

Short communication

## Effect of pre and post emergence herbicides on microbial biomass carbon and dehydrogenase activity in soils

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### Abstract

A pot culture experiment in rice was conducted to study the effect of pre and post emergence herbicides on microbial biomass carbon and dehydrogenase activity in soils. Results indicated that the herbicides had negative effect on microbial biomass carbon. Dehydrogenase activity also showed a decline due to the application of herbicides, but to a lesser magnitude than microbial biomass carbon. The adverse effect was pronounced only at 15 days after application of herbicides and followed the order *viz.*, pendimethalin > bispyribac-sodium > oxyfluorfen > cyhalofop-butyl. The adverse effects were of lower magnitude in the soils of high organic matter content.

**Keywords:** Bispyribac-sodium, Cyhalofop-butyl, Dehydrogenase activity;, Microbial biomass carbon, Oxyfluorfen, Pendimethalin

Rice as a staple food crop plays an important role in food as well as nutritional security particularly for Asian countries. To sustain and safeguard food security in the country, the productivity of rice has to be enhanced under limited resources. Various biotic and abiotic stresses are the limiting factors in enhancing rice productivity. The major stress is imposed by competition due to weeds for water, nutrients, light, and space. Hence, weed management is indispensable in crop production. Due to the scarcity and high cost of labour, weed management with herbicides is widely practiced. The problem associated with herbicides is the persistence of their residues in soil which interact with microorganisms thereby altering the biological activity.

Soil microbial biomass, both the source and sink of available nutrients, plays an important role in nutrient transformations (Singh et al., 1989). The direct and indirect effects of toxic chemicals on soil biology include reduction in microbial population and

reduced mineralization of organic compounds. Dipika (2014) reported that the application of herbicides exerted adverse effect on soil microbial biomass carbon.

Dehydrogenase activity is considered as the most sensitive indicator of soil microbial activity due its association with viable microbial population (Mijangos et al., 2006). Most of the herbicides applied to the soil had inhibitory effect on the enzyme dehydrogenase (Sebiomo et al., 2011). Therefore, the assay of microbial biomass carbon and dehydrogenase activity in the soil will be useful to understand the potential adverse effect of herbicides on soil health and to predict the persistence of herbicide residues in the soil system under rice.

Pendimethalin and oxyfluorfen are effective pre emergence herbicides commonly used in rice. Among the post emergence herbicides, bispyribac-

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**Table 1.** Chemical and biological characteristics of soil samples

Soils / soil sampling sites		pH	Organic carbon (%)	Available N(kg ha <sup>-1</sup> )	Available P(kg ha <sup>-1</sup> )	Available K(kg ha <sup>-1</sup> )	Microbial biomass carbon (µg C g <sup>-1</sup> day <sup>-1</sup> )	Dehydrogenase activity (µg TPF g <sup>-1</sup> day <sup>-1</sup> )
Medium O.M. (S <sub>1</sub> ) soils	Control (rice field)	5.36	0.85	237.98	21.62	124.80	153.31	57.12
	Absolute control (non-cropped area)	5.68	1.08	258.40	23.35	199.36	192.12	61.21
High O.M. (S <sub>2</sub> ) soils	Control (rice field)	5.01	2.47	296.30	27.90	325.61	421.10	85.37
	Absolute control (non-cropped area)	5.20	1.92	269.69	24.76	260.96	322.01	78.43

\*O.M: Organic matter. Control: Rice field with a history of herbicide application. Absolute control: Non-cropped area without a history of herbicide application

sodium and cyhalofop-butyl are widely used in rice tracts of Kerala. These chemicals are recommended at the rate of 1.5 g, 0.15 g, 25 g and 0.08 kg a.i.ha<sup>-1</sup> respectively (KAU, 2016). Hence the present investigation was carried out to determine the effect of the above four herbicides on microbial biomass carbon and dehydrogenase activity in soils with medium and high organic matter content.

The study was conducted in the year 2016-2017 at the Department of Soil Science and Agricultural Chemistry, College of Horticulture, Kerala Agricultural University. Representative soil samples were collected during March-April, 2016 from four sites in Thrissur district, Kerala, India viz., rice field having medium organic matter status with a history of herbicide application: S<sub>1</sub> Control, soil from non-cropped area having medium organic matter status and without a history of herbicide application: S<sub>1</sub> Absolute control, high organic matter soil with a history of herbicide application: S<sub>2</sub> Control, and high organic matter soil without history of herbicide application from non-cropped area: S<sub>2</sub> Absolute control.

Pot culture experiment with rice variety Jyothi was conducted in the *khari*f season of 2016 with twelve treatments and six replications in factorial CRD (four herbicides under two soil types + four controls). Pre emergence herbicides (pendimethalin and oxyfluorfen) were applied at six days after sowing (DAS) and post emergence herbicides (bispyribac-sodium and cyhalofop-butyl) at 16

DAS. Soil samples were analysed at six intervals viz., two hours before herbicide application and then at 7, 15, 30, and 60 days after herbicide spray and at harvest so as to evaluate the changes in microbial biomass carbon and dehydrogenase activity. Microbial biomass carbon was assayed as per the procedure described by Jenkinson and Powlson (1976) and dehydrogenase activity as per the procedure suggested by Casida et al. (1964).

Chemical characteristics of the soil viz., pH, organic carbon and available primary nutrients before the experiment are furnished in Table 1. The soil samples differed in their pH and the values ranged from 5.01 to 5.68. Organic carbon content varied from 0.85 to 2.47 per cent. Available nitrogen, phosphorus and potassium content were higher in Kole land soils due to high organic matter content. All the chemical characteristics were superior in high organic matter soils which favoured the growth of microflora.

The data on the initial biological characteristics of the soil samples viz., microbial biomass carbon and dehydrogenase activity are presented in Table 1. Rice soils of Kole lands recorded the highest microbial biomass carbon, dehydrogenase activity as well as organic carbon content (2.47%). Hojati and Nourbakhsh (2006) also found that microbial biomass carbon and dehydrogenase activity increased with soil organic carbon content.

Table 2. Effect of herbicide application on microbial biomass carbon

Treatments	0 DAHA			7DAHA			15DAHA			30DAHA			60 DAHA			Harvest		
	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean
Pendimethalin	168.10 <sup>d</sup>	453.10 <sup>a</sup>	310.60 <sup>b</sup>	131.00 <sup>b</sup>	422.38 <sup>c</sup>	276.69 <sup>c</sup>	128.00 <sup>b</sup>	425.28 <sup>b</sup>	276.64 <sup>b</sup>	107.45 <sup>b</sup>	412.18 <sup>b</sup>	259.82 <sup>cd</sup>	171.40 <sup>ab</sup>	454.35 <sup>c</sup>	312.88 <sup>d</sup>	91.30 <sup>b</sup>	356.43 <sup>c</sup>	223.87 <sup>d</sup>
Oxyfluorfen	168.10 <sup>d</sup>	453.83 <sup>a</sup>	310.97 <sup>a</sup>	143.03 <sup>b</sup>	436.07 <sup>b</sup>	289.55 <sup>b</sup>	129.33 <sup>b</sup>	429.40 <sup>ab</sup>	279.37 <sup>b</sup>	115.62 <sup>bc</sup>	417.75 <sup>ab</sup>	266.68 <sup>b</sup>	180.37 <sup>ab</sup>	458.47 <sup>c</sup>	319.42 <sup>c</sup>	109.30 <sup>ab</sup>	379.23 <sup>b</sup>	244.27 <sup>b</sup>
Cyhalofop- butyl	168.83 <sup>d</sup>	454.58 <sup>a</sup>	311.71 <sup>a</sup>	153.50 <sup>c</sup>	441.32 <sup>ab</sup>	297.41 <sup>a</sup>	132.00 <sup>b</sup>	430.20 <sup>ab</sup>	281.10 <sup>b</sup>	118.28 <sup>c</sup>	420.85 <sup>ab</sup>	269.56 <sup>b</sup>	185.98 <sup>c</sup>	467.00 <sup>b</sup>	326.49 <sup>b</sup>	117.43 <sup>a</sup>	383.33 <sup>b</sup>	250.38 <sup>b</sup>
Bispyribac-sodium	168.10 <sup>d</sup>	454.40 <sup>a</sup>	311.25 <sup>a</sup>	142.50 <sup>b</sup>	434.18 <sup>b</sup>	288.34 <sup>b</sup>	129.14 <sup>b</sup>	426.63 <sup>b</sup>	277.88 <sup>b</sup>	115.37 <sup>bc</sup>	414.58 <sup>b</sup>	264.98 <sup>bc</sup>	177.83 <sup>b</sup>	457.98 <sup>c</sup>	317.91 <sup>cd</sup>	99.44 <sup>ab</sup>	361.37 <sup>c</sup>	230.40 <sup>cd</sup>
Control	168.83 <sup>d</sup>	455.32 <sup>a</sup>	312.08 <sup>a</sup>	155.58 <sup>c</sup>	447.88 <sup>b</sup>	301.73 <sup>a</sup>	147.92 <sup>b</sup>	433.83 <sup>a</sup>	290.88 <sup>b</sup>	136.40 <sup>a</sup>	424.42 <sup>a</sup>	280.41 <sup>a</sup>	197.33 <sup>b</sup>	502.73 <sup>c</sup>	350.03 <sup>a</sup>	135.43 <sup>a</sup>	418.68 <sup>a</sup>	277.06 <sup>a</sup>
Absolute control	218.28 <sup>c</sup>	358.28 <sup>b</sup>	288.28 <sup>b</sup>	202.97 <sup>c</sup>	348.83 <sup>d</sup>	275.90 <sup>c</sup>	191.53 <sup>d</sup>	334.20 <sup>c</sup>	262.87 <sup>c</sup>	186.67 <sup>d</sup>	322.18 <sup>c</sup>	254.42 <sup>d</sup>	234.47 <sup>c</sup>	369.53 <sup>d</sup>	302.00 <sup>c</sup>	175.10 <sup>c</sup>	293.07 <sup>d</sup>	234.09 <sup>c</sup>
Mean	176.71 <sup>b</sup>	438.25 <sup>a</sup>	311.25 <sup>a</sup>	154.76 <sup>b</sup>	421.78 <sup>a</sup>	288.34 <sup>b</sup>	142.99 <sup>b</sup>	413.26 <sup>a</sup>	288.34 <sup>b</sup>	129.96 <sup>b</sup>	401.99 <sup>a</sup>	264.98 <sup>bc</sup>	191.23 <sup>b</sup>	451.68 <sup>a</sup>	302.00 <sup>c</sup>	121.33 <sup>b</sup>	365.35 <sup>a</sup>	234.09 <sup>c</sup>
CD (0.05)	4.23*	7.33**	5.14	2.72	7.27	6.67	4.71	3.92	6.79	9.60	8.06	5.70	3.29	5.70	5.03	5.03	8.71	12.32
	10.37***																	

Table 3. Percentage reduction in microbial biomass carbon with respect to control at different days after herbicide application

Treatments	0DAHA			7DAHA			15DAHA			30DAHA			60DAHA			Harvest		
	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean
Pendimethalin	0.43	0.49	15.80	5.69	1.97	2.88	13.46	1.97	21.22	2.88	13.14	9.62	32.59	14.87				
Oxyfluorfen	0.43	0.33	8.07	2.64	1.02	1.57	12.56	1.02	15.23	1.57	8.60	8.80	19.30	9.42				
Cyhalofop- butyl	0.00	0.16	1.34	1.47	0.84	1.32	10.76	0.84	13.29	0.84	5.75	7.11	13.29	8.44				
Bispyribac-sodium	0.43	0.20	8.41	3.06	1.66	2.32	12.70	1.66	15.42	2.32	9.88	8.90	26.58	13.69				

CD (0.05)\* : CD for comparing the soil types CD (0.05)\*\* : CD for comparing the treatments CD (0.05)\*\*\*:CD for comparing the interaction

MBC: Microbial biomass carbon in control MBC T: Microbial biomass carbon in herbicide treatments S<sub>1</sub>: Medium O.M. soil S<sub>2</sub>: High O.M. soil

Table 4. Effect of herbicide application on dehydrogenase activity at different days after herbicide application (DAHA)

Treatments	0 DAHA			7DAHA			15DAHA			30DAHA			60 DAHA			Harvest		
	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	Mean
Pendimethalin	61.66 <sup>d</sup>	89.65 <sup>a</sup>	75.66 <sup>a</sup>	63.38 <sup>c</sup>	95.27 <sup>a</sup>	79.33	61.73 <sup>c</sup>	94.21 <sup>c</sup>	77.97 <sup>c</sup>	67.32 <sup>c</sup>	102.56 <sup>c</sup>	84.94 <sup>c</sup>	84.07 <sup>c</sup>	124.69 <sup>b</sup>	104.38 <sup>c</sup>	60.52 <sup>d</sup>	89.72 <sup>a</sup>	75.12
Oxyfluorfen	61.65 <sup>d</sup>	89.67 <sup>a</sup>	75.66 <sup>a</sup>	64.53 <sup>de</sup>	95.58 <sup>a</sup>	80.06	62.54 <sup>c</sup>	94.48 <sup>b</sup>	78.51 <sup>c</sup>	68.15 <sup>c</sup>	103.36 <sup>c</sup>	85.76 <sup>bc</sup>	87.82 <sup>c</sup>	128.14 <sup>b</sup>	107.98 <sup>ab</sup>	61.81 <sup>d</sup>	90.09 <sup>a</sup>	75.95
Cyhalofop butyl	61.67 <sup>d</sup>	89.66 <sup>a</sup>	75.67 <sup>a</sup>	65.37 <sup>d</sup>	95.61 <sup>a</sup>	80.49	63.05 <sup>c</sup>	95.10 <sup>b</sup>	79.08 <sup>c</sup>	69.82 <sup>c</sup>	103.41 <sup>c</sup>	86.62 <sup>b</sup>	88.01 <sup>c</sup>	128.53 <sup>a</sup>	108.27 <sup>a</sup>	62.34 <sup>d</sup>	90.89 <sup>a</sup>	76.62
Bispyribac sodium	61.66 <sup>d</sup>	89.67 <sup>a</sup>	75.67 <sup>a</sup>	64.21 <sup>de</sup>	95.57 <sup>a</sup>	79.89	62.00 <sup>c</sup>	94.37 <sup>c</sup>	78.19 <sup>c</sup>	68.04 <sup>c</sup>	103.33 <sup>c</sup>	85.68 <sup>c</sup>	86.53 <sup>c</sup>	126.84 <sup>b</sup>	106.69 <sup>b</sup>	61.65 <sup>d</sup>	89.83 <sup>a</sup>	75.74
Control	61.65 <sup>d</sup>	89.68 <sup>a</sup>	75.67 <sup>a</sup>	65.48 <sup>d</sup>	95.78 <sup>a</sup>	80.63	67.86 <sup>c</sup>	98.97 <sup>b</sup>	83.42 <sup>b</sup>	70.85 <sup>d</sup>	103.53 <sup>a</sup>	87.19 <sup>ab</sup>	88.36 <sup>c</sup>	128.82 <sup>b</sup>	108.59 <sup>a</sup>	63.06 <sup>d</sup>	91.83 <sup>a</sup>	77.45
Absolute control	65.74 <sup>c</sup>	82.31 <sup>b</sup>	74.03 <sup>b</sup>	69.26 <sup>c</sup>	90.12 <sup>b</sup>	79.69	76.18 <sup>c</sup>	96.59 <sup>b</sup>	86.39 <sup>a</sup>	78.01 <sup>c</sup>	97.92 <sup>b</sup>	87.97 <sup>a</sup>	96.27 <sup>d</sup>	121.23 <sup>c</sup>	108.75 <sup>a</sup>	67.31 <sup>c</sup>	85.43 <sup>b</sup>	76.37
Mean	62.34 <sup>b</sup>	88.44 <sup>a</sup>	75.67 <sup>a</sup>	65.37 <sup>b</sup>	94.65 <sup>a</sup>	80.06	65.56 <sup>b</sup>	95.62 <sup>a</sup>	79.69	70.36 <sup>b</sup>	102.35 <sup>a</sup>	87.19 <sup>ab</sup>	88.51 <sup>b</sup>	126.38 <sup>a</sup>	108.75 <sup>a</sup>	62.78 <sup>b</sup>	89.63 <sup>a</sup>	76.37
CD (0.05)	0.31*	0.53**	0.60	0.60	1.47	1.55	0.90	1.55	1.32	0.54	0.93	1.45	0.83	1.45	1.30	1.30	3.18	NS
	0.75***																	

Dehydrogenase activity (µg TPF g<sup>-1</sup>day<sup>-1</sup>)

Table 5. Percentage reduction in dehydrogenase activity with respect to control at different days after herbicide application

Treatments	Reduction in dehydrogenase activity (%) = $(\text{DH}_c - \text{DH}_t / \text{DH}_c) \times 100$											
	0DAHA		7DAHA		15DAHA		30DAHA		60DAHA		Harvest	
	S <sub>1</sub>	S <sub>2</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>1</sub>	S <sub>2</sub>
Pendimethalin	0.00	0.00	3.21	0.53	9.03	4.81	4.98	0.94	4.86	3.21	4.03	2.30
Oxyfluorfen	0.00	0.00	1.45	0.21	7.84	4.54	3.81	0.16	0.61	0.53	1.99	1.89
Cyhalofop butyl	0.00	0.00	0.17	0.18	7.09	3.91	1.45	0.12	0.40	0.23	1.14	1.02
Bispyribac sodium	0.00	0.00	1.94	0.22	8.64	4.65	3.97	0.19	2.07	1.54	2.24	2.18

CD (0.05)\* : CD for comparing the soil types CD (0.05)\*\* : CD for comparing the treatments CD (0.05)\*\*\*:CD for comparing the interaction  
 DH C: Dehydrogenase activity in control DH T: Dehydrogenase activity in herbicide treatments S<sub>1</sub>: Medium O.M. soil S<sub>2</sub>: High O.M. soil

Microbial biomass carbon (MBC) showed a decreasing trend upto 30 days after herbicide application (DAHA) and thereafter a drastic increase (Table 2) at 60 DAHA in all the interactions studied. This might be due to rhizosphere effect which augmented the native microflora of the root system, as 60 DAHA coincided with the panicle initiation stage. At harvest, microbial biomass carbon decreased probably due to the decreased moisture status of the soil and decline in root activity at late maturity stage of rice. At the harvest stage of rice, a drastic decline in microbial biomass was also reported by Aparna (2000). Microbial biomass carbon was significantly higher in soils having high organic matter content.

The extent of decline in microbial biomass carbon was highest at harvest followed by 30 DAHA. At thirty days after herbicide application and at harvest, reduction in microbial biomass carbon varied from 0.84 to 21.22 per cent and 8.44 to 32.59 per cent respectively (Table 3). Among the herbicide treatments, maximum reduction in MBC was observed in pendimethalin treatment followed by bispyribac-sodium, oxyfluorfen, and cyhalofop-butyl. Mammalian toxicity (oral LD<sub>50</sub>) and persistence of the herbicides in the soil also followed the same order; pendimethalin > bispyribac-sodium > oxyfluorfen > cyhalofop-butyl (RSC, 1987).

Percentage reduction was comparatively lower in high organic matter soils throughout the crop period and the same could be attributed to the buffering action of organic matter. Among the two pre emergence herbicides, adverse effect on microbial biomass carbon was more for pendimethalin than oxyfluorfen. In the case of post emergence herbicides, bispyribac-sodium had more impact on microbial biomass carbon compared to cyhalofop-butyl.

Data on the effects of herbicide application on dehydrogenase activity revealed that it increased upto 60 DAHA with slight variations and declined thereafter registering a peak at 60 DAHA (Table 4). Dehydrogenase activity at 15 DAHA was comparatively lower than activity at seven days after herbicide application in all herbicide treatments. This might be due to the effect of herbicide application on the activity of this intracellular enzyme. Lower activity of dehydrogenase at harvest stage of rice may be due to decline in rhizosphere activity and organic carbon content coupled with dry condition prevailing at harvest of rice. Metabolism and survival of the soil microorganisms are affected by the soil moisture availability (Uhlirova et al., 2005).

High organic matter soils recorded the highest dehydrogenase activity compared to medium organic matter soils. This might be due to enough substrate provided by the soil to support higher microbial biomass, and higher enzyme production. The findings are in close agreement with Yuan and Yue, 2012.

In the pendimethalin treatment, the percentage reduction in dehydrogenase activity at 15 DAHA was 9.03 and 4.81 per cent for S<sub>1</sub> and S<sub>2</sub> soils (Table 5). In cyhalofop-

butyl treatment, the corresponding changes were 7.09 and 3.91 per cent respectively. At harvest, the extent of decline in pendimethalin treatment reduced to 4.03 and 2.30 per cent for S<sub>1</sub> and S<sub>2</sub> soils respectively. Corresponding figures for cyhalofop-butyl treatment were 1.14 and 1.02 per cent respectively. Pendimethalin spray resulted in reduction of enzyme activity probably due to its acute toxicity and long half-life in soil. The oral LD<sub>50</sub> of pendimethalin is 4050 mg kg<sup>-1</sup> and its persistence in soil is three to four months (RSC, 1987). Among the selected herbicides, pendimethalin showed maximum reduction in dehydrogenase activity with respect to control followed by bispyribac-sodium, oxyfluorfen, and cyhalofop-butyl in decreasing order.

Microbial biomass carbon was highly sensitive to herbicide application compared to dehydrogenase activity. Application of pendimethalin exerted adverse effect on microbial biomass carbon and dehydrogenase activity followed by bispyribac-sodium, oxyfluorfen, and cyhalofop-butyl at all the intervals. The adverse effects of herbicides on biological activity were minimal in high organic matter soils.

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