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Biological Degradation of Herbicide (Atrazine) using *Pseudomonas aeruginosa* and *Trichoderma viridae*

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Herbicides are widely used in agriculture. Asia now accounts for a vast majority of the global rice herbicide market. In this study bacteria and fungal species was isolated from the soil samples. Among these isolated colonies *Pseudomonas aeruginosa* and *Trichoderma viridae* were identified based on the cultural, morphological and biochemical characteristics. The herbicide (atrazine) degradation ability of *Pseudomonas aeruginosa* and *Trichoderma viridae* were analyzed. Among the study highest growth were noted in atrazine containing medium for both test organism. Maximum growth indicates the highest degradation of herbicides. The effect of herbicide was noted based on the morphometric and biochemical characteristics of the experimental plant (*Vigna mungo*). The decreased Seed germination, shoot and root length noted in Herbicide containing pot when compared with other treatment. Biochemical compound such as chlorophyll, and total protein were analyzed. Among the study all the biochemical parameters such as chlorophyll, protein content decreased amount noted in Herbicide containing pot, when compared with control treatment (without Herbicide).

Key words: Herbicide, Soil, *Pseudomonas aeruginosa*, *Trichoderma viridae* and Biological degradation.

An herbicide is used to kill unwanted plants in agricultural field. Selective herbicides kill specific targets while leaving the desired crop relatively unharmed. Some of these act by interfering with the growth of the weed and are often synthetic "imitations" of plant hormones (Kellogg *et al.,* 2000). Prior to the widespread use of chemical herbicides, cultural controls, such as altering soil pH, salinity, or fertility levels were used to control weeds. Mechanical control (including tillage) was also (and still is) used to control weeds (Peng *et al.,* 1993).

In 1932, people discovered selective organic herbicide, use it as a contact herbicide for the control of broad leaf weeds in cereals. In the beginning of 40's, chemist synthesizes 2, 4-D it has the selective biological action and its salts and esters are systemic herbicides. After 50age, most organic compounds such as triazines, carbamates, thiocarbamates, ureas have been used as herbicide in china, herbicides were first introduced in the mid 1960's and are now used on 30-40% of total rice area (Naylor, 1996). Asia now accounts for a vast majority of the global rice

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herbicide market. Japan alone accounts for more than 50% of the value of all herbicides sold for rice production (Shibayama, 1996).

The bacterium *Pseudomonas* has multiple applications; in particular, it is known for its capacity to degrade phenolic compounds (Hughes and Cooper, 1996) and other aromatic substances (Eaton and Seliefonov, 1996). Herbicides are frequently detected in shallow ground water of the United States and elsewhere, and the dominant chemicals include atrazine, cyanazine and dicamba (Kolpin *et al.,* 2000). In this present study bacteria and fungal species was isolated from the soil samples. The isolated *Pseudomonas aeruginosa* and *Trichoderma viridae* herbicide degradation also analyzed.

MATERIAL AND METHODS

Collection of Soil Sample

The soil samples were collected from various locations at Pudukkottai district, Tamil nadu in India and the sample were stored in a sterile container for the further studies.

Isolation and identification of Bacteria and fungi

The serial dilution procedures were followed. The nutrient agar medium and potato dextrose agar were prepared and it was poured onto the sterile Petri plates. After solidification, the selected dilution factors 10^{-4} to 10^{-7} and 10^{-2} to 10^{-4} were spread on the medium respectively. Then the plates were incubated for the isolation of bacteria and fungi. The isolated bacteria and fungi strains were identified based on their cultural morphological and biochemical characteristics.

Screening of herbicide degradation (Plate method)

The isolated *Pseudomonas aeruginosa* and *Trichoderma viridae* herbicide degradation ability was screened based on the resistance capability. The various concentrated herbicide containing nutrient agar medium were prepared and the test organism *Pseudomonas areuginosa* were inoculated. A control plate was prepared with nutrient agar medium without herbicide. The plates were incubated at 37°C for 24-48hours. After incubation the growth was observed. The above procedure was followed for *Trichoderma viridae* in the various concentration of herbicide containing potato dextrose agar medium. The plates were incubated at 25-30°C for 48-72hours. After incubation the growth was observed.

Effect of herbicide

Effect of herbicide in the growth of plant was analyzed in pot culture experiment. The pot culture experiment was designed through following manner.

Treatment - I

The seeds were inoculated into sterile soil samples. After 3 days the germination of the seeds were observed.

Treatment - II

Sterile soil sample were added with herbicides (atrazine) in various concentration such as 0.2%, 0.4%, 0.6%, 0.8% respectively. Then the seeds were inoculated in the soil sample and observed the results after the germination of the seeds.

Treatment - III

The sterile soil samples were mixed with the herbicide (atrazine) in various concentrations such as 0.2%, 0.4%, 0.6%, and 0.8%. After that the herbicide containing soil samples were treated with microbial suspension. This setup was incubated at room temperature for 5 days. The growth was observed after the germination of the seeds.

Treatment - IV

The sterile soil samples were mixed with *Pseudomonas aeruginosa* and *Trichoderma viridae* respectively. This setup was incubated at room temperature for 5 days. After the incubation period the seeds were inoculated in the soil samples. The effect of herbicide was analyzed based on the morphometric and biochemical characteristics of each treatment.

Estimation of Morphometric Parameters

Percentage of germination

The percentage of germinating ability was calculated for each treatment by the following formula

$$\% \text{ of Germinating ability} = \frac{\text{Total no. of seed germinate}}{\text{Total no. of seed shown}} \times 100$$

Root and shoot length

Plants were collected from each pot at 7th day. The length of the root and shoot was measured individually for plant and expressed in cm.

Estimation of Biochemical Parameters

Estimation of Total Chlorophyll (Mahadevan and Sridhar. 1974)

One gram of leaf material was collected and measured it with 20ml of 80% acetone. The extract was centrifuged at 5000rpm for 5 minutes. The supernatant was saved and the pellet was re-extracted repeatedly with the same solvent until the residue becomes colourless. The supernatant were collected and made upto 100ml with 80% acetone. Optical density was measured against acetone as blank at 645 and 663 nm. Chlorophyll a, b and total chlorophyll were computed using Arnon's formula (Arnon, 1949).

$$\text{mg chlorophyll a/g tissue} = 12.7\,(A_{663}) - 2.69\,(A_{645}) \times \frac{v}{1000 \times w}$$

$$\text{mg chlorophyll b/g tissue} = 2.39\,(A_{645}) - 4.68\,(A_{663}) \times \frac{v}{1000 \times w}$$

$$\text{mg chlorophyll g tissue} = 20.2\,(A_{645}) + 8.02\,(A_{663}) \times \frac{v}{1000 \times w}$$

Where

A= absorbance at specific wavelengths.

V= final volume of chlorophyll extract in 80% acetone.

W= fresh weight of tissue extracted.

Assay of Total Protein Assay

Total protein was estimated by the method of Lowry *et al.,*(1951) with bovine serum albumin as the standard.0.2 to 1ml of working standard and test samples were taken into a series of test tubes and made up to 1ml with saline. A tube with 1ml of water served as blank. To all the tubes 5ml of alkaline copper reagent was added and kept at room temperature for 10minutes. After 10minutes 0.5ml of Folin's phenol reagent was added and incubated a room temperature in the dark for 30minutes. Blue colour was developed and it was read at 660nm. Standard graph was prepared by plotting the OD values obtained against the concentration of the standard.

RESULTS AND DISCUSSION

In this present study bacteria and fungal species was isolated from the soil samples. Number of bacterial and fungal colonies was isolated from the soil samples. Among these isolated colonies *Pseudomonas aeruginosa* and *Trichoderma viridae* were identified based on the cultural, morphological and biochemical characteristics. The results were presented in the table -1. The isolated *Pseudomonas aeruginosa* and *Trichoderma viridae* herbicide degradation also analyzed. This review is mainly focusing on the pesticide particularly herbicides as a factor affecting the microbial biomass in different soils. The influence of herbicides have various effects associated with the type of herbicides, the concentration of the herbicide, the type of soils, and the conditions of the experiment (temperature, moisture, and the time of incubation etc) (Pugh *et al.,* 1971).

The herbicide (atrazine) degradation ability of *Pseudomonas aeruginosa* and *Trichoderma viridae* were analysed. Based on the ability resistance the results were present in table-2. Among the study highest growth were noted in atrazine containing medium for both test organism. Maximum growth indicates the highest degradation of herbicides. That indicate the *Pseudomonas aeruginosa* and *Trichoderma viridae* moderately degrade the Atrazine. Same result reported by Sethanathan and Siddarmapa, (1978) many herbicides that persist from one season the next can injure sensitive plants.

Table 1. Morphological and Biochemical test for *Pseudomonas aeruginosa*

S. No.	Morphological and biochemical characters	*Pseudomonas aeruginosa*
1	Cultural characters	Phycocyanin pigment production
2	Gram staining	Negative
3	Motility	Motile
4	Shape	Rod
5	Indole test	-
6	Methyl red test	-
7	Voges-proskauer test	-
8	Citrate utilization	+
9	Catalase test	-
10	Oxidase test	+
11	Carbohydrate fermentation	
	a. Sucrose	+
	b. Mannitol	-

'+' - Positive, '-' - Negative

The trazine herbicides (eg., atrazine, simazine) applied as pre-emergent herbicides for selective weed control in corn, sometimes persist and injury sensitive seeding crops the next year.

The effect of herbicide on soil from the growth plant was analyzed in pot culture experiments and herbicide degradation also studied. The effect of herbicide was noted based on the morphometric and biochemical characteristics of the experimental plant (*Vigna mungo*) and table 3. In this morphometeric parameter such as germinating ability, shoot

Table 2. Screening of herbicide degradation ability in microbes

S. No	Test organisms	Control	Concentration of the herbicide atrazine (%)			
			0.2	0.4	0.6	0.8
1.	*Pseudomonas aeroginoasa*	H	H	H	H	M
2.	*Trichoderma viridae*	H	H	H	M	S

H- Heavy growth, M-Moderate growth, S-slow growth.

Table 3. Effect of herbicide in *Vigna mungo* plant

S. No	Morphometric parameter	control	Effect of herbicide in experimental plant (atrazine %) (Mean ± SD)			
			0.2	0.4	0.6	0.8
1.	Germinating ability (%)	100	100	60	50	40
2.	Shoot length (cm)	15±0.1	12±0.2	10±0.1	7±0.1	5±0.2
3.	Root length (cm)	6±0.4	5±0.2	5±.0.2	4±0.1	3±0.2

Table 4. Biochemical contents of herbicide affected *Vigna mungo* leaf sample

S. No	Biochemical content	control	Effect of herbicide in experimental plant (atrazine %)			
			0.2	0.4	0.6	0.8
1.	Chlorophyll A (mg/g fw^{-1})	0.35	0.25	0.18	0.13	0.11
2.	Chlorophyll B (mg/g fw^{-1})	0.39	0.28	0.22	0.18	0.16
3.	Total Chlorophyll (mg/g fw^{-1})	0.74	0.53	0.40	0.31	0.27
4.	Protein (mg/g)	22.07	21.04	18.7	17.2	16.4

Table 5. Morphometric observation of microbes treated herbicide mixed plant sample

S. No	Morphometric parameter	Control	Herbicide degradation ability of microbes in soil (Mean ± SD)							
			Pseudomona aeruginosa (atrazine %)				*Trichoderma viridae* (atrazine %)			
			0.2	0.4	0.6	0.8	0.2	0.4	0.6	0.8
1.	Germinating ability (%)	100	100	80	80	70	100	90	80	70
2.	Shoot length (cm)	15±0.2	15±0.1	14±0.1	12±0.4	12±0.4	15±0.1	14±0.1	15±0.2	12±0.1
3.	Root length (cm)	7±0.4	7±0.2	6±0.3	4±0.2	4±0.1	7±0.2	6±0.1	6±0.1	4±.0.1

length and root length were analyzed in the experimental plants. The results were presented in table 3 and 5. Poor seed germinating ability was noted in the Herbicide alone pot. At the same time Herbicide and test organism mixed pot seed germinating ability similar to the control pot (without Herbicide). The decreased shoot and root length also noted in Herbicide containing pot when compared with other treatment. At the same time similar growth noted organism only treated plant compared with control plant. Herbicides and pesticides affect various soil microbial processes

Table 6. Biochemical contents of microbes treated herbicide mixed plant sample

S. No	Morphometric parameter	Control	Herbicide degradation ability of microbes in soil							
			Pseudomona aeruginosa (atrazine %)				*Trichoderma viridae* (atrazine %)			
			0.2	0.4	0.6	0.8	0.2	0.4	0.6	0.8
1.	Chlorophyll A(mg/g fw^{-1})	0.35	0.35	0.28	0.22	0.19	0.32	0.27	0.25	0.17
2.	Chlorophyll B(mg/g fw-1)	0.39	0.27	0.22	0.19	0.16	0.29	0.28	0.22	0.15
3.	Total Chlorophyll(mg/g fw^{-1})	0.74	0.57	0.50	0.41	0.35	0.61	0.55	0.47	0.32
4.	Protein(mg/g)	29.2	27.2	25.3	19.1	16.7	28.7	23.5	20.2	16.8

(Johnen and Drew, 1977), inhibit decomposition (Pugh and Williams, 1971) and depending upon type and rate of application, can alter the biomass quantitatively and qualitatively in both the short and long term (Andrson and Armstrong, 1981). Biochemical compound such as chlorophyll, and total protein were analyzed. The results were presented in table-4 and 6. Among the study all the biochemical parameters such as chlorophyll, protein content decreased amount noted in Herbicide containing pot, when compared with control treatment (without Herbicide). At the same time all the parameter comes to normal (control plant) in the Herbicide and microbes mixed treatment. In malaysia, *F.miliacea* has been reported to be resistant to 2, 4-D. The resistant *F.miliacea* biotype required 22 times the recommended dose of 2, 4-D for a 50% reduction in growth (Watanabe *et al.,* 1997).

CONCLUSION

This study clearly prove the Herbicide and their residue degradation can be accelerated by employing microbes which can be effectively utilized both as bio control agent and soil cleanser. The present investigation concluded that the *Trichoderma viridae* degraded the atrazine effectively. These microbial consortiums can be effectively used to degrade atrazine from contaminated soils, sediments and waste waters.

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