Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Improving yield-related physiological characteristics of spring rapeseed by integrated fertilizer management under water deficit conditions

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A B S T R A C T

Two separate field experiments were conducted in 2018 and 2019 as split-plot based on randomized complete block design with three replications to evaluate physiological responses of rapeseed to fertilization treatments (control, chemical fertilizer, inoculation of seeds with PGPR, vermicompost and combined fertilizers) under different irrigation levels (irrigation after 70,100, 130, and 160 mm evaporation). Water stress increased the activities of catalase, polyphenol oxidase, peroxidase and superoxide dismutase and the contents of proline, soluble sugars and malondialdehyde and also leaf temperature, but decreased membrane stability index, chlorophyll content, leaf water content, stomatal conductance and grain yield. Application of fertilizers particularly combined fertilizers decreased proline content and leaf temperature, but increased the antioxidant enzymes activities, soluble sugars, chlorophyll content, leaf water content, membrane stability index, and stomatal conductance under different irrigation intervals. These superiorities of fertilization treatments were led to considerable improvement in grain yield. The results revealed that the combined fertilizer application improved most of the physiological parameters. It was deducted that the application of combined fertilizers reduced chemical fertilizer by about 67% and alleviated the deleterious effects of water limitation on field performance of rapeseed.

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1. Introduction

Rapeseed (also called oilseed rape) (Naeem et al., 2010) is known as one of the most momentous oil crops due to the suitable fatty acids and high oil content of grains (Jian et al., 2019). In crop rotation system, spring oilseed rape is a good option for squeezed cropping systems, because of the earlier harvest in comparison with winter cereals (Takashima et al., 2013; Andrade et al., 2017; Menendez et al., 2019). Rapeseed is somewhat tolerant to drought stress (Sadaqat et al., 2003), however, acute drought can decrease the yield of this crop (Mogensen et al., 1997; Godarzi et al., 2017).

Drought as abiotic stress mostly limits the growth and development of crops (Barnabás et al., 2008; Sehgal et al., 2019). Water stress prevents growth by diminishing the water turgor of the plant cells, which adversely affects biochemical and physiological processes in plants (Liang et al., 2019). One of the primary physiological consequences of water deficit is the prohibition of photosynthesis, because of deficit in Ci (intercellular CO_2 concentrations) as a result of chlorophyll destruction, stomatal closure, and disorder of photochemical system (Bohnert and Jensen, 1996; Liu et al., 2016). The production of reactive oxygen species (ROS) is a physiological response of plants to drought stress. Increasing ROS can damage cell membranes by enhancing lipid peroxidation (Gill and Tuteja, 2010; Wang et al., 2017; Zhang et al., 2019). Plants have an extended defensive mechanism for mitigating the harmful effects of ROS via the activation of enzymatic and non-enzymatic antioxidants (Zhang et al., 2019). The enzymes that eliminate ROS include superoxide dismutase (SOD), peroxidases (POX), catalase (CAT) and polyphenol oxidases (PPO) (Sofo et al., 2010; Sharma et al., 2012). The non-enzymatic reaction of plants to water deficit involves the accumulation of osmolytes such as soluble sugar, proline, soluble protein, etc., that are responsible for osmotic regulation under stress (Ashraf and Foolad, 2007; Hasanuzzaman et al., 2019). Ghassemi et al. (2018) reported that drought stress enhanced antioxidant enzymes activities such as POX, CAT and APX and osmolytes in ajowan plant against ROS. Mohammadi et al. (2019) also showed that drought stress increased proline, the antioxidant enzymes (POX, PPO, SOD), and

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Peer review under responsibility of King Saud University.

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malondialdehyde (MDA). Water deficit limits physiological performance of plants by increasing leaf temperature (LT) and decreasing chlorophyll *a* (Chl a), chlorophyll *b* (Chl b), membrane stability index (MSI) (Ghassemi et al., 2018) and chlorophyll content index (CCI) (Ghassemi-Golezani and Afkhami, 2018).

The soil management approach is sometimes affiliating on chemical fertilizers that are harmful to the environment and human health (Ju et al., 2018). Application of bio-fertilizers in plant ecosystems is one of the basic pillars of sustainable agriculture, due to decreasing or eliminating the use of inorganic fertilizers (Shubhra et al., 2004; Rezaei et al., 2018). Bio-fertilizer (vermicompost, plant growth-promoting rhizobacteria, etc.) is a biological product that can be used as fertilizer in the soil (Mulyani et al., 2017) and is effective in enriching the soil micro (Mn, Zn, Fe, etc.) and macro (N, P and K) nutrients through nitrogen fixation, and degradation of organic compounds in the soil. This can lead to better nutrient uptake and improves drought tolerance (Suhag, 2016). Application of bio-fertilizers help to crops to overcome the negative effects of drought (Azab, 2016).

The results of Khalilzadeh et al. (2016) showed that CAT, POX and PPO activities and finally the grain yield of wheat increased as a result of bio-fertilizer application under drought stress. Combined application of chemical fertilizer, PGPR and vermicompost increased the accumulation of osmolytes such as proline and sugar content and also enhanced chlorophyll content (Mondal et al., 2017). Bio-fertilizers such as vermicompost and PGPR increased relative water content, total chlorophyll (Kazeminasab et al., 2016), stomatal conductance and chlorophyll content (Kheirizadeh Arough et al., 2016) under drought stress. Generally, the use of bio-fertilizers (PGPR such as *Pseudomonas flourescens*, *Azotobacter oryzae* and *Azospirillium chroococcum* bacteria and vermicompost) can be an appropriate way of improving crops yield under water stress conditions. Therefore, it would be valuable to assess the physiological responses of rapeseed to integrated fertilizer management under different levels of water supply.

2. Materials and methods

2.1. Experimental conditions

Two field experiments were conducted in 2018 and 2019 at the Research Farm of the University of Tabriz, Iran (Latitude 38° 05′N, Longitude 46° 17′E, Altitude 1360 m above sea level) to investigate the variations in physiological traits and grain yield of rapeseed (*Brassica napus*) in response to water limitation and fertilization. The experiments were laid out as split-plot based on RCB design in three replications, with four irrigation levels (I_1, I_2, I_3, I_4: irrigation after 70, 100, 130, and 160 mm evaporation from class A pan, respectively) in main plots and five levels of fertilizer in sub-plots. Fertilizer levels were: without fertilizer (F_0) as control, chemical fertilizer including N and P (about 300 and 150 kg ha^{-1}, respectively, based on soil analysis, Table 1) (F_1), inoculation of seeds with PGPR (*Pseudomonas flourescens*, *Azotobacter oryzae* and *Azospirillium chroococcum* with populations 2×10^7 CFU ml^{-1} (Biofarm)((F_2), vermicompost amount 10 ton ha^{-1} (F_3) and combined fertilization (1/3 F_1 + 1/3 F_3 + inoculation PGPR) (F_4). Each plot with

a length of 3 m contained 6 rows at a distance of 25 cm from each other. In both years, seeds (cv. Delgan, prepared of Seed and Plant Improvement Institute of Karaj, Iran) were sown in about 1–2 cm depth of a sandy loam soil in May. All plots were irrigated twice after sowing. After seedling emergence and establishment, irrigation intervals were applied according to the treatments. During plant growth and development, hand weeding was carried out frequently.

2.2. Measurements

2.2.1. Nitrogen and phosphorus contents

Leaf nitrogen content was assayed by kjeldahl method (Jones, 1991) and phosphorus content was measured by the yellow method using a spectrophotometer (Model Analytikjena Spekol 1500 Germany) at 430 nm (Shimadzu UV3100, Japan) (Tandon et al., 1968).

2.2.2. Antioxidant enzymes

Several young leaves were separated from three plants of each plot at 50 day after sowing and the method of Kumar and Khan (1982) was applied to assay polyphenol oxidase (PPO) activity. The assay mixture for PPO consisted of 1 ml of 0.1 M catechol, 0.5 ml of enzyme extract and 2 ml of 0.1 M phosphate buffer (pH = 6.0). After incubation of this mixture at 25 °C for 5 min, the reaction was stopped by adding 1 ml of 2.5 NH_2SO_4. The absorbance of the resultant purpurogallin was read at 495 nm. The PPO activity was expressed as Umg^{-1} protein (U = change in 0.1 absorbance min^{-1} mg^{-1} protein). According to Singh et al. (2010), CAT activity was determined by alterations in absorbance at 240 nm (Ug^{-1} FW). The activity of POX was measured by the change of absorption at 470 nm due to guaiacol oxidation. The activity was assayed for 2 min in a reaction solution containing 2.5 ml of 50 mM potassium phosphate buffer (pH = 7.0), 1 ml of 1% guaiacol, 1 ml of 1% H_2O_2 and 0.3 ml of enzyme extract (Gueta-Dahan et al., 1997). The SOD activity was estimated as the volume of enzyme affecting 50% of the maximum inhibition of nitro blue tetrazolium decrease.

2.2.3. Measurement of osmolytes

The method of phenol-sulphuric acid (Kochert, 1978) was followed to estimate the soluble sugar content of leaves. By using the calibration curve of pure glucose, the soluble sugar content of rapeseed leaves was expressed as mg g^{-1} DW.

The proline content of rapeseed leaves was measured according to Bates et al. (1973). About 0.5 g of leaf sample was homogenized in 5 ml of 3% sulfosalicylic acid and after that, 2 ml of the extracted sample was poured into a plastic tube and then 2 ml of glacial acetic acid and 2 ml of ninhydrin were added to this mixture. The samples were then heated for 1 h at 100 °C in a Bain Marie (BM-15 Bain Marie, Magapor SL, Spain). Subsequently, the samples were cooled at room temperature of 22–25 °C and the mixture was extracted with toluene, and the absorbance of the upper phase was recorded at 520 nm. Proline content of leaves was determined by the calibration curve of pure proline and expressed as mg/g fresh weight (FW).

Table 1
Some physical and chemical properties of soil in the experimental area.

Texture	E.C	pH	CaCO$_3$	O.C	N	P	K
	ds/m	–	%			Mg kg^{-1}	
2018	0.77	7.73	14.6	0.08	0.11	13	302.2
2019	0.78	7.24	14.8	0.1	0.13	12.82	298.8

E.C.: Electrical conductivity, CaCO$_3$: Calcium carbonate O.C.: Organic carbon, N: Nitrogen, P: Phosphorus, K: Potassium.

2.2.4. Malondialdehyde

The method of Janero (1990) was used to determine of malondialdehyde content (mmol g^{-1} FW) of leaves. Plant samples (500 mg) were homogenized in 5 ml of 5% trichloroacetic acid. Afterwards, the homogenate samples for the duration of 10 min at 25 °C were centrifuged at 1800 g. The supernatant was added to 2-thiobarbituric acid (TBA), afterward the mixture was heated for the duration 10 min at 98 °C and cooled about at 22–25 °C (room temperature). Finally, the absorbance of the supernatant was recorded at 532 nm.

2.2.5. Chlorophyll content

The Chl a, b and total chlorophyll contents in rapeseed plant leaves were determined by the method of Arnon (1949). The fresh leaf samples (0.2 g each) were cut and placed in tubes containing 10 ml of 80% acetone at −4 °C for 24 h. The absorbance of the supernatant of extracted samples (for 5 min were centrifuged at 10,000 g) was recorded at 645 and 663 nm, using a spectrophotometer (Model Analytikjena Spekol 1500 Germany).

2.2.6. Membrane stability index

Membrane stability index was measured in accordance with Ghassemi-Golezani et al. (2016). First, 100 mg of leaf samples were placed in a falcon with double distilled water (10 ml) and heated at 40 °C for 30 min (C_1). Thereafter, the conductivity was assayed after placing the samples for a duration of 10 min at 100 °C (C_2). The MSI was calculated as:

$$MSI = (EC_1/EC_2) \times 100 \tag{1}$$

2.2.7. Leaf water content

Three plants were harvested from each plot 45 day after sowing. About 0.5 g of fresh leaf sample was weighed (FW), then the leaves were dried at 80 °C for 48 h and reweighed (DW). LWC was calculated as:

$$LWC(\%) = [(FW - DW)/FW] \times 100 \tag{2}$$

where FW is sample fresh weight, and DW is sample dry weight.

2.2.8. Leaf temperature

At the flowering stage (55 DAS), an infrared thermometer (TES-1327) was used to estimate leaf temperature (°C) in lower, middle and upper leaves of three plants from each plot. Subsequently, the mean LT was calculated for each treatment at each replicate.

2.2.9. Stomatal conductance

Stomatal conductance 56 day after sowing was determined by using a porometer system (Porometer AP4, Delta-T Devices Ltd., Cambridge, U.K.).

2.2.10. Grain yield

The rapeseed plants were harvested in 1 m^2 of the middle part of each plot and the grains with about 15–16% moisture content were separated from siliques and weighed.

2.3. Statistical analysis

All the data were analyzed by MSTAT-C and SAS 9.4. Means of the date were compared by Duncan multiple range test at p ≤ 0.05.

3. Results

3.1. Nitrogen and phosphorus contents

The interaction of irrigation × fertilizers was significant for nitrogen and phosphorous contents in leaf tissues. Increasing water limitation interval up to 100 mm evaporation did not change the nitrogen and phosphorous contents in rapeseed plants, but these nutrients were reduced with further increment in irrigation intervals up to 160 mm evaporation. Application of fertilizers improved the contents of nitrogen and phosphorous under all levels of irrigation treatments. The combined application (F_4) of fertilizers showed the highest effect on increasing the contents of nitrogen and phosphorous in rapeseed leaves. These superiorities were greater under severe water deficit (Fig. 1).

3.2. Enzymes activities and MDA

The interaction of water supply × fertilizer treatment was significant for the activities of all antioxidant enzymes and MDA of rapeseed leaves. Decreasing water supply was led to an increase in PPO, CAT, POX, and SOD activities, and MDA content. Treatments of plants with fertilizes under normal (I_1) and mild (I_2) irrigations did not show any significant effect on all of these traits (except POX activity and lipid peroxidation). Chemical fertilizer (F_1) had no effect on enzymes activities under all levels of watering, but it was significant for MDA content. Application of bio-fertilizer, especially combined fertilizer (F_4) under moderate (I_3) and severe (I_4) drought stress increased PPO, CAT, POX, and SOD activities, while reduced MDA content in comparison with control (Table 2).

3.3. Osmolytes

The interaction of water supply × fertilizer was also significant for the osmolytes. The soluble sugars and proline contents increased by decreasing water supply. Application of fertilizers increased soluble sugars, but decreased proline content under all irrigation levels. The effect of F_4 on osmolytes was more than other fertilizers (Table 2).

3.4. Membrane stability index

The irrigation intervals were significantly interacted with fertilizers for membrane stability index (p < 0.01). The MSI was significantly reduced under water stress. Application of fertilizers did not statistically alter MSI value under normal irrigation and mild stress. However, the combined fertilization (F_4) significantly improved the MSI under moderate and severe stresses, compared with the F_0 and F_1. The superiority of chemical fertilizer in comparison with control was only significant under I_3 (Table 2).

3.5. Chlorophyll content

Combined analysis of the data for two years showed significant interaction of irrigation × fertilizer treatments for Chl a, Chl b and total chlorophyll of rapeseed plants. The chlorophylls *a*, b and total chlorophyll were decreased with increasing drought stress. Fertilizer treatments increased chl a and total chlorophyll under all irrigation levels. The highest chl a and total chlorophyll were recorded for F_1, followed by F_4 under I_1 and I_2, but in moderate and severe stress conditions the values of these traits were obtained by application of bio-fertilizers, especially by F_4. There was no statistically significant difference between F_2 and F_3 treatments under all irrigation levels. The fertilizer treatments had no significant effect on Chl b in comparison with control (Table 3).
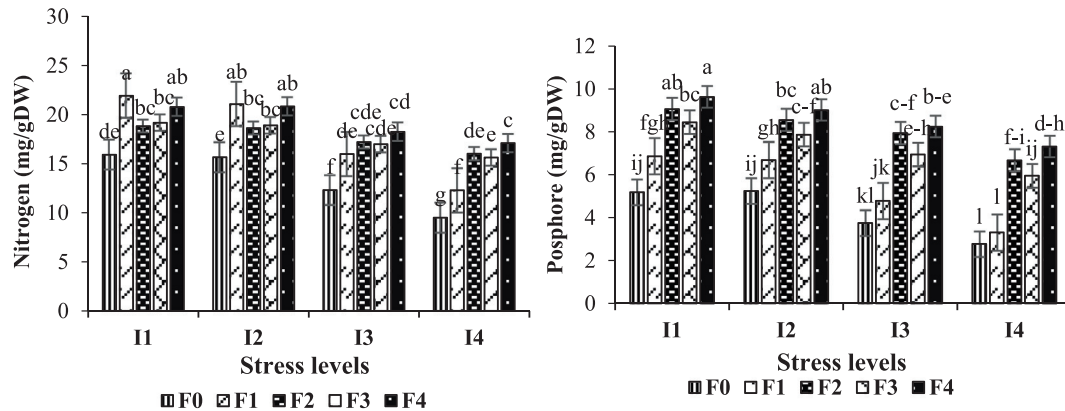
Fig. 1. Mean nitrogen and phosphorus contents of rapeseed for the interaction of irrigation × fertilization. Different letters indicate significant difference at P ≤ 0.05 (Duncan multiple range test). I1, I2, I3, I4: irrigation after 70,100, 130 and 160 mm evaporation. F0: control, F1: chemical fertilizer, F2: inoculation with PGPR, F3: vermicompost and F4: combined fertilization.

Table 2
Changes in PPO, CAT, POX, and SOD activities, MDA content, osmolytes, and MSI in rapeseed leaves affected by fertilizers under different levels of water supply.

Irrigation	Treatments	PPO	CAT	POX	SOD	MDA	Soluble sugars	Proline	MSI
		$(U\ g^{-1}\ FW)$				$(mmol\ g^{-1}\ FW)$	$(mg\ /gDW)$	$(mmol\ g^{-1}\ FW)$	–
I_1	F0	0.48f	0.22 h	0.16f	0.28 g	2.4 j	30.93 ijk	15.3 ij	85.71 ab
	F1	0.55f	0.24 h	0.17f	0.3 fg	2.28 j	29.89 k	15.23 ij	86.93 a
	F2	0.51f	0.25 h	0.17f	0.32 fg	2.27 j	30.53 ijk	15.03 j	86.81 ab
	F3	0.57f	0.24 h	0.18f	0.31 fg	2.28 j	30.3 ijk	15.07 ij	86.45 ab
	F4	0.56f	0.23 h	0.19f	0.31 fg	2.26 j	30.27 jk	15.07 j	87.28 a
I_2	F0	0.78f	0.5 gh	0.35 ef	0.59 efg	3.4 gh	32.7 ij	16.36 hi	84.41 abc
	F1	0.79f	0.54 g	0.87 e	0.63 efg	3.06 hi	31.53 ijk	16.1 ij	86.25 ab
	F2	1.01f	0.65 g	0.94 e	0.74 e	2.65 ij	32.14 ijk	15.8 ij	87.01 a
	F3	1.01f	0.66 g	0.89 e	0.71 ef	2.7 ij	32.77 i	15.54 ij	86.18 ab
	F4	1.07f	1.38 fg	1.56 d	0.79 e	2.44 j	31.87 ijk	15.57 ij	87.08 a
I_3	F0	1.87 e	2.01f	1.66 d	1.29 d	5.9 bc	38.56 h	22.8c	76.61 e
	F1	2.02 e	2.09f	1.85 d	1.32 d	5.28 ed	43.6f	21.8 bc	79.45 d
	F2	2.81 cd	3.16 d	2.68c	2.11c	4.34f	45.64 ef	18.13 fg	81.05 cd
	F3	2.78 d	3.11 d	2.69c	2.14c	4.48f	46.63 e	18.53f	81.35 cd
	F4	3.37 bc	3.56c	2.87c	2.19c	3.72 g	49.07 d	17.33 gh	82.98 bc
I_4	F0	1.8 e	2.74 e	2.83c	1.95c	7.29 a	40.9 g	32.67 a	64.08 g
	F1	2.05 e	2.84 e	3.11 bc	1.89c	6.33b	43.51f	27.43b	65.31 g
	F2	3.53b	4.06b	3.84b	2.94b	5.56 cd	63.35b	22.07 cd	70.28f
	F3	3.29 bcd	4.22 ab	3.86b	2.98b	5.71 cd	58.79c	22.49 cd	70.48f
	F4	4.09 a	4.39 a	4.43 a	3.44 a	4.85 ef	67.61 a	21.3 de	74.48 e
F test	**I × F**	0.602**	0.48**	0.316**	0.332**	0.509**	99.002**	14.494**	14.21**

Different letters in each column indicate significant difference at p ≤ 0.05 (Duncan test).
PPO: polyphenol oxidase, CAT: catalase, POX: peroxidase, SOD: superoxide dismutase, MDA: malondialdehyde, MSI: membrane stability index and I_1, I_2, I_3, I_4: irrigation after 70,100, 130- and 160-mm evaporation. F_0: control, F_1: chemical fertilizer, F_2: inoculation with PGPR, F_3: vermicompost and F_4: combined fertilizers.
** Significant at p ≤ 0.01.

3.6. Leaf water content and leaf temperature

The interaction of water stress × fertilizers was significant for leaf water content and leaf temperature in rapeseed plants. Leaf water content was decreased, while LT was increased under stressful condition, with no significant differences between I_1 and I_2 treatments. The effect of fertilizer treatments on leaf water content and leaf temperature was not significant under normal irrigation and mild stress. However, application of bio-fertilizers especially F_4 significantly enhanced LWC and reduced LT under moderate and severe stresses (Table 3).

3.7. Stomatal conductance

A significant interaction of water supply × fertilizers was observed for stomatal conductance of rapeseed plants. The stomatal conductance was decreased with increasing water stress, with no significant change under I_1 and I_2 treatments. Chemical and bio-fertilizers, especially F_4, increased stomatal conductance of rapeseed leaves under all irrigation intervals (Table 3).

3.8. Grain yield

The interaction of irrigation × fertilizer was also significant for grain yield. Decreasing water supply significantly reduced grain yield of rapeseed. However, fertilizer treatments significantly increased grain yield. The highest grain yield was obtained for F_1 treatment under normal irrigation, with no significant difference with F_4 treatment. Combined fertilizers (F_4) significantly enhanced grain yield under I_3 and I_4, compared with other treatments. The rapeseed grain yield under normal irrigation was higher for F_3 than F_2 treatments. This advantage was declined with increasing drought stress, although there was no significant difference between these two treatments (Table 3).

Table 3
Changes in Chl a, Ch b, total chlorophyll content, LWC, LT and grain yield of rapeseed plants affected by fertilizers under water supply levels.

Irrigation	Treatments	Chl a	Ch b	Total chlorophyll	LWC	LT	Stomatal conductance	Grain yield
		(mg g^{-1} DW)			%	(•C)	(mmol m^{-2} s^{-1})	(gr/m)
I_1	F0	1.44d e	0.835 abc	2.27def	80.16 ab	21.92 ef	142.40 a	187.77 de
	F1	2.17 a	0.86 a	3.03 a	82.5 a	19.6 fg	145.66 a	263.11 a
	F2	1.62 d	0.836 ab	2.46 de	81.48 a	21.58 fg	144.10 a	221.25 bc
	F3	1.65 d	0.843 ab	2.49 cde	81.51 a	21.58 fg	144.57 a	224.66 bc
	F4	1.95b	0.846 ab	2.79b	81.66 a	19.25 fg	146.40 a	249.48 ab
I_2	F0	1.29 fgh	0.79 def	2.03 gh	80.83 abc	25.58c-f	140.83 a	176.35 ef
	F1	1.89 bc	0.813 bcd	2.7 bc	81.83 a	22.25 ef	145.10 a	232.41 ab
	F2	1.49 def	0.79 def	2.28 ef	81.17 a	23.25 def	144.91 a	207.23 cd
	F3	1.51 def	0.8 cde	2.31 def	81.5 a	22.25 ef	143.40 a	205.35 cd
	F4	1.72 cd	0.8 cde	2.53 cd	82.5 a	21.6 fg	143.90 a	221.17 bc
I_3	F0	0,74 ij	0.73 h	1.47 i	74.18 de	29.6b	100.57 cd	132.06 h
	F1	1.17 gh	0.753 fgh	1.92 h	76.15 cd	27.6 cd	106.93 bcd	166.6 fg
	F2	1.17 gh	0.75 gh	1.92 h	78.17 bc	26.6 cde	116.26b	171.03 ef
	F3	1.19 gh	0.763 efg	1.95 gh	78.49 bc	24.9c-f	116.50b	170.88 ef
	F4	1.39 ef	0.766 efg	2.16 fg	78.83 bc	25.91c-f	118.47b	198.11c
I_4	F0	0.48 k	0.676 j	1.16 j	62.48f	34.9 a	71.93 e	70.52 j
	F1	0.65 j	0.706 ij	1.36 ij	65.16f	33.25 ab	77.60 e	89.23 i
	F2	0.84 i	0.693 ij	1.53 i	71.17 e	28.91c	96.66 d	133.03 h
	F3	0.77 i	0.701 ij	1.47 i	71.84 de	28.6c	97.86 d	124.17 h
	F4	1.12 h	0.716 hi	1.83 h	73.5 de	27.9 cd	109.4 bc	156.82 g
F test	$I \times F$	0.133**	0.00031**	0.139**	28.73**	22.45**	142.40 a	1799.26**

Different letters in each column indicate significant difference at p ≤ 0.05 (Duncan test).
Chl a: chlorophyll *a*, Chl b: chlorophyll *b*, LWC: leaf water content, LT: leaf temperature and I_1, I_2, I_3, I_4: irrigation after 70,100, 130 and 160 mm evaporation. F_0: control, F_1: chemical fertilizer, F_2: inoculation with PGPR, F_3: vermicompost and F_4: combined fertilizers.
** Significant at p ≤ 0.01.

4. Discussion

Decrements of nitrogen and phosphate under water stress are related with decreasing water potential in rhizosphere and plant cells. The nitrogen and phosphorous contents were augmented in response to the different fertilizers, especially F_4 (Fig. 1). Adhikary (2012) reported that uptake of macronutrients such as N and P in plants was considerably improved by application of vermicompost, so there was a significant increase in nitrogen and phosphorus contents in plant leaves under water stress. The bacteria can also increase the nutrient uptake with modification of physico-chemical properties of rhizosphere such as increasing cation exchange capacity of soil and some biochemical responses in root tissues. In addition, the PGPRs treatments enhance the photo assimilate translocation to the root tissues and consequently improve the root nutrient absorption power under different environmental conditions such as drought stress (Ansari and Ahmad, 2019). Kohler et al. (2008) reported that infection of Lettuce roots with *Pseudomonas mendonica* significantly improved the phosphatase activity in roots and phosphate accumulation in leaves. So, the increment of phosphatase activity is one of the main mechanisms of action which is augmented by PGPRs such as *Pseudomonas* bacteria. Increment of phosphate content by some PGPR has been related to the solubilization and increased uptake of phosphate (Yang et al., 2009). PGPRs can enhance the activity of some important nitrogen metabolizing enzymes such as nitrate reductase in plant organs, thereby improving the nitrogen content under water stress (Ansari and Ahmad, 2019).

In many plants, water deficit could lead to oxidative stress via increasing ROS (Ghassemi et al., 2018). This stress could lead to stomatal closure and CO_2 decline. Reactive oxygen species including OH (hydroxyl), O^{2-} (superoxide) and H_2O_2 (hydrogen peroxide) radicals produced under stress such as water stress via increased electrons leakage to molecular oxygen (Arora et al., 2002). Excess ROS can cause electrolyte leakage by oxidizing the plant cell membrane (Venkatesh et al., 2012), and photosynthetic inhibition via

attacking the related proteins (Mittler, 2002). Rapeseed plants protect themselves against physiological damages of water stress by increasing activities of polyphenol oxidase (PPO), catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) (Table 2). Increasing PPO, CAT, POX, and SOD activities (Table 3) was also related with the availability of various major and minor elements in bio-fertilizers (Ibrahim et al., 2013). Vermicompost and PGPR provide some microelements (Fe, Zn, etc.) are prosthetic groups for antioxidant enzymes such as POX, CAT and SOD (Hosseinzadeh et al., 2018) that can annihilate ROS in the plant. The increase in antioxidant activity is the result of the affirmative role of vermicompost and PGPR in up-regulating the antioxidant enzymes activities in rapeseed plants under water stress. Application of vermicompost under moderate and severe drought stress increased antioxidant enzymes activities such a SOD and CAT (Kiran, 2019). Agami et al. (2016) reported that activities of CAT, PPO, and POD (peroxidase) in plants treated by PGPR under water stress are more than plants without this treatment. The highest enzymes activity was observed in F_4 treatment, in comparison with F_2 and F_3 (Table 2). This superiority was achieved by the additive effects of PGPR and vermicompost on plants.

The MDA and MSI could be used as markers to estimate the damages of oxidative stress on cell membranes (Esfandiari et al., 2007; Khajeeyan et al., 2019). The enhanced levels of malondialdehyde in stress conditions indicate the membrane damage/membrane sensitivity due to deficit of water (Meena et al., 2016). Drought stress damaged the leaf cellular membranes (Liang et al., 2019), that is communicated by the increasing of malondialdehyde (MDA) content and decreasing membrane stability index (Table 2). Rising lipid peroxidation and decreased MSI with increasing water stress are related to the overproduction of ROS, including O^{2-} and H_2O_2 in plant leaves (Ghassemi et al., 2018). Also, Shanazari et al. (2018) reported that increasing drought stress decreased cell membrane stability and increased malondialdehyde. In this condition, generally crops increase their activities of enzymes for scavenging reactive oxygen species. Bio-fertilizer particularly F_4

under water stress by improving antioxidant activities (Table 2) led to rise of MSI and reductions in MDA content (Table 2). Higher activity of enzymes and lower malondialdehyde content in bio-fertilized plants indicated their improved defense status in destroying ROS harm (Abid et al., 2016). Therefore, bio-fertilizers increase cell membrane stability and decrease lipid peroxidation by increasing the protective mechanism in plant cells.

Accumulation of soluble sugars and proline content with decreasing water supply (Table 2) strongly related to an osmotic adjustment of plants under stress that protects the integrity of macromolecules and membranes when dehydration is very high (Farhoudi et al., 2015). The advantage of higher osmolytes is also reflected in the maintenance of higher leaf water potential and activities of antioxidant enzymes such as polyphenol oxidase, peroxidase, and catalase (Babaei et al., 2017). Greater soluble sugars content concentration in leaves of plants treated with bio-fertilizer (Table 2), might be due to enhanced LWC (Table 3) and leaf area as well as reduced chlorophyll photo-oxidation activity (Salehi et al., 2016). Similarly, results of Mondal et al. (2017) showed that combined fertilization vermicompost + NPK + phosphate solubilizing micro-organism and Azotobacter increased the soluble sugar content (Rao et al., 2007). Because both chlorophyll and proline are synthesized from a similar precursor (glutamate) (Farhangi-Abriz and Ghassemi-Golezani, 2018), proline content was diminished by bio-fertilizer treatments (Table 2) to improving chlorophyll synthesis in plant leaves. Results showed that bio-fertilizer application such as vermicompost in chamomile plants (Salehi et al., 2016) and PGPR in Basil plants (Heidari et al., 2011) reduced the content of leaf proline compared with control, while increased chlorophyll content. Also, reported that of application of bio-fertilizer under stress conditions, especially under moderate and severe conditions reduced proline content compared to the application of chemical fertilizers (Mohammadi et al., 2019).

To determining the tolerance of plants to water stress, chlorophyll content has been introduced as an index (Hosseinzadeh et al., 2018). Reduction in the chlorophyll contents (Table 3) by water deficit is a sign of oxidative stress damage and demolition of chlorophyll by rising activity of chlorophyllase enzymes (Salehi et al., 2016; Agami et al., 2016). Also, acute drought stress in plants can prevent the photosynthesis by affecting on chlorophyll components, changes in chlorophyll content, and damaging the photosynthetic systems and decrease nutrient uptake (Manivannan et al., 2007). Furthermore, the biosynthesis of proline from glutamate precursor can be one of the other reason for decreasing of chlorophyll content under drought (Navari-Izzo et al., 1990). In different studies, chlorophyll content in plants treated with bio-fertilizer was higher in comparison non-treated plants (Belimov et al., 2009). The highest chlorophyll content of rapeseed plants under drought stress was observed in combined fertilizer treatment (Table 3) that could be due positive effects of vermicompost and PGPR such as Azotobacter and Azospirillium bacteria in supply the N and P (Fig. 1). These macro-elements has main role in manufacturing chlorophyll in leaves, cytosine, and oxin, and increased the physiological activity and total chlorophyll. Furthermore, increasing chlorophyll content by bio-fertilizer treatments under stress conditions may be due to increased activity of PPO, CAT, POX, and SOD (Table 2). The enhanced activity of antioxidant enzymes in plants prevents the degradation of chlorophyll molecules, because these enzymes by decreasing the production of reactive oxygen species prevent the damage of proteins and cellular structures (Wu and Tiedemann, 2001).

Reduction in leaf moisture content could be associated with an imbalance between water loss and water uptake by the plants. Decreasing leaf water content due to deficit of water (Table 3) is an indication of the decline in turgor pressure in plant cells and causes growth retardation (Kumar and Sharma, 2010; Ghassemi-Golezani and Afkhami, 2018). Leaf water content in bio-fertilizers treated plants was higher than F_1 and F_0 (Table 3). Similarly, Inoculation by bio-fertilizer such as PGPR under water limitation increased the leaf water content (Kheirizadeh Arough et al., 2016). This could be the result of enhancing root growth by indole-3-acetic acid (IAA) produced by bacteria (Marulanda et al., 2009). Also, vermicompost due to having porous structure, more water holding capacity, organic ions and plant hormones (Beykkhormizi et al., 2016) can improve the leaf water content. On the other, improved the leaf water content (Table 3) by fertilizer treatments can be due increasing osmolytes like soluble sugars content (Table 2).

The leaf temperature can be used as a physiological trait to determine the plant's water condition (Jiménez-Bello et al., 2011). Rising leaf temperature under water deficit (Table 3) is relevant to reducing transpiration and stomatal conductance (Table 2). Commonly, deficient water leading to stomatal closure in crops, and this caused to more temperature in plants leaves (Ghassemi et al., 2018). LT decreased as a result of fertilizer application (Table 3), which could be due to increased stomatal conductance (Table 2) as a result of greater leaf water content (Table 3). Between LT and LWC is a negative relationship, and fertilizers, particularly combined fertilization caused a reduction in LT by increase LWC under water stress. The crop, which shows high vegetative growth shows low canopy temperature because of large leaf water content (Jan and Boswal, 2015). Singer et al. (1998) found that application of bio-fertilizer and organic fertilizers affected various physiological characters like leaf water content and canopy temperature.

Stomata closure is an initial response of plants to water deficit (Pirasteh-Anosheh et al., 2016). When roots expose to water stress generate the chemical signals such as ABA that cased response in the stomatal. On the other hand more ROS lead to increased ABA accumulation, and these excess ABA levels can increase the ROS generation in guard cells, thereby creating positive feedback to stimulate stomatal closure (Mittler and Blumwald, 2015). Root-generated chemical signals and decreasing leaf water content (Table 3), leaf turgor and atmospheric vapor pressure lead to stomatal closure and decreased stomatal conductance in response to drought stress (Table 3). Application of bio-fertilizers, especially combined fertilizer (F_4) enhanced the stomatal conductance (Table 3) as a result of increasing LWC (Table 3) under water deficit. PGPRs produce plant growth hormones that increase activities of nitrate reductase (NR) and the N-use efficiency in plants. Enhance N utilization by plants also help in enhance photosynthesis. Increasing in photosynthesis, transpiration rate, stomatal opening, and reduction stomatal resistance may lead to increased chlorophyll content (Table 3), stomatal conductance (Table 3) and carbon dioxide assimilation (Misratia et al., 2013; Seema et al., 2018). Vermicompost, also because of proper drainage, high ventilation capacity, highly porous texture, and water storage, prevents the stomatal closure under water deficit and, enhances carbon dioxide necessary for photosynthesis (Arancon et al., 2004). Also, as a result of vermicompost application, an increase in stomatal conductance can depend on the increase in leaf water content (Table 3). Similarity result reported by Kiran (2019) in Lettuce plants under water stress.

The highest grain yield of rapeseed plants under normal conditions (I_1) by chemical fertilizer was related to higher Chl a, Chl b and total chlorophyll and LWC (Table 3). The urea as a nitrogen source increases nitrogen supply during flowering and pod filling stages, retards leaf aging and improves photosynthesis (Kulsum et al., 2007). Nitrogen supply during leaf growth also contributes to the formation of chloroplasts, which ultimately increases chlorophyll content (Singh et al., 2016). In general, the amount of chlorophyll increased by enhancing the amount of nitrogen

available to the plant and followed by the ability to absorb sunlight and produce more assimilates and finally to increase growth and yield in plants (Salehi et al., 2016). Decreasing the effect of chemical treatment on grain yield with increasing drought stress (Table 3) is the consequence of decreasing nutrient-uptake, due to water limitation.

Reduction in grain yield by increasing water stress (Table 3) can be due to increasing MDA (Table 2) and LT (Table 3), and decreasing MSI (Table 2), chlorophyll a, b, total chlorophyll contents, LWC, and stomatal conductance (Table 3). Reported that grain yield loss of water-stressed plants could be because of a reduction in chlorophyll content, photosynthesis (Flexas and Medrano, 2008), MSI, Ca and K and increasing MDA (Ghassemi et al., 2018). Improving grain yield of bio-fertilizer treated plants (Table 3) under water stress conditions, is the result of increasing in the PPO, CAT, POX, and SOD activities, MSI (Table 2), chlorophyll contents, LWC and stomatal conductance (Table 3), carbon dioxide assimilation rate, Ci and concentrations of N, K and Ca in leaf tissues (Hosseinzadeh et al., 2018). The application of bio-fertilizer reduces damages of water stress via increasing the activities of antioxidant enzymes (Table 2). In different studies have shown that applicated of the bio-fertilizer because of increasing enzymatic activity, absorption of nutrients and water holding lead to rising in crops production (Lakhdar et al., 2009; Huerta et al., 2010). Also, reported that vermicompost application via soil nutrients supply, microbial biomass, and increase crops biomass increased the beneficial effects of PGPR. Combined application of organic and inorganic fertilizers (vermicompost, PGPR and chemical fertilizer) stimulated the accumulation of some metabolites for suitable plant growth and increased growth and yield of plants via increase activities antioxidant enzymes (Mondal et al., 2017). Adhikary (2012) reported that uptake of macronutrients such as N and P in plants improved considerably by application of vermicompost, so there was significant increase in nitrogen and phosphor contents in plant leaves.

5. Conclusions

Increasing antioxidant enzymes activities and osmolytes did not overcome lipid peroxidation under water stress. Combined fertilizer application considerably enhanced the activities of these enzymes, leading to a reduction in lipid peroxidation, particularly under moderate and severe stresses. The oxidative injuries due to water stress caused a decrease in chlorophyll content, LWC, MSI, and stomatal conductance of rapeseed plants. The injurious effects of stress were reduced by application of vermicompost and PGPR, especially by combined fertilizer under stressful conditions, resulting in higher chlorophyll content, LWC, MSI, stomatal conductance and grain yield. These superiorities were achieved by additive effects of vermicompost and PGPR, reducing the use of chemical fertilizer by 67%.

Acknowledgements

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