Bull. Environ. Contam. Toxicol. (2002) 68:766–770

DOI: 10.1007/s00128-001-0319-0

Bulletin of Environmental Contamination and Toxicology

Persistence and Effect of Butachlor and Basalin on the Activities of Phosphate Solubilizing Microorganisms in Wetland Rice Soil

A. Debnath, A. C. Das, D. Mukherjee

Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur 741 252, India

Received: 15 May 2001/Accepted: 31 December 2001

Herbicides are applied to the crop fields to get rid of unwanted plants. Besides killing weeds, these chemicals sometimes adversely affect the growth and activities of beneficial microorganisms in soil (Kole and Dey 1989; Selvamani and Sankaran 1993). Some reports are also available (Chen et al. 1981; Sandhu et al. 1990) on the stimulating effect of herbicides on microbial populations in soil. Therefore, a thorough investigation is necessary to draw a definite conclusion on the effect of herbicides on growth and activities of soil microorganisms. The aim of the present study was to investigate the persistence and effect of two herbicides, viz., butachlor [N - (butoxymethyl) – 2 - chloro - 2′, 6′- diethyl-acetanilide] and basalin [N - (2 - chloroethyl) - 2, 6 - dinitro - N - propyl - 4 - trifluoromethyl) aniline] at rates of 2.0 and 1.5 kg a.i. ha^{-1} respectively, on the growth and activities of phosphate solubilizing microorganisms as well as the availability of phosphorus in the rhizosphere soil of wetland rice.

MATERIALS AND METHODS

An experiment was conducted in microplots (5m x 3m) with three replications following randomized block design (RBD). The soil belongs to Typic Fluvaquent (USDA 1975) having the general characteristics: sand 20.7%, silt 32.8%, clay 46.5%, water holding capacity 49.7%, pH (1:2.5 w/v) in water 7.1, electrical conductivity 0.35 dS m^{-1}, cation exchange capacity 12.5 cmol (p^+) kg^{-1}, organic C 5.8 g kg^{-1}, total N 0.6 g kg^{-1}, C : N ratio 9.6, exchangeable NH_4^+ 73.3 mg kg^{-1}, soluble NO_3^- 31.6 mg $kg^{-1}$and available P 8.1 mg kg^{-1}. Each plot received N, P and K @ 30, 22 and 42 kg ha^{-1} in forms of urea, single superphosphate and muriate of potash respectively. Twenty-five days old rice seedlings (*Oryza sativa* L. variety IR - 36) were transplanted 2 seedlings $hill^{-1}$, with a spacing 15 cm by 20 cm hill to row. After 10 days of transplanting of the crop, two herbicides viz., butachlor and basalin at rates of 2.0 and 1.5 kg a.i. ha^{-1} respectively, were applied to the crop fields separately by mixing with 600 L ha^{-1} of distilled water. After 21 days of transplanting, another 30 kg ha^{-1} of N as urea was applied to the crop as top dressing. The crop was cultivated following usual agronomic practices.

Rhizosphere soil samples were collected after 0 (1 hour), 10, 20, 30, 45 and 60 days of herbicide application from each plot at random for analysis, keeping side

---

Correspondence to: A. C. Das

by side sets for the estimation of moisture content. The colony forming units (cfu) of phosphate solubilizing microorganisms was enumerated in sucrose-tricalcium phosphate agar medium (Pikovskaia 1948) following serial dilution and pour plate method (Pramer and Schmidt 1964). The phosphate solubilizing capacities of the soils were determined by estimating soluble P in 15 ml Pikovskaia's (1948) broth following the method as outlined by Debnath et al. (1994). The soils were also analyzed for available P in sodium bicarbonate extract colorimetrically following the method of Jackson (1973).

The soils were also analyzed to determine the persistence of butachlor and basalin by extracting the soils in benzene and methanol respectively, following the method as described by Debnath (1997). After extraction, the herbicide residues were estimated through gas-liquid chromatography (GLC) with the help of 5890A (HP) gas chromatograph coupled with 3392A (HP) integrator and equipped with Ni^{63} electron capture detector and a glass column (180 cm x 2 mm i.d.) packed with 3% OV-IOI on 80 to 100 mesh chromosorb - W (HP). The operating temperatures of injector, column and detector were maintained for butachlor at 250 °C, 200 °C and 250 °C, and for basalin at 250 °C, 180 °C and 250 °C, respectively. The flow rates of carrier gas (N_2) were adjusted to 65 and 50 mL min^{-1} for butachlor and basalin, respectively. The recovery rates of fortified controls of butachlor and basalin were 90% and 98%, respectively. The residue values were processed to calculate the half-life ($T_{1/2}$) following the method of Hoskins (1961).

RESULTS AND DISCUSSION

Application of herbicides, in general, significantly augmented the proliferations of phosphate solubilizing microorganisms in the rhizosphere soil of rice (Table 1). Between the two herbicides, basalin was more stimulative as compared to butachlor. The significant rise in the population of phosphate solubilizing microorganisms due to the application of herbicides pointed out that the microorganisms utilized the chemicals and their degraded products as their energy, carbon and other nutrients for their growth and development (Alexander 1977; Cork and Krueger 1991). Moreover, at this period the crop was at flowering stage when the activities of the soil microorganisms in the rhizosphere soil were at optimum level and the crop released more amount of root exudates rich in growth promoting substances (Rovira 1965) which were preferably utilized by the soil microorganisms for their growth and metabolism (Kole and Dey 1989). Sustaining the earlier report (Das and Mukherjee 1994), the proliferation of phosphate solubilizing microorganisms led to the stimulation of phosphate solubilizing capacity of the rhizosphere soils and this microbial activity was concomitant with their proliferation. Incidentally, there was a significant positive correlation ($r = 0.944$) between the population of phosphate solubilizing microorganisms and phosphate solubilizing capacities in soil. The greater solubilization of insoluble phosphates by the increased phosphate solubilizing

Table 1. Effect of herbicides on the population and activities of phosphate solubilizing microorganisms and the availability of phosphorus in the rhizosphere soil of rice.

Sampling days	Treatments		
	Control	Butachlor	Basalin
	Number of P- solubilizing microorganisms (cfu x 10^4 g^{-1} soil)		
0 (1hr)	49.7 ± 6.2	51.2 ± 4.9	52.0 ± 6.2
10	58.9 ± 7.1	65.8 ± 8.8	70.7 ± 5.1
20	71.1 ± 5.2	83.8 ± 8.6	86.4 ± 7.0
30	90.0 ± 8.9	96.3 ± 10.2	102.7 ± 11.1
45	59.7 ± 6.7	65.7 ± 7.2	71.5 ± 6.8
60	37.9 ± 3.2	41.3 ± 5.1	52.2 ± 5.1
Mean	61.2	67.3	72.5
	Amount of P- solubilized (μg g^{-1} soil)		
0(1hr)	157 ± 25	153 ± 23	168 ± 17
10	169 ± 27	173 ± 28	188 ± 25
20	185 ± 16	200 ± 29	212 ± 22
30	204 ± 31	234 ± 34	241 ± 32
45	199 ± 28	222 ± 36	232 ± 29
60	194 ± 22	216 ± 24	224 ± 19
Mean	185	207	211
	Amount of available P content (μg g^{-1} soil)		
0(1hr)	8.1 ± 2.1	8.4 ± 1.1	8.3 ± 2.1
10	9.0 ± 2.1	9.1 ± 2.3	9.5 ± 1.8
20	10.5 ± 1.3	10.7 ± 1.9	12.4 ± 2.3
30	11.4 ± 1.6	12.6 ± 1.8	14.2 ± 2.4
45	9.7 ± 1.7	11.5 ± 2.1	12.8 ± 2.2
60	8.6 ± 0.5	10.3 ± 2.2	11.2 ± 1.7
Mean	9.5	10.4	11.4

microorganisms as well as higher content of organic acids present in the root exudates of the growing plants (Rovira 1965; Alexander 1977) resulted greater release of available phosphorus in the rhizosphere soil. Similar observation was also reported by Isaeva (1967). Between the two herbicides, basalin released more amount of available phosphorus as compared to butachlor.

The persistence of the herbicides in the rhizosphere soil of rice varied (Table 2). Between the two herbicides, the dissipation of basalin was faster than that of butachlor depicting the half-lives ($T_{1/2}$) 5.38 and 6.69 days, respectively. The earlier workers (Duseja 1981; Kulshrestha et al. 1981) also confirmed the persistence of the herbicides in soil. As microbial degradation is one of the main reasons of dissipation of herbicides in soil (Alexander 1977), the results pointed out that the rhizosphere microorganisms utilized the herbicides as well as their degraded products for their growth and development (Cork and Krueger 1991). It was also revealed that 72.79% basalin was dissipated from soil within 10 days of

Table 2. Persistence of herbicides in the rhizosphere soil of rice.

Sampling days	Residues (µg g^{-1} soil)	
	Butachlor	Basalin
0 (1hr)	0.989 ± 0.24	0.283 ± 0.02
10	0.827 ± 0.13	0.077 ± 0.02
20	0.122 ± 0.02	0.005 ± 0.001
30	ND	0.003 ± 0.001
45	ND	0.001 ± 0
60	ND	ND
DL	0.02	0.001
$T_{½}$ (days)	6.69	5.38
r	- 0.902	- 0.958

ND = not detected, Means ± SD, DL = detection limit, $T_{½}$ = half-life, r = correlation coefficient

its application and no residue was detected after 60 days. Butachlor, on the other hand, was degraded very rapidly during 10 to 20 days and no residue was of the herbicide was detected after 30 days of application. The degradation of both the herbicides in soil followed first order reaction kinetics (Duseja and Holmes 1978; Kulshrestha 1987). It was also confirmed by the presence of significant correlation (r = - 0.902 for butachlor and - 0.958 for basalin) between the persistence of herbicide residues and sampling days.

The results of the present investigation thus indicated that application of butachlor and basalin augmented the growth and activities of phosphate solubilizing microorganisms, which in turn, released more amount of available phosphorus in the rhizosphere soil of rice. The results also pointed out that the enhanced microbial population degraded the herbicides for their growth and metabolism resulting in a rapid dissipation of the herbicides in soil system.

REFERENCES

Alexander M (1977) Introduction to soil microbiology. Wiley Eastern Ltd, New Delhi, India

Chen YL, Lin FP, Chen LC, Wang YS (1981) Effects of herbicide butachlor on nitrogen transformation of fertilizers and soil microbes in water logged soil. J Pestic Sci 6: 1-7

Cork DJ, Krueger JP (1991) Microbial transformations of herbicides and pesticides. Adv Microbiol 36: 1- 6

Das AC, Mukherjee D (1994) Effect of insecticides on the availability of nutrients, nitrogen fixation and phosphate solubility in the rhizosphere soil of rice. Biol Fertil Soils 18: 37- 41

Debnath A (1997) Interaction between some herbicides and microflora is soil ecosystem. PhD Thesis. Bidhan Chandra Krishi Viswavidyalaya, India
Debnath A, Das AC, Mukherjee D (1994) Studies on the decomposition of non-conventional organic wastes in soil. Micrbiol Res 149: 195 -201
Duseja DR (1981) Soil dissipation of three herbicides. Proc 8th Asian Pac Weed Sci Soc Confer p 487- 490
Duseja DR, Holmes EE (1978) Field persistence and movement of trifluralin in two soil types. Soil Sci 125: 41 - 48
Hoskins WM (1961) Mathematical treatment of loss of pesticide residues. Plant Prot Bull FAO 9: 163 -168
Isaeva LI (1967) The effect of herbicide application on root nutrient uptake in sod-podzolic soils. Agrokhim 4: 122-125
Jackson ML (1973) Soil chemical analysis. Prentice-Hall of India Pvt. Ltd, New Delhi, India
Kole SC, Dey BK (1989) Effect of aromatic amine herbicides on microbial population and phosphate solubilizing power of the rhizosphere soils of groundnut. Indian Agric 33: 1- 8
Kulshrestha G (1987) Dissipation of herbicide butachlor from different formulation in direct seeded and transplanted rice crop. Pesticides 21: 20 -24
Kulshrestha G, Yaduraja NT, Mani VS (1981) Dissipation of butachlor and propanil herbicides in rice crop. Proc 8th Asian Pac Weed Sci Soc Confer p 469 - 473
Pikovskaia RI (1948) Mobilization of phosphates in soil in connection with the vital activities of some microbial species. Microbiol 17: 362 - 370
Premer D, Schmidt EL (1964) Experimental soil microbiology. Burgess Publishing Co, Minneapolis 15, Minnesota, USA
Rovira AD (1965) Interaction between plant roots and soil micro- organisms. Ann Rev Microbiol 19: 241 - 266
Sandhu KS, Joshi AK, Chahal VPS, Singh HD (1990) Rhizosphere microflora as affected by different herbicidal treatments in carrot crops. J Res Punjab Agric Univ 27: 41 - 45
Selvamani S, Sankaran S (1993) Soil microbial population as affected by herbicides. Madras Agric J 80: 397- 399
USDA (1975) Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys. Agric Hand Book 436. US Govt Printing Office, Washington DC, USA