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Biostimulation of Some Fungal Strains for the Degradation of Atrazine Contaminated Soil

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Abstract- Biostimulatory effects of cow dung on some fungal biodegradation of atrazine. This work was designed to investigate the effect of using a biostimulant (cow dung) on some fungal strains for the possible degradation of atrazine-contaminated soil. The physicochemical characteristics of the soil and cow dung were determined using standard methods. The effects of pre and post biostimulation of fungal species were carried out using enumeration techniques. Fungal strains such as Trichoderma harzianum and Aspergillus niger were identified based on macroscopic and microscopic identification. Atrazine degradation was monitored by assaying the enzymes involved in the degradation and its metabolites using GC-MS techniques. The results of the soil physicochemical characteristics showed slightly acidic pH (6.7±0.99), moderate calcium (6.3±0.19Cmol/kg), high magnesium (5.10±0.38 Cmol/kg), moderate potassium (0.48±0.02 Cmol/kg, low sodium (0.15±0.01 Cmol/kg), moderately low nitrogen (0.16±0.01g/kg), moderate phosphorus (12.22±1.45 g/kg), high O.C(15.38±0.05 g/kg) were obtained respectively.

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Keywords: atrazine, biostimulation, degradation, fungi, contamination, soil.

I. Introduction

Atrazine is a chlorinated systemic selective herbicide widely used globally to kill weeds. It is employed in the cultivation of sugarcane, maize, corn. Atrazine is highly persistence in soil and the average half-life of atrazine ranges from 13 to 261 days (USEPA, 2003) more than 100days in river water (Seiler *et al.,*1992) and around ten days in sea water. Atrazine ($C_8H_{14}ClN_5$) is a pre-emergent herbicide with the systemic action of inhibiting photosynthesis is considered toxic in the short and medium term, and is not easily degraded. Some researchers show that this product causes changes which can acts as an endocrine breaker, affecting reproductive function in vertebrates and teratogenic effects in human (Cooper *et al*., 2007; Raymundo *et al.,*2009).

Soil contamination and its adverse effects on the overall ecosystem is one of the problems we are facing today. Agrochemicals needed to boost agricultural activities especially soil acting herbicides have adverse effects on soil microcosm (ATSDR, 2014). Agrochemical refers to a broad range of insecticides, fungicides, and herbicides, and very large group of substances that are specifically designed to kill biological organisms including weeds, insects and rodents. However, the extensive use of agrochemicals may result in their accumulation in the agricultural soils and produce, thus causing harm to microorganisms and animals. Their low biodegradability in soil has classified these chemicals as persistent toxic substances (Tayade *et al*., 2013).

Biostimulation and bioremediation are common methods employed to reduce the concentration of atrazine and other pollutants in the soils. This process incorporates biological organisms such as microorganisms to a contaminated environment to induce and accelerate the biodegradation process (Gopinath and Sims, 2011). In this regard, Robinson (1996) reported that in systems with a wild population of microorganisms, degradation processes were more effective. Fungi strains such as *Trichoderma harzianum* and *Aspergillus niger* exhibited the ability to transform the atrazine molecule enzymatically, leading to various degradation products such as hydroxyatrazine (HA), Desethylatrazine (DEA) and deisopropylatrazine (DIA) (Govantes *et al*., 2009). The ability of microorganisms to degrade herbicides has stimulated Scientist to develop methods to access their potentials for the transformation of these anthropogenic chemicals via enzymatic route (Ramdas and Gerald, 2011). Hence, the present study is designed to study the possible degradation of atrazine by some fungal strains such as *Trichoderma harzianum* and *Aspergillus niger.*

II. Materials And Methods

a) Chemicals and reagents

All chemicals and reagent were of analytical grades.

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b) Collection and Preparation of Soil Samples

Surface soil (0-20cm depth) samples from six (6) different locations within Teaching and Research Farm of the Faculty of Agriculture, University of Maiduguri, for the isolation of the microorganisms. The soil samples for pot experiment within the same Research Farm, University of Maiduguri, with no prior atrazine application. The soil samples were air-dried, homogenized, passed through a 2.0mm (pore size) sieve mesh to remove stones and plant debris.

c) Treatment and Experimental Design

The contamination with atrazine powder were carried out for six weeks at company recommended rates of 3%w/v per kg of soil. The soil treatment was carried out in triplicates using a completely randomized design. Soil samples (1.6kg) into eight (8) different plastic containers of 3liters volume each labeled from A to H, respectively. The soil in each plastic bin with various concentration of atrazine powder (10mg/ml (1%), 30mg/ml (3%) and 50mg/ml (5%) and thoroughly mixed to achieve complete artificial contamination.

d) Biostimulatory Studies

The cow dung collected was sieved, sterilized, in ahot air oven at 160°C for 1 hour and 400g of cow dung was used to stimulate 1.6kg soil, and contaminate with various concentrations of atrazine using a modified method described by Moorman *et al.* (2001). Soil samples and cow dung (CD), at 80%/20% were used to set up the biodegradation slurries, in a 3L medium. Sterilized deionized water was added to submerge the content of the treatment. An aliquot of 10ml of strains and consortia culture in liquid broth media harvested at late exponential phase, washed and re-suspended into deionized water and was inoculated into biodegradation medium.

e) Characterization of Soil samples and Cow dung

Characterization of soil sample and cow dung was carried out using various analytical methods, and soil particle size was determined using the hydrometer method as described by Bouyoucus (1992). Determination of Phosphorus and Nitrogen were carried out by Bray and Kurtz (1945) and Macro-kjedahl methods respectively. Organic carbon content was determined using the Wakley and Black method (1934).

f) Fungal enumeration and identification

Potato dextrose agar (PDA) was used for the above. Morphological identification was by macroscopic observation using standard fungi atlas. Fungal were characterized as described by Barnett and Hunter, (2006)

g) Statistical Analysis

Descriptive Statistic Mean and Standard error of the mean (Mean ± SEM) and analysis of variance (ANOVA) by *Computer Software, Statistics version 10.0*, Microsoft (2005). Comparisons of means were carried out by Least Significant Difference (LSD) at 5% probability level.

III. Results And Discussion

Biostimulatory effects of a biostimulant (cow dung) on fungal atrazine biodegradation in the soil. The soil samples showed a slightly acidic pH. The acidity might be due to washing away of soil. Many researchers have established the relationship between soil characteristics and atrazine degradation parameters (Zablotowicz *et al*., 2006). Houot *et al.,* (2000)observed that soil pH was a great contributor to atrazine fate in soil, but this can hardly be buttressed as soils studied haveslightly acidic values. The soil nitrogen, organic carbon, and organic matter were low, which indicated a low fertility content. Soil organic matter is one of the most important factors responsible for controlling the fate of pesticides in the soil environment (Umar *et al*., 2012). The bioavailability of most atrazine for microbial biodegradation by sorption of organic matter (Alexander., 1984). However, organic carbon substrate may also affect microbial community structure, and potential for degradation of herbicides such as atrazine (Rhine *et al.,* 2003). The results of the mineral elements such as nitrogen, phosphorus, calcium, and magnesium obtained from cow dung used showed moderate to high values which may be capable of increasing the nutrients for microbial growth in the soil, thereby enhancing the atrazine degradation efficiency.

Table 1: Physicochemical characteristics of the soil and cow dung

Parameter	Soil	Cow-dung
pH	6.70 ± 0.09	6.9
EC	0.12 ± 0.02 dSm-1	-
O.M	1.08 ±0.01 %	
Total N	0.16 ±0.01 %	15.5%
Av P	12.22 ±1.45 mg/kg	10.89mg/kg
C: N	5.13 ± 0.58	
Ca	6.37 ±0.19(Cmol/kg)	3.05 %
Mg	5.10 ± 0.38	0.57 %
K	0.48 ±0.02	2.03 %
Na	0.15 ±0.01	0.54 %

H+ Al	0.53 ±0.03	ND
CEC	12.28±0.22	ND
% Base Saturation	92.44±3.36 %	ND
Zn	ND	10.66 mg/g
Cu	ND	20.29 mg/g
Fe	ND	5.77 mg/g
Mn	ND	5.11 mg/g
Particle Size Distribution (%)		
Clay	20.27±1.07 %	ND
Sand	62.20±1.51 %	ND
Silt	17.53±0.98 %	ND
Textural class	Sandy loam	

Values are Mean ± SEM of three replicates, ND-Not Detected, CEC-Cation Exchangeable Capacity, H+Al –Exchangeable Acidity, O.M-Organic Matter, O.C-Organic Matter, EC-Exchangeable Cations

IV. Effects of Some Biostimulant (Cow-Dung) on Some Fungal Strains

From the results obtained pre and post stimulation studies at various atrazine level provided the basis of understanding changes in soil environment amended with organic wastes. It has been shown that degradation of atrazine by given native microbial population by the presence of required nutrients (Dellile *et al*., 2009). Biostimulation accelerates the decontamination rate, as an addition of one or more limiting nutrients that improve the degradation potentials of inhibiting microbial population (Nikolopoulou *et al.,* 2009).

The results of soil amended with cow dung and inoculated with various fungal strains pre and post biostimulation at 1% atrazine showed an increase in total fungal count, as the CFU/g of fungi increased with amendments. The reason for the higher fungi in the soil amended with cow dung may be as a result of the presence of the appreciable quantities of carbon, nitrogen, and phosphorus in the cow dung, which are necessary nutrients for fungal and bacterial biodegradative activities (Adesodun and Mbagwu, 2008). The results of soil amended with cow dung and inoculated with microorganisms pre and post biostimulation at 3% atrazine level showed a higher total fungal count in treatment containing consortium of three (3) microorganisms (*Trichoderma harzianum, Aspergillus niger,* and *Yeast*), was significantly difference ($P<0.05$) from control group. The reason for the higher counts of fungi in the soil amended with cow dung may be as a result of the presence of appreciable quantities of carbon, nitrogen and phosphorus in the cow dung, which may be the nutrients required for fungal and bacterial biodegradative activities (Ijah and Antai., 2003a; Adesodun and Mbagwu., 2008). There was an increased in fungal population with the highest count in the consortium of microorganisms. The soil used for biostimulation had C: N of 5.13 which is low for effective bioremediation of atrazine, hence the need for an addition of organic wastes as a source of nutrients (N and P).

V. Effect of Biostimulation on Some Fungal Strains

Table 2: a. Fungi counts of 1% Atrazine treated Soil that was Stimulated

Treatment/Organism	Fungi x10^5 (CFU/g)	
	Pre	Post
Un-inoculated	1.10x10^{5e}±14850	1.60x10^{6d}±14813
Trichoderma harzianum	1.50x10^{5de} ±15667	3.50x10^{6c} ±22912
Aspergillus niger	3.00x10^{5c} ± 24500	4.00x10^{6c} ±22912
Yeast	2.00x10^{5cd} ±16667	4.28x10^{6c}±23511
T.harzianum+A.niger	6.00x10^{5b} ±28867	5.85x0^{6b}± 44378
T.harzianum+Yeast	6.00x10^{5b}±28867	6.60x10^{6b}±44378
A. niger +Yeast	7.00x10^{5b}±28867	7.60x10^{6b}±44378
T. harzianum + A. niger+Yeast	9.00x 10^{5a}±44096	8.50x10^{6a}±45092

Means with the same superscript are not significantly different at ($P<0.05$)

Means with different superscript are significantly different at ($P<0.05$)

Table 2: b. Fungi counts of 3% Atrazine treated Soil that was Stimulated

Treatment/Organism	Fungi x 10^5 (CFU/g)	
	Pre	Post
Un-inoculated	$2.00x10^{5e}$±33333	$2.13x10^{6d}$±29627
Trichoderma harzianum	$3.00x10^{5d}$±34433	$7.\ 10^{x}10^{6c}$±37850
Aspergillus niger	$4.00x10^{5cd}$ ±3833	$38.10x10^{6c}$±45825
Yeast	$6.00x10^{5c}$±45335	$8.57x10^{6c}$±47022
T. harzianum+A.niger	$1.37x10^{6b}$ ±57735	$1.17x10^{7b}$±56789
T.harzianum+Yeast	$1.37x10^{6b}$ ±57735	$1.32x10^{7b}$±68738
A. niger +Yeast	$1.57x10^{6b}$ ±57735	$1.52x10^{7a}$±70537
T. harzianum+A.niger+Yeast	$1.80x10^{6a}$±76376	$1.70x10^{7a}$±88192

Means with the same superscript are not significantly different at (P<0.05)

Means with different superscript are significantly different at (P<0.05)

Table 2: c. Fungi count of 5% Atrazine treated Soil that was Stimulated

Treatment/Organism	Fungi x10^5 (CFU/g)	
	Pre	Post
Un-inoculated	$3.00x10^{5e}$ ±12500	$3.20x10^{6d}$±13527
Trichoderma harzianum	$6.00x10^{5d}$±13228	$1.10x10^{7c}$±46789
Aspergillus niger	$9.00x10^{5c}$±36602	$1.20x10^{7c}$±46789
Yeast	$4.50x10^{5cd}$ ±22540	$1.28x10^{7c}$±46789
T. harzianum+ A niger	$1.97x10^{6b}$ ±49999	$1.75x10^{7b}$±52672
T. harzianum +Yeast	$1.97x10^{6b}$ ±49999	$1.98x10^{7b}$±88756
A. niger +Yeast	$2.27x10^{6b}$ ±49999	$2.28x10^{7a}$±90110
T. harzianum + A. niger +Yeast	$2.70x10^{6a}$±86602	$2.55x10^{7a}$±90185

Means with the same superscript are not significantly different at (P<0.05).

Means with different superscript are significantly different at (P<0.0).

Generally, the information obtained from the pre and post-stimulatory studies provided the basis of understanding changes in soil environment amended with organic wastes (Umar *et al*., 2012). It has been shown that degradation of herbicides and pollutants by the native microbial population may be enhanced by the presence of required nutrients (Dellile *et al*., 2009). Isah, D *et al*., (2017) also reported that *Pseudomonas aeruginosa* was capable of degrading atrazine efficiently when stimulated with cow dung.

VI. Conclusion

Atrazine could be persistent in the soil, which may be utilized by fungi strains as a source of carbon and nitrogen. Thus, cow- dung can be used to enhanced microbial population. Fungal strains used such as *T. harzianum, A. niger* were effective in degrading atrazine and may be employed in the bioremediation of atrazine-contaminated soil.

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