Agricultural Sciences Journal
Available online at http://asj.mnsuam.edu.pk/index.php
ISSN 2707-9716 Print
ISSN 2707-9724 Online
https://doi.org/10.56520/asj.v5i2.249

Research Article

IMPACT OF ATRAZINE AND BROMOXYNIL ON THE COLONY FORMING UNITS (CFU) OF SOIL BACTERIA

Rahamdad Khan[1], Saad Jan[1], Roohul Amin[1], Inzimam ul haq[1], Abuzar Shoukat[1], Abbas Khan[1], Muhammad Shabir Akbar[1], Tauseef Ali[1],Osama Younas[1], Syed Majid Rasheed[1]*

[1] *Department of Agriculture, Faculty of Sciences, Bacha Khan University Charsadda.*
**Corresponding author: smrasheed@bkuc.edu.pk*

Abstract

According to overwhelmingly positive effects throughout time, herbicide treatment has become a crucial component of thriving agricultural output worldwide. However, its detrimental effects on non-target soil microorganisms involved in the nitrogen cycle, nutrient degradation, and organic matter breakdown must be considered. In the current study, the consequences of the two (2) herbicides that are most often used in Pakistan, Atrazine and Bromoxynil, were evaluated on soil bacteria over the course of fifteen consecutive days (exposure period). Recommended field rate of herbicide application was followed (i.e. active ingredient of 6.17 mg for Atrazine and 2.4 mg for Bromoxynil per gm of soil). During the investigations half and double recommended doses of these herbicides were used. Time interval of 5, 10 and 15 days were used to determine the bacterial populations. Statistical analysis of the investigation revealed that the bacterial population did not exhibit any appreciable variations in relation to the exposure duration (p 0.05). The Atrazine application along with the herbicide treatments, reduced the bacterial population during all the tested interval durations with just half of the suggested field rate. The current study reflected that apart from the benefit of herbicides in controlling weeds these chemicals also effect bacterial population that is a serious issue for present and future agriculture. Further, the same study needs to be perform under field conditions to confirm the finding of laboratory results before issuing solid recommendations to the farming community.

Keywords: Atrazine, Bromoxynil, Soil, Bacteria population, Colony forming units.
(Received: 29-Mar-2023 Accepted: 20-Jun-23) Cite as: Khan. R., Jan. S., Amin. R., Haq. I. U., Shoukat. A., Khan. A., Akbar. M. S., Ali. T., Younas. O., Rasheed. S. M., 2023 Impact of atrazine and bromoxynil on the colony forming units (cfu) of soil bacteria. Agric. Sci. J. 5(2): 1-7.

1. INTRODUCTION

Herbicide usage in agriculture has made a significant contribution to the production of both food and cash crops around the world, and Pakistan is no exception. But owing to favorable climatic circumstances such as rainfall, sunlight, healthy soil, etc. many common weed species have invaded agricultural fields, damaging the farm productivity industry (Ntow et al., 2006). Manufacturers have responded by dumping a variety of herbicides designed to eradicate various weed types at various phases of development onto the agrochemical business (Sebiomo et al., 2011). The fact that most growers use these insecticides may be due to their effectiveness in managing the desired weeds. The bulk of microbial species, including soil bacteria, fungi, and actinomycetes, whose activities affect soil fertility through organic material degradation, organic matter decomposition, and nutrient cycling, are contained in the soil, where all agricultural wastes are kept (Zain et al., 2013; De-Lorenzo et al., 2001; Hutsch, 2001). Using these chemicals, however, interacts with some of these natural processes and reduces the effectiveness of species that aren't the objective (Subhani et al., 2000). Certain soil organisms, however, utilize the carbon produced when these herbicides break down as a source of energy for their own metabolic processes.

Numerous studies have demonstrated that the factors like the amount and rate of

herbicide application, the persistence of the herbicides in the soil environment and the chemical's toxicity determined how contaminated the soil is with these substances (Anju et al., 2010). Although the majority of these herbicides are made to have the desired weed-controlling effect for a sufficient amount of time (Greer et al., 1990). Transfer and degradation are the two main mechanisms that control what happens to herbicides that are sprayed into the soil environment. The supplied chemicals are transported in the soil environment via processes as precipitation, drainage, flora and fauna absorption, adsorption, and desorption, while retaining their physiological integrity. Some of the pathways for degradation that have been chemically formulated include microbial decomposition, plant detoxification, chemical breakdown, and photodecomposition. These two procedures evaluate the persistence of herbicides, their effectiveness against weeds, and their potential to contaminate soil and groundwater (Subhani et al.,2000). Therefore, in order to implement practical methods to shorten the duration in which herbicide remains persistent in the soil environment, it is necessary to understand the elements influencing the processes by which it degrades.

The majority of people in Pakistan are unable to read and comprehend herbicide labels. Because of this, streams, rivers, and groundwater, a crucial natural resource, have become contaminated (Baran et al., 2007). In addition to endangering the environment and unwanted species, these contaminations also expose people to several health risks. Therefore, research is required to determine how some of these herbicides, which are widely used in Pakistan, affect some of the beneficial microbes in the soil. Mainly, this study was conducted to assess the impact of some common herbicides on the particular bacteria population and to test different doses of these herbicides against bacterial colonies.

2. Materials and Methods

The experiment was carried out at the laboratory of Agriculture department, The Bacha Khan University Charsadda Pakistan. The experimental design used was a completely randomized design with factorial arrangements repeated three times. Further details of the study are given below.

2.1. Soil sampling

Without using herbicides beforehand, samples of the top fertile soil (5 cm depth) was collected from a sugarcane field close to Bacha Khan University in Charsadda. The earth was bulked together after being gathered from various locations around the fields. A quantity was obtained for laboratory examination after it had been fully mixed and shaken. To get rid of plant detritus and stones, a mesh of 2.0 mm size was used to sieve the samples.

2.2. Herbicides selection

The two commonly used herbicides Bromoxynil and Atrazine were obtained from a local agricultural market in Charsadda. The selection of these herbicides were made upon the frequent use by farmers, the data were obtained from the survey of local agriculture dealers. The individual details of both the herbicides are;

a) Bromoxynil

The trade name of this herbicide is Buctril supper manufactured by Bayer Company. It is a hydroxybenzonitrile herbicide used for post-emergent control of broadleaf weeds; on alfalfa, garlic, corn, sorghum, flax, cereals, turf and on pasture and rangelands.

b) Atrazine

The trade name of atrazin is GENGWEI 550 SC by Jaffer group of companies is a pre-emergence herbicide. It is highly effective on annual grasses and some annual broad-leaved weeds growing in crops like Maize, beans, sunflowers, sugar cane, and a wide range of other crops

2.3. Soil treatments

Three concentration of each Bromoxynil and Atrazine were prepared to check their impact on the bacterial colonies. The recommended field dose of Atrazin is (495-727ml/acre) while, for Bromoxinil it is 900

ml/acre. These concentrations include recommended rate (X) of the mentioned herbicides, double (2X) of the recommended dose and half (0.5X) of the recommended dose. The data for bacterial colonies were recorded frequently at five, ten and fifteen days after exposure periods. A control treatment was also added for comparison. For Bromoxynil and Atrazine, the recommended rates of the active ingredient per gram of soil were 2.4 mg and 6.67 mg, respectively. The below formula is used for calculating the treatments/ dose (Zain et al.,2013).

$$Y\,(mg/g) = \frac{RFR\ (g\ a.i\ /\ ha)}{Am.\ AiF\ (g\ a.i\ /\ L) \times 450\ L/ha} X \frac{1000\ mg}{1g}$$

Where,

Y= Chemical (mg) in soil (g)

RFR= recommended field rate

Am. AiF= amount of active ingredient in formulation

2.4. Data recording/ procedure

a. Bromoxynil

In order to compare the bacteria population in the soil to that of soils treated with different herbicides, initially the bacteria number in the soil was first counted at the stage without any chemical treatment. Both prior to and following chemical modification, the organic matter of the soil was measured. The use soil in the study was fine-grained natural clay soil.

b. Bacterial Colonies Counting

i. Agar media preparation

In a beaker, 28 grams of the dehydrated powder or lab-prepared media is added to 1000 milliliters of distilled or deionized water. The suspension is then heated to boiling to dissolve the medium completely. The dissolved medium is then autoclaved at 15 lbs pressure (121°C) for 15 minutes. Once the autoclaving process is complete, the beaker is taken out and cooled to a temperature of about 40-45°C.

c. Calculation of bacterial colony

Pour Plate Counter was used to calculate the bacteria colony. The dehydrated medium (powder) weigh 20.5 g was suspended in 1 liter of distilled water to create the plate count agar. To dissolve the powder, the mixture was heated and boiled for one-minute while being stirred frequently. To sanitize the agar, it was placed in a flask and heated in an autoclave to 1210C. Each treated soil sample weighed out at one gram and was serially diluted. With a sterilized 1 ml pipette, an aliquot of 1 ml was drawn from an inch beneath the surface and put into an empty sterile plate of 15 ml.

In the sterile plate, agar that has been cooled to 45 0C, and extracted diluted sample was added. Before the mixture was incubated under a laminar flow, it was completely mixed, chilled, and allowed to harden on a level laboratory bench. To avoid becoming wet from condensation, these labelled samples were placed upside down. For 24 to 48 hours, incubation was carried out at 25 °C, or room temperature. After that two serial dilutions of 1/100 were use.

$$Total\ Dilution\ Factor = 10 * 100 * 100 = 10\text{^}5$$

$$CFU/mL = cfu/ml = ((no.\ of\ colonies\ x\ dilution\ factor)) / volume\ of\ culture\ plate.$$

Using a colony counter, the total viable colony forming units on each plate were counted, and the data was collected.

d. Statistical Analysis

A statistical transformation was performed on data acquired from a bacterial colony before it was presented in tables. The means of the various herbicide exposure times were compared using the Analysis of Variance (ANOVA). The data was once again subjected to several comparisons of findings in order to highlight the variances between the soil treatments and compare the average values between test days and chemical treatments.

3. Results:

3.1. Bacterial colony forming units (CFU) 5 days after application

The analysis of data revealed that both the tested herbicides and their doses significantly affect the bacterial population/ CFU after 5 days of application. Among the herbicides the maximum (5.67) bacterial CFU was recorded for both X and 2X doses of bromoxynil. However, the minimum

(4.33) bacterial CFU found in the 0.5X dose of herbicide Atrazine (Table 1). Interestingly, the soil sample with no application of any herbicides/ control have the maximum (7.00) bacterial CFU. It can be clearly seen from the results that the herbicide doses affect the bacteria colony as compared to the untreated control. At the initial stage of herbicide application, the bacteria population was comparatively high than the rest of the data intervals i.e., 10 and 15 days.

Table 1. Effect of herbicides and their doses on Bacterial colony forming units after 5 days of herbicides application.

Herbicides doses	**Bacterial colony forming units (CFU/g)**
Bromoxynil 2X dose	5.67± 0.06 a
Bromoxynil X dose	5.67± 0.06 a
Bromoxynil 0.5 X dose	5.33± 0.05bc
Atrazine 2X dose	5.00± 0.04bc
Atrazine X dose	5.00± 0.04bc
Atrazine 0.5 X dose	4.33± 0.03 c
Control	7.00± 0.07 a

LSD for treatment =1.2085

3.2. Bacterial CFU 10 days after application

The analysis of data revealed that both herbicides and their concentration can effect bacterial population 10 days of application. Maximum bacterial population (6.0000) was observed for Atrazine normal dose. While the minimum (3.6667) population was evident for Atrazine 2X dose. After 10 days of exposure, the table 2 exhibited the mean bacterial population of the soil sample that had been treated with atrazine and bromoxynil. The normal dose of recommended field rate for atrazine recorded the highest number of bacterial population followed by baseline determination and Bromoxynil half recommended field rate. On the other hand, a critical analysis of the 2X treatment (Atrazine) demonstrates a progressive decrease in bacterial population, also Bromoxynil double dose, which also showed decline in population from 5 DAT to 10 DAT.A clear decrease was observed in the bacteria population while comparing 10 DAT with 5 DAT.

3.3. Bacterial CFU 15 days after application

The analysis of data revealed that both herbicides and their concentration can effect Bacterial population after 15 days of application. Maximum bacterial population (5.1667) was evident for Atrazine normal dose, while minimum CFU (4.6667) was observed for Atrazine double dose and Bromoxynil normal dose. Table 3 displayed the average number of bacteria in the soil sample after it had been exposed for 15 days to Atrazine and Bromoxynil. Bacterial population of Bromoxynil 0.5X and 2X recommended field rate revealed same

Table 2. Effect of herbicides and their doses on Bacterial colony forming units after 10 days of herbicides application.

Herbicides doses	**Bacterial colony forming units (CFU/g)**
Bromoxynil 2X dose	4.47 ± 0.02ab
Bromoxynil X dose	4.33± 0.13 ab
Bromoxynil 0.5 X dose	4.67± 0.03 ab
Atrazine 2X dose	3.67± 0.04 b
Atrazine X dose	6.00± 0.03 a
Atrazine 0.5 X dose	5.33± 0.04 ab
Control	5.67± 0.12 a

LSD For treatment =1.8627

results after 15 days of exposure period. However, after fifteen days of treatment, a reduction in the number of bacteria is observed with X dose of Bromoxynil and 2X dose of atrazine of company's recommended field rate against baseline determination.

Table 3. Effect of herbicides and their doses on Bacterial colony forming units after 15 days of herbicides application.

Herbicides doses	**Bacterial colony forming units (CFU/g)**
Bromoxynil 2X dose	5.00± 0.02 b
Bromoxynil X dose	4.67± 0.13 b
Bromoxynil 0.5 X dose	5.00± 0.04 b
Atrazine 2X dose	4.67± 0.03 b
Atrazine X dose	5.17± 0.04 b
Atrazine 0.5 X dose	5.17± 0.12 b
Control	6.67± 0.03 a

LSD For treatment =1.3910

4. Discussion

The changes in the microbial population reported in this study were consistent with findings reported by Ayansina and Oso (2006), who found that soils treated with greater concentrations of herbicides had much lower microbial counts than soils treated with approved levels. The rising tendency in the bacterial population in the first and second weeks of the same herbicide treatment was confirmed by Sebiomo et al. (2011), who had found an increased bacterial population after the herbicide treatment. Herbicides could be utilized by bacteria as a source of carbon, according to experiments by Radosevich et al.,(1995). According to Anderson et al., (2000), the rise in bacterial population did, however, become fatal with the ensuing increase in exposure time, which may have contributed to the rapid fall in bacterial population. By 10 days following treatment, the bacterial population inhibition percentages had dramatically decreased. According to Anderson et al (2000), the population expansion of the bacteria became lethal with an increase in exposure duration, which may have caused the bacterial population to decline. At 5DAT, 10DAT, and 15DAT, respectively, there was a continuously dropping bacterial population, similar to the baseline finding in the current experiment. A comparable soil treatment resulted in a free-fall decline in the microbial population, according to a research carried out by Zain et al. (2013).

5. Conclusion

The results of our study indicated the presence of Atrazine and Bromoxynil in the soil exert considerably changes the growth and development of soil microorganism specially the bacteria. Further, the population of bacteria sharply increased with herbicide application initially but soon declined from 5DAT to 15DAT. Moreover, the amount of herbicide's active ingredient, the length of exposure, and various environmental conditions have an impact on the bacterial population.

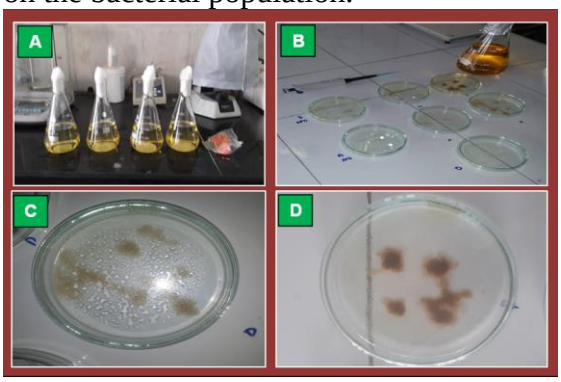

Figure A. The prepared soil bacteria mixture;

Figure B. Petri dish arrangement.

Figure C. Initial stage of bacterial growth;

Figure D. Later stage of bacterial colony growth zones.

Therefore, the herbicide dose calculation is very much important not only for exerting

minimum toxicity to the environment but also to sustain the soil microbial population. Farther studies would be recommended for testing these herbicides under field conditions to conform the laboratory findings and to recommend concrete suggestions for the farming community.

6. Conflict of Interest

The Authors declare that there is no conflict of interest.

7. Authors' Contribution Statements

AK, MSA and TA executed the field activity, AS and IH carried out laboratory analyses, RK and RA conceived the idea and supervised the work, SJ and OY carried out statistical analysis, SMR write up and polishing of article.

8. Acknowledgments

NA

9. REFERENCES

Abbas, Z., M.Akmal, K.S.Khan and F.Hassan. 2014. Effect of Buctril Super (Bromoxynil) Herbicide on Soil Microbial Biomass and Bacterial. Brazilian Archives of Biology and Technology.57(1): 9-14.

Anderson, L.M., B.A. Diwan,N.T. Fear and E. Roman. 2000. Critical windows of exposure for children's health. Cancer in human epidemiological studies and neoplasms in experimental models. Environmental Health Perspectives. (108): 573–594

Anju, A., S.P. Ravi and S. Bechan. 2010. Water pollution with special reference to pesticide contamination in India. Journal of Water Resource and Protection, 2010.

Araújo, A.D., R.T.R. Monteiro andR.B. Abarkeli. 2003. Effect of glyphosate on the micractivity of two Brazilian soils. Chemosphere. 52(5): 799-804.

Ayansina, A.D.V and B.A. Oso. 2006. Effect of two commonly used herbicides on soil micro flora at two different concentrations. African Journal of Biotechnology. 5(2): 129-132.

Baran, N., C. Mouvet and P. Negrel. 2007. Hydrodynamic and geochemical constraints on pesticide concentrations in the groundwater of an agricultural catchment(Brevilles, France). Environmental Pollution. 148 (3): 729-738.

Busse, M.D., A.W. Ratcliff,C.J. Shestak and R.F. Powers. 2001. Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. Soil Biology and Biochemistry. 33(12): 1777-1789.

Cole, M. A. 1976. Effect of long-term Atrazine application on soil microbial activity. Weed Science. (24): 473-476.

De-Lorenzo, M. E.,G.I. ScottandP.E. Ross. 2001. Toxicity of pesticides to aquatic microorganisms: a review. Environmental Toxicology and Chemistry. (20) 84-98.

Estok, D.,B. Freedman andD. Boyle. 1989. Effects of the herbicides 2.4-D, glyphosate, Hexazinone, triclopyr on the growth of three species of ectomycorrhizal fungi. Bulletin of Environmental Contamination and Toxicology. (42): 835-839.

Greer, C.W., J. Hawari and R. Samson. 1990. Influence of environmental factors on 2,4-dichlorophenoxyacetic acid degradation by Pseudomonas cepacia isolated from peat. Archives of Microbiology. 154(4) 317-322.

Haney, R. L.,S.A. Senseman and F.M. Hons. 2002. Effect of Round-up Ultra on microbial activity and biomass from selected soils. Journal of EnvironmentalQuality. 31(3): 730-735.

Hutsch, B.W. 2001. Methane oxidation in non-flooded soils as affected by crop production. Invited paper. European Journal of Agronomy. 14(4): 237-260.

Ntow, W.J.,H.J. Gijzen,P. Kelderman and P. Drechsel. 2006. Farmer perceptions and pesticide use practices in vegetable production in Ghana. Pest Management Science. 62 (4): 365-356.

Radosevich, M., S.J. Traina, Y.I. Hao and O.H. Touvinen. 1995. Degradation and mineralization of atrazine by a soil bacterial isolate. Applied and Environmental Microbiology. 61: 297-302.

Sebiomo, A.,V.W. Ogundero and S.A. Bankole. 2011. Effects of four herbicides on microbial population, soil organic matter and dehydrogenase activity. African Journal of Biotechnology. 10(5): 770-778.

Subhani, A., A.M. EI-ghamry, C. Huang and J. Xu. 2000. Effects on some herbicides on soil microbes effect of pesticides (Herbicides) on soil microbial. Journal of Biological Sciences. 3(5): 705-709.

Zain, N.M.M., R.B. Mohamad, K. Sijam, M.M. Morshed andY. Awang. 2013. Effects of selected herbicides on soil microbial populations in oil palm plantation of Malaysia: A microcosm experiment. African Journal of Microbiology Research, 7(5): 367-374.

Zain, N.M.M., R. B. Mohamad, K.Sijam, M.M. Morshed and Y. Awang. 2013. Effect of selected herbicides In Vitro and in soil on growth and development of soil fungi from oil palm plantation. International Journal of Agriculture and Biology. 15(5): 820-826.