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Effect of *Azospiillum lipoferum* and *Thiobacillus thioparus* on Quantitative and Qualitative Characters of Rapeseed (*Brassica napus* L.) Under Water Deficit Conditions

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Abstract: In order to investigate the effect of *Azospirillum* and *Thiobacillus* bacteria on quantitative and qualitative traits of canola (*Brassica napus* L.) under water deficit condition, a field experiment was carried out as split plot arrange in randomized complete block design (RCBD) with four replications at Research Field of Agriculture and Natural Resources, Islamic Azad University-Karaj Branch, Karaj, Iran, during 2008/9. There were two factors. Irrigation at four levels as main factor: irrigation after depletion of 40% (D_0), 60% (D_1), 75% (D_2) of available soil moisture and stop irrigation (D_3) after flowering stage in main plot and bacteria application with four levels: *Azospiillum lipoferum* (B_1), *Thiobacillus thioparus* (B_2), *A. lipoferum* + *T. thioparus* (B_3) and control (B_0) in sub plot. Some traits such as number of sub shoot per plant, number of sub-sub shoot per plant, length of pod per main shoot, number of pods per plant, number of pods per m^2, seed yield, biological yield, harvesting index and weight of 1000 seeds were measured. The results showed that the simple effect of water deficiency was significant on seed yield, biological yield and 1000 seeds weight in probability of 1%, on number of pods per m^2 in probability of 1% and no significant was observed on other traits. Water stress decreased seed yield by 46% from 2485.8 kg/ha to 1334.6 kg/ha, biological yield from 10403.1 kg/ha to 6285.7 kg/ha, 1000 seeds weight from 5.6 g to 4.9 g, number of pods per m^2 from 6909.2 to 6707.5 in control treatment in comparison with severe stress. Also the simple effect of bacteria on number of sub shoot and sub-sub shoot per plant was significant in probability of 1% and no significant observed on other traits, so the application of *Thiobacillus* bacterium significantly increased these two factors compared with *Azospirillum* bacterium and control treatments. Also the interaction effect of stress and bacteria was significant on number of sub-sub shoot per plant, length of pod per sub shoot and number of pods per m^2 in probability of 5%.

Key words: Oilseed rape · Water deficit · Biological fertilizers · Yield

INTRODUCTION

Abiotic stresses are the main cause of crop failure worldwide, dipping average yields for most major crops by more than 50% [1]. Water deficit more than order stresses limits the growth and the productivity of crops [2]. The average annual yield loss due to drought was estimated between 17 to over 70 percent in the world [3]. Without sufficient water to maintain transpiration, leaf temperature can rise above their optimum for metabolism. Therefore, plants under low water availability are more prone to heat stress, too [4].

Rapeseed is more resistant to water stress in comparison with turnip due to better physiological properties such as high ratio of root: shoot and higher efficiency for material transport to the grains. Winter cultivars of oilseed rape also are more drought resistant than spring cultivars. Under drought condition, the secondary root system of canola change to a short glandular form and will be elongated only after providing moisture, but basically is susceptible to drought during germination and pod growth stage [5]. Mehrnia *et al.* [6] observed that the effect of water stress between early pod formation stage to two weeks after that was significant in

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1% probability and drought stress at flowering and pods filling stages decrease their growth. Also Palomo *et al.* (1999) [7] reported that if drought stress has extended two weeks after pollination with 50% of field capacity, seed yield decrease by 20%. When plant reaches to its final growth stages, decrease the water requirement and if water deficit remove before maturity, there is a little compensation.

Agricultural manipulation of symbiotic and free-living plant-growth-promoting rhizobacteria has become a significant of modern agriculture practice in many countries [8]. Plant growth promoting rhizobacteria (PGPR) are free living soil born bacteria that mostly live in near or in plant roots. They promote plant growth and yield either directly or indirectly. The direct mechanisms of plant growth promotion may involve the synthesis of substances by the bacterium or facilitation of the uptake of nutrients from the environment [9]. In addition, the use of PGPM as inoculants could contribute to minimize the negative effects of several plant stresses, including water stress [10]. In many reports, *Azospirillum* is considered the most important rhizobacterial genus for improvement of plant growth or crop yield worldwide. Evaluation of 20 years of data indicated that 60-70% of field experiments were successful, with yield increase ranging from 5 to 30%. In spite of many intensive studies on physiology and molecular biology of these bacteria, the exact mode of their effect on plants is still unclear [8]. Positive reports of application of biofertilizers (*Azotobacter* and *Azospirillum* and other bacteria) on yield are available on crops like: Indian mustard, cotton, corn, sorghum, wheat, tobacco and barley, which is attributed to the enhancement of factors like N_2 fixation nitrate reductase activity, intake of NO_3, NH_4, H_2PO_4, K, Fe, plant water status and production of phytohormones such as Indol acetic acid [11]. In an experiment, the effect of inoculation with *Azospirillum brasilense* on growth and yield of *Sorghum bicolor* in hydroponic systems was a significant enhancement of dry matter content, leaf area development and grain yield. At later stages of growth, leaf senescence was delayed in inoculated plants, thus favoring dry matter accumulation and grain filling. In addition studies showed that *Azospirillum* promote the growth of tomato, eggplant, pepper, cotton and mustard [12]. Okon and Kapulnik [13] observed improvements in root development and function with *Azospirillum* which lead in many cases to higher crop yield.

Biologic fertilizers containing of *Thiobacillus* are very important in Iran soils. Activities of sulphur oxidizing microorganisms lead to expedition in sulphur oxidation and decrease soil pH. Sulphur biologic fertilizers help plants in sulphur nutrition, increase nutrients absorbtion such as P, Fe and Zn, amendment of saline sodic and sodic soils and subsequent increase crop yield via sulphur oxidation by soil-born sulphur oxidizing microorganisms. The number of *Thiobacillus* genus bacteria in many crop soils is low and is about hundred per 1g dry soil. Even in many cases reported that there is nothing autotrophic *Thiobacillus* in soil [14]. In an investigation indicated that amount of sulphur oxidation in soils inoculated with *Thiobacillus* is 11 times more than non-inoculated soils [15].

Besharati *et al.* [16] observed that utilization 0.5 percent sulphur along with *Thiobacillus* bacteria, increased soil absorption of iron and iron uptake by corn order to control 96.2 and 76.4 percent. Who also concluded that soil micro-organisms with oxidation of sulfur in the soil, reduced the pH of soil and increased solubility of iron compounds. Other study, Siyami *et al*. [17] concluded that in soil inoculated with bacteria *Thiobacillus*, sulfur oxidation rate was significantly increased compared to the control. Also who reported that the use of sulfur along with *Thiobacillus* bacteria increased soil absorbable phosphorus, iron and zinc than control during the period of the experiment, respectively, to 209.6, 166.6 and 74 percent. Baloei [15] observed that utilization of *Thiobacillus* bacteria significantly increase the yield and harvest index compared with no utilization of it. Who observed that in different levels of sulfur, utilization of *Thiobacillus* lead to performance of more seed yield than non-utilization of it and attribution its reason to local decrease in pH of the soil around the roots as the result of Concurrent use of sulfur with *Thiobacillus*.

Concerning to the necessity and importance of development of oilseeds, particularly rapeseed cultivation in Iran and most important problem facing with, mainly drought stress, this project proposed and directed In line with the sustainable and organic agriculture's goals and in order to evaluation the effect of plant growth promoting and sulfur oxidizing bacteria on growth an yield of oilseed rape.

MATERIALS AND METHODS

This study was conducted during 2008/9 in Research Field of Islamic Azad University- Karaj Branch, Mahdasht, Karaj, Iran (35°45´N, 51°06´E, 1313 m). The location has a semi-arid climate with 275 mm annual precipitation in average. The soil was sandy loam with a pH of 7.9 and its

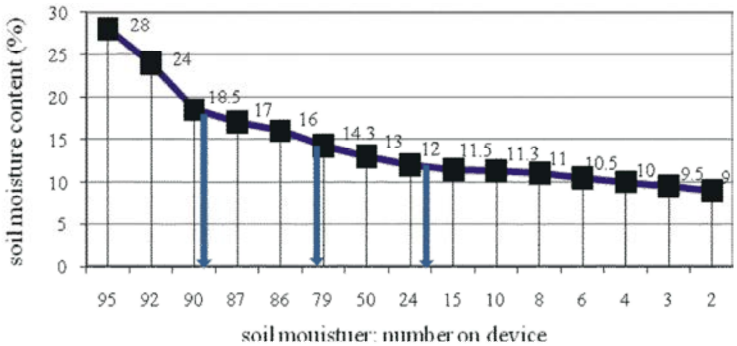

Fig. 1: Calibration curve of these gypsum blocks (Paknejad *et al.* [18])

salinity in 0-30 cm of soil profile was 4.46 dS m^{-1}. The field experiment was conducted as arrange in randomized complete block design (RCBD) with four replications. There were two factors. Irrigation at four levels as main factor: irrigating after depleting 40% (D_0), 60% (D_1), 75% (D_2) of available moisture and stop irrigation (D_3) after flowering stage in main plot and bacteria application with four levels: *Azospiillum lipoferum* (B_1), *Thiobacillus thioparus* (B_2), *A. lipoferum* + *T. thioparus* (B_3) and control (B_0) in sub plot. Each main plot consisted of four sub plot and each sub plot consisted of three rows, 60 cm spaced, which plants distance in row was considered about 4 cm. During planting, 100 kg urea.ha^{-1} was used, based on soil analysis (0-30 cm). In November 2008, canola seeds were sown in a depth of 2 cm.

The utilized canola (var. Okapi) is an intermediate maturity cultivar, which was grown during the months September (2008) to July (2009). Also, the inoculums was as powder containing approximately 10^8 CFU which were isolated and refined by Soil Biology Research Section, Soil and Water Research Institute, Iran. Seeds inoculated with *Azospirillum* by resin. *Thiobacillus* directly applied to soil as combination with sulphur (5 cm under seedbed).

Irrigations were done when needed and all treatments well-watered until the flowering stage. Rill irrigation technique was used. In flowering stage to exert exact water treatments, some gypsum blocks, already calibrated, were installed in plots. Concerning the calibration curve of these gypsum blocks, which was determined previously (Fig. 1), by monitoring soil moisture since flowering until end of growth season. Two rows (120 cm) were left non-planted between each two adjacent main plots to avoid from interference among watering treatments. Also one row (60 cm) was left non-planted between each two adjacent sub plots.

Seed yield was taken at maturity by harvesting the 2.4 m^2 area of the inner row of each plot at the end of June. Ten plants were collected randomly from the central row and the following traits were recorded for each plot; number of sub shoot per plant, number of sub-sub shoot per plant, length of pod per main shoot number of pods per plant, number of pods per m^2, seed yield, biological yield, harvesting index and 1000-seed weight. All data were analyzed with the GLM procedure using the SAS statistical software package. Means were compared using Duncan's Multiple Range test at 5% probability level.

RESULTS AND DISCUSSION

According to analysis of variance, the effect of water stress on canola seed yield was significant at 1% (Table 1). Average comparison results (Table 2) showed that the severe stress treatment (D_3) recorded the lowest seed yield round 1334.6 kg/ha. The highest yield obtained from control treatment with the 2485.5 kg ha that located with low and average humidity stress levels (irrigation after 60 and 75% humidity discharge) in a same statistical group. Severe stress reduced grain yield by 46% than the control treatment. Low and medium stress treatments also led to reducing yield 18% and 10% than the control, respectively. Results indicated that canola is able to withstand against moderate stress levels and repair damages into seed yield. Hasanzadeh *et al.* [19] and Khurgami *et al.* [20] pointed that drought stress as stop irrigation since flowering stage, reduced grain yield and its components. Champolivier and Merrien [21] reported that the seed yield and it's components significantly affected by water shortage since flowering to the end of seed filling stage. The highest yield loss (48%) was observed when only 35% of total crop water requirement was providing during this phase. Ghobadi *et al.* [4] reported that the greatest decrease in yield (30.3%) was related to taking place water stress during flowering and then siliqua development (20.7%) stages. According to results of this study, bacteria hadn't any significant effect on seed yield of canola (Tables 1, 2). This result is

Table 1: Analysis of variance for measured traits

		Mean Square									
SOV	df	Seed yield	Biological yield	HI (%)	1000 seeds weight	Pods/ plant	Pods/m^2	Sub shoot/plant	Sub-sub shoot/plant	Length of main pods	Length of sub-pods
Replication	3	180502.65	0.01	0.004	0.17	4019	13183424.07	0.32	0.35	0.27	0.1
Water stress	3	3938280.84**	0.14**	0.01$^{n.s.}$	1.56**	12270.86$^{n.s.}$	2929529.45*	0.77$^{n.s.}$	0.41$^{n.s.}$	0.27$^{n.s.}$	0.29$^{n.s.}$
Error of stress	9	336258.75	0.01	0.004	0.13	6161.28	6432615.81	0.90	0.27	0.33	0.24
Bacteria	3	118904.93$^{n.s.}$	0.02$^{n.s.}$	0.02$^{n.s.}$	0.02$^{n.s.}$	5476.27$^{n.s.}$	1605246.41$^{n.s.}$	1.27**	1.39**	0.38$^{n.s.}$	0.09$^{n.s.}$
Stress*Bacteria	9	209900.17$^{n.s.}$	0.03$^{n.s.}$	0.005$^{n.s.}$	0.12$^{n.s.}$	1676.82$^{n.s.}$	7656299.44*	0.18$^{n.s.}$	0.35*	0.23$^{n.s.}$	0.36*
Total Error	36	224488.67	0.02	0.006	0.07	2075.90	3523070.5	0.26	0.14	0.18	0.14
C.V. (%)	-	23.38	3.13	16.12	5.24	21.75	22.69	13.47	29.32	6.29	5.51

Table 2: Means comparison of evaluated traits

Treatment	Length of sub-pods (cm)	Length of main pods (cm)	Pods/m^2	Pods/plant	Sub-sub shoot/plant	Sub shoot/plant	1000 seeds weight (g)	HI (%)	Biologic yield (kg/ha)	Seed yield (kg/ha)
D_0	6.73	6.71	9609.2a	246.25	2.54	4.04	5.58a	25.97	10403.1a	2485.5a
D_1	6.98	6.96	9169.2a	212.92	1.74	3.82	5.25b	21.06	9598.1a	2039.6a
D_2	6.73	6.71	7599.6ab	197.34	1.62	3.71	5.02bc	24.84	9714.8a	2247.5a
D_3	6.69	6.68	6707.5b	181.38	1.67	3.51	4.87c	20.77	6285.7b	1334.6b
B_1	6.71	6.83	7833.2	194.40 b	1.54bc	3.45c	5.19	20.36b	9645.2	1977.9
B_2	6.82	6.83	8283.4	215.15 ab	2.66a	4.08a	5.22	27.02a	8430.4	2151.5
B_3	6.87	6.87	8579.3	233.19 a	2.37ab	3.93ab	5.18	21.18b	9899.4	2016.4
B_0	6.73	6.54	8389.7	195.15 b	1c	3.64bc	5.13	24.07ab	8026.7	1961.6
D_0 B_1	6.58bc	6.78	9518.97abc	220.91	1.15c	3.55	5.51	18.40	14337.08	2606.39
D_0 B_2	6.78abc	6.96	7454.77bc	232.77	2.53bc	4.23	5.61	32.63	8343.77	2550.54
D_0 B_3	7.10ab	6.59	9931.26ab	287.62	4.50a	4.48	5.55	26.19	9134.67	2270.43
D_0 B_0	6.47c	6.53	11531.72a	243.68	1.98bc	3.90	5.67	26.67	9797	2514.58
D_1 B_1	6.77	6.80	8372.01	211.63	1.66abc	3.42	5.19	22.71	8802.49	1942.98
D_1 B_2	6.78	7.09	10635.81	226.06	2.85a	4.28	5.44	21.27	10312.80	2180.54
D_1 B_3	7.18	7.34	9720.57	235.43	1.93ab	3.98	5.29	19.44	10398.67	2020.58
D_1 B_0	7.20	6.62	7948.54	178.59	0.53c	3.63	5.09	20.82	8878.37	2014.47
D_2 B_1	7.15a	6.94	6449.74bc	164	1.40bc	3.38	4.93	25.12	9389.85	2180.29
D_2 B_2	6.79abc	6.37	9228.12a	234.44	3.40a	4.25	4.92	27.55	8367.61	2151.69
D_2 B_3	6.42c	6.94	7708.86ab	210.74	1.13bc	3.65	5.23	21.04	13397.12	2657.90
D_2 B_0	6.59bc	6.61	7011.80ab	180.20	0.53c	3.58	5.01	25.64	7704.7	2000.30
D_3 B_1	6.36b	6.82	6991.95	181.08	1.95	3.45	5.14	15.21	6051.22	1181.87
D_3 B_2	6.95a	6.92	5814.79	167.33	1.88	3.55	4.92	26.65	6697.59	1723.05
D_3 B_3	6.78ab	6.60	6956.46	198.96	1.90	3.60	4.66	18.04	6667.27	1116.54
D_3 B_0	6.67ab	6.40	7066.74	178.14	0.97	3.45	4.74	23.18	5726.61	1316.94

Means were compared with duncan's multiple range test at 5% level. In every part of each column, averages with at least one same letter are not significantly different. D_0: full irrigation until flowering stage and then irrigation after 40% water depletion. D_1: full irrigation until flowering stage and then irrigation after 60% water depletion. D_2: full irrigation until flowering stage and then irrigation after 75% water depletion. D_3: full irrigation until flowering stage and then stopping irrigation after it. B_0: without using bacteria. B_1: *Azospirillum* utilization. B_2: *Thiobacillus* utilization. B_3: combined use of *Azospirillum* and *Thiobacillus*

disagree with results of Amooaghayi *et al.* [22]. Noorgholipour *et al.* [23] showed that usage of *Thiobacillus* along with sulfur and phosphate, increased seed yield of soybean rather than usage this treatment without *Thiobacillus*. They stated that this is because the oxidation of sulfur by *Thiobacillus* bacteria, lead to the dissolution of phosphate, increasing uptake of phosphorus and ultimately increasing the yield. Failure to affect of bacteria on seed yield in this study can be a result of unfit and poor soil condition (field testing of soil organic carbon was equal to 0.72). Amooaghayi *et al.* [22] in their study on the impact of both indigenous and non indigenous *Azospirillum* strains on growth and yield of wheat, discovered to the importance of proportionality of bacteria strains with the cultivars and from other researchers have focused on this topic. They pointed that probably strains isolated from each type of crop, have more heterogeneity than other strains to cooperate with the plant and promoting its growth and development. They also indicated that from utilization of local strains with these bacteria can obtained more efficiently results, because nitrogen fixation ability of indigenous strains in the local conditions is better than non-native strains. The *Azospirillum* had not any positive effect on growth characters and yield of canola. It's maybe due to disproportion of bacterial strains and crop species together. Noorgholipour *et al.* [23] stated that non-significant effect of utilization of sulfur along with *Thiobacillus* on increasing some elements' concentration in soil and plant rather than control, can be largely attributed to soil buffering properties. They also reported that environmental conditions such as soil fertility and population of sulfur-oxidizing microorganisms have a significant impact on oxidation of sulfur. And don't be significant of some indices in soil and plant rather than control, can be a result of low sulfur oxidizing, thereby deficiency in soil nutrient and population of sulfur oxidizing microorganisms. Based on this non-significant effect of *Thiobacillus* on seed yield in this study, can be a result of deficiency in Soil organic carbon, low population and concentration of utilized bacteria and insufficient time for increasing bacteria population then sulfur oxidation in soil. These results also showed that interaction effect of stress and bacteria had no significant effect on canola seed yield (Table 2).

According to ANOVA (Table 1), the effect of water stress on canola biologic yield was significant at 1%. Comparison of averages showed that the lowest biologic yield got from severe stress treatment (D_3) with 6285.7 kg/ha which was 40% lower than the control. Biologic yield in irrigation after 60% and 75% water depletion treatments had located with control in a same statistic group. Also showed that similar to yield, this trait had not any significant change until moderate stress levels. But increasing stress level and cutting irrigation after flowering, led to 40% decreasing in biologic yield. These results were similar to the results of Mihrabi *et al.* [24] and Dreccer *et al.* [25] on canola. These results showed that the effect of bacteria and interaction effect of stress and bacteria were not significant on biomass of canola (Tables 1, 2).

The effect of water stress was non-significant on harvesting index of canola. Delkhosh *et al.* [26] reported that water stress as cutting irrigation since stem formation on plant, don't have any important effect on biomass. Mohammadi *et al.* [27] quoted Abdmishani and Shabestari (1989) indicated that HI is an explanatory of genotype's ability for more appropriate of photosynthetic material to economic yield(seed). Therefore no important change in HI at different levels of stress, except severe stress in this study can be a good reason for relative tolerance of used cultivar against dehydration conditions and for plant's effort to repair damages to grain yield. According to ANOVA results, effect of bacteria on HI was not significant (Table 1). Nevertheless differences observed between bacteria treatments (Table 2). *Thiobacillus* bacteria treatment so won the highest harvest index and the lowest was related to the bacterium *Azospirillum* treatment. The results also showed that interaction effect of stress and bacteria was not significant on HI (Table 1).

The analysis of variance showed that the effect of water stress was significant on weight of 1000 seeds in canola (Table 1). Means Comparison (Table 2) showed that stress levels were significantly reduced 1000 seeds weight. The highest seed weight observed in control treatment (5.6 g) and located in top statistical group. The lowest values was obtained from the treatment cutting irrigation since flowering stage which showed 13% reduction compared to the control. Low and moderate stress treatments also had no significant difference together. Hasanzadeh *et al.* [19] concluded that water stress as cutting irrigation since flowering stage led to reducing seed yield and it's components such as 1000 seeds weight and reduces it from 3.5g in control to 2.7 in severe stress treatment. Champolivier and Merrien [21] reported that grain weight in rapeseed is only affected by water stress in initiation phase of pod development to seed coloring stage. Ghobadi *et al.* [4] reported that short-term water stress during seed development reduced seed yield through reducing seed weight. Results showed that bacteria and interaction effect of bacteria and stress had no significant effects on seed weight. Amooaghayi *et al.* [22] and Ghorbani Nasrabadi *et al.* [28] observed respectively the positive effects of *Azospirillum* and *Thiobacillus* on seed weight.

The results showed that water stress reduced number of pods per plant, but this decrease was not significant and all stress treatments were compared in a group (Tables 1, 2). Results obtained is consistent with the results of Vafabakhsh *et al.* [29], Delkhosh *et al.* [26] and Nasri *et al.* [3]. They observed that stress treatments are ineffective on the number of pods per plant. Analysis of variance showed that the effect of bacteria on canola pods per plant was not significant (Table 1). But the means was different between bacteria treatments (Table 2). So the greatest number of pods/plant was obtained from combined treatment using two types of bacteria and the lowest amount was from *Azospirillum* bacteria. Results also showed that the interaction effect of bacteria and stress had no significant effects on number of pods/plant (Table 1).

According to analysis of variance, the effect of stress on number of pods per m^2 were significant at the 5% level (Table 1). The maximum number of pods per m^2 obtained from control, which located with other treatments,except severe stress in a same statistical group. The severe stress treatment (D_3) had get the lowest number of pods/m^2 which was 30% lower in compare with control (no stress treatment) (Table 2). There was no significant effect of bacteria on this trait. But the interaction effect was significant at the 5% level and in a general trend the number of pods per m^2 was decreased with increasing stress intensity. In non-stress conditions, comparison between the levels of bacteria showed that the treatment without the use of bacteria, with 11531.72 pods/ m^2 had obtained the greatest amount, which had significant difference with *Thiobacillus* only. *Thiobacillus* bacterium treatment by average 7454.77 pods/ m^2 was the lowest. So it seems that in the absence of stress, consumption of bacteria is not very useful. Using *Thiobacillus* bacteria alone is not recommended. Because significantly reduced an important component of yield, the number of pods per m^2. But comparing different bacterial treatments in mild stress conditions (irrigation after 60% moisture discharge) indicates that there is no significant difference between bacterial treatments. Although the combined use of two types of bacteria increased the number of pods/m^2, but this increase is not significant. Comparisons between levels of bacteria in the average moisture stress (irrigation after 75% moisture discharge) indicated that *Thiobacillus* bacteria treatment with 9228.12 pods/ m^2 had the highest average which had no significant difference with other bacterial treatments, except treatments of *Azospirillum* bacteria. *Azospirillum* treatment with 6449.74 pods/ m^2 had the lowest number of pods in moderate stress condition. According to the results, *Thiobacillus* increased number of pods, when water stress was moderate. But *Azospirillum* cause a severe decreasing on this trait in this condition. In severe stress condition (D_3), the number of pods per m^2 had no significant differences between bacteria treatments. It seems be that in no stress (D_0) and severe stress (D_3) conditions, *Thiobacillus* had a negative effect on the number of pods/m^2 and cause decreasing in this trait. But in low and moderate stress this bacteria increased the number of pods.

According to the results, effects of water stress, bacteria and interaction of them, was non-significant on the length of pod of main shoot. Delkhosh *et al.* [26] explained that the water stress had no significant effect on pod's length.

Although the effect of water shortage stress and the effect of bacteria wasn't significant on the length of pods in sub shoot, but the interaction effect was significant at 5% level. In control treatment of stress, comparison between bacteria levels observed that by using two kinds of bacteria obtained the highest length of pods in sub shoot which with two other bacteria treatments located in one statistical group, but showed significant difference with no bacteria treatment. Thus it concluded that in ideal moisture conditions, separate or combined usage of bacteria, specially combined usage, can be more efficient than no using bacteria in increasing this trait. In D_1 (full irrigation until flowering stage then irrigation after 60% water depletion since this time) treatment no important difference observed and whole of bacteria located in same statistical group. By comparing bacterial treatments at D_2 (full irrigation until flowering stage then irrigation after 75% water depletion since this time) observed that *Azospirillum* had the highest amount of sub-shoot's pod length and settle with *Thiobacillus* in one statistical group. On the other hand in moderate water stress, separate use of two bacteria, especially *Azospirillum*, increase sub-shoot pod's length. Comparison of bacterial treatments at severe stress condition (full irrigation until flowering stage then cutting irrigation after it) showed that in this conditions *Thiobacillus* act more efficient than other bacteria treatments in increasing this trait and *Thiobacillus* only differ with *Azospirillum* treatment.

Results of this study showed that water shortage stress hadn't significant effect on number of sub-shoots per plant (Table 1). Delkhosh *et al.* [26] performed a study about oilseed rape's cultivars in different levels of irrigation and obtained results similar to this. Analysis of variances in this study showed that the effect of bacteria on number of sub-shoots per plant is significant at 1%. Means comparison showed that the highest amount was seen in *Thiobacillus* with 4.1 and the lowest values was observed in *Azospirillum* with 3.5 which 15% reduced rather than *Thiobacillus*. By attention to results, it transpires that highest number of sub-shoots per plant obtained from treatments containing *Thiobacillus* (separate and combine use) and *Thiobacillus* bacteria - especially in combine use- treatments is obviously effective on this trait. Analysis of variance results showed that the interaction effect of water stress and bacteria on number of sub-shoots per plant is not significant (Table 1).

The results showed that the effect of water stress was insignificant on number of sub-sub-shoot per plant. But bacteria's effect on this trait was significant at 1%. *Thiobacillus* bacteria by obtaining the highest number were located in superior statistic group. After this treatment, the combined use of bacteria treatment got the highest amount. Although both of them were in same group. The lowest number was from control (B_0) (Table 1). Results of ANOVA showed that the interaction effect on this trait was significant at 5%. In non-stress conditions, comparison between the levels of bacteria, showed that the combined of two bacteria treatment, substantially increase the number of sub-sub-shoot per plant. On the other hand, the highest number obtained from no water stress condition by using two kinds of bacteria, comparing different bacterial treatments in mild stress conditions (irrigation after 60% moisture discharge) indicates that the lowest number was related in no bacteria treatment which had significant difference with other levels of bacteria (Table 2). Thus we can say that in mild stress condition, utilization of bacteria in anyway, is so better than no sue of them on produce sub-sub-shoot in plant. Comparisons between levels of bacteria in the average moisture stress (irrigation after 75% moisture discharge) indicated that *Thiobacillus* bacteria treatment recorded the highest number which has significant difference with other bacterial treatments and settle in superior statistic group. Whereas other levels of bacteria located in a same group (Table 2). In a clear expression, the separate usage of *Thiobacillus* was more useful and more efficient than the others on this trait. In severe stress condition (D_3), the number of sub-sub-shoot per plant had no significant differences between bacteria treatments.

CONCLUSION

Severe water shortage stress as cutting irrigation since flowering stage caused 46% decreasing in seed yield. Whereas, seed yield reduction in other levels of stress, was not significant than control. This indicates canola can tolerant water shortage to high extent and repaired damages into yield. But if provide sufficient water, will produce seed yield at most.

The non-significant effect of bacteria on seed yield and on its components such as seed weight and the number of pods per plant can be related with different factors such as soil unfavorable organic matter condition (the organic carbon percent in soil of experimental field was 0.72). Amooaghayi *et al.* [22] in their study on the impact of both indigenous and non indigenous *Azospirillum* strains on growth and yield of wheat, discovered to the importance of proportionality of bacteria strains with the cultivars and from other researchers have focused on this topic. They stated that probably strains isolated from each type of crop, have more heterogeneity than other strains to cooperate with the plant and promoting its growth and development. They also indicated that from utilization of local strains with these bacteria can obtained more efficiently results, because nitrogen fixation ability of indigenous strains in the local conditions is better than non-native strains. Based on this result suggested that in future researches on evaluation the effect of growth promoting bacteria, use from indigenous strains of bacteria in experimental soil and and use from associated strains with evaluated cultivar. Noorgholipoor *et al.* [23] quoted as Agrifacts (2003) that the lowest yield in the treatment of elemental sulfur, are associated with sulfur oxidation time shortage and oxidation low amounts of it. In their study on maize, they expressed that the non-significant effect of use of sulfur and *Thiobacillus* on increasing some elements concentration in soil and plant than control can be largely attributed to soil buffering properties. They noted that environmental conditions such as soil fertility and population of microorganisms have a significant impact on the oxidation of sulfur and don't meaningful some indicators measured in the soil and plant than control also can be attributed with low sulfur oxidation, resulting in low nutrient in soils and low oxidation of sulfur. Therefore non-significant effect of *Thiobacillus* on traits in this research maybe related with low population and concentration of utilized bacteria and insufficient time for increasing bacteria population in soil and thereby sulfurs oxidation. Therefore recommended in future researches, more percentage and population of bacteria to be used and if possible, additional time for express positive effects of sulfur oxidizing bacteria be considered.

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