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OMEGA-3 FATTY ACID IN PURSLANE (*PORTULACA OLERACEA* L.) INTERCROPPED WITH DRAGON'S HEAD (*LALLEMANTIA IBERICA* FISCHER & C.A. MEYER) AS AFFECTED BY MULCHING AND BIOFERTILIZERS

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ABSTRACT

Identifying and studying the environmental and agronomic factors are of major importance in increasing the quantity and quality of medicinal plants. Hence, this study was conducted to evaluate the effectiveness of mulch and biofertilizer application on yield and yield components, oil quantity and quality of purslane in different cropping systems. Two experiments were carried out with factorial arrangement based on randomized complete block design with three replications in 2015 and 2016. The first factor was cropping systems including *Portulaca oleracea* L. and *Lallemantia iberica* Fischer & C.A. Meyer sole cropping, the additive intercropping of purslane/dragon's head (P100/D20, D40 and D60 %); the second factor was mulch application including with (+M) or without (-M) wheat straw mulch; the third factor was two types of fertilization including application of 100% chemical fertilizer (F_1) and 50% chemical + biological fertilizers (F_2). The mulching significantly affected yield, yield components, oil content and omega-3 fatty acid of purslane. The highest value of omega-3 fatty acid was observed in mulched plants. Sole cropping of purslane increased number of capsule and seed yield. The highest land equivalent ratio (1.56) was observed in D40+P100 intercropped with mulch application and 50% chemical + biological fertilizers in 2016. Therefore, the organic intercropped system with mulching is applicable in the medicinal plant production such as purslane in order to reach sustainable agricultural goals.

Keywords: Biofertilizer, intercropping, land equivalent ratio, mulch, omega-3, purslane.

INTRODUCTION

The tendency to produce medicinal plants and the demand for natural products is increasing in the world. Purslane (*Portulaca oleracea* L.) medicinal plant belongs to the Portulacaceae family, which is grown in Eastern Mediterranean countries (Simopoulos et al., 1992). Purslane has been used to feed animals, and is commonly eaten as a vegetable for human food. It is quite high in iron, α-linolenic acid (LNA, 18:3w3) and antioxidants such as vitamins A, C and E and β- carotene. The plant has been used for a wide variety of medicinal purposes. LNA, an omega-3 fatty acid, is an essential fatty acid witch has a major role in human health and disease prevention such as cancer or heart disease (Omara-Alwala et al., 1991; Simopoulos et al., 1995). Humans are unable to synthesize omega−3 fatty acids, but can obtain it through diet including plant and marine oils (Freemantle et al., 2006). Intercropping as a useful strategy carried out in many areas of the world due to its advantages, including higher productivity and profitability per unit area, better efficiency of resources, higher efficiency of land use (Willey, 1979). Enhancing quantity and quality of crop production in intercropping systems are associated with better use of the available resources (Zhang and Li, 2003). Biofertilizers generally are replacing for chemical fertilizers which is able to make moveable main nutrition elements from soil to plants (Wu et al., 2005). In addition to bio-fixing nitrogen, they influence growth, yield quality and quantity of many crops by producing notable amounts of growth simulating hormones (Zahir et al., 2004). Studies of Ghamari et al. (2016a) on purslane/dragon's head intercropping showed that the integrated application of biological and chemical fertilizers increased yield and yield components of purslane. Mulching is a suitable agronomic method for increasing yield by changing microclimate (Shahidul Haque et al., 2003). In addition to increasing yield, mulching can control weeds, moderate the soil temperature, promote the maintenance of soil moisture, control erosion (Sharma et al., 2011; Myburgh, 2013). According to Saxena and Singh (1995) application of mulch and nitrogen fertilizer can enhance essential oil yield and yield of mint (*Mentha arvensis* L.) plants. The environmental conditions influence on oil fatty acid composition so that deficiency of nutrient and water supply could result in decrease seed yield and quality such as oil composition (Roche et al., 2006; Subedi and Ma, 2009). This research was undertaken to investigate the application of mulch and biofertilizer effect on productivity and omega-3 fatty acid of purslane in the purslane/dragon's head cropping system.

MATERIALS AND METHODS

Experimental design

Two experiments were conducted in Research Farm of University of Tabriz in 2015 and 2016. Weather changes during the experiments are shown in Figure 1. Some of the chemical and physical properties of soil are exhibited in Table 1. The experiments were carried out with a factorial arrangement based on randomized complete block design with three replications. The first factor was cropping systems including purslane (*Portulaca oleracea* L.) sole cropping, dragon's head (*Lallemantia iberica* Fischer & C.A. Meyer) sole cropping, the additive intercropping of purslane/dragon's head (P100/D20, P100/D40 and P100/D60 %); the second factor was mulch application including with (+M) or without (-M) wheat straw mulch; the third factor was two types of fertilization including application of 100% chemical fertilizer (F1) and 50% chemical + biological fertilizers (F2). Wheat straw mulch is distributed in plots (2m × 2m) according to the desired treatment rate of 2 t/ha. Chemical and biological fertilizers included Urea (75 kg/ha) and Azoto Barvar-1 (contain free living nitrogen fixing bacteria), respectively. Dragon's head was spaced 20cm × 1cm, 20cm × 5cm, 20cm × 2.5cm and 20cm × 1.66cm for create density of sole cropping, 20%, 40% and 60%, respectively, and purslane was spaced 40cm × 10cm for sole cropping. Sowing date of purslane and dragon's head were 2 May, 2015 and 6 May, 2016. Seeds take 6 to 8 days to germinate. The tandem disk harrow and cultivator were performed in early spring of 2015 and 2016. Primary tillage was carried out in the previous fall. Urea was used as equal split at three stages during the growing season. Before sowing, seeds were sprayed with biofertilizer solution. Hand weeding was performed after sowing and irrigation was carried out as required. Dragon's head and purslane plants were harvested at 12 and 15 weeks after sowing, respectively.

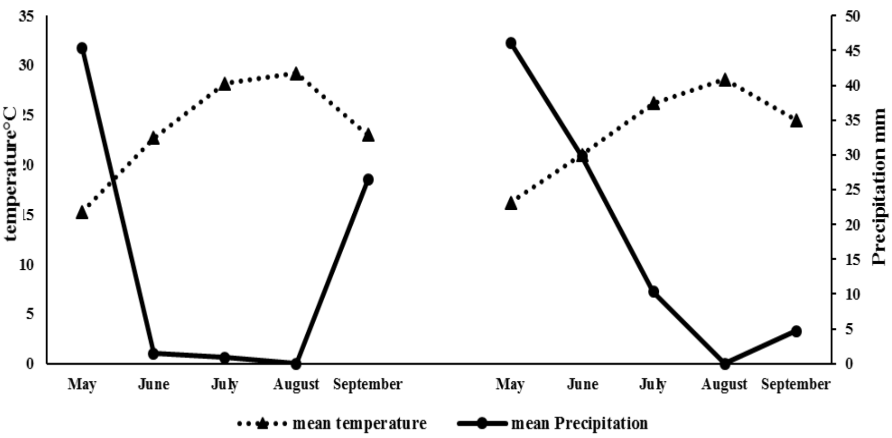

Figure 1. Means of temperature and precipitation recorded during 2015 (left) and 2016 (right).

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

OC (%)	pH	Sand (%)	Silt (%)	Clay (%)	Soil texture	EC (ds/m)	P (mg kg^{-1})	K (mg kg^{-1})	Total N (%)
0.76	7.4	65	20	15	Sandy loam	1.1	16	290	0.15

Plant growth analysis

Ten plants of purslane were collected randomly from each plot to determination of yield components of purslane. Also, plants in $2m^2$ of each plot were harvested and seed yield was recorded.

Quality parameters

Seed samples were powdered and oil content was extracted according to the AOCS (1993) method from the seeds with hexane in soxhlet extractor. Oil yield was determined by following formulas:

$$\text{Oil yield} = \%\ \text{Oil} \times \text{Seed yield} \quad (1)$$

Fatty acids were esterified as methyl esters and analyzed according to the AOCS (1993) method using a gas chromatograph. The apparatus for gas chromatography Varian CP-3800 was equipped with a FID detector (Flame Ionization Detector) and a column

(CPsill-88, 100m×0.25mm ×0/2μm). Helium gas was used as carrier gas with a pressure of 5 bar and a flow rate of 1.3 ml / min. The temperature of the injection site was set at 250 °C. Fatty acid methyl esters were identified by comparing GC retention times with those of a mixture of standard fatty acids. Fatty acids were quantified using peak areas, against internal standard.

Evaluation of intercropping

The land equivalent ratio (LER) is an index for comparing and evaluating the advantage of different ways of managing intercropped plots. The LER is described as follows:

$$LER = P_d/M_d + P_p/M_p \quad (2)$$

Where P_d and M_d are the yields of intercropped and sole dragon's head, and P_p and M_p are the yields of intercropped and sole purslane, respectively. If LER is greater than one, intercropping has the advantage and while it is less than one, the sole cropping is preferred (Vandermeer, 1989).

Statistical analysis

Combined analysis of variance was performed using MSTAT-C software. Means of treatments were compared using the Duncan's multiple range test at the 5% probability level. The data showed normal distribution and no transformation was required.

RESULTS AND DISCUSSION

Yield components

Number of capsule (m^2) and seed number per capsule of purslane were significantly influenced by year and mulching (P≤ 0.01), so that in both years, wheat straw mulch increased number of capsule and seed per capsule compared to no mulch application (Tables 2 and 3). The more soil water content and the reduction of evaporation caused by mulches are the main reasons for increasing the germination, seedling emergence and plant growth (Malhi et al., 2006). Singh et al. (2007) revealed that the mulch application increased the number and length of panicles in rice plant. Also, the effect of cropping system on number of capsule was significant (P≤0.01) (Table 2). Furthermore, 1000 seed weight of purslane was affected by year treatment, such that the highest of 1000 seed weight was observed in the second year of the experiment (Table 3). Similar results reported by Ghamari et al. (2016 b). The average monthly temperature during the growing season in the first year of experiment was more than the second year (Figure 1), therefore, the plant growth in the first year, under the influence of climatic conditions, was worse than the second year.

Table 2. Two-year analysis of variance for measured parameters as affected by mulching, fertilizer and cropping systems.

		Purslane							Dragon's head
S.O.V	**df**	**Capsule number (m^2)**	**Seed per capsules**	**1000 seed weight**	**Seed yield**	**Oil content**	**Oil yield**	**Omega-3**	**Seed yield**
Y	1	810517.59**	111.69**	0.193**	17.306**	24.392**	5.900**	15.593**	17950.08**
Y×B	4	184269.86**	39.10**	0.049	55.437**	15.974**	4.371**	33.13**	2037.94**
C.S	3	276574.61**	0.029	0.028	1302.85**	0.007	26.010**	0.684	33501.64**
M	1	36167.82**	239.18**	0.119	103.045**	92.964**	25.169**	118.659**	5805.90**
F	1	10.71	0.017	0.104	0.120	0.037	0.103	0.991	17.30
Y×C.S	3	391.014	0.004	0.013	11.55**	0.052	0.422**	0.047	213.09**
Y ×M	1	140.94	0.418	0.005	0.331	0.005	0.042	0.005	7.72
C.S ×M	3	1.514	0.040	0.008	0.592	0.024	0.127	0.349	19.21
Y× F	1	1.876	0.218	0.021	0.015	0.007	0.091	0.092	0.064
C.S×F	3	0.158	0.067	0.002	0.028	0.093	0.014	0.084	0.142
M× F	1	0.138	0.003	0.039	0.073	0.011	0.008	0.007	0.088
Y×C.S ×M	3	15.372	0.057	0.001	0.318	0.034	0.007	0.389	34.00
Y×C.S ×F	3	0.272	0.087	0.003	0.008	0.033	0.003	0.004	0.138
Y×M×F	1	0.004	0.006	0.012	0.081	0.001	0.005	0.040	0.004
C.S ×M×F	3	0.226	0.082	0.051	0.012	0.005	0.001	0.013	0.206
Y×C.S ×M×F	3	0.208	0.095	0.001	0.004	0.134	0.026	0.001	0.063
E	60	690.28	0.108	0.034	1.544	0.062	0.047	0.251	13.11
C.V. (%)		2.00	4..44	5.77	3.33	1.78	4.14	1.45	2.11

Notes: *Statistically significant at p ≤ 0.05. **significant at p ≤ 0.01. **Y:** year, **B:** block, **C.S:** cropping system, **M:** mulch, **F:** fertilizer and **E:** error.

Table 3. Measured parameters of purslane and dragon's head as affected by year and mulching.

Treatment	Purslane							Dragon's head
	Capsule number (m^2)	Seed per capsules	Oil content (%)	Oil yield ($gr.m^{-2}$)	Omega-3 (%)	1000 seed weight (gr)	Seed yield ($gr.m^{-2}$)	Seed yield ($gr.m^{-2}$)
Year								
2015	1219.07 b	73.94 b	13.44 b	4.97 b	36.04 b	0.368 b	36.85 b	158.20 b
2016	1402.84 a	76.10 a	14.45 a	5.46 a	37.84 a	0.430 a	37.69 a	185.55 a
Mulch								
-M	1291.54 b	73.44 b	12.96 b	4.70 b	35.32 b	0.351 a	36.24 b	164.09 b
+M	1330.36 a	76.60 a	14.93 a	5.73 a	38.56 a	0.446 a	38.31 a	179.65 a
Fertilizer								
F1	1311.29 a	75.04 a	13.93 a	5.20 a	34.56 a	0.397 a	37.31 a	172.30 a
F2	1310.62 a	75.01 a	13.97 a	5.22 a	34.33 a	0.401 a	37.24 a	171.45 a

Notes: Different letter (s) indicates significant difference at $p \leq 0.05$.

Number of capsule (m^2) was higher in sole cropping than under intercropping systems (Figure 2). In intercropping systems, interspecific and intraspecific competition increases for obtaining of light, water and nutrients. Therefore, with increasing the density of dragon's head in intercropping systems, the capsules number of purslane per unit area decreased compared with the sole cropping of purslane. Kizil and Toner (2005) also reported that the number of capsules in black cumin decreased by increasing plant density. Also, there was no noticeable difference between application of 100% chemical fertilizer and 50% chemical + biological fertilizers (Table 2). In the other words, it is possible to use biofertilizers instead of chemical fertilizers. Shaalan (2005) stated that the use of *Azospirillum* and *Azotobacter* increased the number of capsules in black cumin. It seems that these bacteria can increase water and nutrient uptake because of developing roots and also provide a large part of nitrogen requirements (Zahir et al., 2004).

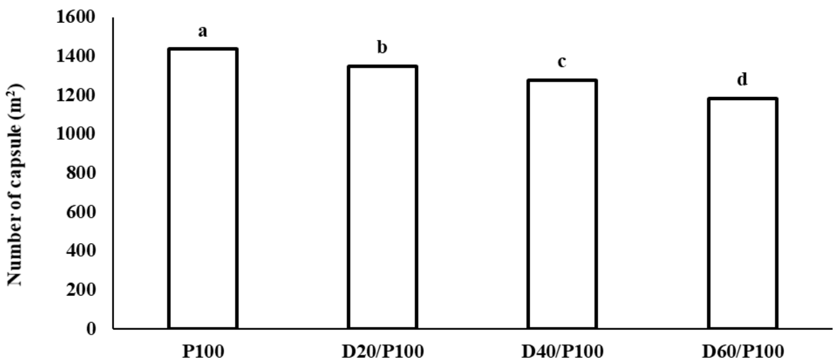

Figure 2. Capsul number (m^2) of purslane affected by cropping systems.

Seed yield

Purslane and dragon's head seed yields were significantly influenced by year, cropping systems, mulch and the interactions of year and cropping systems ($P \leq 0.01$) (Table 2). Sole cropping of both plant increased seed yield, compared with intercropping in second year (Table 4). The yield of each crops was decreased in intercropping systems due to interspecific competition for light, water and nutrients. Also, these results agree with those reported by Lal et al. (2017). The yield of leek (*Allium porrum* L.) intercropped with dyer's woad (*Isatis tinctoria* L.) in the sole cropping system was higher than the intercropping system. It showed that the intraspecific competition in the sole cropping system was lower than the interspecific competition in the intercropping system (Xie and Kristensen, 2017). Furthermore, seed yield of purslane and dragon's head was affected by mulching. The highest purslane (38.31 $g.m^{-2}$) and dragon's head (179.65 $g.m^{-2}$) seed yields were obtained from wheat straw mulched plants (Table 3). Mulch improves the yield and yield components of plants by maintaining soil moisture, reducing soil temperature and evaporation from the soil surface (Limon-Ortega et al., 2002; Sharma et al., 2011). Most reports confirm that the yield of plants has increased due to use of mulch in comparison with non-mulch soils (Bruce et al., 2006; Zhang and Sun, 2007; Chakraborty et al., 2008). Dass and Bhattacharyya (2017) observed that wheat straw mulch application in soybean facilitated in lowering the soil temperature and reducing evapotranspiration, and mulching increased soybean yield and nutrient uptakes. Seed yield was not affected by fertilizer treatment, that is, if the replacement of biofertilizers instead part of the chemical fertilizer, the seed yield does not change much due to nitrogen in urea and Azoto Barvar-1 fertilizers that increase plant growth (Table 3). Abdelaziz et al. (2007) found that the use of biological fertilizers increased the growth and yield of rosemary.

Table 4. Interactive effect of cropping systems and year on yield of purslane and dragon's head.

Treatment		Purslane		Dragon's head
		Oil yield (gr.m^{-2})	Seed yield (gr.m^{-2})	Seed yield (gr.m^{-2})
2015	**D100**	6.09 b	45.01 b	204.92 b
	D20/P100	5.24 c	39.14 c	107.63 h
	D40/P100	4.54 d	33.65 d	154.98 f
	D60/P100	3.98 e	28.65 e	165.27 e
2016	**D100**	6.84 a	47.26 a	223.58 a
	D20/P100	5.91 b	40.66 c	139.58 g
	D40/P100	4.95 c	34.22 d	185.19 d
	D60/P100	4.15 e	29.60 e	193.86 c

Notes: Different letter (s) indicates significant difference at p ≤ 0.05.

Oil content

Oil content of purslane was affected by year and mulching (P ≤0.01) (Table 2). The highest oil content (14.93%) occurred in mulched plants, while the lowest (12.96%) was achieved by no- mulched plants (Table 3). As regards to Table 3, oil content of purslane was higher in the second year of the experiment compared to the first year. Moreover, cropping systems had no significant effect on oil content. Therefore, application of wheat straw mulch makes it possible to retain more moisture in the soil due to reduced evaporation rates. Low moisture of soil reduces the amount of oil content due to disturbance in the metabolic processes of the seed and damage to the transfer of assimilates to the seed (Bouchereau et al., 1996). Like us, Dass and Bhattacharyya (2017) reported that application of wheat-residue augmented oil content and oil yield over no-mulch because of enhanced moisture availability facilitating better nutrient uptake and synthesis of oil. Faradonbeh et al. (2013) and Zamir et al. (2013) have also stated similar results due to the beneficial effect of mulching in quality of different crops. Oil content of purslane was not significantly influenced by fertilizer treatment (Table 2). This means that biofertilizers can be used instead of chemical fertilizers. Basu et al. (2008) and Akbari et al. (2011) reported similar results in confirming the role of biofertilizers in increasing the amount of seed oil. Mekki and Ahmed (2005) reported that application of biofertilizer singly or in combination with chemical fertilizers increased oil content as well as nutrients elements in soybean seeds. In general, biofertilizers can play a very important role in providing plants with essential nutrient elements required for oil formation.

Oil yield

Results showed that oil yield, which is the outcome of oil content and seed yield, was significantly influenced by year, cropping systems and mulching treatment. Also, oil yield of purslane was affected by interactions of year and cropping systems (P≤0.01). However, there was no significant difference between application of 100% chemical fertilizer and 50% chemical + biological fertilizers in terms of oil yield (Table 2). Therefore, in order to reduce the environmental risks, it is possible to replace the part of the chemical inputs with biological inputs. There are many reports indicating bacterial inoculation plus application of chemical fertilizers increase oil yield and oil content in several plants (Kumar et al., 2009; Jahan et al., 2013). In the second year, sole cropping (P100) increased oil yield in purslane compared to intercropping treatment (Table 4). Mulching with wheat straw markedly increased oil yield of purslane compared with no-mulched control (Table 3). In general, mulch application retains more moisture in the soil and elongates purslane maturity which in turn improves growth and development in this plant. Since oil yield is a function of seed yield and oil content, any changes in seed yield and oil content affect oil yield. Dass and Bhattacharyya (2017) also reported that oil yield of soybean was significantly increased with mulch application. Investigations of Lal et al. (2017) on Ethiopian mustard and chickpea intercropping system, showed that sole cropping and application of mulch with the increasing levels of fertility increased oil yield of Ethiopian mustard. Furthermore, Abdelkader and Hamad (2015) also found out that the highest oil yield of safflower intercropped with fenugreek is achieved in pure stand compared to intercropping systems.

Omega-3 fatty acid

The seeds of purslane include considerable amounts of the omega-3 fatty acid LNA (18:3w3), which is essential for human health. According to Table 2, the year and mulching significantly affected the amount of omega-3 fatty acid. However, omega-3 fatty acid was not affected by cropping system and fertilizer treatments. In other words, biological fertilizers plus application of chemical fertilizers by the half of recommended amount compared to 100% chemical fertilizer, resulted in the same effect on amounts of the omega-3 fatty acid (Table 3). Luis et al. (2013) noted that seed treated with plant growth promoting rhizobacteria (PGPR) inoculation enhances fatty acids content of soybean seed. Pazhanivel et al. (2011) also reported that quality of fatty acid contents in *Arachis hypogaea* seed improved by application of biofertilizer. Shehata and El-Khawas (2003) in a sunflower study and Cosge et al. (2007) in a safflower study reported similar results.

There was a significant increase in omega-3 fatty acid (38.56%) because of mulching with wheat straw over the no-mulched control (Table 3). Mulching was more effective in increasing omega-3 fatty acid due to its profitable effect on moisture maintenance, which also improved nutrient availability in the soil. These ranges are similar to those reported by Simopoulos et al. (1992) and Liu et al. (2000) in purslane. The result in Table 3 showed that the omega-3 fatty acid increased in the second year, compared to first year, due to high temperature in the first year of the experiment. Krol and Paszko (2017) showed that there is a relationship between climatic parameters and oil content, oil yield and fatty acid composition. The effect of agronomic practices and environmental factors such as light, water and nutrient were different on the synthesis of fatty acids (Baldini et al., 2000). In the case of medicinal plants, water deficit may cause significant variations in composition of metabolites so that primary metabolites such as lipids and total fatty acids reduced but secondary metabolites (essential oils, phenols) increased (Boschin et al., 2008; Petropoulos et al., 2008). Since the use of mulch helps maintain soil moisture, so, the amount of fatty acids decreased in no mulched plots due to lack of moisture. Our results were in agreement with those obtained by Bettaieb et al. (2009) who reported that water deficit decreased significantly the foliar fatty acid content of *Salvia officinalis* and Laribi et al. (2009) who illustrated a significant decrease in seed total fatty acid contents of caraway (*Carum carvi* L.) under water deficit. In addition, Rotundo Jose and Westgate Mark (2009) found that water deficit during soybean seed filling reduced seed oil content by about 35%.

Land equivalent ratio (LER)

In both years, the land equivalent ratio values were greater than 1 in all intercrops. Furthermore, LER values in the second year were higher than the first year in all of treatment combinations. The highest (1.56) and the lowest (1.38) LER values were observed in D40+P100 intercrop with mulch application and 50% chemical + biological fertilizers in 2016 and D20+P100 intercrop and 100% chemical fertilizer without mulch application in 2015, respectively (Figure 3). Land equivalent ratio is one of the intercropping indices that showed the supernumerary advantage of intercropping over sole cropping systems. When the LER is greater than one, that means high efficiency in utilization of the natural resources such as light, land, nutrients and water in intercropping systems. These results are conformity to those reported by Ghamari et al. (2016 b) who stated that LER of purslane + dragon's head intercropping was higher than the yields obtained by sole cropping these species. The higher LER value in intercropping than sole cropping system was stated by other researchers (Imran et al., 2011; Weisany et al., 2015; Koocheki et al., 2016).

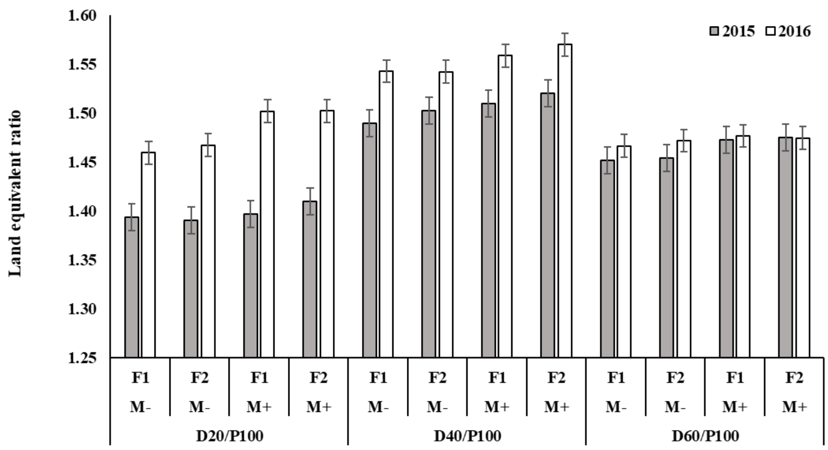

Figure 3. Land equivalent ratio (LER) of purslane and dragon's head as affected by mulching, fertilizer and cropping systems in 2015 and 2016

CONCLUSION

The results of this study indicate that oil content and omega-3 fatty acid in purslane seed increased in mulched plants. Although yield reduction was observed in intercropping treatments rather than sole cropping, but ultimately land equivalent ratio higher than one, it confirmed the more advantage of intercropping system. Wheat straw mulching increased yield and yield components of purslane due to retains more moisture in the soil and reduces soil temperature in mulched plots. Also, quantity and quality of purslane seed was not significantly affected by replacing biofertilizers with chemical fertilizer. In other words, biological fertilizers plus application of chemical fertilizers by the half of recommended amount can be used instead of 100% chemical fertilizers that will be according to sustainable agriculture. The second cropping season was wetter and cooler than in the first year of study that led to improving the measured traits. Hence, intercropping system (D40+P100) with application of 50% chemical + biological fertilizers and mulching is more efficient than other treatments.

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