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EFFECTS OF ENZYMATIC BIOFERTILISER ON GROWTH AND YIELD OF LENTIL GENOTYPES UNDER DEFICIT IRRIGATION

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*Lentil (*Lens culinaris *Medic.) is one of the important pulse crops in semiarid agro-ecological zones with a Mediterranean-type climate. Terminal drought stress and poor plant nutrition are important factors limiting crop under these regions. The effects of enzymatic biofertiliser (MOG) application at sowing time or during reproductive stage on some morphological traits and yield components of eight lentil lines were evaluated under deficit-irrigation conditions at Maragheh (37°23' N; 46°16' E) in northwestern Iran. Results revealed that application of biofertiliser did not significantly affect most of the morphological traits. However, foliar application of MOG during early flowering stage somewhat increased 100-grain weight and grain yield and decreased the number of empty pod per plant. Moreover, the results indicated that there was significant diversity between lentil lines for the investigated traits. The best performance for grain yield was recorded for FLIP 86-35L. The overall lack of considerable response of lentil to the MOG treatments may suggest that the optimal efficiency of biofertiliser cannot be achieved under water scarcity conditions. Improvement in the adaptation of enzymatic fertilisers to semi arid regions with terminal drought stress requires to be increased.*

Key words: *evapotranspiration, grain yield, growing conditions, nutrition, water deficits.*

INTRODUCTION

Lentil (*Lens culinaris* Medic.) is one of the prime cool season grain legumes in Iran and is usually grown on rain-fed areas with Mediterranean climate characterised by irregular and low to medium precipitation. The advantages of grain legumes in cropping systems are well recognised, Lentil is typically a short duration crop and can fix significant amounts of atmospheric N_2, which permits growing in N-poor soils and provides low-cost protein in dry areas.

The area sown under this crop during 2012 was 135 thousands hectares in Iran with total production of 85 thousands tonnes and average yield 629 kg·ha^{-1} (Anonymous, 2012). The yield of lentil in Iran is lower than in many other countries. The main reasons of low yield of lentil are terminal drought stress, nutritional imbalance, cultivation of local cultivars with low potential yield and lack of other production inputs (Sabaghpour *et al*., 2004). Therefore, identifying germplasm sources with acceptable drought tolerance, using morpho-physiological tools, and mitigating the terminal drought stress through some management practices, can be advantageous. Although supplemental irrigation can to some extent alleviate the effects of drought stress, water supply is a major constraint to lentil production in the Mediterranean climate of West Asia. Furthermore, there are significant differences between genotypes of lentil in coping with terminal drought stresses. In lentil, poor phenological adaptation has been shown: relatively long duration from sowing to flowering and podding, and low grain yields (Siddique *et al*., 2001). Accordingly, much attempt has been spent in recent years on the assessment of cool season grain legume species, to address this limitation.

Cropping systems in Mediterranean rain-fed regions have huge unexploited potential and this potential can be realised through improved technologies, reasonable water policy and larger investment (Oweis and Hachum, 2012). The application of water in an amount less than evapotranspiration (ET) requirements is termed deficit irrigation (DI). Irrigation supply under DI is diminished relative to that required to meet maximum ET (English, 1990). Consequently, water required for irrigation can be decreased and the water saved can be diverted for substitute uses.

Furthermore, crop harvests attained in semi arid regions are low and the residue returned to the soil is insufficient, leading to lower rates of nutrient cycling and low amount of soil organic matter. Therefore, the application of the suitable fertilisers could adequately meet the nutrients requirement of the crops. In essence, to achieve optimum production potential, input efficiency and environmental protection

some fundamental principles of using fertilisers, like as right source, right place, right timing and right application method, should be considered (Ryan *et al*., 2012). Biological fertilisers have been found to increase crop yield and prevent micro or macro-nutrients loss, thus encouraging the sustainable development of the agriculture industry. Biofertilisers usually contain some advantageous microorganisms, such as phosphate solubilising bacteria (*Psudomonas* sp., *Bacillus* sp., *Micrcoccus* sp. etc), fungi (*Aspergillus* sp. *Pencillium* sp. etc.) and actinomycetes, which are mostly associated with the plant rhizosphere and convert insoluble inorganic phosphorus into soluble form (Vikram, 2007). Although much effort has been directed to identify the effect of chemical fertilisers under water insufficient conditions (Roberts, 2007; Ryan *et al*., 2008), the influence of biological fertilisers has not been systematically studied. Therefore, the present field study was conducted to evaluate the impact of enzymatic organic biofertliser applied at different phenological stages on growth and yield of lentil under deficit irrigation in northwest Iran.

MATERIALS AND METHODS

The trials presented here were carried out at the research field of the College of Agriculture, University of Maragheh (37°23' N; 46°16' E), Maragheh, in northwest Iran, during late winter and spring 2013. The field was located at altitude 1485 m above sea level, where the climate is semi arid and cold temperate. Maragheh has average annual precipitation of 375 mm, consisting of 73% rain and 27% snow falling through winter and early spring. Rainfall is not generally well-distributed through the year and the occurrence of rainfall during late winter and early spring is frequent, with about ten days per month on average. Rainfall from June to October is relatively, when the highest rate of evapotranspiration occurs. Application of the irrigation is required in that period. Monthly meteorological data (temperature, relative humidity, evapotranspiration, and rainfall) for the growing season is shown in Table 1.

The soil texture of the experimental site is sandy loam, comprising of 53% sand, 31% silt and 16% clay, with pH of 7.45 and EC 0.506 dS m^{-1}.The area was mouldboard-ploughed and disked before planting. The experiments were laid out as split plots (3×8) based on a randomised complete block design with three replicates. Seeds were hand planted in the first week of March in eight rows per plots. The unit plot size was 2.5 m × 2.0 m with plant spacing of 25 cm × 8 cm. Weeds were controlled over the growth period with hand hoeing. The main plots included three treatments of liquid enzymatic fertilizer (MOG): control — no application of fertilizers; sowing — soil application at sowing stage (at concentrations of 5%) and reproductive — foliar application during early flowering stage (at concentrations of 2%). Sub plot treatments were eight lentil (*Lens culinaris* Medic.) lines viz FLIP 86-35L (G1), FLIP 87-22L (G2), FLIP 89-63L (G3), FLIP 90-25L (G4), FLIP 92-12L (G5), FLIP 92-36L (G6), FLIP 96-15L (G7), and FLIP 96-46L (G8). All of these lines were developed at the International Centre for Agricultural Research in Dry Areas (ICARDA), in West Asia. MOG is a liquid enzymatic biofertiliser that is manufactured using some fruit juice and crop residues, and contains 18 enzymes (like alkaline protease, glucamylase, Lipase, lipoxigenas, nitrogenase, and others), natural forms of micro and macro nutrients and vegetable based vitamins. MOG organic fertiliser was provided from Azarabadegan Company (West Azarbaijan, Iran). The MOG liquid biofertiliser consisted of 25% total organic carbon, 4% total nitrogen, 4% K_2O, 0.42% Fe, 0.16% Cu and 13% enzyme. The pH of the MOG was 6.1.

Table 1

METEOROLOGICAL DATA FOR THE LENTIL GROWN SEASON IN 2013 AT MARAGHEH STATION

	Minimum temperature (°C)	Maximum temperature (°C)	Mean temperature (°C)	Precipitation amount (mm)	Mean humidity (%)	Actual crop evapotranspiration (mm)
March	2.4	14.7	9	11.9	45.7	52.38
April	6.6	20	14	20.83	42.8	70.08
May	10.6	23.7	17.7	18.03	43.9	88.39
June	16.6	30.6	24.5	7.2	27.7	107.50
July	18.2	31.2	25.9	0	35	120.74

The Penman-Monteith equation standardised by the Food and Agriculture Organization (FAO56-PM) was used for predicting reference evapotranspiration (ET_0) for 24-h calculation time (Allen 2000). Plants were grown under rain-fed conditions during the vegetative stage and deficit irrigation was applied during the reproductive stage. The amount of water applied for deficit irrigation was 50% of actual crop evapotranspiration (ET_c) and irrigation was repeated when the available soil water at top 60 cm soil dropped below 40% of total available water. In practice, after the estimation of ET_0 rate, the proper crop coefficient (Kc) was applied to estimate actual ET_c (Nikbakht *et al*., 2007).

Lentils were harvested at ground level by hand from late June to early July and some agronomic traits including branches $plant^{-1}$, plant height (cm), pods $plant^{-1}$, grains pod^{-1}, 1000-grains weight (g), root nodules $plant^{-1}$ and rooting depth (cm) were recorded on 15 randomly selected plants in each plot. Grain and biological yield was determined by harvesting the middle three rows of each plot after avoiding border effects. The shoots were dried in hot air dryers at 45 °C for three days and biological yield was recorded. Harvest index (HI) of each plot was calculated according to the following formula: HI = (Grain yield/Biological yield) ×100. For root paramaters, soil samples containing roots were soaked in water overnight in plastic buckets. Soil-water suspensions were made by gently hand crushing the soil clots in buckets filled with water and then roots were washed with water and rooting depth and root nodules $plant^{-1}$ were measured (Kashiwagi *et al*., 2006). Shoot length (plant height) was measured at harvest time

and also ratio of shoot length to rooting depth was calculated. The 1000-seed weight, number of seeds per plant and total seed weight per plant were measured two weeks after harvesting and drying. The data recorded were subjected to statistical analysis using Fisher's analysis of variance methods. The least significant difference (LSD) at 5% was used to compare between means.

RESULTS

The results obtained from the variance analysis of data indicated that the effect of lentil genotypes on morph-physiological characters, like plant height, number of secondary branches, pod number per plant, seed number, number of root nodules per plant, number of empty pod per plant, 100 grain weight, biological yield, grain yield, harvest index and ratio of shoot length to rooting depth were significant (Tables 2 and 3). However, the interaction between biofertiliser × genotype was only significant for plant height and seed number per pod (Table 2).

Maximum plant height was observed for FLIP 86-35L (G_1) with MOG foliar application during reproductive stage. This could be due to higher growth rate or later flowering, in comparison with the other genotypes. These results suggest that genotype improvement efforts for higher seed yield can be achieved through higher growth rate during the early growth stages and better allocation of photoassimilate to reproductive organs. However, dwarf plants were noted in lines FLIP 92-36L, FLIP 96-15L, FLIP 96-46L and FLIP 89-63L, with heights of 22.39, 23.03, 23.39 and 24.75 cm, respectively (Table 2). Although Ayub *et al*. (2001) suggested that shorter varieties with more branches may be considered as a selection index in lentil, in the present study the highest yield was recorded for the taller genotype. The findings of the current study are consistent with the results of Hussain *et al*. (2014). The number of secondary branches per plant (NSB) is an important yield component of lentil, especially in water limited conditions (Ayub *et al*., 2001). In this study statistically significant differences were observed in number of secondary branches per plant between the genotypes at the 5% level (Table 2). The lowest NSB were produced by FLIP 96-46L, FLIP 96-15L, and FLIP 92-36L, respectively (Table 2). The number of pods per plant is one of the most important yield components in lentil, and is strictly related to number of branches (Hussain *et al*., 2014). The highest number of pods generally leads to higher grain yield and vice versa. In this study the highest number of pods per plant (18% higher than the average) was observed in line FLIP 98-35L. The lowest number of pods was recorded for line FLIP 96-46L (27% lower than the average) (Table 2). Highly significant variability in number of pods in lentil germplasm has also been noted by some other scientists (Hegazy *et al*., 2012; Hussain *et al*., 2014). Seed number per pod was highest value for FLIP 92-36L and FLIP 86-35L with foliar application of MOG during early flowering stage. Additionally, soil application of MOG at sowing stage in line FLIP 92-12L showed similar performance of seed number per pod. The lowest seed number per pod was recorded in line FLIP 96-46L with no fertilizer use.

Lines FLIP 86-35L and FLIP 92-12L had the highest number of root nodules and the lowest number was recorded for

Table 2

GROWTH PARAMETERS AND YIELD COMPONENTS AS AFFECTED BY APPLICATION OF BIO-FERTILISER ON LENTIL (*Lens culinaris* Medic) GENOTYPES

Bio-fertiliser	PH (cm)	NSB per plant	PN per plant	SN per pod	Grain yield ($g \cdot plant^{-1}$)	RD (cm)	Root nodules
Control	23.65^{b}	4.91^{a}	26.62^{a}	2.02^{a}	1.70^{b}	21.90^{a}	13.23^{a}
Sowing	25.33^{ab}	5.62^{a}	27.93^{a}	2.19^{a}	1.90^{ab}	23.30^{a}	14.46^{a}
Reproductive	26.88^{a}	5.38^{a}	26.58^{a}	2.32^{a}	2.32^{a}	21.74^{a}	13.70^{a}
Genotypes							
FLIP 86-35L	29.28^{a}	5.91^{a}	31.69^{ab}	2.068^{ab}	2.87^{a}	22.99^{a}	15.43^{a}
FLIP 87-22L	27.28^{b}	5.95^{a}	34.77^{a}	2.154^{a}	2.12^{ab}	22.01^{a}	13.52^{bc}
FLIP 89-63L	24.75^{bcd}	5.94^{a}	25.85^{cd}	2.01^{ab}	1.62^{b}	24.16^{a}	13.01^{bc}
FLIP 90-25L	25.74^{bc}	5.30^{ab}	29.43^{b}	1.92^{b}	2.12^{ab}	17.51^{a}	10.46^{cd}
FLIP 92-12L	26.42^{ab}	5.76^{a}	28.08^{b}	2.22^{a}	2.19^{ab}	23.10^{a}	15.62^{a}
FLIP 92-36L	22.39^{de}	4.66^{bc}	22.94^{d}	2.3^{a}	1.57^{bc}	20.77^{a}	13.70^{bc}
FLIP 96-15L	23.03^{d}	4.78^{b}	23.71^{cd}	2.04^{ab}	1.51^{bc}	21.88^{a}	14.54^{b}
FLIP 96-46L	23.39^{d}	4.09^{c}	19.86^{e}	1.96^{bc}	1.37^{c}	22.68^{a}	13.79^{bc}
Significance level							
Bio-fertilizer (B)	*	ns	ns	ns	*	ns	ns
Genotypes (G)	**	*	**	**	*	ns	*
B×G	*	ns	ns	*	ns	ns	ns

PH = plant height, NSB = number of secondary branches, PN = pod number, SN = seed number and RD = rooting depth. Mean values of the same category followed by different letters are significant at $p \leq 0.05$ level. ** Significant at 0.01 level;* significant at 0.05 level; ns, non-significant.

Table 3

EFFECT OF ENZYMATIC BIO-FERTILISER (MOG) ON MORPHOLOGICAL TRAITS AND YIELD COMPONENTS OF LENTIL (*Lens culinaris* Medic) GENOTYPES

Bio-fertiliser	NEP	100-GW (g)	BY ($kg \cdot ha^{-1}$)	SY ($kg \cdot ha^{-1}$)	GY ($kg \cdot ha^{-1}$)	HI (%)	SL/RD
Control	4.05^{a}	3.02^{b}	2956^{a}	2106^{a}	840^{bc}	28.43^{a}	1.12^{a}
Sowing	2.78^{c}	3.17^{ab}	3093^{a}	2118^{a}	965^{ab}	31.20^{a}	1.18^{a}
Reproductive	3.14^{bc}	3.56^{a}	3164^{a}	1971^{a}	1184^{a}	37.43^{a}	1.30^{a}
Genotypes							
FLIP 86-35L	2.47^{c}	4.29^{a}	3196^{ab}	1781^{a}	1205^{a}	37.64^{a}	1.31^{a}
FLIP 87-22L	2.72^{bc}	2.88^{c}	3345^{a}	2276^{a}	1079^{b}	32.27^{b}	1.33^{a}
FLIP 89-63L	2.84^{bc}	3.29^{bc}	3088^{b}	2235^{a}	849^{cd}	27.53^{c}	1.05^{a}
FLIP 90-25L	3.64^{b}	3.65^{b}	2928^{b}	1893^{a}	1031^{b}	35.27^{ab}	1.51^{a}
FLIP 92-12L	3.43^{b}	3.58^{b}	3203^{ab}	2082^{a}	1116^{ab}	34.86^{ab}	1.16^{a}
FLIP 92-36L	2.89^{bc}	2.94^{c}	2939^{b}	2167^{a}	775^{d}	26.31^{cd}	1.14^{a}
FLIP 96-15L	3.82^{b}	2.85^{c}	2881^{bc}	2195^{a}	690^{de}	23.19^{de}	1.08^{a}
FLIP 96-46L	5.16^{a}	3.52^{b}	2778^{c}	2094^{a}	686^{e}	24.15^{d}	1.05^{a}
Significance level							
Bio-fertilizer (B)	*	*	ns	ns	*	ns	ns
Genotypes (G)	*	**	*	ns	**	**	*
B×G	ns	ns	ns	ns	ns	ns	ns

NEP = number of empty pod per plant, 100 GW = 100 grain weight, BY = biological yield, SY = straw yield, HI = harvest index and SL/RL = ratio of shoot length to rooting depth. Mean values of the same category followed by different letters are significant at $p \leq 0.05$ level. ** Significant at 0.01 level;* significant at 0.05 level; ns, non-significant.

line FLIP 90-25L. The other lines were fairly similar for this trait (Table 2). The number of empty pods per plant was affected by biofertiliser and genotype. Utilisation of MOG at sowing time and reproductive stage decreased the number of empty pods by up to 32% and 21%, compared to the control, respectively. The lowest number of empty pods was recorded in lines FLIP 86-35L, FLIP 87-22L, FLIP 89-63L and FLIP 92-36L. Line FLIP 96-46L had the highest number of empty pods, which was reflected clearly in other traits, such as grain yield and harvest index (Table 3). Al-Ghzawi *et al*. (20112) showed that size of grain is positively associated with grain yield in lentil. Application of biofertliser slightly affected the 100-grain weight: plants treated with biofertiliser had larger grain yield, compared to the control. The highest biological yield was recorded for lines FLIP 86-35L, FLIP 87-22L and FLIP 92-12L, and similarly, these lines had the highest grain yield (Table 3). Application of MOG biofertilier at sowing and reproductive stage improved grain yield by up to 15% and 40%, compared to the control. This may be due to increased grain weight and reduced number of empty pods. The highest grain yield and harvest index was observed for lines FLIP 86-35L and FLIP 92-12L (Table 3).

DISCUSSION

Although application of the biofertiliser slightly improved the grain yield of lentil plants, its effect on growth was not as considerable as for chemical fertiliser, reported by other researchers (Tomar *et al*., 2000; Ryan *et al*., 2012). Limited effectiveness of biofertiliser based on enzymes or growth regulators is probably caused by inappropriate environmental conditions (Ryan *et al*., 1985; Zhu *et al*., 2012). Parrado *et al*., (2008) produced a water-soluble enzymatic hydrolyzate extract from proteinic vegetable by-products and employed it as a biofertiliser on tomato plants (*Licopericon pimpinellifolium* cv. Momotaro). They found that application of hydrolyzate extract-biofertiliser significantly improved plant height, number of flowers per plant, and number of fruits per plant. This could be due primarily to its phytohormonal action. Recently, a novel insoluble P-solubilising fungus, *Pichia farinose* FL7, was isolated from compost and introduced as biofertiliser (Zhu *et al*., 2012). The biofertiliser produced by *P. farinose* when applied to spent mushroom substrate significantly increased phosphate solubilisation and improved growth of soybean in pot experiments. This fungus showed considerable resistance against multiple environmental stresses.

However, it seems that in semi-arid regions, application of biofertilisers alone will not be sufficient and simultaneous application of other nutrients resources seems to be unavoidable. Haque and Khan (2012) reported that lentil seed inoculation with mixed cultures of two Phosphate- solubilising bacteria (PSB) with utilization of both organic and inorganic phosphor source (50%) significantly improved plant height, nodule number plant, straw and grain yields in two locations of Bangladesh. The best biofertilisers are those containing multiple-stress tolerance microorganisms and en-

zymes. Chang and Yang (2009) introduced a multi-functional biofertiliser that contained thermo-tolerant phosphate-solubilising microbes, like as *Bacillus smithii* F18, *Bacillus coagulans* C45 and *Bacillus licheniformis* A3. Activity of amylase, arboxymethyl cellulase, chitinase, pectinase, protease, lipase, and nitrogenase were observed in these microbes. Adding these microbes might improve soil quality and increase the soluble phosphorus content in biofertilisers.

Lack of effectiveness of biofertiliser might be due to soil conditions in arid regions. For example, the dissolved phosphorous moves into soil and some becomes available for plant uptake, but most of it reacts rapidly with compounds such as calcium (also iron and aluminium), which are in high concentrations in alkaline soils of semi-arid areas (Roberts, 2007). Under water limited conditions, earlier maturity due to late season N deficiency reduces lentil biomass, harvest index and yield components (Whitehead *et al*., 2000). In indeterminate crops, such as lentil, water insufficiency can induce N remobilisation from shoots and roots to seeds and initiate senescence, therefore, yield may suffer from terminal drought stress and late season N deficiency. Any N management and terminal irrigation strategy should target reducing the rate of senescence and to improve grain yield.

Although this experiment showed limited effectiveness of MOG biofertiliser under deficit irrigation situations, increasing and extending the role of biofertilisers under favourable moisture conditions still can reduce the need for chemical fertilisers and decrease adverse environmental effects (Werner and Newton, 2005).

This study revealed that biofertiliser application could not considerably affect the growth and yield attributes of lentil during both stages under deficit irrigation. It seems that beneficial effects of biofertilisers are strictly influenced by ecological factors such as water scarcity. Semiarid regions of northwestern Iran often suffer low rainfall, severe high salinity and alkaline pH, which may negatively affect the efficiency of the enzymatic biofertilisers. Also, our results showed that lentil lines developed in the semi-arid region (ICARDA) still showed high diversity for all the characteristics investigated. The best performance was observed for line FLIP 86-35L, which exceeded all other lines by producing the highest growth and yield components.

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ENZIMATISKO BIOMĒSLOJUMU IETEKME UZ DAŽĀDU LĒCU GENOTIPU AUGŠANU UN RAŽU IRIGĀCIJAS TRŪKUMA APSTĀKĻOS

Tika pētīta enzimatisko biomēslojumu ietekme uz astoņu lēcu (*Lens culinaris* Medic.) genotipu morfoloģiskajām pazīmēm un ražas elementiem irigācijas trūkuma apstākļos. Dažādi genotipi atšķirīgi reaģē uz biomēslojuma lietošanu, tomēr kopumā morfoloģiskās pazīmes uz to reaģē vājāk nekā ražas elementi. Optimālais biomēslojuma efekts nav sasniedzams ūdens deficīta apstākļos.