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Improving Lettuce (*Lactuca sativa* L.) Growth and Yield by the Application of Biofertilizers

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ABSTRACT

Biofertilizers, especially Plant Growth Promoting Rhizobacteria (PGPR), are the most reliable replacements for the chemical ones in sustainable agroecosystems nutrient management. To study the effect of Azotobacter, Azospirillum, Pseudomonas (strain 187) and their mixture on lettuce growth, this study was conducted in 2010 at the Research Field of Islamic Azad University, Karaj branch, Iran. The experimental design was randomized complete block design with three replications. Results indicated that inoculating seeds with the biofertilizers significantly increased plant height, the number of leaves, biomass, leaf area index and plant yield, compared with the control (no biofertilizer). Generally, Azospirillum was the best treatment studied in this experiment with the highest value of the measured traits. It increased plant biomass by 43.96% compared with the control.

Key Words: *Azotobacter*, *Azospirillum*, *Pseudomonas*, plant growth promoting rhizobacteria (PGPR).

INTRODUCTION

Lettuce is a salad vegetable; cultivated since 4500 BC in the Mediterranean area. This plant is a source of vitamins and nutrients which are highly required for human health, and because of high cellulose content, it facilitates digestion. Moreover, lettuce contains lactocin and lactucopicrin which improve sleep. Today, lettuce is cultivated mostly in the North America, west Europe, Mediterranean area and Australia [1].

Rhizosphere is a medium for the interaction of soil, plants and microorganisms. The bacteria in the rhizosphere are called rhizobacteria, and rhizobacteria which improve plant growth are called Plant Growth Promoting Rhizobacteria [2]. After the adverse effects of chemical fertilizer were revealed, the biofertilizers which consist of beneficial microorganisms are under attention. The increasing costs of chemical fertilizers and the associated environmental health issues have encouraged researchers to study biofertilizers.

Pseudomonas is one of the most important Plant Growth Promoting Rhizobacteria (PGPR) which improves plant growth directly and indirectly. Suslow and Schroth (1982) evaluated the effect of *Pseudomonas fluorescence* on sugar beet and concluded that root yield was increased by 6.1- 8.6% [3]. Afzal and Asghari (2008) also found that applying *Pseudomonas* significantly increased wheat grain yield [4].

Azotobacter is a free-living nitrogen fixing rhizobacterium which does not form symbiotic relationships with plants [5]. *Azotobacter* is the most commonly used biofertilizer for some plant species like maize, wheat, sorghum and rice [6].

Azospirillum is an associative nitrogen fixing bacterium which promotes plant growth through nitrogen fixation and plant growth promoters' exudation [7, 8]. Ardakani et al. (2011 b) found that inoculating wheat seeds with *Azospirillum* increased plant N absorption by 21.68% [9].

Azotobacter and *Azospirillum* have synergistic relations. Researchers found that co-inoculating wheat and barley seeds with *Azospirillum* and *Azotobacter* increased yield [10].

Regarding the value of the mentioned biofertilizers, this experiment was conducted to see if the biofertilizers can improve lettuce growth and yield in a sustainable agricultural production system.

MATERIALS AND METHODS

This experiment was conducted in 2009 at the Research Field of Islamic Azad University, Karaj branch, Iran. The research was conducted in the randomized complete block design with three replications. Treatments of the experiment were *Azotobacter*, *Azospirillum*, *Pseudomonas* (187 strain), the mixture of the three microorganisms and control (with no biofertilizer).

To inoculate seeds, 2 g of each biofertilizer was used for 8 g of lettuce seeds (*Lactuca sativa* L. cv. Falat) in each plot, except for the control. The inoculation was conducted in dark shadow and then seeds were planted manually. Irrigation was started after planting and repeated every 4-5 days during the growing season. Thinning was carried out at 4-5 leaves stage to reach the desired density (15 plants/m^2).

To study the physiological indices and growth analysis, samples were taken weekly, 35 days after emergence. Totally, eight samplings were conducted and leaf area index, the number of leaves and leaf dry weight were measured. Leaf area index was measured by the use of chequered papers. To determine leaf dry weight, leaves were detached form the harvested plants, dried at 70^{o}C oven for 48 h, and weighted. At the end of the growing season, when plants were fully maturated, samples were harvested to measure the yield.

Finally, data were analyzed using SAS, and means were compared according to Duncan's multiple range test ($P \leq 0.05$)

RESULTS AND DISCUSSION

Analysis of variances indicated the significant effect of biofertilizer on plant height, the number of leaves, yield and leaf dry weight at $p \leq 0.01$, and on leaf area index (LAI) at $p \leq 0.05$ (Table 1).

Table 1. Analysis of variances of the measured traits

SOV	df	Mean Squares (MS)				
		Plant height	Number of leaves	Leaf Area Index	Yield	Leaf dry weight
Replication	2	**	ns	ns	*	*
Treatment	4	**	**	*	**	**
Error	8	0.15	22.56	1.43	2259.16	90.06
CV (%)	-	1.07	8.46	12.74	5.69	5.69

*ns, nonsignificant; **, significant at P≤0.01; *, significant at P≤0.05.*

Results indicated that different biofertilizers increased plant height compared with the control (Figure 1). In 84 days after emergence, plant height was the highest in *Azospirillum* (41.66 cm) and the lowest in the control (30 cm). In other researches, it was indicated that inoculating *Nigella sativa* L. seeds with *Azospirillum*, *Azotobacter* and *Pseudomonas* improved growth factors such as plant height [11, 12]. *Azospirillum* can biologically fix air nitrogen and improve plants N absorption [8]. Ardakani et al. (2011 b) studied the effect of different biofertilizers on the macronutrients absorption by wheat. They concluded that application of *Azospirillum* increased plant N absorption by 21.68% compared with the control [9].

Application of *Azospirillum* resulted in the highest number of leaves, although it had no significant difference from the mixed application of the biofertilizers (Figure 2). *Azospirillum* and the mixed application increased the number of leaves by 46.08% and 42.61%, respectively. The number of leaves was significantly correlated to the dry weight,

total yield and plant height but had no correlation with LAI (Table 2). Hamidi et al. (2006) found that inoculating corn seeds with *Azospirillum* significantly increased the number of leaves [13]. *Azospirillum* establishes associative relation with plants and improves plant growth and yield through the biological N fixation and plant growth promoters' exudation [14].

Table 2. The correlation of the measured traits

	Leaf dry weight	Yield	Leaf area index	Number of leaves	Plant height
Leaf dry weight	1				
Yield	0.99**	1			
Leaf area index	0.46*	0.47*	1		
Number of leaves	0.82**	0.83**	0.29 ns	1	
Plant height	0.76	0.76**	0.70**	0.52*	1

*ns, nonsignificant; **, significant at P≤0.01; *, significant at P≤0.05.*

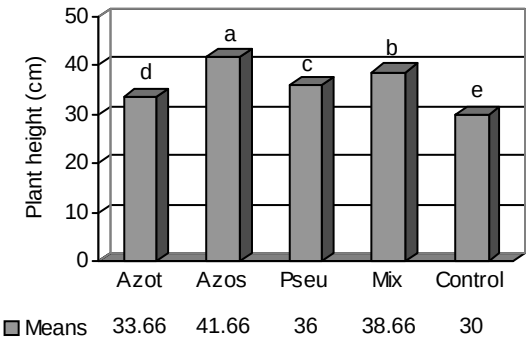

Figure 1. The effect of biofertilizer treatments on plant height.

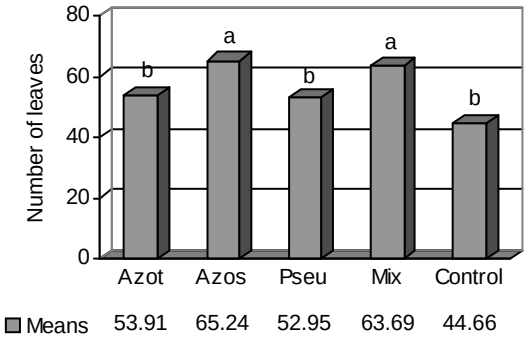

Figure 2. The effect of biofertilizer treatments on the number of leaves.

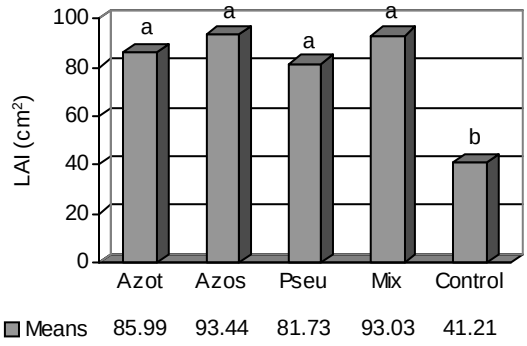

Figure 3. The effect of biofertilizer treatments on leaf area index (LAI).

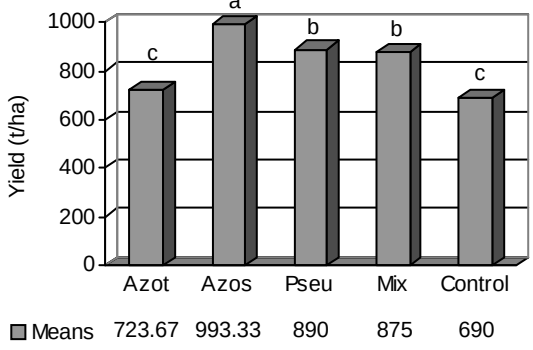

Figure 4. The effect of biofertilizer treatments on yield.

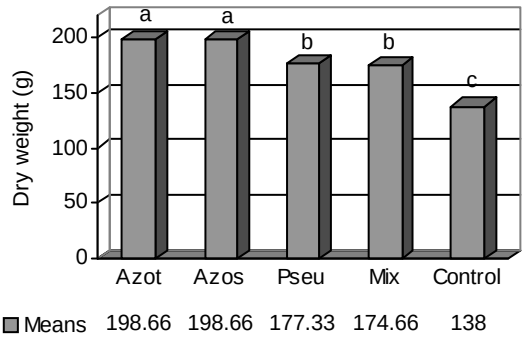

Figure 5. The effect of biofertilizer treatments on leaf dry weight.

Azotobacter, *Azospirillum*, *Pseudomonas* and the mixed application were in the same group for their effect on LAI (Figure 3). The total yield was the highest in *Azospirillum*. Application of *Azospirillum* increased the total yield by 43.96% compared with the control (Figure 4). *Azospirillum* was the best biofertilizer to increase leaf dry weight although it was the same as *Azotobacter* (Figure 5). Both *Azospirillum* and *Azotobacter* increased leaf dry weight by about 43.95% compared with the control. Stancheva et al. (1992) represented that inoculating corn seeds with *Azospirillum* enhanced plant dry weight. *Azospirillum* produces growth promoting hormones such as auxin, gibbereline and cytokinin; affecting plant growth [15]. Moreover, *Azospirillum* improves plants macro and micronutrients absorption. Ardakani et al. (2011 a) showed that application of *Azospirillum* significantly affected micronutrients absorption by wheat ($p \leq 0.01$) [16]. In their experiment, inoculating wheat seeds with *Azospirillum* increased Fe (by 20.14%), Mn (by 26.16%), Zn (by 25.74%) and Cu (by 27.08%) absorption compared with control. Results of this study indicated that biofertilizers can improve lettuce yield. Although all tested biofertilizers in this experiment were effective on the measured traits, but *Azospirillum* was the most effective treatment which increased plant height, total yield and leaf dry weight by 38.87, 43.96 and 43.96%, respectively.

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