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Effect of anilofos and pendimethalin herbicides on N_2-fixing and phosphate solubilizing microorganisms in relation to availability of nitrogen and phosphorus in a *Typic Haplustept* soil

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ABSTRACT

An experiment was conducted under laboratory condition to investigate the effect of two systemic pre-emergence herbicides, viz. anilofos and pendimethalin either alone or in a combination, at their recommended field application rates (400 g and 1.0 kg a.i./ha., respectively) on the growth and activities of aerobic non-symbiotic N_2-fixing bacteria and phosphate solubilizing microorganisms as well as their role in the transformations and availability of nitrogen and phosphorus in *Typic Haplustept* in West Bengal. Application of the herbicides, in general, stimulated the growth and activities of aerobic non-symbiotic N_2-fixing bacteria and phosphate solubilizing microorganisms resulting in an increased availability of nitrogen and phosphorus in soil. Anilofos highly augmented the proliferation and activities of aerobic non-symbiotic N_2-fixing bacteria and accumulated highest amount of available nitrogen and phosphorus in soil. The combined application of herbicides highly stimulated the growth and activities of phosphate solubilizing microorganisms in soil.

***Key words* :** Anilofos, available nitrogen, available phosphorus, herbicides, N_2-fixing bacteria, phosphate solubilizing microorganisms.

The use of herbicides for combating unwanted weeds in the crop fields has been increased steadily day by day. During the application of herbicides, a large portion of these herbicidal chemicals accumulate in the top layer soil (0-15 cm) where most of the microbiological activities occur. Microorganisms, in general, degrade a variety of carbonaceous substances including the accumulated herbicides in soil to derive their energy and other nutrients for their cellular metabolism (Debnath *et al.*, 2002; Das *et al.*, 2003). As a result, amount of microbial biomass increases which favourably influences the transformation of plant nutrients in soil (Sandhu *et al.*, 1990; Das *et al.*, 2003). On the other hand, reports are also available (Kole and Dey, 1989; Selvamani and Sankaran, 1993) on the adverse effect of herbicides on growth and activities of beneficial microorganisms in soil. The objective of the present study was to investigate the effect of two commonly used pre-emergence herbicides viz., anilofos(*S*-[*N*-(4-chlorophenyl)-*N*isopropylcarbamyl]-*0*,*0*-dimethyl phosphorodithioate) (an organophosphate derivative) and pendimethalin [*N*-(1-ethylpropyl)-2,6-dinitro-3,4-dimethyl aniline] (a dinitroaniline derivative) at their recommended field rates on growth and activities of non-symbiotic N_2-fixing bacteria and phosphate solubilizing microorganisms in relation to the availability of nitrogen and phosphorus in soil.

MATERIALS AND METHODS

An alluvial soil (*Typic Haplustept)* having the general characteristics as presented in Table 1, was collected from the Instructional Farm of Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal by taking several slices from the surface soil layer (0-10 cm) by means of a spade as outlined by Jackson (1973). The composite soil samples were air dried at 30-35 ^oC in shade and passed through 2 mm (4-8 mesh/cm) sieve. The processed soils were stored in screw-cap jar and were used for the experiment. Two pre-emergence herbicides viz. anilofos [30% a.i.] and pendimethalin [30% a.i.] at rates of 400 g and 1.0 kg a.i./ha., respectively were mixed thoroughly either separately or in a combination with 250 g air-dired and sieved soil (= 2mm) and were placed in polyethylene pots. The water content of the soil was adjusted to 60% of water holding capacity of the soil and it was maintained throughout the experiment. To avoid photodegradation of the herbicides and evaporation loss of water from the soil, the pots were kept covered with black polythene sheet and were incubated in dark at 30 ± 1^oC for 60 days. All the treatments were replicated three times and separate sets of treatments were maintained for each sampling day.

Sampling of soil

During incubation, soil samples were collected at periodic intervals [after 0 (1h), 15, 30, 45 and 60 days of incubation] from each treatment earmarked for that particular sampling day following the method as described by Das and Mukherjee (2000). The sub-samples were immediately analyzed to determine microbial populations and biochemical transformations. Soil moisture content was measured from the sub-samples.

Microbial analysis of soil

The colony forming units (cfu) of aerobic non-symbiotic N_2-fixing bacteria and phosphate solubilizing microorganisms were enumerated in sucrose-calcium carbonate agar (Jensen, 1930) and sucrose tricalcium agar (Pikovskaia, 1948) media respectively following serial dilution technique and pour plate method (Salle, 1973). The agar plates were incubated at 30 ± 1 °C for 7 days. After incubation, cfu of the microorganisms developed on the respective agar plates were counted following the method as outlined by Salle (1973). Non-symbiotic N_2-fixing capacities of the soils were determined (Das and Debnath, 2006) by incubating 1 g soil from each sample in 50 ml Jensen's broth (Jensen, 1930) containing 2% sucrose in conical flasks at 30 ± 1 °C for 15 days followed by estimation of total nitrogen content (Bremner, 1996) in the broth. Phosphate solubilizing capacities of the soil samples were determined by incubating 1 g soil of each sample in 15 ml Pikovskaia's broth (Pikovskaia, 1948) in culture tubes at 30 ± 1 °C for 15 days followed by estimation of soluble phosphorus (Olsen and Dean, 1982) in the broth.

Soil samples were analyzed to estimate the changes of available inorganic nitrogen (exchangeable NH_4^+ and soluble NO_3^-) in 2*N* potassium chloride extract through distillation (Mulvaney, 1996). Available phosphorus was determined in sodium bicarbonate extract colorimetrically following the method of Olsen and Dean (1982).

RESULTS AND DISCUSSION

Effect on N_2-fixing bacteria and their capacities

Application of anilofos and pendimethalin, either alone or in a combination, increased the growth and multiplication of aerobic non-symbiotic N_2-fixing bacteria in soil (Fig. 1). The stimulative effect was more pronounced with anilofos when it was applied alone. As compared to untreated control, pendimethalin augmented the proliferation of aerobic non-symbiotic N_2-fixing bacteria but the augmentation under this treatment was *at par* with the combined application of the herbicides. It was also revealed that on an average, anilofos, pendimethalin and their combined application stimulated the growth and multiplication of aerobic non-symbiotic N_2-fixing bacteria by 50%, 33.4% and 35.4% respectively as compared to untreated control. This indicated that the microorganisms utilized the herbicides and their degraded products to derive their energy, carbon and other nutrients for their cellular metabolic activities (Kole and Dey,1989; Cork and Kruenger, 1991; Debnath *et al.*, 2002).

Increased proliferation of N_2-fixing bacteria resulted enhancing influence on non-symbiotic N_2-fixing capacity of soils (Fig. 2). Among the treatments, anilofos exerted maximum stimulating influence (23.0%) followed by pendimethalin (18.6%) and their combined application (13.3%) as compared to untreated control. Incidentally, there was a positive correlation (r = 0.932) between the population of aerobic non-symbiotic N_2-fixing bacteria and non-symbiotic N_2-fixing capacity of soil (Table 2). Similar observations on the stimulative effect of different herbicides were also recorded earlier by Kole and Dey (1989) and Das and Debnath (2006).

Effect on phosphate solubilizing microorganisms and their activities

The applied herbicides, either singly or in a combination, significantly accentuated the phosphate solubilizing microorganisms and the stimulation was more pronounced when the herbicides were applied in combination (Fig. 3). As compared to untreated control, maximum stimulation of phosphate solubilizing microorganisms was recorded under combined application of the herbicides (42.5%) followed by anilofos (36.8%) and pendimethalin (26.0%). This indicated that the cited microorganisms utilized the applied herbicides as well as their degraded products to derive their energy, carbon and other nutrients for their cellular metabolism (Das *et al.*, 2003; Das and Debnath, 2006).

The proliferation of phosphate solubilizing microorganism significantly accentuated the phosphate solubilizing capacity of soil (Fig. 4). This was also in accordance with the findings of earlier workers (Debnath *et al.*, 2002; Das and Debnath,

2006) who demonstrated that application of herbicides highly stimulated the solubilization of insoluble tricalcium phosphates by the microbes present in soil. The results of the present investigation pointed out that the proliferation of phosphate solubilizing microorganisms did not always reflect to their efficiency. On an average, the highest amount of phosphate solubilization was recorded with the application of pendimethalin either singly or in a combination with anilofos.

Effect on available nitrogen and phosphorus in soil

The greater microbial population as well as

Table 1. General characteristics of the soil used in the experiment

	Soil characteristics	:	Results
1.	Soil taxonomy (USDA, 1975)	:	*Typic Haplustepts*
2.	Texural class	:	Clay loam
3.	Sand (%)	:	27.7
4.	Silt (%)	:	40.7
5.	Clay (%)	:	31.6
6.	Bulk density (g/cm^3)	:	1.04
7.	Water holding capacity (%)	:	53.1
8.	pH (1:2.5 w/v) in water	:	6.4
9.	Cation exchange capacity [cmol (p$^+$)/kg]	:	11.15
10.	Electrical conductivity (dS/m)	:	0.21
11.	Organic carbon (%)	:	0.60
12	Total nitrogen (%)	:	0.072
13.	C : N ratio	:	8.33
14.	Exchangeable NH_4^+ (mg/kg)	:	97.7
15.	Soluble NO_3^- (mg/kg)	:	43.9
16.	Available phosphorus (mg/kg)	:	25.0
17.	Total phosphorus (g/kg)	:	0.525
18.	Aerobic non-symbiotic N_2-fixing bacteria (cfu ´ 10^5/g)	:	29.3
19.	Phosphate solubilizing microorganisms (cfu ´ 10^5/g)	:	111.7
20.	Non-symbiotic N_2-fixing capacity (mg/g)	:	9.6
21.	Phosphate solubilizing capacity (mg/g)	:	1.52

Table 2. Correlation coefficients (r) among different parameters studied for determining the effect of herbicides in soil

Parameters	Available phosphorus	N_2 – fixing bacteria	N_2 – fixing capacity	P – solubilizing microorganisms	P – solubilizing capacity
Available N	0.827**	0.667**	0.706**	0.658**	0.605**
Available P	—	0.536*	0.575**	0.600**	0.554*
N_2 – fixing bacteria	—	—	0.932**	0.697**	0.393
N_2 – fixing capacity	—	—	—	0.643**	0.379
P – solubilizing microorganisms	—	—	—	—	0.271

*, ** significant at 0.05 and 0.01 level respectively

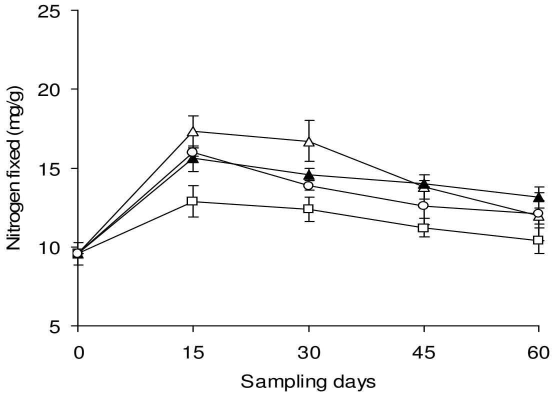

Fig. 1. Effect of herbicides on the population of aerobic non-symbiotic N_2-fixing bacteria in soil.

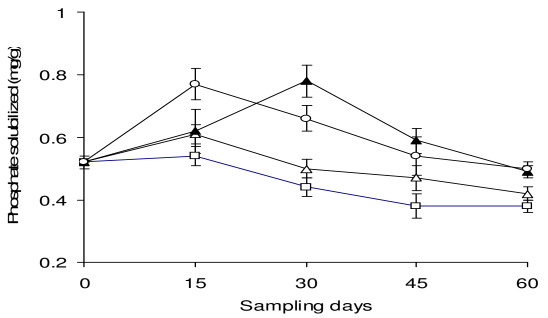

Fig. 4. Effect of herbicides on phosphate solubilizing capacities of soil.

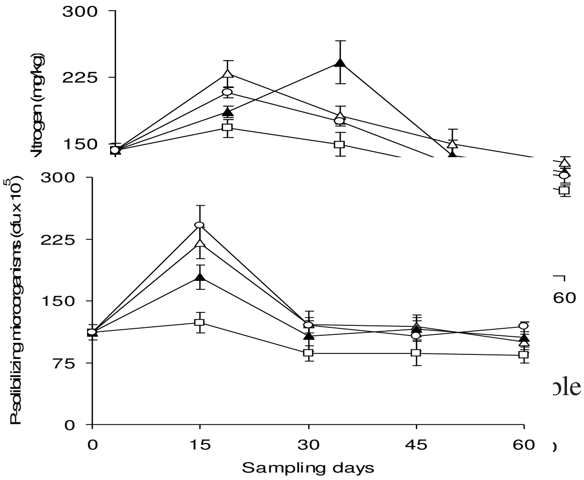

Fig. 2. Effect of herbicides on non-symbiotic N_2-fixing capacities of soil.

Fig. 3. Effect of herbicides on the population of phosphate solubilizing microorganisms in soil.

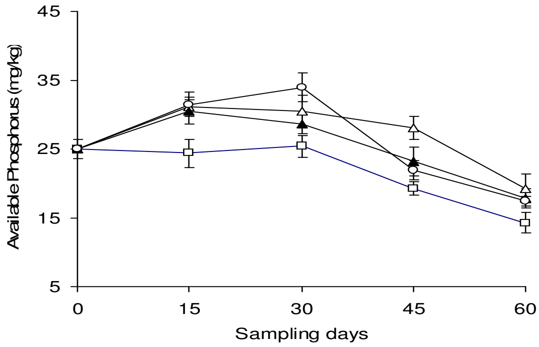

Fig. 6. Effect of herbicides on the changes of available phosphorus content in soil.

their activities stimulated the availability of both nitrogen and phosphorus in the soil (Fig. 5 and 6). Application of anilofos accentuated the highest amount of available nitrogen on 15th day of incubation while pendimethalin exerted greatest stimulation on the 30th day of incubation. This indicated that there was greater mineralization of organic nitrogen by ammonifying and nitrifying microorganisms as compared to control soil. Comparing with untreated control, highest accumulation of available nitrogen was recorded with anilofos (32.4%) followed by pendimethalin (21.9%) and their combined application (17.6%). Similar observation was also reported earlier by Das and Debnath (2006).

Sustaining the earlier reports (Dey and Dutta, 1981; Debnath *et al.*, 2002; Das and Debnath, 2006), the greater microbial activities due to application of herbicides significantly stimulated the availability of phosphorus in soil and the stimulation was more pronounced with anilofos as compared to pendimethalin. Incidentally, available phosphorus was positively correlated with phosphates solubilizing microorganisms ($r = 0.6$) and phosphate solubilizing capacity ($r = 0.554$) [Table 2]. This indicated that the active microorganisms were highly stimulated due to the assimilation of these herbicides as well as their degraded products (Das and Debnath, 2006) during the early days of incubation.

The results of the investigation revealed that application of anilofos and pendimethalin, either alone or in combination significantly augmented the growth and multiplication of non-symbiotic N_2-fixing bacteria and phosphate solubilizing microorganisms and their respective capacities in soil. It was also revealed that the availability of nitrogen and phosphorus was positively correlated with the proliferation and activities of the microorganisms in soil. So, the cited herbicides at their recommended field rates can be applied safely to eradicate the unwanted weeds to maintain soil fertility.

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