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# Effect of six herbicides on soil microbial population and yield in direct seeded rice

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### Abstract

The use of herbicides in direct-seeded rice may affect the biological equilibrium of the soil and thus influence the nutrient status, health and productivity of the soil. To study the effect of herbicides on soil microbial population of direct-seeded rice, a field experiment was conducted with fourteen treatments at Agriculture Research Farm, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India during Kharif 2016 and 2017. The results revealed that viable microbial population was influenced to varying degrees with different weed control treatments during both the years. The application of treatment (T9) Bispyribac-Na 30 g a.i. ha-1 PoE, significantly increased by 121.71% and 134.08% actinomycetes population when compared with the treatment weed free and weedy check, respectively at 90 days after sowing. Similarly, the application of herbicides treatment ( $T_1$ ) Pendimethalin 1000 g.a.i ha<sup>-1</sup> (PE) also significantly increased actinomycetes count by 74.59% and 91.33% when compared with the treatment weed free and weedy check, respectively at 90 days after sowing. Application of treatment (T<sub>10</sub>) Bispyribac-Na 15 g a.i. ha<sup>-1</sup> fb one hand weeding at 40 days statistically influenced the grain and straw yields and harvest index over all othertreatments. Highest grain (66.67 q ha<sup>-1</sup>) and straw (97.77 q ha<sup>-1</sup>) yields and harvest index (40.54%) was observed under (T<sub>10</sub>) Bispyribac-Na 15 g a.i. ha<sup>-1</sup>fb one hand weeding at 40 days and was statistically at par with Azimsulfuron 17.5 g a.i. ha<sup>-1</sup>fb one hand weeding at 40 days after sowing  $(T_{12})$ . The herbicides, viz. Pendimethalinand Pyrazosulfuronas pre-emergence and Almix, Ethoxysulfuron, Bispyribac-Naand Azimsulfuron as post-emergence were safe for soil microbial populations at recommended rate.

Keywords: Ainomycetes, bacteria, direct-seeded rice, fungi, herbicides

### Introduction

Rice (Oryza sativa L.) is predominantly grown by transplanting seedlings, this practice consumes about 150 ha-cm of water and engagement of labour for transplanting and weeding (Mahajan and Chauhan 2016)<sup>[16]</sup>. Manual transplanting is labour cumbersome and scarcity of labour during peak season force to shifting of crop establishment methods from transplanting to direct-seeded rice (DSR) (Choudhary 2017, Choudhary et al. 2017) <sup>[7, 8]</sup>. It has several advantages such as requirement of 35-57% less water and 67% less labour expenses over transplanting rice. Apart from these, DSR requires less use of machine, and have lesser methane emissions (Chauhan et al. 2012)<sup>[6]</sup>. However, weeds are major biological constraint in DSR, mainly due to absence of impounding of water at crop emergence, hence, production and weed management are crucial for increasing the productivity of rice (Chauhan 2012) <sup>[6]</sup>. The extent of yield reduction of rice due to weeds has been estimated up to 95% in India (Naresh et al. 2011)<sup>[18]</sup>, 71-96% in the Philippines (Chauhan and Johnson 2011)<sup>[5]</sup>. To meet the global rice demand, it is estimated that about 114 million tonnes of additional milled rice need to be produced by 2035 which is equivalent to an overall increase of 26 per cent in the next 25 years (Kumar and Ladha 2011)<sup>[13]</sup>. To sustain present food self-sufficiency and to meet future food requirements of the country, India has to augment its rice productivity by 3% per annum but the possibility of expanding the area under rice in the near future is inadequate. There has, however, been stagnation in rice productivity in recent years and long-term experiments showed a dilapidated trend in rice yield. Due to receding water table, rising costs of labour for transplanting of paddy and the adverse effects of puddling on soil properties;

direct-seeded rice (DSR) is gaining popularity in the country. But, weeds are the main constraint for farmers who practicing direct- seeding rice cultivation so use of herbicides both preand post-emergence is required for good crop and its productivity. An unintended consequence of the application of herbicides is that it may lead to significant changes in the populations of microorganisms and their activities thereby influencing the microbial ecological balance in the soil (Min et al. 2002, Saeki and Toyota 2004) [17, 20] and affecting the productivity of soils. When herbicides are applied in soil, they may exert certain side effects on non-target organisms. Therefore, there has been considerable interest on the influence of herbicides on the soil microflora and microbially mediated processes. The effects of these chemicals on certain variables are associated with microbial biomass and their activity (Wardle and Parkinson 1991) <sup>[26]</sup>. The increasing reliance of rice cultivation on herbicides has led to concern about their ecotoxicologicalbehaviour in the rice field environment. Soil health and microbial diversity have become vital issues for the sustainable agriculture. Loss of microbial biodiversity can affect the functional stability of the soil microbial community and soil health. Generally, there are some negative effects of herbicides on the population level or composition of species. The impact of applied herbicides on the soil microbial populations were studied which included analysis of bacteria, actinomycetes and fungi counts. In Bihar, two pre-emergence and four post-emergence herbicides are being used in direct-seeded rice for chemical weed control, therefore, this work was carried out to estimate the counts of these microbes at different period of crop growth after their application.

### Methods materials

A field experiment was conducted at Agriculture Research Farm, Bihar Agricultural University, Sabour, Bhagalpur, Bihar (longitude 87o2'42" East and latitude 25o15'40" North at altitude of 46 meters above mean sea level in the heart of the vast Indo-Gangetic plains of North India.) during Kharif Season 2016 and 2017. The soil of the experimental site was loamy sand in texture having normal soil reaction (pH 7.39) and electrical conductivity (0.25 dsm<sup>-1</sup>), low in organic carbon (0.53%) and available N (183.61 kg ha<sup>-1</sup>) and medium in available P (21.65 kg ha<sup>-1</sup>) and K (208.88 kg ha<sup>-1</sup>). The experiment comprised of 14 weed control treatments, viz. alone application of Pendimethalin and Pyrazosulfuron were applied as pre-emergence while other herbicides as postemergence at 20 days after sowing of crop *i.e.* Pendimethalin 1000 g a.i. ha<sup>-1</sup> (T<sub>1</sub>), Pendimethalin 500 g a.i. ha<sup>-1</sup>fb one hand weeding at 40 days after sowing (T<sub>2</sub>), Almix 4 g a.i. ha<sup>-1</sup> (T<sub>3</sub>), Almix 2 g a.i. ha<sup>-1</sup>fb one hand weeding at 40 days after sowing (T<sub>4</sub>), Ethoxysulfuron 15 g a.i. ha<sup>-1</sup> (T<sub>5</sub>), Ethoxysulfuron 7.5 g *a.i.* ha<sup>-1</sup>fb one hand weeding at 40 days after sowing (T<sub>6</sub>), Pyrazosulfuron 25 g a.i. ha<sup>-1</sup> (T<sub>7</sub>), Pyrazosulfuron 12.5 g *a.i.* ha<sup>-1</sup>*fb* one hand weeding at 40 days after sowing (T<sub>8</sub>), Bispyribac-Na 30 g a.i. ha<sup>-1</sup> (T<sub>9</sub>), Bispyribac-Na 15 g a.i. ha<sup>-1</sup>fb one hand weeding at 40 days after sowing (T<sub>10</sub>), Azimsulfuron 35 g a.i. ha<sup>-1</sup> (T<sub>11</sub>), Azimsulfuron 17.5 g *a.i.* ha<sup>-1</sup>fb one hand weeding at 40 days after sowing  $(T_{12})$ , weed free  $(T_{13})$  and weedy  $(T_{14})$ . The experiment was laid out in randomized block design (RBD) with three replication. Rice variety 'Rajendra Mahsuri-1' was seeded on 17th June 2016 and 16th June 2017 with tractor drawn conventional drill using with seed rate of 30 kg ha-1 in rows spaced at 20 cm. The recommended dose of fertilizers and plant protection measures for insect-pest and

disease control were applied. Herbicides was sprayed byknapsack sprayer fitted with flat fan nozzle using 300litres of water per hectare.

The composite soil samples were taken at 90 days after sowing of the crop and at harvest whereas yield data calculated at harvest stage on the basis of net plot area to convert in ha<sup>-1</sup> grain and straw yield separately. Four samples of rhizospheric soil under each treatment were taken from 0-15 cm soil depth and mixed so as to have a representative sample of the treatment. The 10 g of soil samples were placed in an Erlenmeyer flask containing 90 ml of sterilized distilled water, and shaken for 30 min. Ten-fold series dilutions were prepared, and appropriate dilutions were plated in specific media. For the isolation of bacteria, fungi and actinomycetes, the Plate Count Agar, Czapek-Dox Agar (Thom and Raper, 1945)<sup>[25]</sup> and Kenknight and Munaier's Medium, respectively were used. The numbers of colony forming cells were determined in each plot by serial dilution pour plate method (Subba, 1986)<sup>[24]</sup>. The obtained field experiment data were analyzed by using standard procedure for Randomized Block Design (RBD) with the help of a computer applying analysis of variance (ANOVA) technique (Snedecor and Cochron, 1971) [23]. The differences among treatments were compared by applying "F" test of significance at 5 per cent of probability and P values was used to examine differences among treatment means.

### **Result and Discussion**

## Effect of various herbicides treatments on microbial population

The counts of fungi and actinomycetes were significantly affected by different herbicides treatments at 90 days after sowing of the crop whereas that of bacteria remained unaffected (Table 1). Among different herbicides treatments, there were significantly lower counts of fungi, actinomycetes and bacteria were found in the weed free and weedy check. Significantly higher microbial populations in the herbicidal treatments at both the stages of observation might be due to healthy and conducive environment for the microorganisms as compared to the control and also more root exudation which are the carbon source for microbial multiplication and their growth. There was no particular pattern of the microbial counts was observed among herbicide treatments but the microbial counts were significantly lower in control plots. It may be concluded that there was increase in the biological properties of the soil in well aerated aerobic soil conditions found in direct seeded rice hence might be ascribed to the improvement in the nutrient status as well as physical conditions of the soil which resulted in better growth of the microorganisms. It could be further inferred that the microbial population started to regain after the weeds were also killed by the herbicides and got mixed in the soil during this period and these might have served to increase the nutrients. The degradation of herbicides may be serving as carbon source for growth of microbes. Bera et al. (2013) <sup>[2]</sup> reported that microorganisms are able to degrade herbicides and utilize them as a source of biogenic elements for their own physiological processes. However, before degradation, herbicides have toxic effects on microorganisms, reducing their abundance, activity and consequently, the diversity of their communities.

The application of treatment T<sub>9</sub> Bispyribac-Na 30 g a.i. ha<sup>-1</sup> PoE, significantly increased by 121.71% and 134.08% actinomycetes population when compared with the treatment weed free and weedy check, respectively at 90 days after

sowing. Similarly, the application of herbicides treatment T<sub>1</sub>Pendimethalin 1000 g.a.i ha<sup>-1</sup> (PE) also significantly increased actinomycetes count by 74.59% and 91.33% when compared with the treatment weed free and weedy check, respectively at 90 days after sowing. It may be due the The degradation of herbicides may be serving as carbon source for growth of microbes and the growth of plants also vigorous having more root biomass that leads to secrete more carbon compounds from the root system. According to Lynch (1983) <sup>[15]</sup>, microbes degrade herbicides in the course of metabolic (when adaptation phenomena take place) and co-metabolic processes. New compounds are formed from herbicide metabolites. Herbicides may be a source of nutrition for microbes (Cook and Hutter, 1981)<sup>[9]</sup>, in which case they significantly affect microbial growth and multiplication. However, herbicides also affect the microbes physiologically: a) by changing their biosynthetic mechanism (a change in the level of protein biosynthesis is reflected on the ratio of extracellular and intracellular enzymes); b) by affecting protein biosynthesis (induction or repression of synthesis of certain enzymes); c) by affecting the cellular membranes (changes in transport and excretion processes); d) by affecting plant growth regulators (transport of indolacetic acid, gibberellin synthesis and ethylene level); e) applied in high doses, they may kill microorganisms (Kaur *et al.*, 2014)<sup>[11]</sup>. The microbial count was found less at harvest stage when compared with the 90 days after sowing of the crop. It might be due to utilization carbon from the degradation of herbicides in early stages and minimum secretions of root exudates which acts as a source of carbon for the growth and multiplication of microorganisms in the rhizosphere. At harvest stage the microbial viz., bacteria, actinomycetes and fungi population under all herbicides treatments were found more when compared with weed free and weedy treatments. It

might be due to the more availability of carbon from the root exudation and from the degradation of herbicides. It could be further inferred that the microbial population started to regain after the weeds were also killed by the herbicides and got mixed in the soil and these might have served to increase the nutrients (Bhatt et al., 2014 and Omara and Ghandor, 2018)<sup>[3,</sup> <sup>19]</sup>. The degradation of herbicides may be serving as a carbon source for growth of microbes. These results were in tune with finding of Jarvan et al. (2014) [10]. The bacterial population in herbicidetreated plots was more or less similar to the unsprayed control plots in later stages indicating that herbicides have no detrimental effect on soil health at applied doses. Anderson (2003) <sup>[1]</sup> reported that herbicides generally appear to have no adverse effect on total bacterial population in soil except at concentrations exceeding recommended rates. However, among all herbicides treatments, application of treatment  $T_{10}$ Bispyribac-Na 15 g *a.i.* ha<sup>-1</sup>*fb* one hand weeding at 40 days after sowing produced higher grain yield to the tune of 104.6 per cent over weedy check and was statistically at par with Azimsulfuron 17.5 g *a.i.* ha<sup>-1</sup>fb one hand weeding at 40 days after sowing  $(T_{12})$  and was significantly superior over rest of the treatments. Similarly results were in conformity with the findings of Sanodiya and Singh (2017) <sup>[21]</sup> and *Yadav et al.* (2009) <sup>[27]</sup>. The increase in yield in  $T_{12}$ treatment was to the tune of 93.3 per cent over weedy check (T<sub>14</sub>), respectively. Pyrazosulfuran 25 g a.i. ha<sup>-1</sup> (PE) (T<sub>7</sub>), Pendimethalin 500 g a.i. ha<sup>-1</sup> (PE) fb one hand weeding at 40 days after sowing (T<sub>2</sub>), Pyrazosulfuran 25 g a.i ha<sup>-1</sup> (PE) fb one hand weeding at 40 days after sowing  $(T_8)$  and almix 4 g ha-1 PoE (T<sub>3</sub>) exhibited statistical parity with each other in terms of lower grain yield among herbicides treatment and produced 39.0, 44.6, 50.7 and 52.6 per cent more yield than that of weedy check ( $T_{14}$ ). The lowest grain yield (32.58 q ha<sup>-</sup> <sup>1</sup>) was noticed in weedy check ( $T_{14}$ ).

Treatments	Bacteria (CFU×10 <sup>6</sup> g <sup>-1</sup> soil)		Actinomycetes (CFU×10 <sup>5</sup> g <sup>-1</sup> soil)		Fungi (CFU×10 <sup>4</sup> g <sup>-1</sup> soil)	
	90 DAS	At harvest	90 DAS	At harvest	90 DAS	At harvest
T <sub>1</sub> Pendimethalin 1000 g a.i. ha <sup>-1</sup> (PE)	13.21	13.78	38.62	15.61	24.74	14.50
T <sub>2</sub> Pendimethalin 500 g a.i ha <sup>-1</sup> (PE) <i>fb</i> 1 HW 40 DAS	11.98	12.55	32.55	12.96	19.37	12.70
T <sub>3</sub> Almix 4 g ha <sup>-1</sup> (PoE)	12.64	13.55	36.45	13.89	23.37	12.30
T <sub>4</sub> Almix 2 g a.i ha <sup>-1</sup> (PoE) <i>fb</i> 1 HW 40 DAS	12.78	13.35	34.78	12.03	21.17	11.53
T <sub>5</sub> Ethoxysulfuron 15 g a.i ha <sup>-1</sup> (PoE)	13.01	13.92	37.38	14.53	23.73	14.82
T <sub>6</sub> Ethoxysulfuron 7.5 g a.i ha <sup>-1</sup> (PoE) <i>fb</i> 1 HW 40 DAS	12.24	12.82	34.95	12.30	20.97	12.16
T <sub>7</sub> Pyrazosulfuran 25 g a.i ha <sup>-1</sup> (PE)	13.08	14.15	38.37	15.24	22.80	14.58
T <sub>8</sub> Pyrazosulfuran 12.5 g a.i ha <sup>-1</sup> (PE) <i>fb</i> 1 HW 40 DAS	11.68	12.25	34.85	13.70	21.01	12.74
T9 Bispyribac-Na 30 g a.i ha <sup>-1</sup> (PoE)	13.04	13.95	39.63	16.14	22.60	13.72
T <sub>10</sub> Bispyribac-Na 15 g a.i ha <sup>-1</sup> <i>fb</i> 1 HW 40 DAS (PoE)	11.96	10.53	34.10	13.59	21.68	11.50
T <sub>11</sub> Azimsulfuron 35 g a.i ha <sup>-1</sup> (PoE)	13.30	13.88	38.03	15.37	22.86	14.83
T <sub>12</sub> Azimsulfuron 17.5 g a.i ha <sup>-1</sup> fb 1 HW 40 DAS (PoE)	11.43	12.00	33.63	13.60	21.32	12.07
T <sub>13</sub> Weed Free	10.94	10.52	17.87	12.05	14.17	11.43
T <sub>14</sub> Weedy	10.54	10.12	16.93	11.10	12.93	11.48
SEm ±	1.12	1.14	1.51	1.41	0.80	1.24
CD at 5%	NS	NS	4.29	NS	2.27	NS

Table 1: Effect of herbicides treatments on microbial population of soil under direct-seeded rice (Pooled data of 2016 and 2017)

 Table 2: Effect of different weed management treatments on Grain yield, Straw yield and harvest index in direct seeded rice (Pooled data of 2016 and 2017)

Treatments	Grain yield (q ha <sup>-1</sup> )	Straw yield (q ha <sup>-1</sup> )	harvest index
T <sub>1</sub> Pendimethalin 1000 g a.i. ha <sup>-1</sup> (PE)	40.34	60.40	40.07
T <sub>2</sub> Pendimethalin 500 g a.i ha <sup>-1</sup> (PE) fb 1 HW 40 DAS	47.14	70.56	40.03
T <sub>3</sub> Almix 4 g ha <sup>-1</sup> (PoE)	49.74	74.07	40.16
T <sub>4</sub> Almix 2 g a.i ha <sup>-1</sup> (PoE) <i>fb</i> 1 HW 40 DAS	54.91	81.38	40.29
T <sub>5</sub> Ethoxysulfuron 15 g a.i ha <sup>-1</sup> (PoE)	50.54	75.90	39.97
T <sub>6</sub> Ethoxysulfuron 7.5 g a.i ha <sup>-1</sup> (PoE) fb 1 HW 40 DAS	61.00	89.98	40.40
T <sub>7</sub> Pyrazosulfuran 25 g a.i ha <sup>-1</sup> (PE)	45.31	67.47	40.20

T <sub>8</sub> Pyrazosulfuran 12.5 g a.i ha <sup>-1</sup> (PE) <i>fb</i> 1 HW 40 DAS	49.12	72.91	40.25
T <sub>9</sub> Bispyribac-Na 30 g a.i ha <sup>-1</sup> (PoE)	54.41	80.55	40.32
T <sub>10</sub> Bispyribac-Na 15 g a.i ha <sup>-1</sup> fb 1 HW 40 DAS (PoE)	66.67	97.77	40.54
T <sub>11</sub> Azimsulfuron 35 g a.i ha <sup>-1</sup> (PoE)	53.11	79.12	40.16
T <sub>12</sub> Azimsulfuron 17.5 g a.i ha <sup>-1</sup> fb 1 HW 40 DAS (PoE)	62.99	93.40	40.28
T <sub>13</sub> Weed Free	67.74	98.38	40.78
T <sub>14</sub> Weedy	32.58	49.08	39.92
SEm ±	1.49	2.49	0.37
CD at 5%	4.69	7.09	NS

Weed free check  $(T_{13})$  also recorded highest straw yield (98.38 q ha<sup>-1</sup>) followed by Bispyribac-Na 15 g a.i ha<sup>-1</sup>*fb* 1 HW 40 DAS (PoE) (T<sub>10</sub>) and Azimsulfuron 17.5 g a.i ha<sup>-1</sup>*fb* 1 HW 40 DAS (PoE) (T<sub>12</sub>). The lowest straw yield was recorded in weedy check (T<sub>14</sub>) which noted lower value which was mainly due to reduced dry matter accumulation in plant. Similarly results were in conformity with the findings of *S*ingh *et al.* (2010) <sup>[22]</sup>.

Harvest index did not differ significantly due to integrated weed management practices. Harvest index of direct seeded rice varied from 39.92 to 40.78%. The highest harvest index (40.78%) was recorded in weed free treatment (T<sub>13</sub>) and lowest harvest index (39.92%) was recorded in weedy plot (T<sub>14</sub>). Among herbicides, highest harvest index (40.54%) was recorded under Bispyribac-Na 15 g *a.i.* ha<sup>-1</sup>*fb* one hand weeding at 40 days after sowing (T<sub>10</sub>) and Azimsulfuron 17.5 g *a.i.* ha<sup>-1</sup>*fb* one hand weeding at 40 days after sowing (T<sub>12</sub>), respectively. Similarly results were in conformity with the findings of Sanodiya and Singh (2017) <sup>[21]</sup>.

Based on above findings it may be concluded that, treatment  $T_{10}$  Bispyribac-Na 15 g *a.i.* ha<sup>-1</sup>*fb* one hand weeding at 40 days after sowinggave highest grain, straw yield and harvest index statistically at par with Azimsulfuron 17.5 g *a.i.* ha<sup>-1</sup>*fb* one hand weeding at 40 days after sowing ( $T_{12}$ ) whereas, microbial populations in the herbicide treated plots were more or less similar to the unsprayed control plots thus indicating that herbicides have no detrimental effect on soil health at the applied doses.

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