



## Changes in microbiological characteristics of rice soil by post-emergence herbicides

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### ABSTRACT

A field study was conducted in an Inceptisol with summer season rice to evaluate the degradability of different post-emergence herbicides applied alone or in combinations in terms of microbiological characteristics of rhizosphere soil. Five different herbicides (bispuryribac-sodium, metamifop, chlorimuron-ethyl, metsulfuron-methyl and cyhalofop-butyl) were applied as an individual or in combination at post-emergence stage (15 DAS). A wetting agent (agrisol) was also used with some herbicides to increase the effectivity of applied herbicides. Application of different herbicides had inhibitory effect on microbial population and their activities in soil after application, but their effects were not pronounced and the soil properties were restored. Although herbicides applied at different rates but their degradability was almost equal and they took only 30 days or less period for complete degradation except cyhalofop-butyl and bispuryribac-sodium + metamifop, which were applied 80 g and 140 g/ha, respectively and found completely degraded before harvest.

**Key words:** Degradability, Herbicides, Microbes, Rice, Soil, Wetting agent

In general weeds reduce the crop yield by 31.5% (22.7% in winter and 36.5% in summer and *Kharif* seasons) (Vivek Mishra *et al.* 2015). The use of chemical herbicides is probably the most important component of weed management system. Generally herbicides are not harmful when the one applied in recommended levels in soil. Application of pre-emergence and post-emergence herbicide reduced the microbial population and their activities in soil from its application from 3 to 22 days after sowing, to 35 days after sowing of the crop, thereafter it became normalize due to degradation of applied herbicides (Mishra *et al.* 2015). According to Radivojevic *et al.* (2004), even though the herbicidal treatments brought higher weed control efficiency, it could not increase the microflora population due to the strong chemicals, which decrease the bacterial population to a great extent. Different micro-organisms are efficient decomposers of herbicides which are generally aliphatic, hydroxyl and aromatic compounds. They easily decompose the aliphatic and hydroxyl group but they decompose aromatic substances at a slower rate. The fate and persistence of newly introduced herbicides in soil are almost unknown. In this experiment, a new combination of post-emergence herbicides was tested comparing with existing herbicides with and without wetting agent with respect to their ultimate effect on soil microflora.

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### MATERIALS AND METHODS

A field study was conducted during summer 2013 at Instructional cum Research farm, IGKV, Raipur in an Inceptisol with summer season rice to evaluate the effect of different post-emergence herbicides applied alone or in combinations in terms of microbiological and biochemical characteristics of rhizosphere soil. Five different herbicides (bispuryribac-sodium, metamifop, chlorimuron-ethyl, metsulfuron-methyl and cyhalofop-butyl) were applied as an individual or in combination at post-emergence stage (15 DAS). A wetting agent (Agrisol) was also used with some herbicides to increase the effectivity of applied herbicides. The experiment included 13 treatments, viz. bispuryribac-sodium + metamifop 42, 56, 70 and 140 g/ha ha, chlorimuron-ethyl + metsulfuron-methyl 20% WP 4 g/ha, cyhalofop-butyl 10% EC 80 g/ha, bispuryribac-sodium 10% SC 20 g/ha + wetter (Agrisol), metamifop 10% SE 50 g/ha + wetter (Agrisol), bispuryribac-sodium 4% + metamifop 10% SE (alone) 70 g/ha, bispuryribac-sodium 10% SC 20 g/ha, metamifop 10% SE 50 g/ha, wetter (Agrisol), unsprayed control. The experiment was conducted on summer rice (*Oryza sativa* L.) with test variety 'MTU-1010'. All the above herbicides were applied at post-emergence stage of crop i.e. 15 DAS of the crop. The soil was Inceptisol (pH: 6.29, EC: 0.20 dS/m, organic carbon: 22.33%, available N: 238.34 kg/ha, available P: 9.24 kg/ha, and available K: 252.71 kg/ha). The treatments were

replicated thrice under randomized block design. Rhizosphere soil was collected at a depth of 7.5-15 cm from 12 locations at different stages of crop growth from the same plot and pooled together for the purpose of analysis. Soil sampling was done at 0, 7, 15, 30, 50 days after sowing of crop and harvest stage of crop. The soil samples were subjected to analysis for microbial biomass carbon, basal soil respiration rate, population of total bacteria, actinomycetes and fungi.

In this experiment different post-emergence herbicides were tested as and individual or in combination of two herbicides to evaluate their toxicity level with respect to microbial properties of rhizosphere soil of rice.

## RESULTS AND DISCUSSION

### Basal soil respiration

All the herbicides including wetting agent significantly inhibited the basal soil respiration (BSR) soon after their application. The degree of inhibition was carried as per the toxicity of the herbicides. Combined application of bispyribac-sodium + metamifop at different concentration reduced the BSR with their increasing levels (**Table 1**). Among different herbicides, cyhalofop-butyl 10% EC exhibited maximum inhibition of BSR and minimum by metamifop 10% EC. Cyhalofop-butyl inhibited the BSR value from 0.339 (7 DAS) to 0.297 mg (15 DAS) whereas metamifop was found rather safe and reduced the value from 0.367 to 0.345 mg within the same period. Wetting agent Agrisol also reduced the

above activity significantly at 15 DAS. The above observations were in close agreement with De Lonezo *et al.* (2001).

At 30 DAS, all the herbicides treatment significantly reduced basal soil respiration over control, except the wetting agent which was found at par with unsprayed control. At 50 DAS, all the herbicides treatments found at par with unsprayed control except cyhalofop-butyl and bispyribac-sodium + metamifop applied (140 g/ha). These herbicides started to degrade at 30 DAS but not degraded completely up to 50 DAS. The results are in conformity with the findings of Mishra *et al.* (2015) who stated that the amount of released CO<sub>2</sub> (as a measure of the intensity of soil microbiological activity) was reduced from 0 DAS to 35 DAS after the application of butachlor in pre-emergence and fenoxapropp-ethyl and ethoxysulfuron in post-emergence stages, respectively. The values then gradually increased upto 50 DAS. In case of hand weeding and weedy check, the BSR rate continuously increased from 0 to 50 DAS and there after narrowed down.

### Microbial biomass carbon

Content microbial biomass carbon (MBC) reduced significantly due to application of bispyribac-sodium, metamifop, cyhalofop, chlorimuron, + metsulfuron alone or in different combinations and doses but their degree of reduction varied. Cyhalofop reduced the MBC severly *i.e.* from 90.28 µg (7 DAS) to 75.22 µg (15 DAS), whereas singal application of

**Table 1. Effect of post-emergence herbicides on basal soil respiration rate (mg CO<sub>2</sub>/h/100 g) and microbial biomass carbon content (µg/g) of rhizosphere soil at different growth stages of rice**

Treatment	Days after sowing											
	0		7		15		30		50		At harvest	
	BSR	MBC	BSR	MBC	BSR	MBC	BSR	MBC	BSR	MBC	BSR	MBC
Bispyribac-sodium + metamifop 42 g/ha + wetter (Agrisol)	0.332	82.46	0.361	94.16	0.335	81.04	0.317	71.10	0.427	179.01	0.322	82.56
Bispyribac-sodium + metamifop 56 g/ha + wetter (Agrisol)	0.327	81.78	0.354	92.99	0.325	79.46	0.305	69.09	0.423	174.24	0.309	81.76
Bispyribac-sodium + metamifop 70 g/ha + wetter (Agrisol)	0.321	80.34	0.347	91.07	0.315	77.02	0.292	66.17	0.419	170.10	0.305	80.56
Bispyribac-sodium + metamifop 140 g/ha + wetter (Agrisol)	0.319	79.87	0.345	90.33	0.307	76.16	0.282	65.10	0.417	169.34	0.304	80.24
Cholrimuron-ethyl + metasufuron-methyl 20% WP 4 g/ha	0.331	82.06	0.359	93.52	0.331	80.28	0.312	70.19	0.426	176.75	0.318	82.34
Cyhalofop-butyl 80 g/ha	0.315	79.34	0.339	90.28	0.297	75.22	0.269	64.98	0.415	169.02	0.301	80.21
Bispyribac-sodium 20 g/ha + wetter (Agrisol)	0.334	82.89	0.362	94.83	0.337	82.08	0.320	72.32	0.430	180.66	0.324	83.42
Metamifop 50 g/ha + wetter (Agrisol)	0.336	83.24	0.364	95.51	0.341	83.01	0.326	73.48	0.432	181.13	0.328	83.84
Bispyribac-sodium + metamifop 70 g/ha	0.326	81.56	0.354	92.48	0.325	78.62	0.304	68.02	0.420	172.15	0.307	81.32
Bispyribac-sodium 20 g/ha	0.339	83.68	0.366	96.24	0.343	84.00	0.329	74.60	0.435	182.47	0.329	84.12
Metamifop 50 g/ha	0.339	84.21	0.367	97.04	0.345	85.01	0.333	75.77	0.438	183.85	0.330	84.78
Wetter (agrisol): A-150K 500 ml/ ha	0.342	85.53	0.370	99.76	0.350	91.41	0.375	143.87	0.456	186.62	0.334	89.12
Unsprayed control	0.343	86.64	0.371	100.37	0.382	126.51	0.406	154.34	0.461	189.30	0.336	89.36
LSD (p=0.05)	NS	NS	NS	NS	0.031	8.66	0.034	10.49	0.043	19.76	NS	NS

BSR – Basal soil respiration, MBC – Microbial biomass carbon

metamifop reduced the MBC content least (**Table 1**). The reduction due to metamifop can be estimated as from 97.04 µg (7 DAS) to 85.01µg (15 DAS) .Other herbicides reduced the MBC values intermediately between the peak values. Agrisol wetter also imparted effect on MBC by reducing the content significantly at 15 DAS. However, it degraded at a faster rate and found ineffective at 30 DAS. This observation was in close agreement with Baboo *et al.* (2013). At 50 DAS, all the applied herbicides found to be degraded completely except cyhalofop and bispyribac-sodium + metamifop mixture (140 g/ha), which were still showed their presence in the rhizosphere by narrowing the MBC content significantly over control. Result of the investigation is in confirmation with the findings of Bolter *et al.* (2006). The decrease in MBC may be due to the adsorption of small amount of pesticides on organic matter that mask the effects of these agrochemicals on soil microbial biomass, and subsequently led to lysis of microbial cells (Jayamadhuri and Rangaswamy 2005). Herbicides affect various soil microbial processes, inhibit decomposition, which depends upon the type and rate of application that can alter the microbial biomass quantitatively and qualitatively (Mishra *et al.* 2013).

### Bacterial population

Soil bacteria significantly affected by all the organo chemicals used in the experiment (**Table 2**).

**Table 2. Effect of post-emergence herbicides on soil bacterial (x10<sup>5</sup>/g), actinomycetes (x10<sup>4</sup>/g) and fungal count (x10<sup>3</sup>/g) in rhizosphere soil at different growth stages of rice**

Treatment	Days after sowing																	
	0			7			15			30			50			At harvest		
	TB	Act	Fun	TB	Act	Fun	TB	Act	Fun	TB	Act	Fun	TB	Act	Fun	TB	Act	Fun
Bispyribac-sodium + metamifop 42 g/ha + wetter (Agrisol)	52.6	74.5	35.2	57.4	81.6	39.2	50.3	74.7	34.5	47.5	69.2	31.2	98.0	110.9	73.0	52.2	74.3	32.7
Bispyribac-sodium + metamifop 56 g/ha + wetter (Agrisol)	52.9	75.3	35.7	57.6	82.4	39.5	49.2	72.5	33.2	45.3	64.5	29.5	97.4	109.8	72.3	51.6	73.7	31.9
Bispyribac-sodium + metamifop 70 g/ha + wetter (Agrisol)	52.5	75.1	34.9	57.4	82.3	38.7	48.2	71.7	31.4	43.1	63.3	26.5	96.3	109.0	70.8	50.8	72.7	31.5
Bispyribac-sodium + metamifop 140 g/ha + wetter (Agrisol)	52.5	75.2	35.1	57.5	82.5	39.2	46.3	70.5	31.2	40.5	61.2	25.3	95.4	108.5	70.1	50.6	72.4	31.3
Cholrimuron-ethyl + metasufuron-methyl 20% WP 4 g/ha	52.6	74.4	34.3	57.7	81.7	38.2	49.7	73.5	32.3	45.8	66.5	29.7	97.8	110.3	72.7	51.8	73.9	32.3
Cyhalofop-butyl 80 g/ha	52.7	74.3	34.5	57.7	81.6	38.6	45.5	68.3	30.4	38.6	58.5	24.5	94.7	108.2	69.7	49.9	71.5	31.2
Bispyribac-sodium 20 g/ha + wetter (Agrisol)	52.7	74.5	34.7	57.6	81.6	38.8	51.7	75.2	34.7	49.1	71.5	31.6	98.3	111.2	73.8	52.6	74.6	33.4
Metamifop 50 g/ha + wetter (Agrisol)	52.6	74.4	34.6	57.5	81.7	38.7	52.2	75.4	35.7	50.3	71.7	31.9	98.6	111.7	74.2	52.7	74.9	33.7
Bispyribac-sodium + metamifop 70 g/ha	52.8	74.7	34.7	57.8	81.9	38.9	48.6	71.7	31.7	43.4	63.7	26.7	96.9	109.3	71.4	51.3	73.2	31.7
Bispyribac-sodium 20 g/ha	52.7	74.7	34.9	57.7	81.8	39.2	52.9	75.7	35.9	51.4	71.7	32.4	99.0	112.5	74.9	52.9	75.0	34.2
Metamifop 50 g/ha	52.5	75.5	34.5	57.4	81.5	38.6	53.3	75.9	36.2	53.1	72.0	33.7	99.5	113.7	75.2	53.2	75.7	34.6
Wetter (Agrisol): A-150K 500 ml/ ha	52.8	75.2	35.1	57.7	82.4	39.3	63.2	76.4	36.7	77.8	98.9	63.6	103.4	118.3	75.4	53.4	75.9	34.4
Unsprayed control	52.7	75.3	35.3	57.6	82.5	39.6	68.9	89.3	49.4	83.4	104.3	67.2	106.7	121.5	78.6	54.8	77.2	35.3
LSD (p=0.05)	NS	NS	NS	NS	NS	NS	5.1	7.2	3.6	5.7	6.3	3.6	10.70	12.7	8.1	NS	NS	NS

TB – Total bacteria, Act – Actinomycetes, Fun - Fungi

The bacterial population ranged between 45.50 to  $52.90 \times 10^5$  due to application of different herbicides. Highest reduction in population was noticed due to cyhalofop-butyl where population got reduced from  $57.7 \times 10^5$  (7 DAS) to  $45.5 \times 10^5$  (15 DAS), whereas minimum inhibition of population was observed in plots where metamifop was applied. In these plots, population decrement was found from  $57.4 \times 10^5$  to  $53.3 \times 10^5$  within the same period. At 30 DAS, except wetting agent, all the treatments have shown the inhibition of bacterial population in comparison to 15 DAS. At 50 DAS, the population of bacteria increased in comparison to 30 DAS and most of the herbicides treated plots have shown at par values of bacterial population to that of unsprayed control except cyhalofop and bispyribac + metamifop (140 g/ha). These findings were in accordance with that of Chowdhury *et al.* (2008) and Baboo *et al.* (2013).

The fate of the herbicide residues in soil is a matter of great concern since they would persist on top soil (Ayansina *et al.* 2003). At harvest population of bacteria in all the treatments found slightly lower than 50 DAS but all were found at par with unsprayed control, which seems that all the herbicides degraded completely before harvesting of the crop. Unsprayed conditions facilitated the growth of bacteria as indicated by the population recorded during the experimentation. Under this treatment, the bacterial

population gradually increased from 0 DAS to 50 DAS followed by a slight declination at harvest.

### **Actinomycetes population**

Actinomycetes population severely affected by singal and dual application of herbicides soon after their application *i.e.* 15 DAS (**Table 2**). The actinomycetes population severely affected by cyhalofop-butyl and least affected by metamifop. The wetting agent also imparted inhibitory effect on actinomycetes population. Cyhalofop reduced the population from  $68.3 \times 10^4$ (15 DAS) to  $58.5 \times 10^4$ (30 DAS) where as metamifop reduced the population from  $75.9 \times 10^4$ (15 DAS) to  $72.0 \times 10^4$ (30 DAS). At 30 DAS the wetting agent found at par with unsprayed control treatment.

At 50 DAS, comparatively higher population of actinomycetes was noticed in comparison to 30 DAS. In this stages all the treatments showed at par values to that of control, which indicated the non existence of respective applied chemicals, except cyhalofop and higher dose of bispyribac-sodium and metamifop which were shown their existence in rhizosphere. This is in agreement with works of Baboo *et al.* (2013) who reported the actinomycetes count (ACT) at different days after herbicide application showed significant differences, with the highest on 28th day and lowest on 7th day after herbicide treatment.

### **Fungal population**

Fungal population is reduced at 15 DAS after application of different post emergence herbicides, which were applied as single or in combination (**Table 2**). Among different herbicides treatment, cyhalofop-butyl inhibit the fungal growth at maximum level. It reduced the population from  $38.6 \times 10^3$ (7 DAS) to  $30.4 \times 10^3$ (15 DAS) per gram of soil. Contrary to this, metamifop as an individual reduced the fungal growth a minimum. It narrowed the population from  $38.6 \times 10^3$ (7 DAS) to  $36.2 \times 10^3$ (15 DAS) per g of soil. Other herbicides used in the experiment exhibited intermediate effect on fungus, between two extremes mentioned above. Wetting agent Agrisol also showed anti-fungal activity as it also significantly reduced the fungal population in soil soon after its application at 15 DAS. At 30 DAS, all the herbicides exhibited their anti-fungal effect and significantly reduced the fungal population, hence showed their presence in rhizosphere. Only wetting agent was completely degraded and found ineffective at this stage. At 50 DAS, effect of all the herbicides found in effective to narrowed down the population comparison to unsprayed control except cyhalofop and bispyribac + metamifop which were applied 80 g and 140 g/ha.

This indicated their incomplete degradation and presence in the rhizosphere soil of rice. Result of the investigation is in confirmation with the findings of Dsehmukh *et al.* (2013) who also observed lowest fungal count in all the treatments of herbicide application over initial values. At harvest, all the applied herbicides did not show their existence. This proved at par values of fungal population in all the herbicides treated plots in comparison to the value of control plot. In control plots, the fungal population gradually built-up in soil from 0 to 50 DAS and then slightly reduced at harvest. Tu *et al.* (2001) also mentioned herbicides have varying effect on soil microbial populations depending on herbicide concentrations and the microbial species present. Low residue levels can enhance population while higher levels can cause population declines.

It was concluded that application of different herbicides had inhibitory effect on microbial and biochemical activities of soil but their effects were not pronounced and the soil properties were restored.

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