh, 2016):

$$\mathrm{RWC} = \frac{(\text{fresh weight} - \text{dry weight})}{(Saturatedleafweight - dryweight)} \times 100$$

Statistical methods and data analysis

All analyses were performed based on a factorial experiment in a completely randomized design. Each data point was the mean of three replicates (n=3) and the least significant difference (LSD) option of the Statistical Analysis System software (Version 9.4; SAS Institute; USA). The data recorded on seed germination, germination rate, radicle elongation, plumule elongation, dry weight seedling, vigor index, radicle length, seedling length, mean germination time, and germination rate were pooled to get a mean value and analyzed statistically. The F-test was considered significant at $P<0.05$ between treatments for halo-priming and bio-priming.

Results

The shoot length

Results showed no significant difference between the effects of different levels of priming and shoot length. Salinity (2.5 $dS.m^{-1}$) resulted in the maximum mean shoot length (3.9 cm) while at salinity level (distilled water) also led to the minimum mean shoot length (2.5 cm) (data not shown). Also, seed priming with fungi increased the shoot length to %40 as compared with the control (distilled war). According to index correlation table (Table 4), the shoot length showed a significant positive correlation with seedling indices (r=0.1635), germination speed coefficient (f=0.364), germination uniformity (r=0.419), the seedling vigor index (r=%631), and relative water content (r=%346). This index also showed a significant negative correlation with germination variance (r=%615).

Table 2
One-way ANOVA for the effect of bio-priming and salinity treatment on various parameters in germination of canola

Parameter	Bio-priming	Salinity	Salinity × Bio-priming	Error	Coefficients of Variation (%)
Plumule length	1.51**	3.19**	0.38ns	0.28	0.53
Radicle length	3.61ns	30.53**	5.95**	1.65	1.28
Length seedling	7.12**	47.89**	6.95**	1.67	1.29
Seedling dry weight	84.20**	29.58**	51.68**	6.18	2.48
Germination percentage	32.15*	73.01**	62.71**	10.04	3.16
Mean germination time	0.084ns	0.055ns	0. 242*	0.096	0.310
Mean daily germination	2.009 *	4.553**	3.915**	0.630	0.45
Germination rate	90.52ns	50.47ns	212.35**	41.74	6.46
Coefficient of germination rate	60.49ns	9.01ns	117.71**	28.19	5.3
Uniformity of Germination time	21685.37ns	4230.98ns	12290.42ns	7901.08	8.88
Longitudinal index Seedling vigor	467.21**	1160.55**	464.21 **	86.83	9.31
Germination rate	0.009ns	0.005ns	0.021**	0.004	0.06
Variance germination	0.196ns	0.094ns	0.085ns	0.081	0.28
Relative water content	280.71ns	1742.19**	305.46ns	314.11	17.72

ns, * and **respectively significant and non-significantat probability level is 5 and 1 %

Rootlet length

According to the results of variance analysis (Table 2), the main effects of salinity and interactions of salinity and priming on rootlet length were significant (P≤0.01). Table 3 shows that the level of fungus priming bacteria with 7.5 dS.m^{-1} salinity produced maximum rootlet length (11.5 cm); on the other hand, the difference was not significant with the same level of priming with 5 dS.m^{-1} salinity while a significant difference was observed with other levels. The minimum mean of this attribute (3.2 cm) was recorded for the fungus level in priming bacteria with a level of non-salinity. As Table 3 shows, a significant correlation was observed between the rootlet length and some germination traits.

The seedling length and seedling dry weight

All study treatments including priming, salinity, and the interaction between salinity and priming had statistically significant effects (P≤0.01) on the seedling length and seedling dry weight (Table 2). The maximum length of seedling (15.6 cm) was obtained with the combination of priming treatments of the fungus level in priming bacteria and the 7.5 dS.m-1 salinity, which was in the same category as fungus treatment with 7.5 dS.m^{-1} (Table 3). This suggests that increased seedling length in the study depended on the increased rootlet length. Table 4 shows that seedling length with the coefficient of germination rate (r=0.425*) and water RWC (r=0.416*) had significant positive correlation while it had a significant negative correlation with germination variance (r=-0.233**).

As for the dry weight of seedlings, the result of the mean comparison (Fig. I) showed that priming seeds with fungi in 2.5 dS.m^{-1} salinity produced maximum dry weight (34.5 milligrams), which differs from other levels. Also, the mean comparison of this attribute showed that rising salinity to 7.5 dS.m^{-1} caused a decrease in this attribute to 13.5 milligrams under this combined treatment. The dry weight of seedlings showed a positive correlation with an average time of germination (r= 0.135**) and RWC (r= 0.198**) while its correlation with the coefficient of germination rate (r = -0.128**) was negative (Table 4).

Germination percentage

The germination percentage was affected by priming (P≤.05), salinity (P≤0.01), and the interaction of priming and salinity (P≤0.01) (Table 1). Considering the mean comparison Table (Table 2), the maximum percentage of germination (100 percent) was under the effect of priming treatment with fungi and 2.5 dS.m^{-1} salinity. The minimum mean germination percentage (75.81 percent) was recorded in priming control with bacteria (Table 3).

Table 3
Comparison of the mean characteristics of canola germination influenced by the interaction of salinity and bio-priming

Treatment		Rootlet length (Cm)	Seedling length (Cm)	Germination percentage (%)	Mean germination time (day)	Mean daily germination (number)	Germination coefficient	Seedling length vigor index	Germination rate
Bio Priming	Salinity ($DS.m^{-1}$)								
Control	0	4.73^{g}	6.59^{f}	$96.07^{a..d}$	2.22^{a}	$24.02^{a..d}$	45.03^{bc}	49.28^{g}	0.45^{b}
	2.5	7.27^{def}	11.10^{cde}	$95.67^{a..d}$	1.97^{a}	$23.91^{a..d}$	51.74^{bc}	55.87^{cde}	0.50^{b}
	5	8.67^{bcd}	11.11^{cde}	$89.83^{c..e}$	2.39^{a}	$22.46^{c..f}$	41.89^{c}	41.75^{efg}	0.42^{b}
	7.5	$8.47^{b..e}$	11.51^{dc}	85.89^{f}	2.14^{a}	21.47^{f}	46.74^{bc}	46.14^{defg}	0.46^{b}
Fungi	0	5.92^{efg}	8.47^{ef}	87.79^{ef}	2.34^{a}	21.95^{ef}	45.84^{bc}	33.63^{fg}	0.46^{b}
	2.5	4.65^{fg}	8.81^{def}	100^{a}	2.07^{a}	25.02^{a}	48.65^{bc}	43.02^{efg}	0.48^{b}
	5	$9.93^{a..d}$	14.69^{ab}	$93.16^{b..e}$	1.79^{ab}	$23.29^{b..e}$	55.73^{b}	78.21^{ab}	0.55^{b}
	7.5	10.77^{ab}	14.91^{a}	$93.94^{a..e}$	1.83^{ab}	$23.48^{a..e}$	54.55^{bc}	76.39^{ab}	0.54^{b}
Bacteria	0	7.48^{de}	10.20^{cde}	75.81^{g}	1.28^{b}	19.70^{g}	78.36^{a}	62.97^{bcd}	0.68^{a}
	2.5	8.01^{cde}	11.58^{c}	$94.44^{a..e}$	2.26^{a}	$23.61^{a..e}$	44.15^{bc}	48.25^{def}	0.44^{b}
	5	7.65^{de}	11.07^{cde}	96.66^{ab}	2.29^{a}	24.16^{ab}	44.97^{bc}	49.36^{def}	0.45^{b}
	7.5	7.60^{de}	11.43^{cd}	88.48^{ef}	1.82^{ab}	22.12^{ef}	55.05^{bc}	55.52^{cde}	0.55^{b}
Fungi and bacteria	0	3.60^{g}	6.61^{f}	$9016^{b..f}$	2.24^{a}	$22.54^{b..f}$	45.48^{bc}	27.04^{g}	0.45^{b}
	2.5	7.92^{cde}	11.99^{bc}	89.44^{def}	1.93^{ab}	22.36^{def}	51.78^{bc}	55.54^{cde}	0.52^{b}
	5	10.52^{abc}	14.54^{ab}	96.31^{abc}	1.89^{ab}	24.08^{abc}	52.94^{bc}	74.13^{abc}	0.53^{b}
	7.5	11.5^{a}	15.61^{a}	96.66^{ab}	1.80^{ab}	24.16^{ab}	55.32^{bc}	83.44^{a}	0.55^{b}

In each column means having at least one same letter were not significantly different according to Duncan's multiple range test ($p \leq 0.05$)

Mean germination time

Among all treatments under experiment, only the interaction of priming in salinity was significant (Table 2). The seeds in priming bacteria growth from non-salinity (control) took less time to germinate. So the minimum mean germination time (1.28 days) was obtained in this combined treatment. The maximum average of this attribute (2.39 days) was obtained for non-priming in 5 $dS.m^{-1}$ salinity (Table 3).

Mean daily germination

The mean daily germination is an indicator of daily germination rate. As the analysis of variance shows (Table 2), the effects of priming treatment ($P \leq 0.05$), salinity ($P \leq 0.01$), and the interaction of priming and salinity ($P \leq 0.01$) on mean daily germination were significant. The priming treatment with 2.5 $ds.m^{-1}$ salinity produced maximum mean daily germination rate (25.05) (Table 3) while the minimum mean (19.07) was recorded for priming with bacteria in salinity control. The mean daily germination showed a negative correlation with the germination rate (r = -0.508**) (Table 4).

Germination coefficient and the coefficient of velocity

Analysis of variance (Table 2) showed that the interactions of priming and salinity for both germination means and coefficient of velocity ($P \leq 0.01$) were significant so that unlike the mean germination time, the maximum germination mean (78.36) and germination rate (61.74) were observed in priming treatment under salinity control while minimum means for both attributes (41.89) and (37.61) were obtained in priming control in 5 $ds.m^{-1}$ salinity (Table 3 and Fig. II). Also, the coefficient of velocity showed a positive relationship with radicle length (r= 0.364**) and seedling length (r=0.425*) while its correlation with the dry weight of rootlet was negative (Table 4).

Table 4
Pearson correlation coefficients between traits related to germination of canola plants affected by bio-priming and salinity

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Shoot length	1												
2. Rootlet length	396.0^{ns}	1											
3. Seedling length	635.0 **	960.0^{ns}	1										
4. seedling dry weight	117.0^{ns}	-023.0^{ns}	015.0^{ns}	1									
5. Germination percentage	299.0^{ns}	006.0^{ns}	095.0^{ns}	-121.0^{ns}	1								
6. Mean germination time	302.0-^{ns}	-363.0^{ns}	-397.0^{ns}	135.0**	381.0^{ns}	1							
7. Mean daily germination	298.0^{ns}	005.0^{ns}	095.0^{ns}	000.1^{ns}	381.0^{ns}	-121.0^{ns}	1						
8. Germination coefficient	178.0^{ns}	288.0^{ns}	296.0^{ns}	-078.0^{ns}	-505.0^{ns}	-958.0^{ns}	-505.0^{ns}	1					
9. Coefficient of velocity	364.0**	375.0^{ns}	425.0*	-128.0 **	-150.0^{ns}	-948.0^{ns}	-150.0^{ns}		1				
10.Uniformity of germination	419.0**	057.0^{ns}	175.0^{ns}	098.0-^{ns}	427.0^{ns}	-082.0^{ns}	427.0^{ns}	-043.0^{ns}	140.0^{ns}	1			
11. Seedling length vigor index	631.0**	871.0^{ns}	925.0^{ns}	-030.0^{ns}	039.0^{ns}	-675.0^{ns}	038.0^{ns}	592.0^{ns}	731.0^{ns}	205.0^{ns}	1		
12. Germination rate	177.0^{ns}	921.0^{ns}	298.0^{ns}	-073.0^{ns}	-508.0^{ns}	-956.0^{ns}	-508.0**	999.0^{ns}	923.0^{ns}	-041.0^{ns}	592.0^{ns}	1	
13. Germination variance	415.0**-	-821.0**	-233.0**	228.0^{ns}	-330.0^{ns}	215.0^{ns}	-330.0^{ns}	-062.0^{ns}	218.0-^{ns}	-878.0^{ns}	266.0^{ns}	-061.0^{ns}	1
14. Relative water content	346.0*	370.0**	416.0*	198.0 **	190.0^{ns}	-040.0^{ns}	190.0^{ns}	006.0^{ns}	095.0^{ns}	097.0^{ns}	339.0^{ns}	003.0^{ns}	159.0*

ns, *, and ** non-significant, significant at 5%, and 1%, respectively

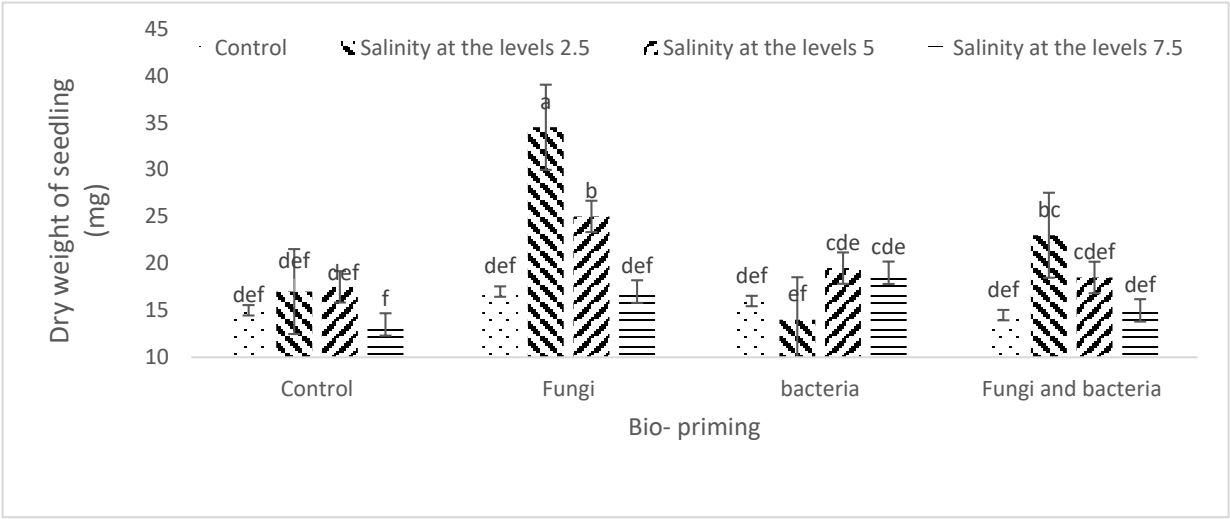

Fig. I. The effects of bio-priming techniques and salinity levels on dry weight of canola seedling

Seedling length vigor index

The seedling length vigor index was significantly affected by priming, salinity, and interaction of priming in salinity (P≤0.01) (Table 2). The result of the mean comparison (Table 3) showed that priming treatment with fungus in bacteria under salinity with 7.5 $dS.m^{-1}$ produced maximum seedling length vigor index (83.44) while for fungus treatment in bacteria with salinity control level showed the minimum (27.04) seedling length vigor index (Table 3 and Fig. III). Seedling length vigor index showed positive relationship with radicle length (r=0.631**) (Table 4).

Germination rate

Interaction of priming and salinity significantly affected the germination rate (P≤0.01). The Table 2 shows that the maximum (0.068) and minimum (0.42) germination rates were recorded in bacteria priming treatment

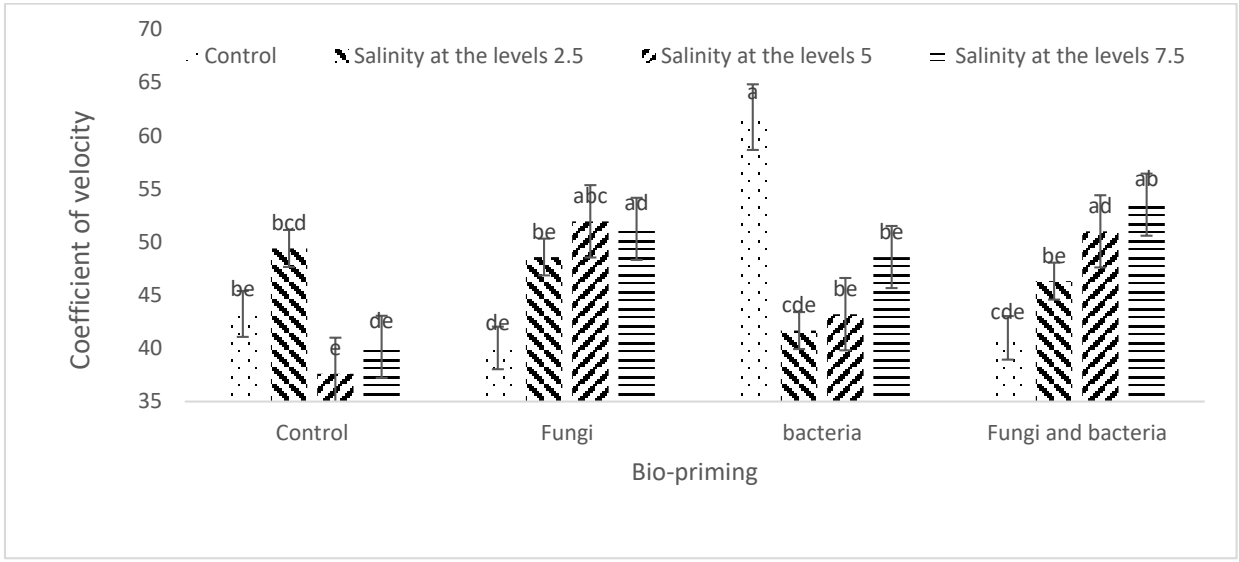

Fig. II. The effects of bio-priming techniques and salinity levels on germination of coefficient of canola

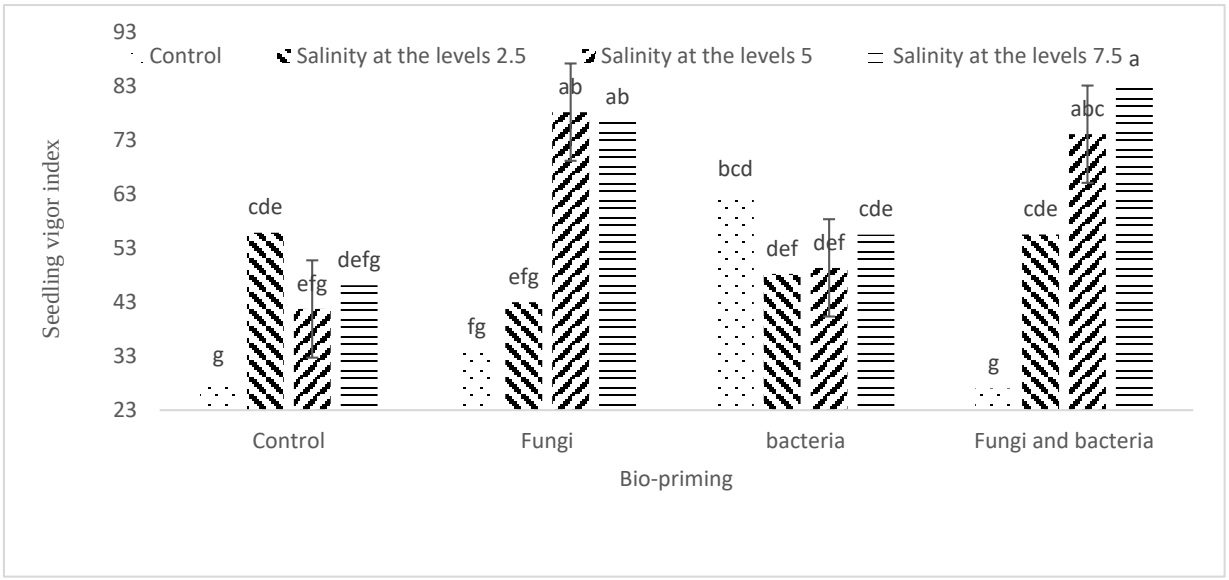

Fig. III. The effects of bio-priming techniques and salinity levels on the seedling vigor of canola

under salinity control and priming control at 5 $dS.m^{-1}$ salinity (Table 3 and Fig IV).

Leaf relative water content

Among the experimental treatments, the effect of salinity on leaf relative water content ($P \leq 0.01$) was significant so that 2.5 $dS.m^{-1}$ salinity level resulted in more relative water content percent than other treatments. The mean percentage of leaf relative water content of this treatment was 90.5% which was at the same category as 5 and 7.5 $dS.m^{-1}$ salinity level while the minimum mean of this attribute (55.5%) was observed under salinity control (Fig. V).

Discussion

As one of the main environmental limiting factors, salinity has a negative impact on the growth and yield of plants. Results of the present work showed that canola could tolerate salinity stress up to 7.5 $dS.m^{-1}$. However, most of the study traits drastically decreased when the stress severity rose up to 7.5 $dS.m^{-1}$ (Table 3). Likewise, studies have shown that salinity has had a negative effect on barley (Sing and Sharma, 2016), rapeseed (*Brassica napus*) (Ataie Somagh et al.,

2016), and corn (*Zea maize* L.) (Akhtar, et al., 2015).

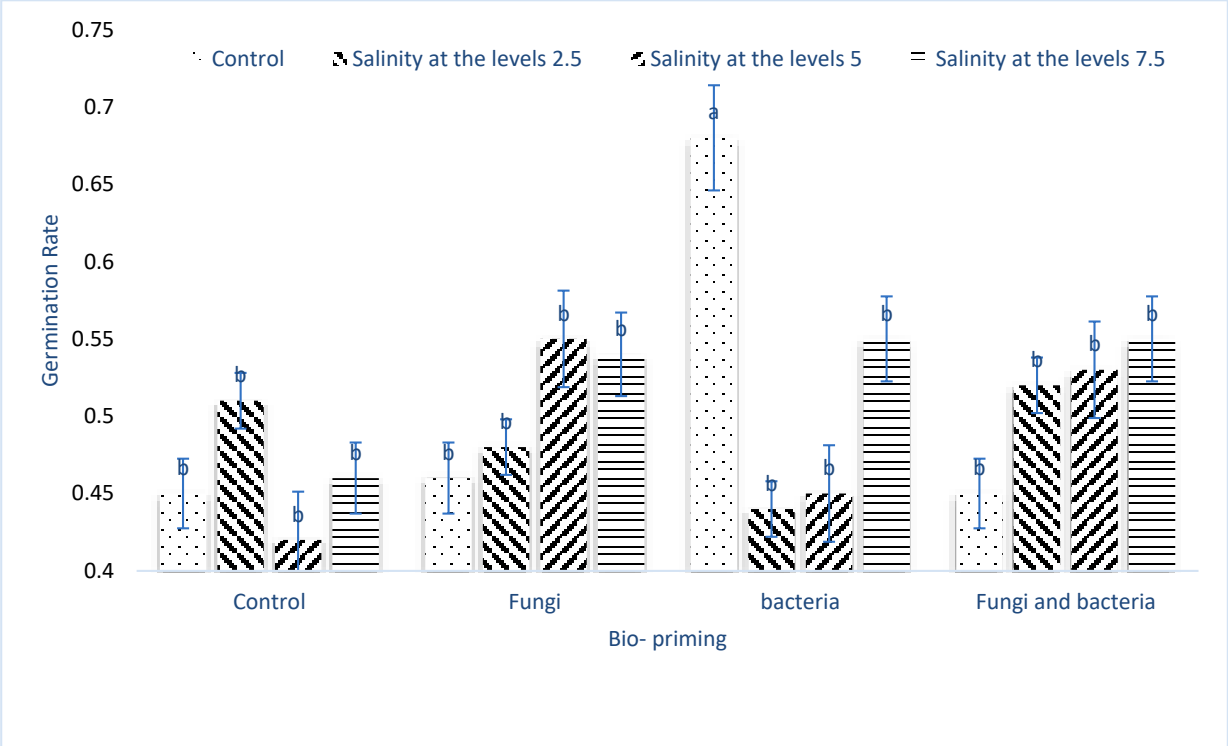

Fig. IV. The effects of bio-priming techniques and salinity levels on the germination rate of canola

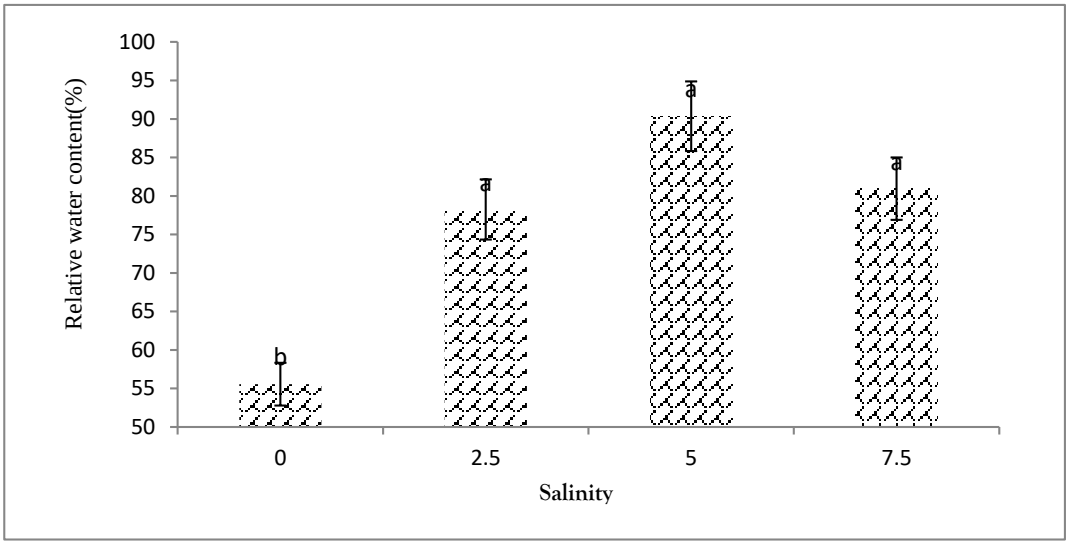

Fig. V. The effect of salinity on the relative water content of canola shoots

In this study, salinity stress had a more negative effect on radicle weight (RW) than shoot weight (SW) (Table 3) which was in contrast with the results reported by (Ataie Somagh et al., 2016). Previous researchers have shown that reduction in RW and SW was mainly due to the ionic effects especially the proportional increase in Na^+ concentrations. Moreover, the presence of Na^+ and Cl^- ions in the cells may induce changes in protein activity because ions affect the structure of the hydration water which surrounds the protein molecule (Sing and Sharma, 2016).

In this study, salt stress remarkably inhibited root development showing that inhibition occurred at root cell proliferation (Ataie Somagh et al., 2016). There are some mechanisms at work including high concentrations of NaCl in the environment leading to a decrease in water

potential. Consequently, plant cells are faced with some difficulties to absorb external water.

The data presented in the study indicate that all studied traits reduced with an increase in salinity concentration. However, priming increased their values in the presence of salinity stress. At the salinity level of 7.5 $dS.m^{-1}$, inoculation of canola seeds with B. subtilis and M. *phaseolina* increased seed vigor index (SVI) up to 36% and 14%, respectively as compared with control. It seems that a significant increase in germination percent (GP) compared to control was the main reason for this phenomenon. Although root length decreased at 5 and 7.5 $dS.m^{-1}$ levels of salinity, the germination percent (GP) was still considerably high for the seeds inoculated with *M. phaseolina* (Tables 2 and 3). As shown in Table 2, seed inoculation with fungi promoted GP and at the same time reduced the radicle length (RL). One possibility could be that the increase in seedling density as a result of increased GP reduced RL, because in the presence of higher seed germination, the total amount of water absorbed by seedlings would be higher and this results in a reduced amount of available water. Thus, less available water along with salt treatment reduced the root length.

In conclusion, findings of the present work showed that bio-priming with the plant growth-promoting microorganism (PGPM) was an effective technique to improve germination percentage and rate in canola (*Brassica napus* L. var. Hyola 401) seeds under saline conditions (Table 3 and Fig. I). Plant growth-promoting regulators (PGPR) refer to bacteria inhabiting around/on the root surface that promotes plant growth and development. In this study a fungus strain was also applied; therefore, we used the term plant growth-promoting microorganism (PGPM) rather than plant growth-promoting regulators (PGPR). Moreover, most of the studies about growth promotion due to bio-priming with PGPM have been conducted under normal conditions while we have investigated the effect of PGPM under salinity stress. Therefore, the results of this study may provide useful information concerning the reduction of undesirable effects of salinity on canola seed germination.

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