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Residual effects of wet season rice herbicides on soil enzymes and microbial biomass carbon in succeeding green gram sown with rice stubble mulch and minimum tillage

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Abstract
Though herbicides are efficient enough to control weeds, but may also have detrimental effects on the soil environment affecting the activity of soil microorganisms for a long time even in the succeeding crop. Therefore an experiment was conducted in the wet and dry seasons of 2018-19 and 2019-20 at ICAR – National Rice Research Institute, Cuttack, Odisha which was laid out in randomized complete block design comprising of ten weed management treatments in transplanted rice in wet season and the residual effects of the same treatments in green gram in dry season after harvest of the rice replicated thrice. The ten treatments were three herbicide mixtures i.e. flopyrauxifen-benzyl + cyhalofop-butyl at (25+125) g ha^{-1} at 18 days after transplanting (DAT) (W_1), fenoxaprop-p-ethyl + ethoxysufuron at (50+15) g ha^{-1} at 18 DAT (W_2) and cyhalofop-butyl + penoxsulam at (100+30) g ha^{-1} at 18 DAT (W_3); three sequential application of herbicides i.e. bispyribac-sodium at 30 g ha^{-1} at 9 DAT *fb* ethoxysulfuron at 15 g ha^{-1} at 21 DAT (W_4), flucetosulfuron at 25 g ha^{-1} at 9 DAT *fb* ethoxysulfuron at 15 g ha^{-1} at 21 DAT (W_5) and cyhalofop-butyl at 100 g ha^{-1} at 9 DAT *fb* ethoxysulfuron at 15 g ha^{-1} at 21 DAT (W_6); two herbicide checks i.e. bensulfuron-methyl + pretilachlor at (60+600) g ha^{-1} at 4 DAT (W_7) and bispyribac-sodium at 30 g ha^{-1} at 2 leaf stage of weeds (W_8); one weed free check (Hand weeding at 20, 40 and 60 DAT) (W_9) and one weedy check (Untreated) (W_{10}). The dry season green gram was sown with the preceding season rice stubble mulch and minimum tillage. Soil environmental parameters *viz.* FDA hydrolase activity, dehydrogenase activity, β-glucosidase activity and microbial biomass carbon were analyzed at 30, 45 and 60 days after sowing of green gram. The soil environment parameters in the green gram were not significantly influenced by the weed management treatments of rice crop; and the minimum tillage and stubble mulch facilitated to gradually increase the activity of soil microbes and enzymes as the crop matured.

Keywords: Green gram, herbicides, microbial biomass carbon, minimum tillage, soil enzymes, stubble mulch

Introduction

Rice-pulse cropping sequence is practically feasible, economically viable, eco-friendly and water saving technology for sustaining soil fertility and increasing productivity (Hegde 1992). India is the highest producer as well as consumer of pulses in the world contributing 25.5% of total global pulse production (GOI 2013) [5]. Green gram provides 347 calories 100 g^{-1} and is the third important pulse crop of India covering 8% of the total pulse production area of the country. In Odisha, green-gram is cultivated in an area of 0.836 million ha with a production of 0.362 million tonnes and productivity of only 434 kg ha^{-1} (OAS 2017-18) [11]. Rice-green gram is a prominent cropping system of Eastern India especially for the state of Odisha. Many farmers of the region grow green gram as a catch crop after harvest of wet season rice. Being a short duration crop, green gram not only utilizes the residual soil moisture but also increases the productivity of the land adding biologically fixed nitrogen to the soil. Moreover, crop rotation has traditionally been seen as one of the cheapest and effective method of weed control as the crop ecology changes as the crop changes (Froud-Williams 1988; Leibman and Dyck 1993) [4, 8]. Out of different biotic stresses a crop experiences, weed infestation is one of the most important factors to reduce the crop yield to a large extent. Therefore in every crop husbandry, weed management aspect has a major role to protect the crop from weed menace and reduce the yield loss. Among different methods of weed control in rice, herbicidal control is getting growing acceptance among the farmers as it is the most labour, time and cost saving and economic method. However, herbicides may cause qualitative and quantitative alterations in the soil microbial populations and their enzymatic activities (Xia *et al*. 2011) [19]. Several literatures reported that herbicides are not harmful when applied at recommended doses (Selvamani and Sankaran 1993) [12] but some herbicides may affect non-target organisms including microorganisms (Latha and Gopal 2010) [7]. Even so, some herbicide may stimulate the growth and activities of the soil microbes (Wardle and Parkinson 1990) [18]. However, information regarding the effects of different herbicides applied in wet season rice, on soil enzymes in succeeding green gram crop is limited. Considering the aforementioned facts, the present experiment was conducted to study residual effect of wet season rice herbicides on soil environment in succeeding green gram.

Materials and Methods

The field experiment was conducted at ICAR – National Rice Research Institute, Cuttack, Odisha in wet and dry seasons of 2018-19 and 209-20. The experiment was laid out in Randomized Complete Block Design with ten weed management treatments in wet season transplanted rice (TPR) and the residual effects of the same treatments were studied in the succeeding green gram crop in the dry season with three replications. The green gram crop was sown after harvest of TPR with resource conservation technologies i.e. minimum tillage and rice stubble mulch. The ten weed management treatments in rice were three herbicide mixtures i.e. flopyrauxifen-benzyl + cyhalofop-butyl at (25+125) g ha^{-1} at 18 days after transplanting (DAT) (W_1), fenoxaprop-p-ethyl + ethoxysufuron at (50+15) g ha^{-1} at 18 DAT (W_2) and cyhalofop-butyl + penoxsulam at (100+30) g ha^{-1} at 18 DAT (W_3); three sequential application of herbicides i.e. bispyribac-sodium at 30 g ha^{-1} at 9 DAT *fb* ethoxysulfuron at 15 g ha^{-1} at 21 DAT (W_4), flucetosulfuron at 25 g ha^{-1} at 9 DAT *fb* ethoxysulfuron at 15 g ha^{-1} at 21 DAT (W_5) and cyhalofop-butyl at 100 g ha^{-1} at 9 DAT *fb* ethoxysulfuron at 15 g ha^{-1} at 21 DAT (W_6); two herbicide checks i.e. bensulfuron-methyl + pretilachlor at (60+600) g ha^{-1} at 4 DAT (W_7) and bispyribac-sodium at 30 g ha^{-1} at 2 leaf stage of weeds (W_8); one weed free check (Hand weeding at 20, 40 and 60 DAT) (W_9) and one weedy check (Untreated) (W_{10}).

Soil samples from each plot consisted of composite samples were collected with a sample probe augur (0-15 cm) at 30, 45 and at 60 days after sowing (DAS) of dry season green gram. Collected soil was thoroughly mixed and composite samples were prepared.

Fluorescein diacetate (FDA) activity

FDA hydrolase activity was measured by the potassium phosphate buffer method (pH 7.6) followed by extraction with chloroform/methanol (2:1 v/v) as described by Adam and Duncan (2001) [1].

Dehydrogenase activity (DHA)

Dehydrogenase activity (DHA) was determined by reduction of triphenyl tetrazolium chloride (TTC) (Casida *et al*. 1964) [2]. Soil samples were treated with $CaCO_3$ and TTC and incubated for 24 h at 37 °C. The triphenyl formazan (TPF) was extracted from the reaction mixture with methanol and assayed at 485 nm.

β-Glucosidase activity

β-Glucosidase activity was assayed by treating soil sample with toluene, modified universal buffer (pH 6.0) and *p*-nitrophenyl-β-d-glucoside solution (Eivazi and Tabatabai 1977) [3]. After 1 h of incubation at 37 °C, 0.5 M $CaCl_2$ and 0.1 M Tris (hydroxymethyl) amino methane buffer pH 12 was added. The suspension was filtered and the filtrate was measured at 420 nm.

Soil microbial biomass carbon (MBC)

Soil microbial biomass carbon (MBC) was measured by modified chloroform fumigation extraction method (Vance *et al*., 1987) [17]. 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.

Results and Discussion

FDA hydrolase activity

FDA is considered as a tool for measuring the early detrimental effect of xenobiotics on soil microbial biomass. It is a sensitive and nonspecific test and able to depict the hydrolytic activity of soil microbes and is considered as an accurate expression of total microbial activity (Nayak *et al*. 2007) [10]. Data regarding FDA hydrolase activity as influenced by different treatments are presented in Table 1. As the data depict, the weed management treatments of wet season rice though influenced FDA hydrolase activity in succeeding green gram, but there was no significant difference among the effects of the treatments during both the years of experiments. However, it can be noticed that the FDA hydrolase activity gradually increased from 30 DAS to 60 DAS and the activity was highest in weed free and weedy check treatments. The treatments had no detrimental effects on FDA hydrolase activity of the succeeding green gram.

Table 1: Residual effects of wet season rice herbicides on FDA hydrolase activity in succeeding green gram

Treatments	FDA hydrolase Activity (μg g^{-1} soil h^{-1})								
	30 DAS			45 DAS			60 DAS		
	2018-19	2019-20	Mean	2018-19	2019-20	Mean	2018-19	2019-20	Mean
W_1	3.21	3.27	3.24	4.53	4.68	4.60	6.25	6.36	6.30
W_2	3.20	3.24	3.22	4.51	4.63	4.57	6.23	6.30	6.26
W_3	3.17	3.23	3.20	4.47	4.62	4.55	6.17	6.29	6.23
W_4	3.25	3.31	3.28	4.58	4.73	4.66	6.32	6.44	6.38
W_5	3.11	3.20	3.16	4.39	4.58	4.48	6.05	6.22	6.14
W_6	3.21	3.25	3.23	4.53	4.65	4.59	6.25	6.32	6.28
W_7	3.11	3.18	3.15	4.39	4.55	4.47	6.05	6.18	6.12
W_8	3.36	3.39	3.38	4.74	4.85	4.79	6.54	6.59	6.57

W 9	3.45	3.52	3.49	4.86	5.03	4.95	6.71	6.85	6.78
W 10	3.41	3.48	3.45	4.81	4.98	4.89	6.64	6.77	6.70
S.Em±	0.10	0.14	0.08	0.15	0.14	0.12	0.27	0.21	0.16
CD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS

*All the treatments are described in the materials and methods; **NS=Non-significant; ***DAS=Days after sowing

Dehydrogenase activity (DHA)

Soil dehydrogenase activity is considered as a valuable parameter for assessing the impact of herbicide treatments on the soil microbial biomass (Sheeja *et al*. 2015) [14]. Data regarding DHA as influenced by different treatments are presented in Table 2. There was no significant difference observed among the effects of the wet season rice weed management treatments on DHA in succeeding green gram. However the overall DHA increased from 30 to 60 DAS and the rate of increase of the DHA was more in 30-45 DAS than 45-60 DAS. The treatments had no detrimental effects on DHA of the succeeding green gram.

Table 2: Residual effects of wet season rice herbicides on Dehydrogenase activity (DHA) in succeeding green gram

Treatments	Dehydrogenase activity (DHA) (mg g^{-1} soil h^{-1})								
	30 DAS			45 DAS			60 DAS		
	2018-19	2019-20	Mean	2018-19	2019-20	Mean	2018-19	2019-20	Mean
W1	139.76	145.35	142.56	171.91	180.29	185.88	191.48	199.97	195.72
W 2	139.33	143.51	141.42	171.37	178.02	184.45	190.88	197.44	194.16
W 3	138.02	141.89	139.95	169.77	176.01	182.55	189.09	195.21	192.15
W 4	141.51	147.17	144.34	173.15	181.59	187.72	193.86	202.47	198.16
W 5	135.41	140.23	137.82	165.69	173.04	179.27	185.51	192.93	189.22
W 6	139.76	144.79	142.28	171.01	178.67	185.07	191.48	199.21	195.34
W 7	135.41	140.55	137.98	165.69	173.43	179.47	185.51	193.37	189.44
W 8	146.29	151.53	148.91	180.73	188.78	194.60	200.42	208.47	204.45
W 9	150.21	156.47	153.34	185.57	194.93	200.36	205.79	215.27	210.53
W 10	148.47	154.36	151.41	183.42	192.30	197.85	203.41	212.36	207.88
S.Em±	5.29	5.48	5.39	6.49	6.78	7.02	7.25	7.54	7.39
CD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS

*All the treatments are described in the materials and methods; **NS=Non-significant; ***DAS=Days after sowing

β-Glucosidase activity

The hydrolysis products of β-glucosidase usually serve as energy sources for microorganisms in soil. The data regarding β-glucosidase activity in the experiment are presented in Table 3. The treatments did not have significant effects on the β-glucosidase activity of the succeeding green gram crop but the activity increased gradually from 30 to 60 DAS. The higher activity of β-glucosidase might be attributed to decomposition of rice stubbles and minimum tillage which facilitates higher microbial activity (Sharma *et al*. 2020) [13]. The treatments had no detrimental effects on β-glucosidase activity in the succeeding green gram.

Soil microbial biomass carbon (MBC)

Among different microbial parameters, MBC is considered to be one of the most responsible parameters for regulating nutrient cycling (Singh *et al*. 2014) [15] and is closely linked to the primary productivity of an ecosystem (Marcel *et al*. 2008) [9] and soil health (Sparling 1997) [16]. The MBC data are presented in Table 4 which depict that the biological property of soil was not significantly affected by different weed management treatments of preceding rice crop. The rate of increase of MBC from 30-45 DAS was higher than that of 45-60 DAS. The treatments had no detrimental effects on MBC in the succeeding green gram.

Table 3: Residual effects of wet season rice herbicides on β-Glucosidase activity in succeeding green gram

Treatments	β-Glucosidase activity (μg g^{-1} soil h^{-1})								
	30 DAS			45 DAS			60 DAS		
	2018-19	2019-20	Mean	2018-19	2019-20	Mean	2018-19	2019-20	Mean
W1	57.92	58.65	58.28	78.44	81.92	80.18	122.71	128.15	125.43
W 2	56.34	53.86	55.10	75.74	78.35	77.05	117.74	121.78	119.76
W 3	55.54	52.61	54.07	74.55	76.97	75.76	115.73	119.48	117.61
W 4	58.64	61.38	60.01	79.42	82.94	81.18	124.24	129.75	126.99
W 5	55.52	52.05	53.78	74.94	77.94	76.44	116.91	121.59	119.25
W 6	57.36	53.48	55.42	77.45	80.58	79.02	120.86	125.74	123.30
W 7	55.84	53.78	54.81	75.52	78.72	77.12	117.99	122.99	120.49
W 8	60.00	56.97	58.49	81.02	84.27	82.64	126.40	131.47	128.93
W 9	62.49	59.37	60.93	84.74	88.64	86.69	132.71	138.82	135.77
W 10	61.47	56.75	59.11	83.23	86.90	85.06	130.18	135.91	133.04
S.Em±	2.45	2.29	2.37	3.31	3.45	3.38	5.18	5.40	5.29
CD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS

*All the treatments are described in the materials and methods; **NS=Non-significant; ***DAS=Days after sowing

Table 4: Residual effects of wet season rice herbicides on microbial biomass carbon (MBC) in succeeding green gram

Treatments	Microbial biomass carbon (MBC) (μg g^{-1})								
	30 DAS			45 DAS			60 DAS		
	2018-19	2019-20	Mean	2018-19	2019-20	Mean	2018-19	2019-20	Mean
W_1	134.06	138.43	136.24	159.39	165.79	162.59	175.68	181.21	178.44
W_2	133.72	136.98	135.35	158.97	164.01	161.49	174.68	179.23	176.95
W_3	132.70	135.71	134.20	157.71	162.44	160.07	174.89	177.48	176.18
W_4	135.43	139.84	137.64	160.36	166.81	163.58	175.77	183.16	179.47
W_5	130.65	134.41	132.53	154.52	160.11	157.31	168.69	175.69	172.19
W_6	134.06	137.99	136.03	158.69	164.52	161.60	172.85	180.61	176.73
W_7	130.65	134.67	132.66	154.52	160.42	157.47	169.54	176.04	172.79
W_8	139.18	143.26	141.22	166.30	172.44	169.37	182.53	187.87	185.20
W_9	142.25	147.13	144.69	170.09	177.26	173.67	185.56	193.19	189.37
W_{10}	140.88	145.48	143.18	168.41	175.20	171.80	183.29	190.91	187.10
S.Em±	5.08	5.23	5.15	6.02	6.25	6.13	6.59	6.84	6.71
CD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS

*All the treatments are described in the materials and methods; **NS=Non-significant; ***DAS=Days after sowing

Conclusion

The herbicide mixtures, sequential application of herbicides and single herbicide used in the experiment were found to have no detrimental effect on the soil microbial biomass and enzymes in succeeding green gram crop. The resource conservation technologies i.e. minimum tillage and rice stubble mulch, may have very good effect facilitating the degradation of rice herbicides also not only maintaining but increasing the microbial biomass carbon, dehydrogenase activity, FDA hydrolase activity and β-glucosidase activity in the succeeding green gram in the dry season after harvest of wet season rice.

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