INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 6, No. 1, p. 293-297, 2015

RESEARCH PAPER OPEN ACCESS

Atrazine biodegradation by stimulating the activity of soil bacterial population in maize field

Shahram Chegini, Behzad Sani, Hossein Hassanpour Darvishi

Department of Agronomy, College of Agriculture, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran

Key words: Bioremediation, *Pseudomonas fluorescence*, *Pseudomonas putida*.

http://dx.doi.org/10.12692/ijb/6.1.293-297 Article published on January 10, 2015

Abstract

Nowadays, environmental pollution is a worldwide issue. Uncontrolled application of chemical inputs to agricultural fields has contaminated soil and water resources; endangering the health of all life forms on earth. One of the most important, cost-effective and safest methods of removing herbicide residues from soil is bioremediation which means using microorganisms such as plant growth promoting rhizobacteria to biodegrade chemicals residue. Application of bacteria to increase the decomposition rate of chemical herbicides in soil is a reliable method; which is the objective of this experiment. So, an experiment was conducted in factorial in the form of a randomized complete block design with three replications in a maize field in Shahriar, Iran. Treatments included bacterial species in four levels (control, *Pseudomonas fluorescence*, *P. putida* and combination of *P. fluorescence* + *P. putida*) and atrazine concentration in four levels (0, 1, 2 and 3 kg/ha). HPLC was used to analyze samples. Results showed that both bacteria species had the ability to biodegrade all three atrazine concentrations in soil. Atrazine biodegradation rate had direct relation with atrazine concentration. Biodegradation capability of *P. putida* (64.13%) was higher than *P. fluorescence* (60.28%); it was the highest in the combined treatment (73.92%).

* **Corresponding Author:** Behzad Sani ✉ dr.b.sani@gmail.com

Introduction

Nowadays, environmental pollution is a worldwide issue. Uncontrolled application of chemical inputs to agricultural fields has contaminated soil and water resources; endangering the health of all life forms on earth (Haghnia and Razavi, 2008).

Biologic methods are one of the promising methods of increasing the sustainability of environment, plants growth and removing chemical inputs residues from soil (Zhou and Song, 2004). According to the researches, promoting the activity of soil microbial population through providing their foods and increasing their population by manmade inoculation, it is possible to boost up the decomposition rate chemical in soil, such as the herbicides; this method is called bioremediation. Microbial degradation may be initiated as soon as the herbicide contacts the microorganisms. In this mechanism, herbicide acts as the source of energy for 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).

Atrazine is a selective herbicide controlling broad leaf weeds mainly in maize fields and some other cereals. Atrazine is considered as a environmental pollutant because it is a persistent chemical and also has the potential to contaminate soil and underground water resources (Sene *et al.*, 2010).

Pseudomonas bacteria, because of their ability to decompose organic and non-organic pollutants such as petroleum derivative, polycyclic aromatic hydrocarbons, pesticides etc., have attracted the attention of researchers (Jilani and Khan, 2006). In different bioremediation studies, Kim and Hao (1999), Lee and Gibson (1996), Lee *et al.* (1995) and Shields *et al.* (1991) reported the effects of Pseudomonas bacteria on the biodegradation of pollutants.

Although there are various reports about Atrazine and its residues; however, more researches are required to understand the complexities of this subject in different climatic conditions. So, the objective of this experiment was to evaluate the bioremediation of atrazine by the application of *Pseudomonas* bacteria in maize cropping system.

Materials and methods

Site and treatments

This experiment was conducted in 2013 in a field in Shahriar, Iran (50° 59´ E, 35° 34´ E, 1100 m above the sea level). The area is located in semi-arid to arid climate with an average annual precipitation of 200-230 ml. The soil at the test site was average. The experiment was conducted in factorial in the form of a randomized complete block design with three replications and two treatments:

Atrazine concentration

In four levels including 0 (A_1), 1 (A_2), 2 (A_3) and 3 (A_4) kg/ha.

Bacterial 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).

Field operations

Filed preparation was started on mid April 2013 and the furrows were formed with the interval of 75 cm and length of 5 m. Maize seeds were planted manually on the rows with the interval of 20 cm, on May 5 and 6, 2013. Planting density was set on 8.32 plants/m^2. Quickly after seeding, the field was irrigated. Atrazine herbicide (trade name: Gesaprim; purity: 99.5%; product of Merck, Germany) was also mixed with irrigation water in the required concentrations.

Bacteria preparation

The bacteria were obtained from Microbe Bank of Soil Biology Research Department, Iranian Soil and Water Research Institute.

Sampling

Sampling was conducted every 15 days; five samples were taken from each plot in different depths to measure herbicide residue in soil. 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.

Statistical analysis

Finally, data were analyzed using M-STATC and means were compared according to the Duncan's multiple range test.

Results and discussion

Results

Analysis of variance indicated the significant effect (P≤0.01) of atrazine concentration, bacteria species and their interaction on the biodegradation of atrazine in soil (Table 1).

Table 1. Analysis of variance of the effect of treatments on atrazine biodegradation.

SOV	df	Mean Square
Replication	2	ns
Atrazine concentration (A)	3	**
Bacteria species (B)	3	**
A × B	9	**
Error	32	0.52
CV (%)	-	11.1

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

Results indicate that both *Pseudomonas* species decomposed atrazine in soil; however, the decomposition rate was higher in *P. putida* (64.13%) than *P. fluorescence* (60.28%); it was the highest (73.92%) in the combined treatment (Table 2; Figure 1).

Mean comparison of the interaction of the two factors showed that the highest residue of atrazine in soil (28.6) was related to 1 kg atrazine × non-inoculated treatment, and the lowest residue (5.1) was related to 3 kg atrazine × *Pseudomonas fluorescence* + *P. putida* (Table 3; Figure 2).

Table 2. Atrazine decomposition percentage 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

Discussion

Results showed that atrazine decomposition was directly related to atrazine application rate. It means that decomposition rate was higher when application rate was higher. This relation may be attributed to the number of microorganisms, enhancement of their

colonies and increased availability of sources of C and N, because atrazine has C and N in its chemical structure (Anonymous, 2001). Soil bacteria use atrazine as the source of energy, C and N (Behiki and Khan, 1986). A large number of factors such as soil moisture, texture, organic matter content, pH, bacterial population and activity play role in atrazine biodegradation (Khan, 1980; Perruci *et al.*, 2000; Theng *et al.*, 2000). Haghnia and Razavi (2008) and Rousseaux *et al.* (2001) reported that enhancement of atrazine concentration in soil results in the enhancement of C and N; activating genes bacteria plasmids which are responsible for the production of atrazine decomposing enzymes.

Table 3. The effect of interaction of atrazine concentration × bacterial treatments on atrazine residue in soil.

	0 kg/ha	1 kg/ha	2 kg/ha	3 kg/ha
Non-inoculated control	0	28.6	28.2	28.2
P. fluorescence	0	10.2	14.4	9.5
P. putida	0	10.9	11.2	8.7
P. fluorescence + *P. putida*	0	9.7	7.6	5.1

Many researchers have tested the effect of *Pseudomonas* bacteria on atrazine decomposition in soil. Mandelbaum *et al.* (1995) reported that *Pseudomonas* ADP has high potential to mineralize atrazine. Behiki and Khan (1986) also reported that *Pseudomonas* strains had the ability to grow in a culture medium containing atrazine.

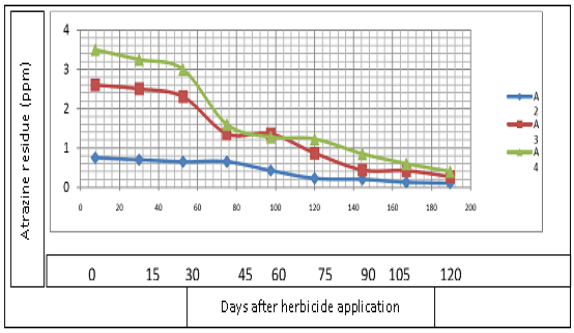

Fig. 1. Atrazine concentration changes in soil in days after herbicide application, in different atrazine concentrations.

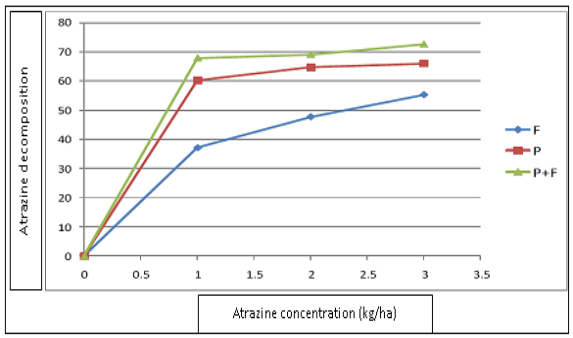

Fig. 1. The effect of interaction of atrazine concentration × bacterial treatments on atrazine decomposition percentage.

Each pesticide is decomposed by a special group of soil microorganisms. When a herbicide is applied to a soil for the first time, the decomposition takes place slowly because of low population of the decomposing bacteria. This was also observed in our experiment. Khan (1980) and Hunter and Shaner (2009) found that application rate and concentration of herbicide or any other chemicals in soil is directly related to its leaching to the lower ground layers and its biodegradation.

Results of our experiment generally indicated that both *Pseudomonas* species had the ability of producing enzymes which are responsible for atrazine decomposition. This ability was higher in *P. putida* than in *P. fluorescence*; the highest decomposition rate happened when both species were applied together.

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