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Short Research Article

Alachlor and Metribuzin Herbicide on N_2-fixing Bacteria in a Sandy Loam soil

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Abstract

Herbicides interact with soil organisms and their metabolic activities and may alter the physiological and biochemical behavior of soil microbes. Some microbial groups are capable of using applied pesticide as a source of energy and nutrients to multiply, whereas the pesticide may be toxic to other organisms. Laboratory experiment was conducted to investigate the effect of two selective systemic herbicides *viz.*, alachlor and metribuzin, at their recommended field rates (2.0 and 0.4 kg a.i. ha^{-1}, respectively) on the growth and activities of non symbiotic N_2-fixing bacteria in relation to mineralization and availability of nitrogen in a sandy loam soil of India. Both the herbicides, either singly or in a combination, stimulated the growth and activities of N_2-fixing bacteria resulting in higher mineralization and availability of nitrogen in soil. The single application of alachlor increased the proliferation of aerobic non-symbiotic N_2- fixing bacteria to the highest extent while that of metribuzin exerted maximum stimulation to their N_2-fixing capacity in soil. Both the herbicides, either alone or in a combination, did not have any significant difference in the stimulation of total nitrogen content and availability of exchangeable NH_4^+ in soil while the solubility of NO_3^- was highly manifested when the herbicides were applied separately in soil. The effect of combined application of the herbicides was more or less at par with their single application.

1. Introduction

A weed is a wild plant growing where it is not wanted and in competition with cultivated plant. Herbicide(s), also commonly known as weed killers, are chemical substances used to control unwanted plants. Herbicides are frequently used by the farmers in their crop fields to eradicate the unwanted weeds for better crop growth. The use of herbicides for combating unwanted weeds in crop fields has been increased steadily. When we spray herbicides in the field a large portion fall into the soil surface. After then it goes downward due to rain fall and ultimately a lot of microbes present within the soil are largely affected or sometimes the physiology of that microbes changes (Das and Mukherjee, 2011 & 2012a). As a result of this physiological changes, the amount of microbial biomass changes (Gebendinger and Radosevich, 2009). This favourably influences the transformations of plant nutrients in soil. Herbicides that disrupt the activities of soil microorganisms may affect the nutritional quality of soils, resulting in serious ecological imbalance (Menon et al., 2004; Das and Mukherjee, 2012b). Hence, it is important to avoid serious injury to the soil microflora, whose functions are vital in maintaining the soil fertility (Kumar et al., 2012; Das and Mukherjee, 2014). Herbicides also influences soil biochemical processes driven by microbial and enzymatic reactions. The microbial mineralization of organic compounds and associated biotransformation such as nutrient dynamics and their bioavailability are also more or less adversely affected by the herbicides (Demanou et al., 2006; Mahia et al., 2008; Niewiadomska, 2005). Soil microorganisms play an important role in degradation of herbicide and are vital in ecosystem processes (Maisnam et al., 2009). They are responsible for decomposition of organic material and available nutrient cycle. Adverse effect of herbicides on growth and activities of many beneficial microorganisms in soil has been reported elsewhere (Malkomes, 2005; Kinney et al., 2005). These deleterious effects of herbicide application on soil microorganisms vary depending on the type of that herbicides and also microorganisms present within that soil (Aggarwal, 2005). Therefore, it becomes necessary to study the effect of different herbicides application on microbial transformation of nutrient elements in soil, to determine nutrient availability for higher crop growth. Keeping the adverse effect of herbicides application on soil biology laboratory study was conducted during 2012 on effect of alachlor and metribuzin herbicide on N_2-fixing bacteria in a sandy loam soil. The objective of the present study was to investigate the effect of two widely used selective systemic herbicides, viz., alachlor [(2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide] and metribuzin [4-amino-6-tert-butyl-3-(methythio)-1,2,4-triazin-5-one], either alone or in a combination, at their recommended field rates (2.0 kg and 400 g active ingredient ha^{-1}) on growth and activities of aerobic non-symbiotic N_2-fixing bacteria in relation to mineralization and availability of nitrogen in soil.

2. Materials and Methods

The experiment was conducted during the year 2012 at the experimental farm of Vivekananda Institute of Hill Agriculture, located in the Indian Himalayan region at Hawalbagh (29°36´N and 79°40´E with 1250 m amsl), in the state of Uttarakhand, India. The climate is subtemperate, characterised by moderate summer (May–June), extreme winter (Dec–Jan) and general dryness, except during the southwest monsoon season (June–Sept). Soil samples used for this experiment (alluvial soil) were collected at a depth of 0–15 cm (plough layer) with no history of herbicide application. Samples were collected in polythene bags, then dried in shade, ground, sieved through a 2 mm sieve and stored at room temperature (31±1 °C). Different physico-chemical properties were analysed with standard method (Jackson, 1973). Organic carbon measured by using the Walkley and Black method (1965), clay, sand and silt measured by employing the Bouyoucos hygrometer (Bouyoucos and Cook, 1967). The electrical conductivity was measured using conductivity meter. Soil pH (1:2.5 H_2O), cation exchange capacity [by 1 M NH_4OAc (pH 7) extraction], Olsen P (by 0.5 M $NaHCO_3$ extraction), sulfate [by 0.04 M $Ca(H_2PO_4)_2$ extraction], were measured according to Black (1965). Acid oxalate-extractable Fe and aluminum (Al), and exchangeable potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) were determined for the initial soil samples by the standard methods of Blakemore et al. (1987). The physico-chemical properties alluvial soil is given in Table 1.

Two herbicides viz., alachlor [20% a.i. in EC formulation – UPL Ltd.] and Metribuzin [40% a.i. in WP formulation - Dhanuka Pesticide Ltd.] at their recommended field rates (2.0 kg and 0.4 kg a.i. ha^{-1}, respectively) were mixed thoroughly, either separately or in combination, with 250 g air-dried and

Table 1: Physico-chemical properties of alluvial soil

Physico-chemical properties	values
pH	6.9
Sand, 0.02-2 mm (%)	58.3
Silt, 0.002-0.2 mm (%)	22.6
Clay, <0.002 mm (%)	19.1
Organic carbon (%)	0.43
CEC (meq100 g^{-1})	9.59
Surface area (m^2 g^{-1})	44.1
EC (mS m^{-1})	0.3
Specific gravity	2.09
Olsen P (mg kg^{-1})	13.48
SO_4 (mg kg^{-1})	14.37
Acid oxalate Al%	0.31%
Acid oxalate Fe%	0.08%
K (cmol Kg^{-1})	0.83
Ca (cmol Kg^{-1})	9.84
Mg (cmol Kg^{-1})	1.49
Na (cmol Kg^{-1})	0.37

sieved soil (≤2 mm) and were placed in polyethylene pots. To avoid/minimize the photodegradation of the herbicides, the pots were kept covered with black polyethylene sheets. This is due to the fact that light penetration under black polythene is negligible. Then the fortified pots were incubated at 25±1 °C for about 60 days. Separate sets of treatments were maintained for each sampling day under incubation. Three replications for each treatment were used for this experiment. During the time of incubation study, soil samples were collected at certain periodic intervals viz., after 0 (1 h), 15, 30, 45 and 60 days from each treatments with three replication. After sub sampling they were immediately analyzed to determine the proliferations of aerobic non-symbiotic N_2-fixing bacteria. Besides, the activities of aerobic non-symbiotic N_2-fixing bacteria along with biochemical transformations were also analyzed. The colony forming units which is expressed as cfu of the aerobic non-symbiotic N_2-fixing bacteria was enumerated in sucrose-$CaCO_3$ agar medium (Jensen 1930). This was done by following serial dilution technique along with pour plate method developed by Salle, 1973. After incubating the agar plates at 25±1 °C for 7 days, cfu of the microorganisms grown on the respective agar plates were counted. Non-symbiotic N_2-fixing capacities of the soil samples were determined by incubating 1 g soil from each sample in 50 ml Jensen's broth (Jena, 1987) containing 2% sucrose in conical flasks at 27±1 °C for 15 days Then total nitrogen was estimated by the method of Bremner 1996 in the broth. To estimate the changes of total N in soil samples, soil were analyzed following the method described by Jackson (1973). Besides, exchangeable NH_4^+ and soluble NO_3^- were measured in 2N KCl extract through distillation technique as described by Mulvaney (1996).

3. Results and Discussion

Growth and multiplication of aerobic non-symbiotic N_2-fixing bacteria in soil as affect by herbicides has been presented in Table 2. After analysis of sample, results of this study revealed that application of herbicides increased the proliferation of aerobic non-symbiotic N_2-fixing bacteria in soil. It was found that the stimulation was more pronounced with alachlor followed by its combined application (alachlor+metribuzin) and then with metribuzin. After 15th days of experiment for all the treatment microbial stimulation increased significantly. Higher stimulation of microbial population under alachlor up to 45th day (then decrease) and under metribuzin and its combination with alachlor up to 15th day (then decrease). This result pointed out that soil microorganism utilized the herbicides along with their degraded metabolites as their energy, carbon and other nutrients sources for their cellular metabolism and growth (Khan, 2006).

Change in aerobic non-symbiotic N_2-fixing capacities of soil has been presented in Table 3. The proliferation of N_2-fixing bacteria significantly stimulated the non-symbiotic N_2-fixing capacities of the soil as affected by herbicide application. Mahia et al. in 2008 also reported same results in atrazine herbicides. The higher N_2-fixation was observed under Metribuzin from 30th to 60th day of sampling. But it was observed that lower number of bacteria was present in metribuzin treatment as compared to alachlor and its combined application. This finding indicated that Metribuzin induced the efficiency of the bacteria rather than their growth and multiplication (Jena and Rao, 1987; Debnath et al., 2002). Among the herbicides, metribuzin exerted highest stimulation as compared to untreated control soil also.

Table 2: Growth and multiplication of aerobic non-symbiotic N_2-fixing bacteria in soil as affect by herbicides

Number of aerobic non-symbiotic N_2-fixing bacteria (cfu×10^5 g^{-1} soil)					
Sampling days	Treatments Control	Metribuzin (Mb)	Alachlor (Ac)	Mb+Ac	Mean
0 (1 h)	30.4	30.4	30.4	30.4	30.4
15	74.0	129.7	130.9	131.3	116.5
30	59.0	94.8	105.1	91.7	87.7
45	59.7	91.4	107.8	88.3	86.9
60	50.4	59.0	69.6	73.2	63.1
Mean	54.7	81.1	88.8	83.0	

LSD (p<0.05); Treatment, 12.7; Sampling date 15.3; Interaction, NS; cfu colony forming unit; *p* level of significance; NS: No significance

Table 3: Changes in aerobic non-symbiotic N_2-fixing capacities of soil as affected by herbicides

Amount of nitrogen fixed (mg g^{-1} soil)					
Sampling days	Treatments Control	Metribuzin (Mb)	Alachlor (Ac)	Mb+Ac	Mean
0 (1 h)	10.7	10.7	10.7	10.7	10.7
15	14.0	16.7	17.1	17.3	16.3
30	13.5	15.7	13.2	14.4	14.2
45	12.3	15.1	12.6	11.8	13.0
60	11.5	14.1	12.9	12.7	12.8
Mean	12.4	14.5	13.3	13.4	

LSD (*p*<0.05; Treatment 0.57; Sampling date 0.75; Interaction 1.29; *p* level of significance

Changes of total nitrogen and available mineral nitrogen in soil as affected by herbicides have been presented in Table 4 and 5. The stimulation of microorganisms and their activities in soil due to the application of both the herbicides brought about greater mineralization of organic nitrogen. This resulting in greater amount of available nitrogen (Snapp and Borden, 2005) followed by loss of nitrogen from soil due to volatilization process. In general, application of metribuzin and alachlor either alone or in combination retained higher amount of total nitrogen as compared to untreated control (Table 4). But metribuzin non-significantly retained higher total nitrogen. Results of this study also recorded that both the herbicides significantly increased the availability of mineral nitrogen (exchangeable NH_4^+ and soluble NO_3^-) in the soil (Table 5). Soluble NO_3^- was more influenced by application of alachlor than combined application and metribuzin alone. The increase

Table 4: Changes of total nitrogen in soil as affected by herbicides

Amount of total nitrogen (g kg^{-1})					
Sampling days	Treatments Control	Metribuzin (Mb)	Alachlor (Ac)	Mb+Ac	Mean
0 (1 h)	0.83	0.83	0.83	0.83	0.83
15	0.95	1.09	1.06	1.10	1.05
30	0.93	1.03	1.01	1.01	1.00
45	0.91	0.97	0.96	0.95	0.95
60	0.77	0.83	0.83	0.83	0.82
Mean	0.88	0.95	0.94	0.94	

LSD (*p*<0.05); Treatment 0.02; Sampling date 0.04; Interaction NS; *p* level of significance; NS: No significance in both the form of available nitrogen can be explained by the fact that soil microflora accelerate both ammonification and nitrification resulting in higher release of mineral nitrogen in soil (Subrahmaniyan et al., 2008). We know that soil retained more exchangeable NH_4^+ than soluble NO_3^-. This suggested that the process of ammonification was faster and nitrification was slower. There was no significant difference in the accumulation of exchangeable NH_4^+ in all the herbicide treatment. There was a significant positive correlation (r=0.824) between the population of non-symbiotic N_2-fixing bacteria and their activities in soil (Table 6).

Table 5: Changes of available mineral nitrogen in soil as affected by herbicides

Sampling days	Treatments Control	Metribuzin (Mb)	Alachlor (Ac)	Mb+Ac	Mean
Amount of exchangeable NH_4^+ (mg kg^{-1})					
0 (1 h)	98.8	98.8	98.8	98.8	98.8
15	106.0	106.8	161.3	145.5	129.9
30	96.6	168.8	107.5	125.4	124.6
45	78.5	86.5	92.5	87.5	86.3
60	63.7	73.0	65.1	73.7	68.9
Mean	88.7	106.8	105.0	106.2	
LSD (p<0.05); Treatment 5.1; Sampling date 6.8; Interaction 11.4					
Amount of soluble NO^{-3} (mg kg^{-1})					
0 (1 h)	45.0	45.0	45.0	45.0	45.0
15	63.3	81.0	90.8	69.7	76.2
30	54.5	75.1	70.1	58.0	64.4
45	45.9	52.7	57.3	54.3	
60	35.1	46.9	47.8	41.6	42.9
Mean	48.8	60.1	62.2	53.7	

LSD (p<0.05); Treatment 1.5; Sampling date 2.8; Interaction 3.4; p level of significance

Table 6: Linear relationships (r values) among different parameters

Parameters	N_2-fixing capacity	Total N	Exch. NH_4^+	Soluble NO_3^-
N_2-fixing bacteria	0.837**	0.909**	0.542*	0.817**
N_2-fixing capacity	–	0.871**	0.561*	0.820**
Total N	–	–	0.741**	0.881**
Exch. NH_4^+	–	–	–	0.757**

LSD (**p<0.01)

4. Conclusion

Application of both herbicides either alone or in combination stimulated growth and activities of aerobic non-symbiotic N_2-fixing bacteria resulting in higher retention and availability of nitrogen in soil. Application of alachlor highly stimulated proliferation of non-symbiotic N_2-fixing bacteria while metribuzin augmented their activities to the highest extent. Both herbicides when applied separately accumulated maximum amount of soluble NO_3^- in soil solution. No significant difference in retention of total-N and exchangeable-NH_4^+ by the herbicides either applied alone or in combination.

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