Full Length Article

Response of Soil Microorganisms and Enzymes Activity to Application of Buctril Super (Bromoxynil) under Rainfed Conditions

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Abstract

A field experiment was conducted for two years to assess the impact of buctril super herbicide application on soil enzymes activity and microbial population. The hypothesis of the study was that the herbicide has negative effects on soil microbes and enzymes. The experiment was laid out in randomized complete block design using five herbicide treatments: 375 mL ha^{-1}, 750 mL ha^{-1}, 1500 mL ha^{-1}, 2250 mL ha^{-1} and control with four replications. Wheat variety Chakwal-50 was sown as a test crop and the herbicide was applied seven days after sowing. The soil samples were taken at 0, 7, 15, 30 and 60 days after herbicide application. Results showed 30% decrease in bacterial population, 23.4% decline in actinomycetes population, 34% decrease in fungi population, 30% reduction in urease activity, 36% inhibition in dehydrogenase activity and 34% decline in alkaline phosphatase activity with bromoxynil herbicide application at 2250 mL ha^{-1} during the year 2011-2012. However, 36, 27.7, 38.6, 31, 36 and 31% decline in bacteria, actinomycetes, fungi population, urease, dehydrogenase and alkaline phosphatase activity was observed, respectively where the herbicide was applied at 2250 mL ha^{-1} during the year 2012-2013. Overall bacteria, actinomycetes and fungi populations showed decreasing trend with herbicide application up to the day-15 and then increasing trend was observed till day-60. Decrease in dehydrogenase and alkaline phosphatase activities was experienced with herbicide application upto day-7, thereafter the activities of these enzymes showed increasing tendency up to day-60 during both years. Buctril super herbicide had negative effects on soil microbes and enzymes activity showing significant reduction in these parameters up to two weeks after its application, which later on subsided. © 2015 Friends Science Publishers

Keywords: Bromoxynil; Soil microbial population; Enzymes activity; Rainfed condition

Introduction

Shortage of water, imbalanced use of fertilizer and weed infestation are main reasons of low crop production in Pakistan. As much as 20 - 45% reduction in wheat yield has been reported due to weeds (Shah *et al*., 2005). Hand weeding, tillage and application of herbicides are commonly used methods for weed control. Due to adoption of no till practice for conservation of soil moisture, the application of herbicides has become unavoidable (Trigo and Cap, 2003). Out of several herbicides being used in Pakistan, buctril super (bromoxynil) is the most commonly used one (Aslam *et al*., 2007). Regardless of the benefits of this herbicide it may also have detrimental effect on soil microbes and enzymes which are essential component of the soil system. Microbes perform essential role in soil and act as marker of soil health and quality. Various researchers (El-Ghamry *et al*., 2000; Pampulha and Oliveira, 2006) have reported adverse effects of herbicides on soil microorganisms. Significant decrease in soil microbe's population has been reported due to high dose (3.0 μg g^{-1} soil) of bromoxynil herbicide (Omar and Abdel-Sater, 2000). Similarly, nitrifying bacteria have been found extremely sensitive to bromoxynil herbicide (Topp *et al*., 1992). Enzymes perform numerous functions in soil. For example urease is involved in hydrolysis of urea; phosphatases change the organic phosphorus into inorganic form and make it available to plants (Schneider *et al*., 2001). Dehydrogenase is connected with oxidation reduction processes occurring in soil (Quilchano and Maranon, 2002). The impact of herbicide on soil enzymes is a key factor which describes the potential toxicity of herbicide in soil (Quilchano and Maranon, 2002). Nutrients mineralization and enzymes activity in the soil performed by soil microbes are affected by herbicides application and indicate signal of stress (Anderson and Domsch, 1980).

The present study was conducted to evaluate the effect of buctril super herbicide on soil bacteria, fungi and actinomycetes population and also on soil urease, dehydrogenase and alkaline phosphatase activity.

Materials and Methods

Field Experiment

A field experiment was conducted for two years at PMAS-Arid Agriculture University (Rawalpindi) Research Farm at Koont during 2011-2012 and 2012-2013. The experimental site was in the Pothwar plateau of northern Punjab commonly called as rainfed potohar region. In summer the mean temperature at the experimental site varies from $36^{o}C$ to $42^{o}C$ with extremes from time to time as high as $48^{o}C$ (Nizami *et al.,* 2004). The experiment was laid out in randomized complete block design with five herbicide treatments and four replications. Wheat variety Chakwal-50 was sown in October every year and buctril super herbicide was applied at 375, 750, 1500, 22500 mL ha^{-1} and control (no herbicide). The soil of experimental field had pH 7.8, organic matter 0.89% and the texture was clay loam.

Soil Enzymes Activity Assay

The activity of urease was assayed with urea solution being utilized as a substrate by incubating the fresh sample at 37°C for 2 h. Ammonium librated was determined at 690 nm on spectrophotometer and expressed as μg NH$_4$-N g^{-1} dry weight 2 h^{-1} (Kendeler and Gerber, 1988). Activity of dehydrogenase was measured by using triphenyl tetrazolium chloride (TTC) as substrate by incubating the sample at 30°C for 24 h. The TPF produced was estimated colorimetrically at 546 nm and expressed as μg TPF g/24 h (Thalmann, 1968). Alkaline phosphatase activity was assayed using *p*-nitrophenyl phosphate as a substrate and incubation of sample was done for 1h at 37°C. The *P*-nitrophenol produced was estimated colorimetrically at 400 nm and expressed as μg Phenol g^{-1} h^{-1} (Eivazi and Tabatabai, 1977).

Soil Microbial Population Count

Determination of total population of bacteria and colony forming units of actinomycetes and fungi was done by soil sample's serial dilutions using fresh soil. About 10^{-4} to 10^{-10} series of dilutions were plated as the exact number of microbes is generally unknown. The counting of bacteria was done on Tryptone Soya Agar (TSA) amended with cyclohexamide 0.1 g L^{-1}, fungi count was done with Rose Bengal Agar modified with 30 mg L^{-1} streptomycin sulphate. Actinomycetes counting was done on Glycerol Casein Agar adjusted with 0.05 g L^{-1} cyclohexamide. A volume of 0.1 mL soil suspension was spread on to the solidified medium and the plates were incubated for 5-7 days at 25°C for bacteria and fungi and for 10 day for actinomycetes (Williams and Wellington, 1982).

Results

Bacterial Population

The highest bacterial population was 1.50×10^{7} cfu g^{-1} soil in control followed by 1.38×10^{7} cfu g^{-1} soil, where herbicide was applied at 375 mL ha^{-1}. Overall 1500 and 2250 mL ha^{-1} herbicide application caused 23.3% and 30.0% reduction in bacterial population during 2011-2012 and 25.4% and 36.0% decrease during 2012-2013, respectively as compared to control (Fig. 1). The fluctuation in bacterial population was high significant (P ≤ 0.005) with the passage of time. At day-60 the average bacterial population was maximum, while minimum was recorded at day-7, irrespective of the treatments. The interactive effect of treatment and sampling days showed statistically significant difference (P≤0.05). During 2011-2012 maximum population was recorded in control at day-7 and at day-60 where herbicide was applied at 2250 mL ha^{-1}. (In control at day-7 the population was maximum because of absence of herbicide, while at day-60 the population was maximum because of adoption of the microbes to the herbicide and they used it as a source of carbon and energy). Minimum population was found in 2250 mL ha^{-1} dose at day-7 indicating 60% decline in bacteria at day-7 by this dose. The interactive effect of treatment and sampling days during 2012-2013 showed maximum population (1.24×10^{7} cfu g^{-1} soil) at day-60 and minimum population (0.39×10^{7} cfu g^{-1} soil) at day-7 by the application of herbicide at 2250 mL ha^{-1} resulting 68.5% less population at day-7 as compared to day-60 by this dose.

Actinomycetes Population

Impact of herbicide on actinomycetes population during 2011-2012 and 2012-2013 is given in (Fig. 2). Highest population was found in control which was 8.1×10^{5} cfu g^{-1} soil followed by 7.1×10^{5} cfu g^{-1} soil at 375 mL ha^{-1} application rate and lowest was 6.2×10^{5} cfu g^{-1} soil at 2250 mL ha^{-1}. In general 1500 mL ha^{-1} and 2250 mL ha^{-1} treatments showed 18.5% and 23.4% decline in actinomycetes population during 2011-2012, while 23.4% and 27.7% decrease during 2012-2013 in actinomycetes population as compared to control. Decrease in actinomycetes population was found from day-7 to day 15 while increase was observed from day-30 to day-60 during both years. The interactive effects of sampling days and treatment during 2011-2012 showed maximum actinomycetes population (8.7×10^{5} cfu g^{-1} soil) at day-7 in control and minimum population (4.2×10^{5} cfu g^{-1} soil) at day-15 in 2250 mL ha^{-1} treatment indicating 52% decline in 2250 mL ha^{-1} treatment at day-15 as compared to control at day-7. During 2012-2013 the interactive effect of treatment and sampling days showed maximum actinomycetes (7.8×10^{5} cfu g^{-1} soil) in control at day-15 and minimum population (3.9×10^{5} cfu g^{-1} soil) was recorded on same day in 2250 mL ha^{-1} treatment indicating 50% less actinomycetes population in 2250 mL ha^{-1} treatment at day-15 as compared to control at same day.

Fungal Population

Fungal population response to applied herbicide during 2011-2012 and 2012-2013 is given in Fig. 3. Results
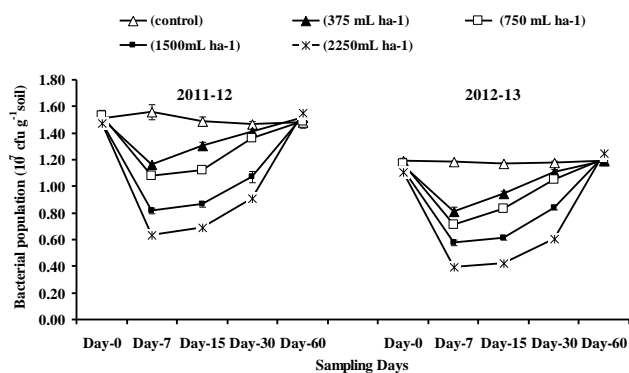
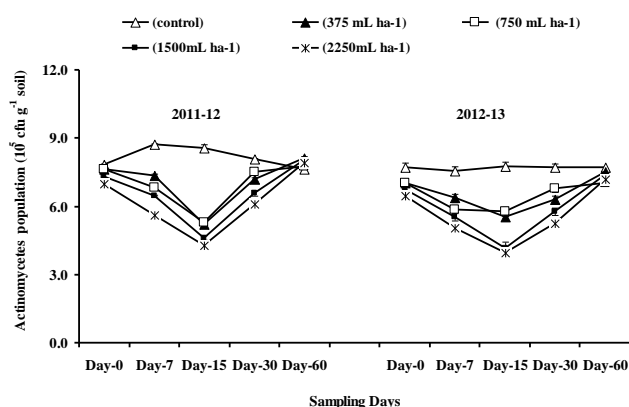**Fig. 1:** Effect of buctril super herbicide on bacterial population

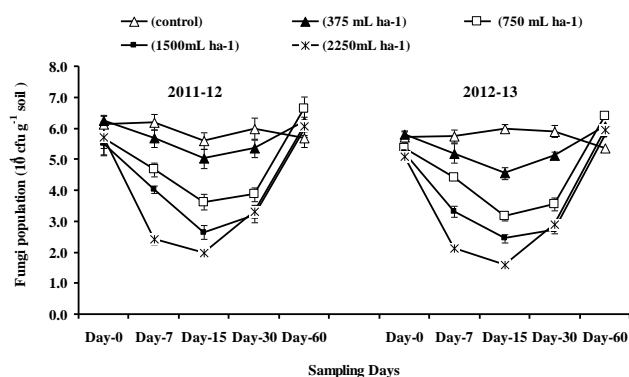**Fig. 2:** Effect of buctril super herbicide on actinomycetes population

Fig. 3: Effect of buctril super herbicide on fungi population

showed highest fungi population (5.9×10^4 cfu g^{-1} soil) in control and lowest (3.9×10^4 cfu g^{-1} soil) where the herbicide was applied at 2250 mL ha^{-1}. On the whole 1500 mL ha^{-1} and 2250 mL ha^{-1} treatments caused 29% and 34% reduction during 2011-2012, whereas 31.5% and 38.6% decrease during 2012-2013 in fungal population, respectively over control. Both years showed peak population of 6.1×10^4 cfu g^{-1} soil and 5.9×10^4 at day-60 while minimum fungi population of 3.8×10^4 cfu g^{-1} soil and 3.6×10^4 cfu g^{-1} soil

was recorded at day 15. After that population showed increasing trend and reached to maximum at day-60. The interaction between treatment and sampling days showed highest population of fungi (6.3×10^4 cfu g^{-1} soil) in 375 mL ha^{-1} treatment at day-0 and lowest (2.0×10^4 cfu g^{-1} soil) in 2250 mL ha^{-1} treatment at day-15 indicating 68% decline in 2250 mL ha^{-1} treatment at day-15 as compared to 375 mL ha^{-1} at day-0 during 2011-2012. But during 2012-2013 maximum population was found in 750 mL ha^{-1} treatment at day-60, while minimum population was experienced in 2250 mL ha^{-1} treatment at day-15 resulting 75% decrease in 2250 mL ha^{-1} treatment at day-15 in comparison to 750 mL ha^{-1} treatment at day-60.

Urease Activity

Buctril super herbicide impact on urease activity is presented in Fig. 4. Highest urease activity values of 299 µg NH$_4$-N g^{-1} dwt 2 h^{-1} was observed in control followed by 291 µg NH$_4$-N g^{-1} dwt 2 h^{-1} in 375 mL ha^{-1} treatment and lowest 210 µg NH$_4$-N g^{-1} dwt 2 h^{-1} in 2250 mL ha^{-1} treatment during 2011-2012. While maximum urease activity of 275 µg NH$_4$-N g^{-1} dwt 2 h^{-1} in control followed by 257 µg NH$_4$-N g^{-1} dwt 2 h^{-1} in 375 mL ha^{-1} treatment and lowest 190 µg NH$_4$-N g^{-1} dwt 2 h^{-1} in 2250 mL ha^{-1} treatment during 2012-2013. Generally 1500 mL ha^{-1} and 2250 mL ha^{-1} treatments showed 22% and 30% decrease during 2011-2012, while 25.4% and 31.0% decrease in urease activity during 2012-2013 in comparison to control. The interactive effect of sampling days and treatment exhibited statistically significant difference. Highest urease activity of 303 µg NH$_4$-N g^{-1} dwt 2 h^{-1} was observed in control at day-7 and lowest urease activity of 134 µg NH$_4$-N g^{-1} dwt 2 h^{-1} was observed in 2250 mL ha^{-1} treatment at day-7 showing 56% decrease in urease activity at day-7 in 2250 mLha^{-1} treatment during 2011-2012. Also interactive effect of treatment and sampling days showed highest urease activity of 285 µg NH$_4$-N g^{-1} dwt 2 h^{-1} in 2250 mL ha^{-1} treatment at day-60, whereas lowest urease activity of 122 NH$_4$-N g^{-1} dwt 2h^{-1} was observed in 2250 mL ha^{-1} reatment at day-7 showing 57% decrease at day-7 in 2250 mL ha^{-1} treatment during 2012-2013.

Dehydrogenase Activity

Impact of bromoxynil herbicide on dehydrogenase activity is shown in Fig. 5. Highest dehydrogenase activities of 31.3 µg TPF g^{-1} 24 h^{-1} and 36.0 µg TPF g^{-1} 24 h^{-1} were observed in control followed by 27.9 µg TPF g^{-1} 24 h^{-1} and 30.5 µg TPF g^{-1} 24 h^{-1} in 375 mL ha^{-1} treatment and least 20 µg TPF g^{-1} 24 h^{-1} and 23.1 µg TPF g^{-1} 24 h^{-1} in 2250 mL ha^{-1} treatment for two seasons. About 31% and 36% decline in dehydrogenase activity during 2011-2012, while 28% and 36% decline during 2012-2013 in 1500 mL ha^{-1} and 2250 mL ha^{-1} treatments were observed, respectively as compared to control. The activity of dehydrogenase was highest at day-0 during 2011-2012, while it was highest
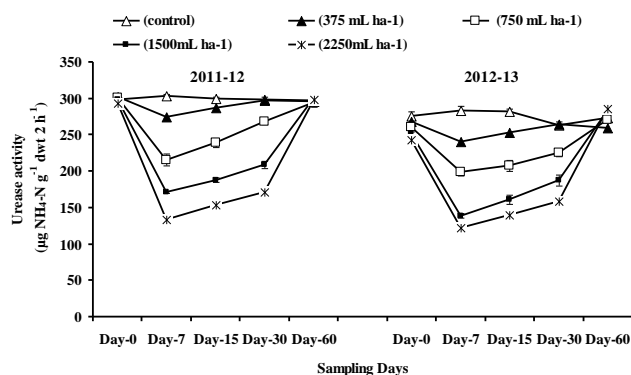
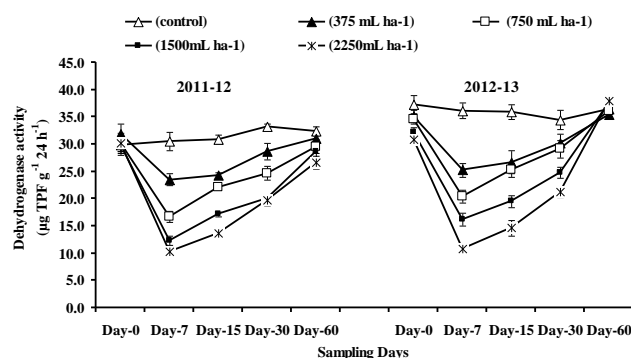**Fig. 4:** Effect of buctril super herbicide on urease activity

Fig. 5: Effect of buctril super herbicide on dehydrogenase activity
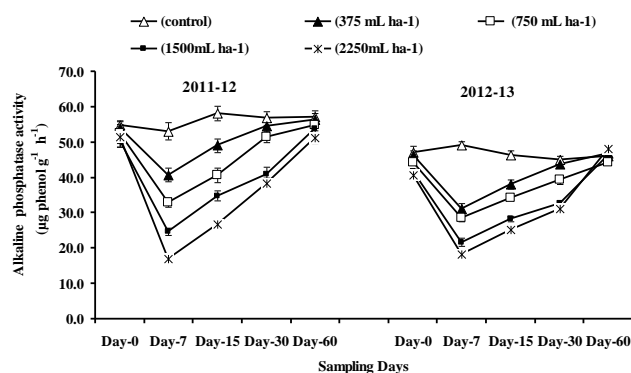

Fig. 6: Effect of buctril super herbicide on alkaline phosphatase activity

at day-60 during 2012-2013. At day-7 minimum activity of said enzyme was recorded during both years. Afterwards increasing trend was observed up to day-60. The interactive effects of sampling days and treatments during first year showed highest dehydrogenase activity of 33.2 µg TPF g^{-1} 24 h^{-1} in control at day-30, while lowest activity of 10.2 µg TPF g^{-1} 24 h^{-1} in 2250 mL ha^{-1} treatment at day-7 indicating 69.2% inhibition in dehydrogenase activity as compared to control at day-30. During second year maximum dehydrogenase activity (37.9 µg TPF g^{-1} 24 h^{-1}) was observed in 2250 mL ha^{-1}

treatment at day-60, while lowest activity was found in 2250 mL ha^{-1} at day-7 showing 71.5% decline in 2250 mL ha^{-1} at day-7.

Alkaline Phosphatase Activity

The impact of Buctril super on alkaline phosphatase activity is given in Fig. 6. Results showed that the activity of alkaline phosphatase was significantly different in all herbicidal treatment and in the order of; Control > 375 mL ha^{-1} > 750 mL ha^{-1} > 1500 mL ha^{-1} > 2250 mL ha^{-1}. Maximum alkaline phosphatase activities of 55.9 and 47.1 µg Phenol g^{-1} h^{-1} were noticed in control followed by 50.9 and 41.2 µg Phenol g^{-1} h^{-1} in 375 mL ha^{-1}, while the lowest alkaline phosphatase activities of 36.9 and 32.7 µg Phenol g^{-1} h^{-1} in 2250 mL ha^{-1} In general 1500 mL ha^{-1} and 2250 mL ha^{-1} showed 27% and 34% decrease in alkaline phosphatase activity during 2011-2012, while 27.6% and 30.6% decrease in alkaline phosphatase activity during 2012-2013 as compared to control. Minimum alkaline phosphatase activities were found at day-7 during 2011-2012 and 2012-2013 then illustrated increasing tendency up to day-60. The interactive effects of treatments and sampling days showed maximum alkaline phosphatase activity (58.2 µg Phenol g^{-1} h^{-1}) in control at day-15, while minimum activity (17.0 µg Phenol g^{-1} h^{-1}) was found in 2250 mL ha^{-1} treatment at day-7, showing 71% inhibition at day-7 in 2250 mL ha^{-1} treatment in contrast to control at day-15 during 2011-2012. The interactive effects of treatments and sampling days showed maximum alkaline phosphatase activity (49.2 µg Phenol g^{-1} h^{-1}) in control at day-7, while minimum activity (18.3 µg Phenol g^{-1} h^{-1}) was found in 2250 mL ha^{-1} at same day, showing 62.8% decline in it at day-7 in 2250 mL ha^{-1} treatment in contrast to control at day-7 during 2012-2013.

Recovery of Bromoxynil Residues

Bromoxynil residues recovery from soil after herbicide treatment is given in Fig. 7. The residues were found up to day-60 after herbicide treatment. Highest residues (1.79 mg kg^{-1}) were found in 2250 mL ha^{-1} treatment followed by 1500 mL ha^{-1} treatment (1.28 mg kg^{-1}), while lowest residues (0.44 mg kg^{-1}) were recovered from 375 mL ha^{-1} treatments. At day-0, maximum residues (1.60 mg kg^{-1}) were recovered followed by (1.37 mg kg^{-1}) at day-7 and lowest residues (0.11 mg kg^{-1}) were found at day-60 during 2011-2012. During 2012-2013 highest residues (1.37 mg kg^{-1}) were found in 2250 mL ha^{-1} treatment followed by 0.94 mg kg^{-1} in 1500 mL ha^{-1} treatment, while lowest residues 0.36 mg kg^{-1} recovered from 375 mL ha^{-1} treatment. At day-0 maximum residues 1.34 mg kg^{-1} were recovered followed by 1.0 mg kg^{-1} at day-7, and lowest residues 0.09 mg kg^{-1} were found at day-60.

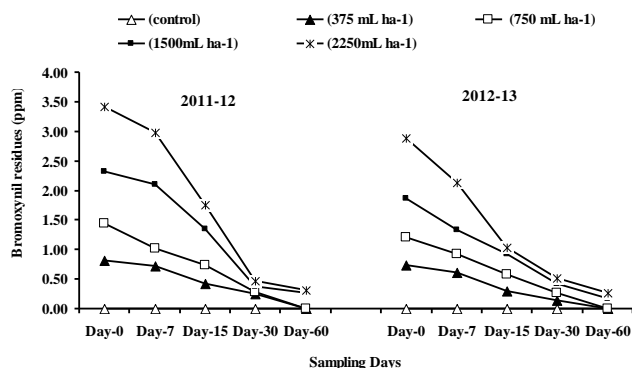Pearson's correlation coefficients between bacteria,

Fig. 7: Recovery of bromoxynil residues from buctril super herbicide applied soil
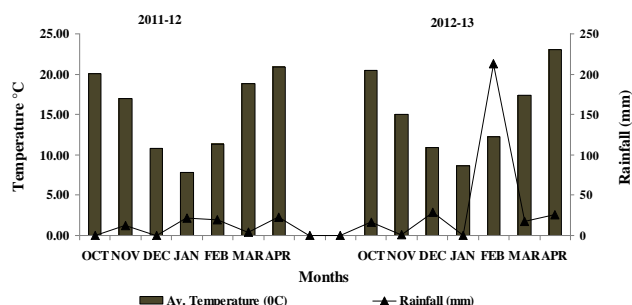

Fig. 8: Meterological data during the experimental period

actinomycetes, fungi population, dehydrogenase, alkaline phosphatase and urease activity are given in Table 1. Highest positive correlation was found between bacteria and actinomycetes population (r=0.82), bacteria and fungi population (r=0.79), bacterial population and alkaline phosphatase activity (r=0.72), bacteria population and urease activity (r=0.71), actinomycetes and fungi population (r=0.81), actinomycetes and urease activity (r=0.61), fungi population and urease activity (r=0.65), alkaline phosphatase and urease activity (r=0.91).

The treatment wise Polynomial second order regression analysis of sampling days with herbicide concentrations for microbial population (bacteria, fungi and actinomycetes) and enzymes activity (urease, dehydrogenase and alkaline phosphatase) was done which is presented in Table 2 and Table 3.

Discussion

In the present study maximum bacterial population (even higher than that of control) was found where the herbicide was applied at 375 mL ha^{-1}. Pampulha and Oliveira (2006) observed enhancement in nitrite oxidizing bacteria (NOB) by the treatment of soil with bromoxynil herbicide and also reported increase in total number of bacteria. The population of bacteria was lowest (0.63×10^7 cfu g[-1] soil) in 2250 mL ha^{-1} treatment at day-7. However, Singh and Dileep (2005) reported 14.4% increase in bacterial population at 15th day,

while 42.9% increase at 60th day after application of dizinon to soil at 800 g a.i ha^{-1}. But Allievi and Gigliotti (2001) reported decline in bacterial population due to sulfonyle urea herbicide and concluded that this herbicide had disrupted the amino acid assimilation ability of nitrifiers leading to their death, which ultimately decreased their number. Reduction of growth of nitrifying bacteria might be due to bromoxynil resulting decrease in their population (Ratnayak and Audus, 1978). A considerable increase in actinomycetes population at lower dose of herbicide was found in the present study. Milosevic and Govedarica (2002) reported increase in the population of actinomycetes at low concentration of herbicide because actinomycetes have used the herbicide as carbon source. Omar and Abdel-Sater (2001) studied the effect of bromoxynil herbicide on actinomycetes and observed increase in actinomycetes number at low concentration. Application of bromoxynil at 2250 mL ha^{-1} exhibited minimum actinomycetes at day 15. Omar and Abdel Sater (2001) reported significant decline in actinomycetes population at higher concentration of brominal herbicide. In our study highest fungal population was observed in control. Lowest fungal population was found where herbicide was applied at 2250 mL ha^{-1}. Except cellulolytic fungi all other groups of fungi are very sensitive to bromoxynil so application of high dose of this herbicide leads to the death of fungi and as a result their population decreased (Pampulha and Oliveira, 2006). Omar (1994) showed a significant suppression in osmophilic fungi population due to bromoxynil and profenofos herbicides at 0.3 and 6 ppm concentration.

The results of our study showed highest inhibition in urease activity at day-7 in all herbicide treatments as compared to control. The treatments receiving 375 mL ha^{-1}, 750 mL ha^{-1}, 1500 mL ha^{-1} and 2250 mL ha^{-1} herbicide showed 9.2%, 28.7%, 43.5% and 55.7% decline in urease activity compared to control at day-7. Weaver *et al.* (2004) reported inactivation of most soil enzymes, because of herbicide attachment on the active site of enzyme and thus preventing substrate attachment to the enzyme. Increasing trend in the activity of urease was observed from day 15 to day 60. This reduction in inhibitory effect was due to soil microbes recovery with the passage of time due to which urease activity increased. As our results showed that microbes (bacteria, fungi and actinomycetes) had overcome the detrimental effects of herbicide after 15-days of its application and maintained their growth to their initial level. A similar trend was reported by Vekova *et al.* (1995) who showed rapid degradation of bromoxynil herbicide and recovery of microbes with time as a consequence enzymes activity attained initial level. Dehydrogenase enzyme is used for the measurement of electrons transfer during utilization of carbon substrate and hence reflects cumulative biological activity in soil (Weaver *et al.,* 2004). During 2011-2012 and 2012-2013 the activity of dehydrogenase enzyme showed significant decline in soil where herbicide was applied at 2250 mL ha^{-1} as compared to control. Our results showed

Table 1: Correlation (r-values) for microbiological properties of soil exposed to buctril super (bromoxynil) herbicide

	Bacterial population	Actinomycetes Population	Fungi population	Dehydrogenase activity	Alkaline phosphatase activity	Urease activity
Bacterial population	-	0.82	0.79	-0.033	0.72	0.71
Actinomycetes Population		-	0.81	-0.097	0.57	0.61
Fungi population			-	0.0193	0.56	0.65
Dehydrogenase activity				-	-0.0103	-0.003
Alkaline phosphatase activity					-	0.91

Table 2: Regression analysis about the effect of buctril super herbicide on microbial population

	Effect of buctril super herbicide on bacterial population					
	2011-2012			2012-2013		
Treatments	Equation	R^2	Treatments	Equation	R^2	
Control	$y = 4E\text{-}05x^2 - 0.0031x + 1.5381$	0.4834	Control	$y = 2E\text{-}05x^2 - 0.0012x + 1.19$	0.8062	
375 mL ha^{-1}	$y = 0.0001x^2 - 0.0061x + 1.3817$	0.3587	150 mL ha^{-1}	$y = 0.0001x^2 - 0.0042x + 1.0197$	0.3475	
750 mL ha^{-1}	$y = 0.0003x^2 - 0.0132x + 1.3592$	0.3871	300 mL ha^{-1}	$y = 0.0002x^2 - 0.0096x + 0.9941$	0.425	
1500 mL ha^{-1}	$y = 0.0006x^2 - 0.0294x + 1.2669$	0.6439	600 mL ha^{-1}	$y = 0.0005x^2 - 0.0228x + 0.9424$	0.6488	
2250 mL ha^{-1}	$y = 0.0008x^2 - 0.0405x + 1.2153$	0.6927	900 mL ha^{-1}	$y = 0.0007x^2 - 0.0367x + 0.8898$	0.7476	
	Effect of buctril super herbicide on actinomycetes population					
	2011-2012			2012-2013		
Treatments	Equation	R^2	Treatments	Equation	R^2	
Control	$y = -0.2166x^2 + 1.1929x + 6.965$	0.8699	Control	$y = 0.0036x^2 - 0.0114x + 7.693$	0.0583	
375 mL ha^{-1}	$y = 0.4657x^2 - 2.7173x + 10.131$	0.6285	150 mL ha^{-1}	$y = 0.3814x^2 - 2.2071x + 8.977$	0.9301	
750 mL ha^{-1}	$y = 0.4186x^2 - 2.4154x + 9.649$	0.6075	300 mL ha^{-1}	$y = 0.2825x^2 - 1.6025x + 8.212$	0.7536	
1500 mL ha^{-1}	$y = 0.6014x^2 - 3.4501x + 10.331$	0.8165	600 mL ha^{-1}	$y = 0.6129x^2 - 3.5296x + 9.772$	0.91	
2250 mL ha^{-1}	$y = 0.6839x^2 - 3.8731x + 10.275$	0.9247	900 mL ha^{-1}	$y = 0.6129x^2 - 3.5296x + 9.772$	0.91	
	Effect of buctril super herbicide on fungi population					
	2011-2012			2012-2013		
Treatments	Equation	R^2	Treatments	Equation	R^2	
Control	$y = 0.0179x^2 - 0.2121x + 6.365$	0.4134	Control	$y = -0.1071x^2 + 0.5829x + 5.175$	0.8013	
375 mL ha^{-1}	$y = 0.2768x^2 - 1.6982x + 7.755$	0.9394	150 mL ha^{-1}	$y = 0.3089x^2 - 1.8061x + 7.37$	0.9427	
750 mL ha^{-1}	$y = 0.7411x^2 - 4.4389x + 9.43$	0.9418	300 mL ha^{-1}	$y = 0.6554x^2 - 3.8096x + 8.8$	0.8849	
1500 mL ha^{-1}	$y = 0.7411x^2 - 4.4389x + 9.43$	0.9418	600 mL ha^{-1}	$y = 0.7893x^2 - 4.6857x + 9.275$	0.9628	
2250 mL ha^{-1}	$y = 0.9893x^2 - 5.7757x + 10.355$	0.9845	900 mL ha^{-1}	$y = 0.9905x^2 - 5.6962x + 9.73$	0.9965	

Table 3: Regression analysis about the effect of buctril super herbicide on soil enzymes activity

	Effect of buctril super herbicide on urease activity					
	2011-2012			2012-2013		
Treatments	Equation	R^2	Treatments	Equation	R^2	
Control	$y = -0.611x^2 + 2.9178x + 297.42$	0.6204	Control	$y = -0.8882x^2 + 2.8218x + 276.65$	0.2823	
375 mL ha^{-1}	$y = 3.6099x^2 - 20.576x + 313.28$	0.4303	150 mL ha^{-1}	$y = 3.1507x^2 - 18.402x + 277.84$	0.2998	
750 mL ha^{-1}	$y = 16.318x^2 - 94.01x + 366.13$	0.7403	300 mL ha^{-1}	$y = 16.078x^2 - 91.734x + 331.08$	0.9292	
1500 mL ha^{-1}	$y = 31.688x^2 - 185.93x + 442.82$	0.9238	600 mL ha^{-1}	$y = 30.127x^2 - 169.63x + 381.98$	0.9351	
2250 mL ha^{-1}	$y = 40.814x^2 - 240.31x + 482.22$	0.932	900 mL ha^{-1}	$y = 35.345x^2 - 199.92x + 400.86$	0.9532	
	Effect of buctril super herbicide on dehydrogenase activity					
	2011-2012			2011-2012		
Treatments	Equation	R^2	Treatments	Equation	R^2	
Control	$y = -0.0693x^2 + 1.171x + 28.578$	0.7727	Control	$y = 0.3579x^2 - 2.4882x + 39.523$	0.7225	
375 mL ha^{-1}	$y = 1.7815x^2 - 10.304x + 39.179$	0.7869	150 mL ha^{-1}	$y = 2.293x^2 - 13.209x + 44.925$	0.8766	
750 mL ha^{-1}	$y = 2.4193x^2 - 13.852x + 39.582$	0.6964	300 mL ha^{-1}	$y = 2.9636x^2 - 16.598x + 46.279$	0.8018	
1500 mL ha^{-1}	$y = 3.5745x^2 - 20.892x + 44.967$	0.8129	600 mL ha^{-1}	$y = 4.2708x^2 - 23.694x + 50.144$	0.9303	
2250 mL ha^{-1}	$y = 4.0413x^2 - 24.005x + 47.63$	0.8043	900 mL ha^{-1}	$y = 5.4509x^2 - 30.262x + 53.911$	0.9411	
	Effect of buctril super herbicide on alkaline phosphatase Activity					
	2011-2012			2012-2013		
Treatments	Equation	R^2	Treatments	Equation	R^2	
Control	$y = -0.1897x^2 + 1.988x + 52.109$	0.4621	Control	$y = -0.038x^2 - 0.3718x + 48.316$	0.3877	
375 mL ha^{-1}	$y = 1.9585x^2 - 9.8992x + 59.115$	0.5524	150 mL ha^{-1}	$y = 2.5015x^2 - 13.69x + 54.799$	0.6043	
750 mL ha^{-1}	$y = 3.4794x^2 - 18.546x + 63.85$	0.6484	300 mL ha^{-1}	$y = 2.9065x^2 - 16.364x + 55.255$	0.7055	
1500 mL ha^{-1}	$y = 5.2351x^2 - 29.122x + 70.686$	0.7814	600 mL ha^{-1}	$y = 4.6569x^2 - 25.926x + 60.71$	0.8637	
2250 mL ha^{-1}	$y = 6.8972x^2 - 39.266x + 78.875$	0.7739	900 mL ha^{-1}	$y = 5.5549x^2 - 30.531x + 63.177$	0.9002	

that higher concentration (2250 mL ha^{-1}) of bromoxynil herbicide induced decline in actinomycetes and bacterial population ultimately leading to decreased dehydrogenase activity because dehydrogenase activity has direct relation with microbial population. Radivojevic *et al.* (2012) reported 42.7% inhibition in dehydrogenase activity by the

application of nicosulfuron herbicide at 3 mg concentration. Zahir *et al.* (2001) described mortality of weed roots by herbicide application as a result root exudates that contain auxin and gebrillin declined. As auxin and gebrillin are involved in enzymes activity enhancement so their suppression contribute toward this decline in dehydrogenase activity. From day-15 to 60 increase in dehydrogenase activity was found. It is found by our results that soil microorganisms recover themselves with time due to which dehydrogenase activity increased. Věková *et al.* (1995) reported recovery of dehydrogenase with the passage of time due to decrease in herbicide concentration and recovery of soil microorganisms. In this present study maximum alkaline phosphatase activity was found in control because of no harmful effect of herbicide. High activity at day-60 was because of degradation of the herbicide by microbes. During early 7 days of herbicide application significant decline in phosphatase activity was recorded. Tu (1981) observed decrease in phosphatase activity by using 2, 4-D herbicide at 10 mg kg^{-1}. They found that this decrease was due to hindrance caused by said herbicide in p-nitrophenol release from p-nitrophenyl phosphate. Xiaohua *et al.* (2005) reported decrease in the activity of alkaline phosphatase due to acetamiprid application because this herbicide had altered the membrane permeability of phosphate solublizers that release phosphatase enzymes. Voets *et al.* (1974) reported 61.8% decrease in phosphatase activity due to atrazine.

Rainfall and temperature data during the crop seasons for both years is given in Fig. 8 which showed normal trends and no extreme was found indicating that this rainfall and temperature has no significant effect on soil enzymes activity and microbial population. Findings of (Gianfreda *et al.*, 2002; Gianfreda and Ruggiero, 2006) indicated that soil enzymes exhibit peculiar characteristics and are resistant to different agents that cause their deactivation such as air, moisture and temperature. So in most cases no considerable reduction in the activity of soil enzymes occur after their exposure to those agents.

Our results showed positive correlation between fungi population and urease activity, between dehydrogenase and alkaline phosphatase activity, between urease activity and population of bacteria, between urease activity and fungal population. Sharma and Mishra (1992) reported positive correlation between fungi population and dehydrogenase activity, between fungi population and alkaline phosphatase activity, between urease activity and fungi population, between bacteria population and urease activity.

Conclusion

Soil microorganisms and the activities of soil enzymes are the essential component of soil environment. The activities of soil enzymes, population of soil microorganisms and biodiversity are the best sign of balanced agro-ecosystem. Our study revealed that recommended dose of buctril super (bromoxynil) herbicide had slightly adverse effect on soil microorganisms and enzymes activity. But higher application rates exerted strong toxic effects on these parameters. So we stressed the role of herbicide concentration, its time of exposure for the estimation of the influence of this herbicide on soil microbiological parameters. From this two year field study it is evident that buctril super (bromoxynil) herbicide is injurious for soil microorganisms and enzymes activity. Hence great care is required while applying this herbicide to soil.

Acknowledgements

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