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Herbicide mixture mediated soil environment analysis in wet direct-sown rice

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Abstract

Direct-sown rice is the possible alternative to the conventional puddled transplanted rice as it saves resources *viz.* water, labour and time. Pre-germinated (sprouted) seeds are sown on puddled soil in wet direct-sown rice. But weed infestation is the major yield compromising factor under this environment as the weeds emerge simultaneously with the rice and compete from the beginning. Among the weed control methods, herbicide-based weed control is the most effective and efficient method. However single herbicide application in a long run leads to weed flora shift and development of herbicide resistance in weeds. In contrast, herbicide mixtures with different mode of action are the possible alternative to reduce the herbicide resistance and to broaden the weed control spectrum season long. But soil environment aspect is important for sustainable rice farming using herbicide mixtures. This investigation was done in Research farm of ICAR – National Rice Research Institute, Cuttack, Odisha in wet season of 2016. The experiment was laid out in randomized complete block design with nine treatments *viz.* herbicide mixture florpyrauxifen-benzyl + cyhalofop-butyl in four doses i.e. 120, 150, 180 and 360 g/ha; florpyrauxifen-benzyl in two doses i.e. 25 and 30 g/ha; bispyribac-sodium at 30 g/ha; weed free and weedy check; replicated thrice. Soil environment parameters *viz.* microbial-biomass carbon, FDA hydrolase activity, dehydrogenase activity and urease activity were studied before, 4, 10, 20 and 30 days after herbicide treatments. The herbicide mixture florpyrauxifen-benzyl + cyhalofop-butyl at 150 g/ha (4.91 t/ha) was found to be the most effective herbicide showing no detrimental effect on soil environment of wet direct-sown rice after 10 days of application and yielding at par with the weed free (5.27 t/ha).

Keywords: Herbicide mixture, microbial biomass carbon, soil enzymes, wet direct-sown rice

Introduction

There are two major global issues; one is food security and another one is environmental sustainability. Rice (*Oryza sativa* L.) is an obvious target for attaining food security as it is staple food for more than half of the world population. The conventional method of rice crop husbandry is transplanting which is a water and labour intensive method^[11]. On the contrary, direct-sown rice (DSR) is advantageous over it being water and labour saving and having 8-10 days of earlier maturity of the crop; hence costs less^[3, 21]. Therefore, DSR could be a possible alternative to save this huge amount of resources. But, DSR is very prone to weed infestation which compromises the yield even up to 100%^[20]. Among different weed control methods, chemical weed control has been proved to be the smartest, economic, most effective and efficient method under growing labour scarcity scenario^[5]. But use of single herbicide with same mode of action in long run may lead to weed flora shift and development of herbicide resistance in weeds. Similarly use of single herbicide seldom proves to be satisfactory in season long weed control due to its narrow spectrum^[18]. So use of herbicide mixture with different mode of action is the possible alternative which broadens the weed control spectrum delaying in the appearance of the resistant weed species^[7].

However, herbicide mixtures persist in soil which may have some non-target effects such as the effect on soil microorganisms^[9]. Soil microbial activities are important soil fertility indicators and soil microorganism mediated biochemical transformations of organic matter that support essential ecosystem functions *viz.* decomposition and mineralization of nutrients.

Herbicide mixtures may affect the soil microbial communities which are responsible for different soil enzyme dynamics^{16, 21} which necessitates the need to assess the risk of application of herbicide mixtures on soil microbial properties. On this background, an attempt was made to assess the soil environmental impacts of the new herbicide mixture i.e. floryprauxifen-benzyl + cyhalofop-butyl. Cyhalofop-butyl, one of the popular post emergence herbicides in rice from aryloxyphenoxy propionate group having acetyl CoA carboxylase (ACCase) inhibitor mode-of-action is combined with the new herbicide, floryprauxifen-benzyl from arylopicolinate group of synthetic auxin herbicide having disrupters of plant cell growth mode-of-action for broad spectrum weed control in direct-sown rice.

Materials and Methods

The experiment was conducted at Institute Research Farm of ICAR-National Rice Research Institute, Cuttack, Odisha (20°27'10"N, 85°56'9"E; 24 m above mean sea level) in *Kharif* season of 2016 where adequate irrigation and drainage facilities are available. The experiment soil was sandy clay loam with pH 7.8 with low available N (215.4 kg/ha), medium available P (48 kg/ha), high available P (322.8 kg/ha) and medium organic carbon (0.52%). The experiment was laid out in Randomized Complete Block Design with nine treatments *viz.* four herbicide mixtures (floryprauxifen-benzyl+cyhalofop-butyl 12% EC (w/v) at 120 (20+100) g/ha, floryprauxifen-benzyl+cyhalofop-butyl 12% EC (w/v) at 150 (25+125) g/ha, floryprauxifen-benzyl+cyhalofop-butyl 12% EC (w/v) at 180 (30+150) g/ha, floryprauxifen-benzyl+cyhalofop-butyl 12% EC (w/v) at 360 (60+300) g/ha), three alone herbicides (floryprauxifen-benzyl 2.5% EC (w/v) at 25g/ha, floryprauxifen-benzyl 2.5% EC (w/v) at 30 g/ha, bispyribac-sodium 10% SC at 30 g/ha), one weed free and weedy check, replicated thrice. The gross and net plot size were 6.0 m x 5.0 m and 5.0 m x 4.0 m, respectively. The test variety '*Naveen*' (115 days duration, Indica type) was sown manually at 20 cm rows apart with a seed rate of 80 kg/ha on 13th June and harvested on 6th October, 2016.

Soil samples were collected diagonally from each plot before treatment of herbicides and at 4, 10, 20 and 30 days after treatment of herbicides for laboratory analysis of microbial biomass carbon (MBC) and soil enzymatic activities (Dehydrogenase activity, FDA hydrolase activity, Urease activity). The following methods were followed for analysis.

Microbial Biomass Carbon

Soil microbial biomass carbon (MBC) was measured by modified chloroform fumigation extraction method¹²⁴. It was assayed by treating 10 g of fresh soil sample with 2 mL ethanol free chloroform in the soil sample and incubated for 24 hrs. In another set, soil was kept in similar condition except for chloroform treatment. After incubation, the lids of the container were opened to remove the chloroform vapors. 40 mL of 0.5 M K₂SO₄ was added to it. The content was shaken for at least 1 hr. The suspension was filtered and the

filtrate was measured at 280 nm in UV-Visible spectrophotometer (Specord 200, Analytik Jena, Germany).

FDA hydrolase activity

FDA hydrolase activity was measured by the potassium phosphate buffer method followed by addition of chloroform/methanol (2:1 v/v) as described by¹¹. Soil samples (2 g) were treated with 15 ml of 60 mM potassium phosphate buffer (pH 7.6) and 0.2 ml of 1,000 µg FDA. After shaking for 20 min at 30°C, 15 ml of chloroform/methanol (2:1 v/v) was added and was centrifuged at 2,000 rpm for 3 min. FDA hydrolase activity was assayed at 490 nm and expressed as µg fluorescein per gram of dry soil per hour.

Dehydrogenase activity

Dehydrogenase activity (DHA) was determined by reduction of triphenyl tetrazolium chloride (TTC)¹⁴. Each soil sample (3 g) was treated with 0.1 g CaCO₃ and 1 mL aqueous 0.18 M TTC and incubated for 24 h at 37 °C. The triphenyl formazan (TPF) was extracted from the reaction mixture (pH 5.3–5.6) with methanol and assayed at 485 nm. Dehydrogenase activity was expressed as milligrams of TPF formed per gram of dry soil per hour.

Urease activity

Twenty grams of soil samples from each treatment were mixed with urea (20 mL) to provide a final concentration of 2,000 µg/g soil, and the suspensions were incubated for 5 h. The amount of residual urea present in the soil suspension upon incubation was determined by non-buffer method¹²⁵. Urease activity was expressed as micrograms of urea hydrolyzed per gram of dry soil per hour.

Statistical analysis

The data were subjected to the Analysis of Variance using the Statistical Analysis System (SAS) and significant differences among the treatment means tested Fisher's protected Least Significant Difference (LSD) test at $p \leq 0.05$.

Results and Discussion

Microbial Biomass Carbon

Among different microbial parameters, soil microbial biomass carbon (MBC) is considered to be responsible for regulating nutrient cycling¹¹⁹ and is closely linked to the primary productivity of an ecosystem¹²¹ and soil health¹²². The MBC data presented in Table 1 depicted that the biological property of soil was significantly affected by different weed management treatments. Among all treatments, the weed free and the weedy check did not show significant variation having higher MBC content in all stages of observations as they had not been applied with herbicides. There was a significant reduction in MBC content at 4 days after treatment (DAT) of the herbicides except bispyribac-sodium at 30 g/ha (84.40 µg g⁻¹) where the MBC content is higher than before treatment.

Table 1: Effect of different weed management treatments on soil microbial biomass carbon (MBC)

Treatments	Dose (g/ha)	MBC ($\mu\text{g/g}$)				
		BT	4 DAT	10 DAT	20 DAT	30 DAT
Florpyrauxifen-benzyl + cyhalofop-butyl	120	68.58	47.61	51.62	63.96	59.26
Florpyrauxifen-benzyl + cyhalofop-butyl	150	68.58	40.69	43.98	62.10	58.72
Florpyrauxifen-benzyl + cyhalofop-butyl	180	68.58	24.77	32.73	53.26	57.36
Florpyrauxifen-benzyl + cyhalofop-butyl	360	68.58	21.16	24.66	52.24	60.28
Florpyrauxifen-benzyl	25	68.58	46.26	49.20	66.20	61.39
Florpyrauxifen-benzyl	30	68.58	42.75	46.52	64.97	55.67
Bispyribac-sodium	30	68.58	84.40	89.55	61.68	49.97
Weed Free	-	68.58	86.72	102.11	51.10	70.72
Weedy (Control)	-	68.58	82.27	92.32	40.78	65.81
SEm \pm		-	2.34	2.33	2.44	2.50
CD (P=0.05)		-	7.02	7.02	7.34	7.51

*BT: Before treatment

**DAT: Days after treatment

After 4 DAT, among the herbicide treatments, under florpyrauxifen-benzyl + cyhalofop-butyl at 120 g/ha (47.61 $\mu\text{g/g}$) highest MBC was observed which was at par with florpyrauxifen-benzyl at 25 g/ha (47.61 $\mu\text{g/g}$), florpyrauxifen-benzyl at 30 g/ha (42.75 $\mu\text{g/g}$) and florpyrauxifen-benzyl + cyhalofop-butyl at 150 g/ha (40.69 $\mu\text{g/g}$). The lowest MBC was observed under florpyrauxifen-benzyl + cyhalofop-butyl at 360 g/ha (21.16 $\mu\text{g/g}$) that was at par with florpyrauxifen-benzyl + cyhalofop-butyl at 180 g/ha (24.77 $\mu\text{g/g}$). Similar trend was also observed at 10 and 20 DAT whereas the MBC contents of all treatments decreased at 30 DAT.

It can be observed that herbicide mixture florpyrauxifen-benzyl + cyhalofop-butyl reduced the MBC content severely whereas there was a less reduction where florpyrauxifen-benzyl alone had been applied and there was least effect of bispyribac-sodium on soil MBC content. Short-term effects in

response to the use of herbicides are related to disturbances of chemical and biological balance in soil, and are known to selectively suppress the activity of soil microorganisms. The decrease in MBC might be due to the adsorption of small amount of herbicides on organic matter that affected the soil microbial biomass, leading to lysis of microbial cells [14 & 8].

FDA hydrolase activity

FDA was considered as a tool for measuring the early detrimental effect of xenobiotics on soil microbial biomass. FDA is a sensitive and nonspecific test and able to depict the hydrolytic activity of soil microbes [13]. FDA hydrolase is considered as an accurate expression of total microbial activity. Data regarding FDA hydrolase activity as influenced by different treatments are presented in Table 2.

Table 2: Effect of different weed management treatments on Fluorescein Diacetate Hydrolase activity (FDA)

Treatments	Dose (g/ha)	FDA (μg fluorescein/g of dry soil/h)				
		BT	4 DAT	10 DAT	20 DAT	30 DAT
Florpyrauxifen-benzyl + cyhalofop-butyl	120	3.319	4.195	2.050	2.456	3.402
Florpyrauxifen-benzyl + cyhalofop-butyl	150	3.319	3.494	1.875	2.749	3.597
Florpyrauxifen-benzyl + cyhalofop-butyl	180	3.319	3.585	1.872	2.389	3.407
Florpyrauxifen-benzyl + cyhalofop-butyl	360	3.319	3.919	2.310	2.476	3.426
Florpyrauxifen-benzyl	25	3.319	3.937	1.980	2.339	3.232
Florpyrauxifen-benzyl	30	3.319	4.997	2.084	2.628	3.563
Bispyribac-sodium	30	3.319	4.396	1.663	3.057	3.867
Weed Free	-	3.319	4.741	1.801	2.414	3.425
Weedy (Control)	-	3.319	5.933	1.557	2.761	3.566
SEm \pm		-	0.306	0.134	0.353	0.243
CD (P=0.05)		-	0.918	0.402	NS	NS

*BT: Before treatment

**DAT: Days after treatment

***NS = Non-significant

The data revealed that by application of herbicides, FDA hydrolase activity of soil was increased up to 4 days. At 4 DAT, the FDA hydrolase activity was highest under the untreated weedy check (5.933 μg fluorescein/g of dry soil/h) followed by florpyrauxifen-benzyl at 30 g/ha (4.997 μg fluorescein/g of dry soil/h) which was at par with the weed free (4.741 μg fluorescein/g of dry soil/h), bispyribac-sodium at 30 g/ha (4.396 μg fluorescein/g of dry soil/h) and florpyrauxifen-benzyl + cyhalofop-butyl at 120 g/ha (μg fluorescein/g of dry soil/h) and the lowest found in florpyrauxifen-benzyl + cyhalofop-butyl at 150 g/ha (3.494 μg fluorescein/g of dry soil/h) which was at par with florpyrauxifen-benzyl + cyhalofop-butyl at 180 g/ha (3.585 μg fluorescein/g of dry soil/h), florpyrauxifen-benzyl + cyhalofop-butyl at 360 g/ha

(3.919 μg fluorescein/g of dry soil/h) and florpyrauxifen-benzyl at 25 g/ha (3.937 μg fluorescein/g of dry soil/h). The FDA hydrolase activities of all treatments decreased up to 10 DAT and increased between 10 and 30 DAT. At both 20 and 30 DAS the treatment effects on FDA hydrolase was non-significant. This result indicated that the herbicides had no detrimental effect on FDA hydrolase of soil.

Dehydrogenase activity

Soil dehydrogenase activity is considered as a valuable parameter for assessing the impact of herbicide treatments on the soil microbial biomass [17]. Data regarding dehydrogenase activity (DHA) as influenced by different treatments are presented in Table 3.

Table 3: Effect of different weed management treatments on Dehydrogenase activity (DHA)

Treatments	Dose (g/ha)	DHA (mg TPF/g of dry soil/h)				
		BT	4 DAT	10 DAT	20 DAT	30 DAT
Florpyrauxifen-benzyl + cyhalofop-butyl	120	8.561	5.765	19.463	32.529	28.202
Florpyrauxifen-benzyl + cyhalofop-butyl	150	8.561	5.093	22.579	36.342	30.432
Florpyrauxifen-benzyl + cyhalofop-butyl	180	8.561	4.961	17.028	27.495	24.489
Florpyrauxifen-benzyl + cyhalofop-butyl	360	8.561	3.370	14.035	25.552	20.426
Florpyrauxifen-benzyl	25	8.561	9.505	15.297	29.271	24.849
Florpyrauxifen-benzyl	30	8.561	9.313	14.201	28.962	22.499
Bispyribac-sodium	30	8.561	8.777	16.799	21.024	19.431
Weed Free	-	8.561	8.421	11.222	18.061	16.341
Weedy (Control)	-	8.561	8.392	12.024	19.251	17.438
SEm±		-	0.477	0.955	2.015	1.803
CD (P=0.05)		-	1.431	2.863	6.042	5.406

*BT: Before treatment

**DAT: Days after treatment

The data revealed that on 4 DAT of herbicides, the DHA content of weed free (8.421 mg TPF/g of dry soil/h), weedy check (8.392 mg TPF/g of dry soil/h) and bispyribac-sodium at 30 g/ha (8.777 mg TPF/g of dry soil/h) remained almost unaffected whereas, there was a slight increase in florpyrauxifen-benzyl at 25 g/ha (9.505 mg TPF/g of dry soil/h) and florpyrauxifen-benzyl at 30 g/ha (9.313 mg TPF/g of dry soil/h) and sharp decrease in herbicide mixture treatments. The highest decrease occurred in florpyrauxifen-benzyl + cyhalofop-butyl at 360 g/ha (3.370 mg TPF/g of dry soil/h) followed by florpyrauxifen-benzyl + cyhalofop-butyl at 180 g/ha (4.961 mg TPF/g of dry soil/h), florpyrauxifen-benzyl + cyhalofop-butyl at 150 g/ha (5.093 mg TPF/g of dry soil/h) and florpyrauxifen-benzyl + cyhalofop-butyl at 120 g/ha (5.765 mg TPF/g of dry soil/h). The finding is in agreement with the finding of Sebiomo *et al.* [16] who observed that the application of herbicides to the soils led to a significant drop in DHA with respect to untreated soil samples.

However, after 4 DAT of herbicides, there was a notable increase in DHA content in the herbicide treated soil than the untreated weed free and the weedy check up to 20 DAT, which might be due to the greater availability of carbon source for the growth and activity of micro-organisms. This result is in close agreement with the findings of Rao *et al.* [15].

The lower dehydrogenase activity in the untreated plots might be attributed to the lower availability of substrate for growth of soil microbes. At 30 DAT, there was a higher dehydrogenase activity observed in the soil as compared to the weed free and weedy check.

Urease activity

Urease catalyzes the hydrolysis of urea to ammonium and carbon dioxide. Ammonium formed represents a bioavailable form of nitrogen for plant uptake; has a primary role in the cycling of nitrogen. Data regarding urease activity as influenced by different treatments are presented in Table 4.

A reduction in the urease activity was observed at 4 DAT which might be attributed to the changes in soil properties like pH and also due to the temporary inhibition caused by the metabolites produced as a result of degradation of herbicides by the micro-organisms as reported by Sheeja *et al.* [17]. As time advanced there was an increase in the urease activity might be due to the fact that the temporary inhibition of enzyme activity was overcome by the increased microflora with the increase in microbial population. More the microbial biomass, more the exudation resulted in higher enzymatic activity [10]. With the advancement of time, all the herbicide treatments except the highest dose i.e. florpyrauxifen-benzyl + cyhalofop-butyl at 360 g/ha.

Table 4: Effect of different weed management treatments on urease activity

Treatments	Dose (g/ha)	Urease (μg of urea hydrolysed/g of dry soil/h)					Grain Yield (t/ha)
		BT	4 DAT	10 DAT	20 DAT	30 DAT	
Florpyrauxifen-benzyl + cyhalofop-butyl	120	349.60	325.90	326.98	365.62	351.36	4.68
Florpyrauxifen-benzyl + cyhalofop-butyl	150	349.60	268.50	253.33	350.75	368.42	4.91
Florpyrauxifen-benzyl + cyhalofop-butyl	180	349.60	341.97	309.72	354.28	365.33	4.17
Florpyrauxifen-benzyl + cyhalofop-butyl	360	349.60	334.08	263.72	325.85	337.14	3.72
Florpyrauxifen-benzyl	25	349.60	334.94	291.88	355.06	367.85	4.46
Florpyrauxifen-benzyl	30	349.60	336.75	285.68	364.77	358.27	4.74
Bispyribac-sodium	30	349.60	318.57	347.00	359.62	345.63	4.36
Weed Free	-	349.60	281.03	264.78	359.10	366.95	5.27
Weedy (Control)	-	349.60	333.37	329.82	327.12	361.78	3.14
SEm±		-	6.19	9.98	4.77	4.74	0.17
CD (P=0.05)		-	18.56	29.92	14.32	14.21	0.52

*BT: Before treatment

**DAT: Days after treatment

Grain yield

All the herbicide treatments significantly influenced the grain yield (Table 4). The highest grain yield was obtained under weed free (5.27 t/ha) which was at par with florpyrauxifen-benzyl + cyhalofop-butyl at 150 g/ha (4.91 t/ha). The treatments florpyrauxifen-benzyl at 30 g/ha (4.74 t/ha),

florpyrauxifen-benzyl + cyhalofop-butyl at 120 g/ha (4.68 t/ha), florpyrauxifen-benzyl at 25 g/ha (4.46 t/ha) and bispyribac-sodium at 30 g/ha (4.36 t/ha) recorded at par grain yield. The lowest grain yield was obtained under weedy check (3.14 t ha⁻¹) followed by florpyrauxifen-benzyl + cyhalofop-butyl at 360 g/ha (3.72 t/ha) and florpyrauxifen-benzyl +

cyhalofop-butyl at 180 g/ha (4.17 t/ha). At par yield in both 150 and 180 g/ha doses of florypyrauxifen-benzyl + cyhalofop butyl herbicide mixture was reported in aerobic rice ^[23].

Conclusions

All the herbicide treatments significantly affected the soil environment of wet-DSR. At 4 days after herbicide application, approximately all the soil environmental parameters were decreased except FDA hydrolase activity. There was a drastic reduction in soil microbial-biomass carbon in all the herbicide treatments except bispyribac-sodium where an increased value was observed at 4 days after herbicide treatment. After 4 days of herbicide treatment, dehydrogenase activity increased gradually. Similarly the urease activity showed at par result in all the plots except the highest dose of herbicide. All the four parameters i.e. microbial-biomass carbon, FDA hydrolase activity, dehydrogenase activity and urease activity showed normal behaviour after 10 days of herbicide treatments. The grain yield was highest in florypyrauxifen-benzyl + cyhalofop-butyl at 150 g/ha being at par with the weed free showing no detrimental effect on soil environment, hence may be recommended for sustainability in wet direct-sown rice.

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