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Plant growth-promoting rhizobacteria improved growth, yield and yield components of Lentil (*Lens culinaris* Medic) under shading growing conditions

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Abstract

A farm experiment was conducted to observe the effects of Azosspirillum strain Brasilience inoculation and artificial shading on the yield and yield components of lentil. The seeds of lentil were incubated in flasks by shaking at 80 rpm for 2 h at 28 °C to coat the seeds with the rhizobacteria. In this experiment two factors including five shading levels (control (no shading), 25%, 50%, 75% and 100% shading) and bio-priming with plant growth-promoting rhizobacteria (seed inoculation with Azospirillum and control) arranged in a factorial experiment based on randomized complete block design with three replications. Shading had significant effect on all traits including number of filled pod, grain number per plant, 100 grain weight, harvest index (HI), biomass yield and grain yield. Also, bio-priming had significant effect on all mentioned traits except harvest index and their interaction were significant ($P< 0.01$) on all traits except 100 grain weight. The results showed that the highest grain yield (2612.4 kg per ha.), HI (33.9), grain number per plant (43.67), number of filled pod (35.5), resulted from no shading (control) and bio-priming (seed inoculation with Azosspirillum). The lowest amounts of mentioned traits resulted from 100% shading and without bacterial application. Although, Maximum biomass yield achieved from 100 shading and bacterial application. The results of this study suggest that seed inoculation with Azosspirillum have the potential to increase the growth and yield of lentil plant under shading growing conditions.

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Introduction

Lentil (*Lens culinaris* Medic) is a member of the leguminosae (*fabaceae*) family and an important pulse crop grown in Iran. Growth of lentil plant is highly sensitive to environmental conditions, especially solar radiation, high temperature and water availability. One of the important reasons for unstable lentil yield is the indeterminate growth habit of lentil plants. Extensive vegetative growth, lodging, pod abortion due to limited light interception in the lower part of the canopy, excessive flower and pod shedding, and competition between pods and vegetative parts for photosynthates are all the consequences of indeterminacy and late maturity. Radiation (sunlight) is one the limiting factors in mixed and agroforestry cultivation systems. Crop yield is a function of radiation intercepted over the growing season, the efficiency of converting the intercepted radiation to biomass and the partitioning efficiency of biomass to seed yield (Sinclair, 1993). So, in agroforestry production systems, maximizing the limited solar radiation with improved crop management practices such as seed inoculation with Plant Growth Promoting Rizobacteria (PGPR) could lead to yield improvement.

Using bio-priming and biological fertilizers has a special importance in yield improvement and soil conservation (Sharma, 2003). Inoculating crops seeds and seedlings with various PGPR is an alternative option to alleviate stress effects in crop plants (Tank and Saraf, 2010). For example Glick *et al* (1998) showed that under different stresses using PGPR can alleviate the adverse effects of stress on plant growth. These bacteria naturally are present within soil yet not in high number, thus seed inoculation may increase population of them (Cakmacki *et al* 2007). The influence of PGPR on plant growth and yield production depends on variables such as plant species Bacteria strain, soil properties and environmental conditions (Murthy and Ladha, 1998).

PGPR represent a wide variety of soil bacteria which, when grown in association with a host plant, result in stimulation of growth of their host (Zahir *et al*., 2004). PGPR include several genera of bacteria, such as Azospirillum, Pseudomonads and Azotobacter. Azosprilium is one of the PGPR which considerably affects plant growth because of its N2 biological fixation ability, phosphate mobilization within soil, its effect on plant hormones regulation particularly auxin, gibberellin and cytokinin (Zahir *et al*., 2004). PGPR can affect plant growth by different direct and indirect mechanisms (Glick 1995; Gupta *et al*. 2000). Some examples of these mechanisms, which can probably be active simultaneously or sequentially at different stages of plant growth, are (1) increased mineral nutrient solubilization and nitrogen fixation, making nutrients available for the plant; (2) repression of soil borne pathogens (by the production of hydrogen cyanide, siderophores, antibiotics, and/or competition for nutrients); (3) improving plant stress tolerance to drought, salinity, and metal toxicity; and (4) production of phytohormones such as indole-3-acetic acid (IAA) (Gupta *et al*., 2000; Cassan *et al.,* 2009).

Interest in study of PGPR has recently increased, due to the potential for improving growth and yield of various crops. Significant yield increases in many other crops have also been reported in response to inoculation with PGPR (Javed *et al*., 1996; Khalid *et al*., 1997; Zahir *et al*., 1998).

In present study the influence of bio fertilizers, limited radiation and their interaction was probed.

Materials and methods

A field experiment was conducted at the Agroforestry Farm of Ilam Agricultural University, during the period from 2nd of March to 28th of June, 2013. This region is found at latitude 33° and 27' N and longitude 46° and 27'E; its height is 1174 above the sea level. The climate of the region is cold and semidry. According to the 16-year statistics, the average rainfall amount and the area temperature are 600 mm and 14°C, respectively. Soil chemical and physical properties are shown in Table 1.

In this experiment two factors including five shading

levels (control (no shading), 25%, 50%, 75% and 100% shading) and bio-priming with plant growth-promoting rhizobacteria (seed inoculation with Azospirillum and control) arranged in a factorial experiment based on randomized complete block design with three replications.

The seeds were surface-disinfected to avoid the presence of any saprophytic and/or pathogenic microorganisms on the seed surface by dipping the seeds for 3 min in 3% sodium hypochlorite and washing 4 times in sterilized water. Seeds were left to dry on sterile Whatman filter papers overnight in a laminar flow hood. The seeds were incubated in the flasks by shaking at 80 rpm for 2 h at 28 °C to coat the seeds with the bacteria. After shaking, the seeds were air-dried on sterile Whatman filter papers overnight in the laminar flow hood.

Lentil Seeds (*Lens culinaris Medik*.) cultivar ILL4400 were sown on 21 February, 2013. 5 rows with 25 cm width and 2 m long designed in a 2×1.3 m plots and seeds planted with 2 cm intervals in North to South direction. Special net cloth with exact thickness was used for shading. The nets were cut and attached on 2×2 m frames according to plot size and placed 1 m height on plots. For 25% shading, 50%, 75% and 100% shading, nets consisted of clothes with 1:4, 2:4 3:4 and 4:4 were used, respectively. During the growth season, hand weeding was done in necessary times, too. Samplings were included of yield per square meter, biomass yield, harvest index, 100 grain weight, number of pod per plant and number of filled pod. For determining grain per plant and number of filled pod, 10 plants per plot sampled randomly with leaving border and their means recorded. For biomass yield, 1 m of each plot harvested and oven dried for 72 h in 72 degree centigrade, then dry matter determined with digital balance. Economic yield determined as grain yield produced at 1 m^2 area. Harvest index calculated as dividing economic yield by biomass yield percent. 100 grain yield determined after separation of grains. For analysis of variance SAS version 9.1software was used and graphs charted with excel.

Results and discussion

Grain yield

Shading, bio-priming and their interaction on grain yield were significant (Table 2). Means comparison showed that the greatest yield was obtained from no shading (control) and bio-priming (seed inoculation with Azosspirillum) treatment (Table 3). Shading during flowering stage reduced photosynthesis rate and led to lower grain yield. Shading reduced amount of RUBP synthesis which has a high correlation with yield (Blenkinsop, 1974). In addition, under shading condition, NR activity also reduced which induces lower carbohydrates and energy in plant (Touchette and Burkholder, 2000). Shading during grain filling reduced soybean, corn and sunflower yield (Andrade, 1995). Using PGPR may improve growth and yield in legumes (Mohammadzade *et al.*, 2012). Seed inoculation by PGPR increases the bacteria population in soil. Higher minerals nutrition availability and uptake, root healthy during growth are the main reasons for improved growth induced by PGPR (Hassanabadi *et al.,* 2010). In legumes, it's already reported that bio-fertilizers significantly increase grain yield in vetch (Mohammadzade *et al.*, 2012). However at higher levels of shading grain yield reduced but all corresponded bio-primed treatments exhibited higher yield (Fig.1). PGPR inoculation may partly cover yield loss resulted from shading.

Table 1. Soil chemical properties of the test.

Soil texture	Acidity	Electrical conductivity	organic carbon %	Phosphorus (ppm)	Absorption	Potassium Absorption(ppm)	Total nitrogen %
loam	0.62	7.32	1.4	14.6		601	12%

Harvest index

Harvest index is part of biological yield that allocated to economic parts of plant. Higher partitioning of dry matter to economic parts of plant lead to higher HI (Maghsoudi *et al.,* 2013). Our data showed that shading and its interaction with bio-priming had

significant effect on HI (Table 2). The greatest HI was observed in from no shading (control) and bio-priming treatment and the lowest was related to 100 shading and without bacterial application (Fig.1). Means comparison clarified that bio-priming increased HI at all levels of shading except control (Table 3). Lower radiation and photosynthesis under shading condition in one hand and providing nutrition for bacteria in the other hand reduce HI. Under shading condition plant increase shoot tissues to gather more light for growth then vegetative tissues are the first sink in such condition. Promoting effect of bio-priming on vegetative and natal growth is predictable. PGPRs affect dry matter partitioning and increase HI (Maghsoudi *et al.*, 2013).

Table 2. Analysis of variance for some traits of lentil under different levels of artificial shading and bio-priming.

S. O. V.	df	Number of filled pod	Grain number per plant	100 grain weight	Harvest index	Biomass yield	Grain yield
Replication	2	6.53^{ns}	11.633^{ns}	0.045^{ns}	5.053^{ns}	^{ns} 148952.6	8417^{ns}
Shading	4	**234.62	**701.637	**0.41	**217.85	**4255673.5	**192593.95
Bio-priming	1	**88.408	5.63^{ns}	**1.1	9.76^{ns}	39540229.4^{**}	**185750.8
shading× Bio-priming	4	**193.95	**275.4	0.113^{ns}	**180.13	**3007616.9	**110639.87
Error	18	6.87	12.28	0.046	180.1	232889.8	22183.809
C.V. (%)		11.97	13.72	5.74	11.88	4.51	8.5

df: degree of freedom; MS: mean of square; *,** significant at 0.05 and 0.01 probability levels, respectively. ns; non significant.

Biomass (biological yield)

The effects of Bio-priming, shading and their interaction were significant on biomass ($P<0.01$) (Table 2). Means comparisons showed that 100 shading and bacterial application had the highest biological yield and the lowest value was observed in control condition. Inoculated seeds exhibited higher biological yield compared with non-inoculated ones (Table 3). This topic may be resulted from higher dry matter allocation to vegetative tissues which makes higher using of sunlight for photosynthesis possible. PGPR have a positive impact on synthesis and regulation of plant hormones, leads to increases in root depth, water and mineral uptake by these bacteria. This mentioned factor can be caused increases in biological yield and eventually grain yield (Molla *et al.*, 2001). Also reported that inoculated seeds by PGPR have higher EY, BY and HI (Maghsoudi *et al.*, 2013). Wadud *et al* (2002) reported that, among all levels of shading the highest shoot biomass belonged to full sunlight (100%) and the lowest to 25% sunlight. Generally, in our study, biomass production increased by increasing shading especially in PGPR inoculated seeds.

Grain number per plant

Grain numbers within plant is one of the important indexes about grain yield. Higher grain numbers induces bigger sink for sap and increase yield (Bazdar *et al.*, 2014). In current study, shading and its interaction with bio-priming had significant effect on grain numbers ($P < 0.01$) (Table 2). Bio-priming at control and 25% shading conditions led to higher grain number but in other shading levels bio-priming reduced the negative effect of shading (Fig.1). Light stress during flower differentiation significantly reduced the allocation of sap to apex which may reduce the number and size of florets. Small florets results from light stress during flowering stage (Cantagallo and Hall, 2002). Since the passed light through canopy is critical for plant growth during flowering stage. Grain number/m2 exhibits linear

correlation with cumulative PAR (Kobata *et al.*, 2000). Cantagallo and Hall (2002), also reported that grain number is so sensitive to shading especially after flowering. Synthesized hormones by PGPR increase lateral roots growth, leaf weight, sap production and improve plant vegetative and economic growth.

Table 3. Comparison of the effects of artificial shading and bio-priming on agronomic traits of lentil.

	Number of filled pod	Grain number per plant	100 grain weight (gr)	Harvest index (%)	Biomass yield (Kg/ha)	Grain yield (Kg/ha)
		Shading				
(control)	31.92^{a}	40.83^{a}	4.16^{a}	25.68^{a}	9728.4^{c}	2326.89^{a}
25%	21.67^{a}	32.92^{b}	3.68^{b}	20.84^{b}	10072.6^{bc}	1972.6^{b}
50%	19^{b}	17.33^{c}	3.47^{b}	18.46^{b}	10550.9^{b}	1842.57^{b}
75%	21.83^{b}	16.42^{c}	3.62^{b}	12.21^{c}	11401.3^{a}	1386.33^{c}
100%	15^{c}	20.17^{c}	3.73^{b}	11.3^{c}	$11693.^{a}$	1228.56^{c}
		Bio-priming				
(control)	20.17^{b}	25.1^{a}	3.54^{b}	18.27^{a}	1672.7^{b}	1672.7^{b}
Azospirillum	23.6^{a}	25.97^{a}	3.92^{a}	17.13^{a}	1830.08^{a}	1830.08^{a}

Means within rows not followed by the same letter differ significantly at $P < 0.05$.

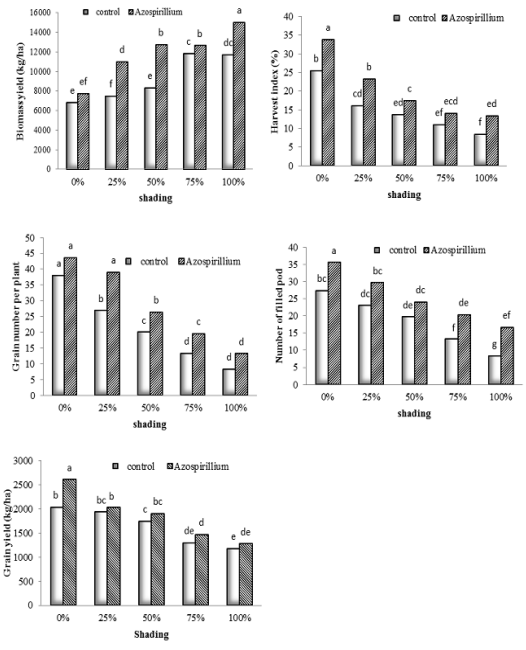

Fig. 1. Effect of artificial shading and bio-priming on agronomical traits of Lentil.

100 Grain weight

Grain weight is another part of grain yield and the effect of bio-priming and shading on this parameter was significant ($P< 0.01$) (Table 2). There was no significant difference among shading levels and control treatment exhibited the highest grain weight. Inoculated seeds had the greater grain weight than non-inoculated ones (10.73%) (Table 3). Bio-fertilizers promote water and nutrition uptake and increase photosynthesis and assimilate partitioning. Using bio-fertilizers provides better condition in soil for plant. Reduced seed filling duration resulted from shading also reduced grain weight in pea (Verghis *et al.*, 1999).

Number of filled pod

Shading, bio-priming and their interaction had significant effect on number of filled pod per plant (Table 2). The highest number of filled pod per plant was observed in control + bio-primed plants (Table 3). Bio-priming reduced the negative effect of shading as under 25% shading number of filled pod per plant was similar to control (Fig. 1). It's already reported that number of filled pod per plant has a positive effect on Lentil yield (singh *et al.*, 1998) and generally development of subsidiary branches compared with artificial shading expose to higher limitation (Wang and Ronald, 1998). Seed priming by PGPR improved seedling establishment and vigor of plants and increased number of filled pod per plant (Mohammadzade *et al.*, 2012). Rokhzadi *et al* (2008)

also reported that seed inoculation by PGPR increased number of filled pod per plant in pea.

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