

# Effects of water deficit stress and symbiosis with *Micrococcus yunnanensis* at the reproductive stage on yield and seed composition of *Camelina sativa*

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## Abstract

*Camelina sativa* is an important oilseed due to its potential in the production of biodiesel and bioproduct. To investigate the water deficit– induced effects and PGPB on the growth and seed composition of *Camelina* “Soheil cultivar”, an experiment was programmed in three levels 100, 75 and 50%FC and the presence of *Micrococcus yunnanensis* as plant growth– promoting bacteria during the reproductive phase. The results showed under water deficient silique and seed number and silique length decrease coincided with the increase of seed weight. Also the decrease of oil content associated with the increased protein and total carbohydrate content. Carbon to nitrogen ratio and oil content were in a line and the nitrogen to sulphur ratio positively correlated to protein content. In seeds, the increase of Mn and P associated with the decrease of Fe and Zn. The highest proportion of fatty acid was related to poly unsaturated fatty acid in particular linolenic acid. The increase of poly unsaturated fatty acid and saturated fatty acid coincided with the decrease of mono unsaturated fatty acid under water deficit stress. The antioxidant capacity and total phenol content had an increasing trend with limited water. PGPB application resulted in the increase of seed weight, seed and silique number, nutrients uptake and the increase of protein. Also, PGPB increased antioxidant capacity and total phenol. PGPB decreased oil content but it had various effects on fatty acid profiles. In general, PGPB had significant effect on remobilization nutrients from soil to developing seed and following metabolites syntheses

## Abstract

Water deficit stress during the reproductive stage considerably obstructs in the desired crop productivity. *Camelina sativa* is an important oilseed due to its potential in the production of biodiesel and bioproduct. To investigate the water deficit– induced effects and PGPB on the growth and seed composition of *Camelina* “Soheil cultivar”, an experiment was programmed in three irrigation levels (well water: 100% FC, mild water deficit: 75%FC and severe water deficit: 50%FC) and the presence of *Micrococcus yunnanensis* as plant growth– promoting bacteria during the reproductive phase. The results showed that under water deficit stress, silique and seed number and silique length decrease provided it coincided with the increase of seed weight. The water deficit resulted in the noticeable alternation in the carbon and nitrogen partitioning to developing seed and subsequently the decrease of oil content associated with the increased protein and total carbohydrate content. Carbon to nitrogen ratio and oil content were in a line in all of the treatment. Additionally, the nitrogen to sulphur ratio positively correlated to protein content. Phosphorous content significantly increased under water deficit stress. In seeds, the increase of Mn associated with the decrease of Fe and Zn was seen when plants treated with drought stress. The obtained results of GC showed that the highest proportion of fatty acid was related to poly unsaturated fatty acid (55.12 to 65.66%) in particular linolenic acid. The increase of poly unsaturated fatty acid and saturated fatty acid coincided with the decrease of mono unsaturated fatty acid under water deficit stress. The antioxidant capacity and total

phenol content had an increasing trend with limited water. PGPB application resulted in the increase of weight seed, seed and silique number, nutrients uptake and subsequently the increase of protein. Also, PGPB increased antioxidant capacity and total phenol capacity. PGPB decreased oil content but it had various effects on fatty acid profiles. In general, water deficit stress and PGPB have significant effect on remobilization nutrients from soil to developing seed and following metabolites synthesis

**Keywords :** water deficit stress, *Camelina sativa* , plant growth promoting bacteria, fatty acid profile, seed composition.

**Introduction** Water deficit stress is a major abiotic threat affecting directly on gene expression, cell metabolism, plant physiology, morphology, and biochemistry feature results in the reduction of plant growth and development and poor crop productivity (Reddy et al., 2004; Guo et al., 2018; You et al., 2018). Water deficit stress during the life cycle particularly in the reproductive stage could encounter plant with a more drastic problem. As, water deficient in seed filling stage were led to decline of seed size following disturbance of storage reserves accumulation (Kok et al., 2013). Several studies indicated that the flowering and seed filling stage is the most susceptible stage to water deficit stress (Sehgal et al., 2018). During the reproductive stage, water deficit stress remarkably affects the nutrients allocation and remobilization of photosynthetic assimilates conducting alternation of seed size, seed number, the weight of seed and seed composition (Hussain et al., 2018; Sehgal et al., 2018). The nutrients management strategy is the most major target of the plant to cope with stresses. It is well documented that water deficit effect on the source-to-sink nutrients partitioning in particular nitrogen (N) and carbon (C) during seed development (Durand et al., 2018). In the seed filling stage, carbohydrates (photosynthesis assimilates) in both leaves (Singh et al., 2016) and silique wall (Li et al., 2016; Ni et al., 2019) act as a carbon supply source for developing seed. On the other hand, water stress-accumulated carbohydrates applied for the biosynthesis compatible soluble in developing seeds (Thalmann and Santelia 2017). The various reports indicated that the developing oilseed C:N ratio act as an effective indicator in suitable partitioning of resources for the oil and protein biosynthesis. Additionally, this ratio alters under stress conditions which can be due to the decrease of photosynthesis and the rate increase of seed development (Gan et al., 2004; Hussain et al., 2019; Lee et al., 2020). In oilseeds, C and N content have a strongly positive correlation with oil and protein content respectively. As oil synthesis is parallel with carbon accumulation and nitrogen limitation (Jaradat and Rinke 2010; Calvery et al., 2016). In oilseed corps, sulphur requirement is higher than other crops for the synthesis of proteins containing s-amino acids and s-containing secondary metabolites (Poisson et al., 2019; Dhillon et al., 2019). Even though sulphur proportion in the seed is considerably lower than nitrogen but its importance is as well as nitrogen as so far as N:S ratio can be suitable indicator assessing of growth and seed yield and its quality (Poisson et al., 2019). In the last decades, the global change of climate and subsequent drought appearance have become a serious problem and it is important acquiescence to an efficient approach with the object of sustainable agriculture under stress condition. It is well documented that plant growth-promoting bacteria (PGPBs) is an all-purpose option in growth, development, and increase of the crop yield. Also, PGPBs handles biotic and abiotic stress-induced climacteric conditions. PGPB-induced physiological, biochemical and morphological changes in the host result in various mechanisms such as to provide N<sub>2</sub> for plants, generation of the plant growth-modulators materials including cytokines, gibberellins, auxins, abscisic acid and ethylene, production of siderophores and higher acquisition to nutrients by solubilization and mineralization (Marulanda et al., 2009; Etesami and Maheshwari, 2018). Some reports have shown the significant effects of PGPB on physical (weight and number seed), and chemical (oil, protein, and mineral elements) trait oilseeds (Olivera et al., 2018; Etesami and Maheshwari, 2018). The antioxidants in oilseed are a determinant indicator of oil stability for oxidative-induced factors. Phenolic compounds possessing multifunction act as important antioxidant (Ali et al., 2012; Kurasiak-Popowska et al., 2019). The importance of oilseeds as one of the essential sources of oil, protein and mineral has been recognized for both of the nutritional and non-nutritional uses (Poisson et al., 2019) *Camelina sativa* L. Crantz (or false flax) an oilseed crop belonging to the Brassicaceae family has a notable potency for the cultivation in the different climates in the world. Great agronomical attributes of camelina which had lionized in last decades are low-demand nutrients, short-term duration of growth, nutrient retention (Anderson et al., 2019) and high resistance to biotic and

abiotic stress (Soorni et al., 2017; Yuan et al., 2017). As for high genetic conservation ([?] 80%) (Lohaus et al., 2020) camelina with *Arabidopsis*, researchers recognized camelina as a suitable genetic model in the study of genes function and transgenic plants (Heydarian et al., 2016). Camelina seed is a rich source oil, protein and mineral nutrients. Based on obtained studies, camelina oil with the relatively high level of unsaturated fatty acid content ([?]85%) and desirable level of erucic acid ([?]3%) is highlighted as a superior vegetable oil with multiple potential in the industries such as, feedstock, biodiesel, cosmetic, medicine, and biopolymers (Waraich et al., 2013; Obour et al., 2017; Jankowsky et al., 2019; Wiwart et al., 2019). Although obtained studies showed that, camelina plant is low- demand water and can acclimate to water deficit but there is not information not only on effect water deficit during the reproductive phase but also the effect of PGPB symbiosis on growth, seed quality and seed composition. The objective of the present experiment is to evaluate the effect of water deficit stress on Camelina “Soheil cultivar in 75 and 50% FC levels from bud to seed maturation stage and also the influence of *Micrococcus yunnanensis* on growth, physical trait seed, protein and oil content, fatty acid profile and some minerals of seed.

## 2. Materials and Methods

### 2.1. Growth conditions and treatments

This research was organized at the Research Greenhouse of Biology Department, Shiraz University, Shiraz, Iran from December to February 2018. The Camelina “Soheil cultivar” was selected as plant material and obtained from Bisetoon Shafa Co., Kermanshah, Iran. Before planting, the soil samples were canvass with a 2 mm sieve and autoclaved for 3h. The results of soil analysis are presented in Table 1. According to these results, essential elements were added to all the pots with 5kg soil. Firstly, all of the pots were divided into two groups on the basis presence and/or not presence of PGPB and their humidity was daily monitored by field capacity. To prepare PGPB suspension, the bacteria *Micrococcus yunnanensis* was supplied from Department of soil science, Shiraz University. The bacteria was incubated in nutrient broth medium at 28°C for 24h on shaker continuously. When sowing, seeds were treated with 1.0 ml bacteria suspension ( $9 \times 10^7$  CFU ml<sup>-1</sup>) in the inoculated groups. Water stress was applied to each pot at the levels of 75 and 50 % FC at the budding stage to full maturity. Camelina silique yield was harvested at the maturity stage for analysis of seed quality and biochemical responses in the treatment of water stress and PGPB.

### 2.2 Determination of seed quality

After measuring crop physical parameters (length of silique, number of silique, number of seed per siliques, number of seed per plant and 1000-seed weight) seeds from each group were used for future analyses.

#### 2.2.1 Seed mineral elements

The mature seeds were randomly harvested from each treatment. P, Zn, Fe, and Mn contents were obtained by atomic absorption spectroscopy Shimadzu A-670. Nitrogen (N), carbon (C), sulfur (S) and hydrogen (H) concentrations were determined by CHNS elemental analyzer (ECS4010, Costech Company, Italy).

#### 2.2.2 Determination of antioxidant capacity and total phenolic content

Radical- scavenging activity of Camelina seed was detrmined by 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) assay according to Hanato et al., 1998 method. In short, the different concentrations of methanol extraction (2ml) were added to the 200μM DPPH solution. The mixture was incubated in room temperature for 30 min. The absorbance value of solution was determined at 517nm using Shimadzu UV- Vis spectrophotometer, Japan. The standard curve of Trolox was prepared for the measurement of antioxidant capacity.

The total phenolic (TPC) content was measured by the Folin-Ciocalteu method (Velioglu et al., 1998). In short, the TPC was extracted from seeds (0.1 gr powder) with 10 ml 80% methanol at 70°C for 3h. 2.5ml of Folin-Ciocateu reagent was added to 0.5 ml methanolic extract and incubated in room temperature for 10 min and centrifuged in 5000 rpm for 20 min. Supernatant was incubated at 90°C for 5min when 2.5

ml  $\text{Na}_2\text{CO}_3$  solution (7.5%) was added. The absorbance value of mixture read at 765 nm and TPC was determined by a standard curve of Gallic acid.

### 2.2.3 Total soluble carbohydrate

Total soluble carbohydrate (TSC) was extracted thrice from 100 mg of mature seed using extraction soluble including glacial acetic acid, methanol, and water. It centrifuged at 3000 rpm for 10 min. The mixture of 0.5 mL extraction with 0.5 mL of 5% (v: v) phenol solution and 2.5 mL purred sulfuric acid were heated at 90°C for 30 min (Dubois et al., 1965). The absorbance was read at 490 nm. TSC content was recorded as  $\text{mg}^{-1}$  dry weight.

### 2.2.4 Oil and protein content

Oil and protein content were measured using the Near Infrared Reflectance spectrometer (NIR). It used an advanced optics based on diode array (7200) technology collecting data simultaneously at all wavelengths (450- 1650 nm).

### 2.2.5 Fatty acid profile

Seed fatty acid contents were determined using gas chromatography (GC) following trans- methylation of fatty acids and production of fatty acid methyl ester (FAME) by the modified method of Xue et al., 2013. In short, dried seeds (300 mg) in 500  $\mu\text{l}$  of methanol containing  $\text{H}_2\text{SO}_4$  (49:1 v,v) were heated at 80°C for 2h. After the mixture was cooled at room temperature, FAMEs separated with 150  $\mu\text{l}$  hexane and 100  $\mu\text{l}$  %0.9 NaCl. After vortexing and centrifuging at 3000rpm for 5min, supernatants (hexane phase) were injected to GC analyzer using a DB-225 column (30m  $\times$  250 $\mu\text{m}$   $\times$  0.25  $\mu\text{m}$ ) on Agilent 7890B.

## 2. 3 Statistical analysis

This work was conducted in a randomized complete design with a factorial arrangement and three replicates. The presented results were statistically analyzed by analysis of variance for all traits using the SAS software version 9.4 and LSD ( $p= 0.05$ ) test was performed for means comparisons.

## 3. Result

### 3.1. Growth and yield quality

To evaluate the effect of water stress levels in the reproductive phase, we defined three levels of drought (75, 50, 25% FC) for oilseed Camelina in first year. Ultimately results showed that high level of stress (25% FC) disrupted many flower buds and weren't collected enough seed for all of our analyses. So, we followed our experiment without this level of drought.

Analysis variance indicated that water-deficient during the reproductive phase significantly affected growth and seed traits (Table 2). Water stress showed no significant difference in the plant length. The number of branches increased by approximately 91.7% in B1D2 but remained the same in the other treatments compared with control. The strongest decrease in seed number (17%), silique number (9%) and silique length (12.5%) were found under stress treated-plant in comparison to control. As shown in Table 2, the highest increase in seed number and silique number were with 10.3, 10.41% respectively in PGPB treated-plants. It shows that both of the seed and silique number are in a line under water deficit and inoculation with PGPB.

Water deficient adversely increased seeds' weight. As the highest seed weight is due to inoculated (40%) and non-inoculated (31.8%) plants under the water deficit (Table 2). As illustrated in Fig 1A, there is a negative correlation between oil content and seed weight. Adversely, seed weight with protein and TSC was in a line (Fig 1B, C).

### 3.2 Seed mineral elements

Obtained data from the CHNS analyzer showed that water deficit reduced the content of carbon, sulphur, and hydrogen. This reduction arranged from 57% to 54.19, from 0.047 to 0.004 and from 0.304 to 0.247 for C,

S, and H respectively. However, the decrease of H concentration was relatively small and no significant. The results of Table 3 showed that a decrease of C concentration considered with the increase of N concentration under water stress. The highest variation of C and N concentration by -4.7% and +27.6 % respectively was in B1D1 in comparison with the control (Table 3). Compared to the relatively low variation C: N ratio, N: S ratio was very high. Insofar as, the highest decrease C: N (by 1.3 fold) considered with the highest increase N: S (by 8.5 fold) in B1D1 (Table 3). In Fig 2A, B was detected a positive correlation between oil content and C: N and a negative correlation between C: N ratio with protein. A significant increase was observed in P concentration with water stress and PGPB treatment (Table 3). The highest P content was related to B1D1 (up to 2 fold) in comparison with control (Table 3). The effect of water deficit and PGPB on micronutrients (Fe, Zn, and Mn) were displayed in (Table 3). The enhancement of Fe and Zn concentration in mild water deficit stress were considerable in inoculated (by 2.5, and 1.5 fold respectively) beside non-inoculated plants (1.9 and 1.2 fold). At all drought levels, enhancement of the micronutrients concentration was higher in inoculated plants than no- inoculated plants

### 3.3 Determination of the antioxidant and total phenolic content

Based on the results attained from the DPPH method, the increase of water stress coincided with the increase of free radical scavenging potential. The highest of antioxidant potential was observed in B1D2 with a 2.4 fold increase in comparison to control. The results of Table 4 have demonstrated that symbiosis with PGPB had a relatively small effect on the increase of the antioxidant potential of Camelina at the different levels of water stress. As illustrated in Table 4 TPC was arranged from 10.46 to 13.79 for non-inoculated plants and 10.50 to 14.34mg/g DW for inoculated plants. It indicated that water deficit significantly increased TPC by about 17.5, 31.83%, in B0D1 and B0D2 respectively in addition 34.22 and 37.09 in B1D1 and B1D2 respectively

### 3.4 Total soluble carbohydrate content

Water stress exhibited a significant increase in TSC content. The enhancement of the TSC content coincided with the decrease in oil content (Fig3). Presented results showed that drought stress increased TSC content by 52.3% and 46.6% in B0D2 and B1D2 respectively. Also, results showed that PGPB treatment merely in mild stress (75% FC) increased in comparison with its parallel level in water stress.

### 3.5 Oil and protein content

The effect of water deficit stress on oil and protein content in inoculated and no-inoculated plants with PGPB has been illustrated in Fig 3. Based on obtained data from NIR, a negative correlation was observed between oil and protein content in all of the treatments (Fig4). Oil content was significantly lower under water stress but the symbiotic association had no significant impact on it. The highest oil content was recorded with 31.94% and 31.28% for control and B1D0 treatment respectively. Adversely, the highest protein content related to B1D2 (27.16%), Also, PGPB treatment had a significant effect on the enhancement of protein content (Fig4).

### 3.6 Fatty acid profile

The effect of water stress and PGPB treatment on the obtained fatty acid profiles in gas chromatography is depicted in Table 5. Water stress increased the main saturated fatty acids (SFA) content including palmitic acid (C16:0) and stearic acid (C18:0) in no-inoculated and inoculated plants, but the content of palmitic acid and stearic acid decreased in all of the inoculated plants in comparison with the no-inoculated plant. As illustrated in the Table 4 the highest palmitic acid and stearic acid (9.99 and 3.69% respectively) in no-inoculated plants were related to B0D2 and in inoculated plants, it was related to B1D1 which accounted 9.18 and 3.49% respectively for palmitic acid and stearic acid content. The effect of water stress and PGPB on monosaturated fatty acids (MUFA) were significant. Oleic acid (18:1), eicoseonic acid (20:1) and erucic acid (22:1) content significantly declined by 46, 22.5 and 45.9 % under water stress respectively. In inoculated plants, the highest oleic acid and eicoseonic acid content were seen in mild water stress (B1D1). In comparison with control, the highest and the least erucic acid content is related to B1D0 and BD2 respectively. As the

increase of erucic acid content coincided with the increase of drought stress levels. The highest proportion of fatty acids was related to polyunsaturated acid (PUFA) including linolenic acid (C18:3) and linoleic acid (C18:2) content. The water-deficit stress in particular (75% FC) increased linoleic acid content in both of the inoculated and no-inoculated plants. The PGPB treatment significantly decreased linoleic acid content. The linolenic acid content ranged from 33.40 to 43.44%. Both of the PGPB and water deficit treatment increased the linolenic acid content. According to Table 5 linolenic acid (18:3) content negatively correlated with linoleic acid (18:2) in the inoculated plants. As the highest linolenic acid (43.44%) coincided with the least linoleic acid (18.53%) content. In general, water-deficit stress increased the total SFA content (6.04%) and total PUFA (<0.1%) content. Adversely, a total MUFA reduced in stressed plants, as the highest MUFA content (30.38%) related to B1D0. The Fig 5 A-C showed the correlation between oil content with the total content of SFA, MUFA, and PUFA in the all of treatments, as there was a severely positive correlation between oil and MUFA and adversely for SFA and PUFA content.

#### 4. Discussion

Although there is documented that *Camelina* can well adapted to semi-arid environmental condition but the present results showed that severe water deficit (25% FC) in particular during the reproductive phase significantly disrupted flower bud and declined seed yield. Based on the recent study, PGPBs are pivotal candidates in sustainable agricultural management under water deficit conditions. Our results showed that water deficit and symbiosis association significantly changed the physical parameters of camelina seed. Exposure to Water deficit during the reproductive phase resulted in a significant decline in silique number and length. It can be a part of the stress tolerance mechanism for the appropriate allocation of assimilate supply to seeds. Seed number strongly affected by water stress. It related to drought effects on inaccurate fertility and abortion of flower and or premature seed production and finally decreasing the yield (Aslan et al., 2009; Rad and Zandi, 2012). Similar results were recorded for *Carthamus tinctorius* (Istanbuluoglu et al., 2009), *Brassica napus* (Hatzig et al., 2018), *Brassica gunica* (Gan et al., 2019). The drought- induced improvement of seed weight was observed in both no-inoculated and inoculated plants (Table 2). Seed filling is strongly dependent on interplant competitions and nutrient accumulation for the synthesis of metabolites. There was a considerable association between seed weight and complement of oil and protein content (Fig1A, C). Additionally, during water deficit stress, the TSC content increased as compared to control. The increase of seed weight with an associated increase in protein content and TSC (Fig1B) can be a sustainable characteristic of water deficit tolerance in plants.

The results of the present study showed the reduction of carbon content and associated increase nitrogen content in seed grown under mild water stress in inoculated and non-inoculated plants. Not only, the C: N ratio is a sustainable indicator in oilseeds to identify the production rate of protein and oil (Jaradat and Rinke, 2010) but also, the C: N balance illustrates the rate growth and development of plants (Otori et al., 2017). There was a positive correlation between C: N ratio and oil accumulation (Fig 2A). Similar to the present study, reported in *Vicia faba* (Kabbadgi et al., 2017), *Tephrosia apollinea* (Hussain et al., 2019) that C: N ratio decreased along with oil accumulation under water deficit stress. Nitrogen accumulation is one of the defense mechanisms in response to the water deficit stress in the seed filling stage. Nitrogen not only tends to mobility from vegetative tissues to seed but also to derivate from free amino acids (Kinugasa et al., 2012). As in seeds developed underwater deficit, the rate of protein synthesis infrequently range and or possibly increase. A positive correlation between N content and protein content in camelina was reported by Jiang and his workers (2014). Conversely, the reduction of carbon supply in developing seed under stress is due to the reduction of the photosynthetic carbon fixation in vegetative tissues and less remobilization of carbohydrates (Wang and Frei, 2011; Thalmann and Santelia, 2017; Sehgal et al., 2018). Based on numerous studies, supplied carbon to developing seeds regulated with carbon partitioning between oil and carbohydrate synthesis a much higher rate of carbohydrates than oil synthesis (Schwender et al., 2015). It is in agreement with our results that water deficit stress- induced alternation of carbon and nitrogen content coincided with the decrease of oil and increase of protein. Additionally, rising TSC content is considerable for acclimation to drought stress and as the main resource for oil synthesis in the seed (Ni et al., 2019). Results presented in *Brassica napus* (Aslam et al., 2009), *Camelina sativa* (Obour et al., 2017; Hossein et

al., 2019), *Brassica junica* (Elferjani and Soolanayakanahally 2018) *Brassica napus*, and *Brassica junicea* are similar to the present report for positively correlation protein with TSC content and negatively correlation oil with both protein and TSC content under stress and or non-stress. Additionally, a positive correlation between seed weight and nitrogen and phosphorus concentration was detected in our study. It can be related to phosphorous and nitrogen partitioning for seed protein synthesis. Nakagawa and workers (2018) has reported that water deficit during the reproductive stage resulted in the reduction of oil and protein content and the increase of carbohydrate in soybean seed. It is related to the increase of genes expression involving in lipid degradation along with the decrease of gene expression being active in lipid biosynthesis.

S (sulphur) is a required constituent for plants in particular Brassicaceae family due to participate in the structure of S-containing proteins and secondary metabolites (like glucosinolates) (Poisson et al., 2019). The present study found a severe reduction in (10 fold) S concentration under water stress conditions in the inoculated plants. The previous finding showed that the reduced S content long with an increase of N content leads to an increase in the protein. Based on these facts, the N: S ratio is a suitable criterion for assay of the S-containing metabolites and oilseed quality (Dhillon et al., 2019, Poisson et al., 2019) possessing in various environment condition (Sutradhar et al., 2017). Our results illustrated a positive relationship between protein content and the N: S ratio under water stress.

Phosphorus (P) is one of the critical nutrient, responsible for plant growth and development with the supply of energy and as a macro-molecule structural component (Cetinkaya et al., 2016). The present study showed that the enhancement of P content is associated with water stress. The higher partition of P content in seed under stress may indicate the possible involvement of P in supplying energy which in turn decreasing seed filling time. Additionally, our finding showed that the increasing trend of P in seed was in a line with protein content and subsequently 1000- weight seed similarity nitrogen. It is suggested that water stress- induced enhancement of phosphorous supply energy for the synthesis of proteins. Our results confirmed the work of Gaspar and workers (2017). One well-known effect of PGPB is the improvement of phosphorus solubility and mobility from soil to root, leave and seed (Etesami and Maheshwari 2018; Taliman et al., 2019). Our finding is in agreement with results reported by Khan et al., 2019 and Mogal et al., 2019.

In as much as the main resources of hydrogen (H) are polymers such as carbohydrates and proteins, hereupon a positive correlation exists between C and both termed polymers (Chávez-Mendoza et al., 2019; Reshad et al., 2019). It is in agreement with our report and Chávez-Mendoza et al., 2019 for hydrogen content. In the present study, drought and PGPB had no significant effect on H content.

The antioxidant capacity of camelina seed by methanol extract was assessed by scavenging DPPH\* radicals value and measuring TPC. The present results showed that antioxidant capacity was enhanced under drought stress with significantly higher rate to enhance in inoculated than that in no- inoculated plants. The present study consisted with those reported in *Cuminum cyminum* (Rebey et al., 2012). Data on the total phenolic content in Table 4 showed that under water deficit stress, TPC increased in inoculated and non-inoculated plants. It is in agreement with this was reported by Rehman et al., 2018 for wheat when PGPR induces phenolic content. In plants, accumulation of phenolic features is a potential response to overcome abiotic and biotic stresses through to remove reactive oxygen species (ROS) and improve nutrients uptake (Sharma et al., 2019). Researchers have reported that PGPRs enhance phenolic content in plants and subsequently increase of nutrients uptakes (Etesami and Maheshwari, 2018).

The presence of Fe, Mn and Zn in oilseeds are consequential in translocation of assimilates and enzymatic reactions of oil, protein and carbohydrate biosynthesis (Goli et al., 2018). The concentration of Fe, Mn, and Zn of Camelina seeds is presented in Table 3. During water deficit stress, the enhancement of Zn, Fe, and Mn content was well known with a higher rate to improve in inoculated than non-inoculated plants. Our results showed a relatively positive correlation between micronutrients and protein content. It is in agreement with this is reported for *Phaseolus vulgaris* (Ghanbari et al., 2013). In previous studies, it has been documented drought-induced decrease of nutrients availability was alleviated by plant- associated bacteria through siderophores production (high affinity to  $\text{Fe}^{3+}$ ) and to convert  $\text{Mn}^{4+}$  to  $\text{Mn}^{2+}$  and improvement of Zn availability (Etesami and Maheshwari, 2018). Reports on the effects of water deficit on micronutrients

content are quite varied depending on the plants potential to stress tolerance (Samarah et al., 2004). Wijewardana and workers (2018) has reported enhancement of Fe and Mn content during drought stress and termed as osmoregulation of developing seeds.

A part of the water deficits tolerance mechanism in plants is the alternation of membrane structure and fluidity with remodeling oil content and fatty acids profile (Mohamed and Latif 2017). The decrease of photosynthesis and carbon remobilization lead to the reduction of seed oil content under drought stress (El Sabagh et al., 2019). The present study confirmed previous findings indicating the effect of water deficit stress on the significant reduction of Camelina oil by about 14.74% (Rebey et al., 2012; Elferjani and Soolanayakanahally 2018; Hatzig et al., 2018). Additionally, the decrease of Camelina oil coincided with the increase SFA (C16:0, C 18:0 and C20:0) and PUFA (C18:2 and C18:3) and the decrease MUFA (C18:1, C20:1). In the Camelina FAMES, the most abundant fatty acid was linolenic acid at 43.44- 33.40%. Our finding coincides with the previous results that showed water deficit stress at the reproductive phase increase linoleic and linolenic acid (Aslan et al., 2009; Gharechaei et al., 2019). The increase in unsaturated fatty acid (linoleic acid and linolenic acid) could be due to the activation of the lipase enzyme and followed developing membrane fluidity for the adaptation to water deficit stress (Upchurch 2008). On the other hand, desaturase enzymes activating in PUFA synthesis have stability under the biotic and abiotic stress (Nayeri and Yarzade 2014). As illustrated in Table5 the increase of the proportion of SFA under stress condition is due to palmitic acid, stearic acid and arachidic acid content having a negative correlation with oil content. The stress-induced increase trend in C16:0 and C18:0 was quite similar in inoculated and non-inoculated plants. These results were the agreement with the obtained report by Laribi et al., 2009. Based on previous findings in soybean (Mohamed and Latif 2017) and sunflower (Petcu et al., 2001) increase of palmitic acid coincided with the decrease in stearic acid under water deficient stress. In the present study, oleic acid, gadeolic acid, and erucic acid were detected as MUFA. In our experiment, MUFAs content correlated positively to Camelina oil under drought stress. It is suggested that a remarkable decrease in oleic acid content may be due to water deficit stress-induced reduction of  $[\Delta]^{9}$  desaturase activity and related – gene expression in the synthesis pathway of oleic acid from stearic acid. Additionally, a decrease of oleic acid along with increase linoleic acid and linolenic acid could be due to the higher enhancement of  $[\Delta]^{6}$  and  $[\Delta]^{12}$  desaturase than  $[\Delta]^{9}$  desaturase activity for strong tolerance to drought stress. Similar results reported for canola under drought stress (Aslam et al., 2009). On the other part, there is a significant decrease in gadeolic acid content in the stressed plant compared to control. It may be due to associate the desaturase activity with elongase enzyme which convert oleic acid to linoleic acid and or gadeolic acid respectively. Erucic acid (C22:1), one of the considerable fatty acids in the Brassicacea family (Velioglu et al., 2017) detected in our experiment. It was showed that the most abundant erucic acid by about 3.03% in B1D0 is still lower than limited content for food consumption. Both of water deficit stress and PGPB decreased C22:1 content by about 1.64%. The present study showed that seed inoculation by *Micrococcus yunnanensis* resulted in the different alterations of seed composition. According to Table 5 the decrease of oil content coincided with the increase of PUFA and decrease of SFA and MUFA in the inoculated in comparison to no-inoculated plant.

Of unsaturated acids fatty acids (USFA), oleic acid and linolenic acid contents increased with PGPB while linoleic acid decreased. Results are in a line with those has reported Silva et al., 2013 in soybean seed and Sharifi et al., 2019 in safflower seed about the effect of PGPB on the reduction of SFA (palmitic acid and stearic acid ) and enhancement of USFA (oleic acid and linolenic acid) in soybean seed. Abased on obtained reports, PGPBs increase seed filling duration increasing involvement of the nutrients in the synthesis of composition seed (Sharifi et al., 2019).

## 5. Conclusion

In this study, we focused on growth and seed yield when Camelina was grown in water deficit condition and symbiosis with *Micrococcus yunnanensis* . From our finding, it can be concluded that Camelina yield is significantly affected by water-deficit stress in the reproductive stage. The enhancement of seed weight coincided with the increase of protein and carbohydrate content when Camelina was disposed of water deficit while the decrease of the seed and silique number was associated with water deficit compared with control.



It confirmed the previous finding that the proportion of oil and protein alter adversely in the developing seed due to disparate partition of nutrients in particular carbon and nitrogen in the various environmental condition. In the fatty acid profile, the most proportion was related to PUFA in particular linolenic acid (18:3) by 50%. Saturated fatty acid and polyunsaturated fatty acid content were relatively parallel when Camelina was grown in water deficit stress. Similar to oil, monounsaturated fatty acid content reduced in stressed plants. In Camelina seed, phosphorus content and N: S ratio positively correlated to protein content and increase of antioxidant capacity in the response to water deficit stress. Besides, micronutrients (Fe, Mn, and Zn) significantly increased under water-limited conditions. Based on the science of the PGPB exhibited various changes in the growth and the physical and biochemical trait Camelina in both of the well-watered and water-limited.

## Declaration of Interest

The authors declare no conflicts of interest

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