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The Effect of *Pseudomonas* Bacteria on Maize Growth and Atrazine Biodegradation in Soil

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ABSTRACT: To study the effect of *Pseudomonas* bacteria on maize growth and atrazine biodegradation, this experiment was conducted in 2013 in a field in Shahriar area, Iran. The experiment was conducted in factorial in the form of a randomized complete block design with three replications and the two factors: (1) atrazine concentration in four levels (0, 1, 2 and 3 kg/ha atrazine herbicide), and (2) *Pseudomonas* species in four levels (non-inoculated control, *Pseudomonas fluorescence, P. putida* and combination of *P. fluorescence* + *P. putida*). Measured traits included: number of row / ear, number of kernel / row, stem diameter, grain yield, and decomposition percentage of atrazine. Results showed that atrazine biodegradation was significantly affected by bacteria; the highest biodegradation rate was achieved when both species were applied together (73.92%). Moreover, results showed the significant effect of atrazine concentration, bacteria and their interaction on all measured traits. Mean comparison of different bacterial treatments showed that all measured traits were the lowest in the control and the highest in the dual inoculation of *Pseudomonas fluorescence* + *P. putida*. Studying the mean comparison of the interactions indicated that grain yield was the highest in 3 kg/ha atrazine × dual bacterial inoculation (12.86 t/ha).

Keywords: Gesaprim, *P. fluorescence*, *P. putida*, *Zea mays*.

INTRODUCTION

Maize is one of the most important food crops which is in the third rank after wheat and rice. Maize had been cultivated about 4500 B.C. in America; then it was introduced to Europe and nowadays it is being cultivated all around the world, especially in warmer climates. In Iran, it is being cultivated in nearly all areas because of sunny weather. Maize plays vital role in human and livestock food production and is also used for various industrial purposes. Maize (*Zea mays* L.) belongs to the Poaceae family. It is a C_4 plant which benefits from warm and sunny climates (Mazaherilaghab, 2008; Nesbitt, 2005; Staller, 2010; Winch, 2006).

Like other crops, maize growth and yield is seriously sensitive to weed infestation. Using chemical herbicides to control weeds is the most prominent method of weed control all over the world. Atrazine is a selective herbicide controlling broad leaf weeds in cereals and especially maize. It was first used in 1950s. It is an inhibitor of photosystem II and prevents electron transfer in sensitive weeds. The half-life of atrazine in soil varies from 4-54 weeks; it is considered a persistent herbicide which can pollute soil and water resources and cause health problems to human and animals (Khan, 1980; Sene *et al.,* 2010; Yaron *et al.,* 1996). The persistency and the associated problems of atrazine require researchers to work on the methods to accelerate atrazine degradation in soil. Biological methods are the most environmentally friendly and cost-effective methods for this purpose (Theng *et al.,* 2000; Zhou and Song, 2004).

It is proved that the activity of soil microbial population can accelerate the degradation process of the chemicals. It is possible to increase the decomposition rate of chemicals in soil, such as the herbicides by promoting soil microbial population activity through providing their foods and increasing their population via manmade inoculations. This method is called bioremediation. Biodegradation could be started as soon as the herbicide contacts the microorganisms. In fact, herbicide acts as the source of energy for soil microbial population; so, the process results in the degradation of herbicide and enhancement of soil microbial population / activity (Abdelhafid *et al.,* 2000; Behiki and Khan, 1986).

Among different microorganisms, *Pseudomonas* bacteria are under attention for bioremediation studies because they can decompose organic and non-organic pollutants such as petroleum derivative, polycyclic aromatic hydrocarbons, pesticides etc. (Jilani and Khan, 2006). The effect of *Pseudomonas* bacteria on the biodegradation of pollutants including atrazine is reported by other researchers (Kim and Hao, 1999; Lee and Gibson, 1996; Shields *et al.,* 1991).

Behiki and Khan (1986) reported that various *Pseudomonas* strains had the ability to grow in a culture medium containing atrazine and reduced the concentration of the herbicide. Ramos *et al.* (1991) conducted and experiment to test the effect of a resistance strain of *Pseudomonas putida* on atrazine concentration reduction in soil. This strain was genetically manipulated to add a plasmid containing the genes that could produce atrazine decomposing enzymes. Results showed that the added plasmid enabled the bacteria to reduce the concentration of atrazine quickly in soil. Rezaei *et al.* (2011) also studied the biodegradation of different concentrations of atrazine by two *Pseudomonas* species bacteria and reported that both species had the ability to decompose atrazine in different concentrations within 48 hours. The decomposition rate was higher when higher atrazine concentration was applied.

Another function of *Pseudomonas* bacteria, which is the primary function, is the improvement of plant growth and yield; some species of *Pseudomonas* bacteria are considered as the plant growth promoting rhizobacteria (PGPR) but some other species may act as a pathogen to plants. Pseudomonas bacteria improve plant growth and yield through direct and indirect mechanisms such as biological nitrogen fixation, enhancement of soil P availability to plant root, improvement of plant root system development, and inhibition of the activity of plant pathogens (Crowley *et al.,* 1991; German *et al.*, 2000). Shaterabadi *et al.* (2011) tested the effect of different *Pseudomonas* strains on sorghum (*Sorghum bicolor* L.) reported that all strains had the ability to improve sorghum yield and yield components including plant height, number of leaves, fresh footage yield and dry forage yield.

Regarding the benefits of *Pseudomonas* bacteria for plant growth and yield, and their effect on the reduction of chemical residues in soil, this experiment was conducted to test the effect of *P. fluorescence P. putida* on yield and yield components of maize, and to evaluate their effect on the biodegradation of atrazine herbicide residues in soil.

MATERIALS AND METHODS

In order to study the effect of *Pseudomonas* bacteria on maize growth and atrazine biodegradation in soil, this experiment was conducted in 2013 in a field in Shahriar area, Iran (50° 59´ E, 35° 34´ N, 1100 m above the sea level). The area is known to have semi-arid to arid climate with an average annual precipitation of 200-230 ml.

Experimental design was factorial in the form of a randomized complete block design with three replications. The two factors of the experiment included:

Atrazine concentration. In four levels including 0 (A_1), 1 (A_2), 2 (A_3) and 3 (A_4) kg/ha atrazine herbicide. Atrazine (trade name, Gesaprim; purity, 99.5%; product of Merck, Germany) was mixed with the irrigation water in the required concentrations.

***Pseudomonas* species.** In four levels including non-inoculated control (B_1), *Pseudomonas fluorescence* (B_2), *P. putida* (B_3) and combination of *P. fluorescence* + *P. putida* (B_4). The bacteria species were obtained from the Microbe Bank of Soil Biology Research Department, Iranian Soil and Water Research Institute, Karaj, Iran. The bacteria were inoculated in BHI broth nutritional solution to be activated. After 24 h in 37°C incubator, the bacteria were transferred to the BHI Agar culture medium.

Filed operations were initiated on the middle of April 2013. The field was prepared using moldboard plow, disk and leveler. The furrows were formed by furrower with the length of 5 m in interval of 75 cm. Then, seeds were planted manually on the rows with 20 cm gap, on May 5 and 6, 2013. Planting density was 8.32 plants/m^2. Quickly after that, the field was irrigated while the atrazine was mixed with the water. During the growing period, irrigation was conducted every seven days.

To measure atrazine biodegradation in soil, sampling was conducted every 15 days. Five soil samples were taken from each plot in different depths, to obtain atrazine residues. Samples were kept in plastic bags, in ice flasks and were transferred to laboratory and were held in -20°C. Then, samples were located in open air until the ice melts down and samples dry. After that, samples were grinded to reach a homogenous form, and were passed through a 1.2 mm sieve. To extract the residue of the herbicide, 50 g of the soil samples were weighted and poured in 250 ml flasks; 100 ml methanol + distilled water (70:30 ratio) was added. Samples were shook for 2 h in room temperature on a horizontal 230 rpm shaker. The mixture was passed through Whatman no. 42 filter paper. Prior to injecting the solution to HPLC, 0.5 ml of it was passed through a 0.2 µm filter syringe. The detector type was UV-VIS Spectrophotometric Detector SPD-2AS at the wavelength of 220 nm.

Other measured traits included: plant height, the number of rows, the number of kernels in row, stem diameter and grain yield. These traits were measured at the end of the growing period.

Data analysis was conducted using M-STATC and means were compared according to the Duncan's multiple range test.

RESULTS AND DISCUSSION

A. Atrazine biodegradation studies

Analysis of variance showed that atrazine biodegradation was significantly affected by bacteria species (data not shown). Mean comparison indicated that among the two species, biodegradation rate was higher in *Pseudomonas putida* (64.13%) and lower in *P. fluorescence* (60.28%). Results showed that the highest biodegradation rate was achieved when both species were applied together (73.92%) (Table 1).

Different groups of chemicals are decomposed in soil by different group of microorganisms. So, when a herbicide is applied to soil for the first time, the lack of the related microorganisms may results in a very slow biodegradation rate. Soil microorganisms use the chemicals such as atrazine as the source of energy and nutrients such as C and N (Behiki and Khan, 1986). In addition to the population and activity of soil microorganisms, other factors have also role on the decomposition rate of chemicals including soil moisture, soil texture, soil organic matter content, soil pH, etc. (Khan, 1980; Theng *et al.,* 2000). Behiki and Khan (1986) tested the response of *Pseudomonas* bacteria to atrazine and reported that the bacteria could survive in a culture medium containing atrazine, and reduce the concentration of the herbicide. Rousseaux *et al.* (2001) application of atrazine in soil results in the enhancement of C and N level which consequently results in the enhancement of the activity and population of bacteria; reducing the concentration of the herbicide.

Table 1: Percentage of atrazine biodegradation in different bacterial treatments.

Treatment	Decomposition percentage (%)
Non-inoculated control	0
P. fluorescence	60.28
P. putida	64.13
P. fluorescence + P. putida	73.92

B. Plant growth studies

Analysis of variance showed the significant effect of atrazine concentration, bacteria and the interaction of the two factors on all measured traits including plant height, the number of row / ear, the number of kernel in row, stem diameter and grain yield (Table 2). Mean comparison of the atrazine concentration levels (Table 3) indicated that the lowest values of all measured traits were achieved in the control (0 kg/ha atrazine). The highest values of the measured traits were achieved in 3 kg/ha atrazine, except for the plant height which was the highest in 2 kg/ha atrazine. Increasing the concentration of atrazine from 0 to 3 kg/ha resulted in the enhancement of plant height and grain yield by about 12% and 53%, respectively (Table 3).

Table 2: Analysis of variance of the effect of treatments on the measured traits.

SOV	df	Mean Squares (MS)				
		Plant height	Number of row / ear	Number of kernel / row	Stem diameter	Grain yield
Replication	2	ns	ns	ns	**	**
Atrazine (A)	3	**	**	**	**	**
Bacteria (B)	3	**	**	**	**	**
A × B	9	*	**	**	**	**
Error	30	1.52	0.21	6.53	0.10	0.55
CV (%)	-	0.6	3.01	5.92	5.41	1.2

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

Table 3: The effect of different atrazine concentrations on the measured traits.

Treatments	Plant height (cm)	Number of row / ear	Number of kernel / row	Stem diameter (mm)	Grain yield (t/ha)
0 kg/ha	184.09c	14.23d	41.96c	20.58d	8.25b
1 kg/ha	193.24b	15.02c	47.37b	21.86c	9.96c
2 kg/ha	208.1a	15.56b	51.35a	25.06a	11.58b
3 kg/ha	207.54a	15.98a	51.65a	25.22a	12.62a

Means in a column followed by the same letter are not significantly different at P≤0.05.

Table 4: The effect of different bacterial treatments on the measured traits.

Treatments	Plant height (cm)	Number of row / ear	Number of kernel / row	Stem diameter (mm)	Grain yield (t/ha)
Control	194.86c	14.76a	47.22b	21.43a	9.98a
P. fluorescence	197.21b	15.31a	47.53b	22.87d	11.79c
P. putida	200.56a	15.42a	46.82b	24.49c	12.26b
fluorescence + putida	201.56a	15.78b	50.73a	24.89b	12.76a

Means in a column followed by the same letter are not significantly different at P≤0.05.

Studying the mean comparison of different bacterial treatments (Table 4) showed that all measured traits were the lowest in the control and the highest in the dual inoculation of *Pseudomonas fluorescence* + *P. putida*. Among the two bacteria species, *P. putida* was more effective on the measured traits compared with the *P. fluorescence*. In the dual inoculation of *P. fluorescence* + *P. putida*, plant height and grain yield were about 3% and 28%, respectively, compared with the control (Table 4).

Mean comparison of the interactions (Table 5) indicated that plant height was the highest (211 cm) in the interaction of 3 kg/ha atrazine × no bacterial inoculation and the lowest (181.4 cm) in no atrazine × no bacterial inoculation.

The number of row / ear was the highest (16.75) in 3 kg/ha atrazine × *P. fluorescence* and the lowest (14) in 0 kg/ha atrazine × *P. putida*. Results showed that the highest number of kernel / row was achieved in 3 k/ha atrazine × dual bacterial inoculation (53.16) and the lowest number of kernel / row was achieved in no atrazine × no bacterial inoculation (39.14). Stem diameter was the highest in 2 kg/ha atrazine × no bacterial inoculation (27 mm) and the lowest in no atrazine × no bacterial inoculation (20.82 mm). Finally, mean comparison indicated that grain yield was the highest in 3 kg/ha atrazine × dual bacterial inoculation (12.86 t/ha) and the lowest in no atrazine × no bacterial inoculation (8.68 kg/ha) (Table 5).

Table 5: The effect of interaction of atrazine × bacterial treatments on the measured traits.

Treatments	Plant height (cm)	Number of row / ear	Number of kernel / row	Stem diameter (mm)	Grain yield (t/ha)
A_1B_1	181.40e	14.40g	39.14ef	20.82h	8.68f
A_1B_2	187.00f	14.65eg	40.75a	21.45f	8.88h
A_1B_3	183.49e	14.00fg	42.52de	22.50de	8.98g
A_1B_4	189.36e	14.66g	46.54bcd	22.50de	9.28f
A_2B_1	198.35d	15.52cd	42.61de	23.40e	9.25d
A_2B_2	189.68d	14.85def	50.45ab	20.85g	10.35g
A_2B_3	194.18a	15.21de	45.65cd	21.00fg	9.96e
A_2B_4	194.85b	14.50efg	50.73ab	22.15e	9.98d
A_3B_1	210.68b	15.47cd	53.04a	27.00a	9.35b
A_3B_2	204.83a	15.14de	50.85ab	22.35de	10.33c
A_3B_3	206.68a	16.34ab	49.92c	24.30b	11.16b
A_3B_4	209.85a	15.22de	52.54a	26.50a	10.68ab
A_4B_1	211.00a	15.43cd	52.62a	26.50a	9.68a
A_4B_2	208.61b	16.75a	50.65ab	22.82d	11.33c
A_4B_3	206.46b	16.12ab	50.19ab	24.70b	11.35b
A_4B_4	210.22a	15.45cd	53.16a	26.85a	12.86a

A_1, 0 kg/ha atrazine; A_2, 1 kg/ha atrazine; A_3, 2 kg/ha atrazine; A_4, 3 kg/ha atrazine.
B_1, no bacteria; B_2, *P. fluorescence*; B_3, *P. putida*; B_4, *P. fluorescence* + *P. putida*.
Means in a column followed by the same letter are not significantly different at $P \leq 0.05$.

Results of this experiment proved the effect of bacterial inoculation with *Pseudomonas fluorescence* and *P. putida* on the growth and yield of maize plant; the dual inoculation of *P. fluorescence* + *P. putida* increased plant height and grain yield by about 3% and 28%, respectively, compared with the control. Although some species of *Pseudomonas* bacteria are pathogen to plants; however, some other species of are considered as the plant growth promoting rhizobacteria (PGPR). *Pseudomonas* bacteria improve plant growth and yield through direct and indirect mechanisms (Crowley *et al.*, 1991; German *et al.*, 2000).

Pseudomonas bacteria produce siderophores under Fe deficiency conditions. Siderophores are organic molecules which act like chelates and transport Fe into plant roots; this is a noticeable mechanism for Fe absorption under low Fe conditions (Sharma *et al.*, 2003). Alipour and Sobhanipour (2012) tested the effect of different bacterial treatments on maize and found that *Pseudomonas* application significantly affected plant growth and yield, Fe uptake and chlorophyll content; increasing plant Fe content from 91.02 to 110.16 mg/kg and plant biomass from 49.50 to 79.25 g/pot. *Pseudomonas* is also a phosphate solubilizing and nitrogen fixing bacterium. Eftekhari *et al.* (2012) reported that application of *Pseudomonas* significantly increased barley grain yield by 10.7% and P content by 23.35%, compared with the control. Alikhani *et al.* (2006) found that *Pseudomonas fluorescence* and some other microorganisms such as *Bacillus* sp. and *Rhizobium leguminosarum* had the ability in solubilizing the mineral P in soil and increase its availability to plant roots.

Pseudomonas bacteria also improve plant root system development. Hasanabadi *et al.* (2010) conducted experiments to test the effect of Pseudomonas inoculation on barley root system development and reported that the inoculation increased root dry weight and fresh weight and root length.

Samavat *et al.* (2012) also found that inoculating common bean with *Pseudomonas fluorescence* increased root length compared with the non-inoculated control.

Waller and Cook (1982) observed that inoculating wheat seeds with *Pseudomonas fluorescent* resulted in the enhancement of wheat yield by 27%. Haghighi *et al.* (2014) tested the effect of different PGPR treatments including *Pseudomonas* on wheat and observed that the treatment significantly affected all measured traits including plant height, biomass and grain yield. Samavat *et al.* (2012) conducted experiments to find the effect of *Pseudomonas fluorescence* on common bean and reported the improvement of growth and yield as the result of inoculation. They found that the inoculation increased plant height, shoot dry weight, root length and leaf chlorophyll content. Shaterabadi *et al.* (2011) also tested the effect of different *Pseudomonas* strains on sorghum (*Sorghum bicolor* L.) reported that all strains had the ability to improve sorghum yield and yield components including plant height, number of leaves, fresh footage yield and dry forage yield.

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