



RICE STUBBLE DECOMPOSITION BY CELLULOSE DEGRADING MICROBE AND YOGURT WITH GLYPHOSATE UNDER RAINFED UPLAND ECOSYSTEM

BORAH NILAY^{*1}, BARUA R.¹, PATHAK P.K.², BARUA I.C.³, HAZARIKA K.⁴ AND PHUKAN A.¹

¹Department of Soil Science, Assam Agricultural University, Jorhat-13, Assam, India

²Directorate of Research (Agri), AAU, Jorhat-13, Assam, India.

³DWR Centre, Department of Agronomy, AAU, Jorhat-13, Assam, India

⁴ICR Farm, AAU, Jorhat-13, Assam, India

*Corresponding Author: Email-nilayborah@rediffmail.com

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Abstract- The residues of crop and weeds in rainfed upland rice ecosystem need rapid, viable utilization before establishment of the succeeding crop. Enhanced decomposition reducing the volume and C:N ratio of the biomass may be a good proposition towards efficient residue management facilitating tillage and decomposition. Cellulose degrading microbe (CDM) or commercial yogurt was evaluated with glyphosate for *in situ* decomposition of rice stubbles after harvest. A field experiment was conducted by spraying glyphosate, and CDM or yogurt with or without sugar on rice stubbles after harvest of the crop. Glyphosate and, CDM or yogurt significantly reduced the dry weight, carbon and nitrogen content of stubbles at 25, 50 and 75 days after spray compared to untreated plot or spraying glyphosate and bacterial cultures separately, and the corresponding organic carbon content and C:N ratio of the stubble reduced significantly. The effect of adding sugar with the spray solution was not significant. The species diversity of the weeds for grass and sedge was unaffected by the treatments, but number of broadleaved weed species significantly increased due to spraying of glyphosate and, CDM or yogurt.

Keywords- Cellulose degrading microbe, Yogurt, rice stubble, Glyphosate, Upland rice, Rainfed.

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Introduction

Rainfed upland rice constitutes about little more than one tenth of the total rice growing areas of India [1]. Rice-based cropping system under rainfed upland situation is an important contributor to the livelihood of the farming community with small to medium holding of marginal land in Asia [2] and many parts of Africa [3]. Increased cropping intensity without appropriate nutrient management may reduce nutrient availability in rainfed rice soils [3], which rely on crop residues recycling for replenishment of carbon lost due to cropping [4, 5]. Incorporation of crop residues and weeds as such is not a suitable option owing to high stubble C:N ratio [6], short time left for the succeeding crop, and other undesirable effects. Rapid *in situ* aerobic method of composting involves energy consuming processes of periodical moisture spraying and materials mixing [7]. Incorporation or mulching of fallow season residues provided no significant yield advantage as compared to burning in upland rice [8], while incorporation with N-fertilizer before sowing of succeeding wheat crop caused highest loss of fertilizer nitrogen [9].

The hurdles of decomposing cellulose-rich compounds may be overcome through consolidated bio-processing (CBP), where cellulose degrading microbes or their consortium could convert the biomass into desired products in a single process step [10]. Use of microbial consortium through sequential or combined application during co-composting [11] and effort to characterize their maturity index [12] had been reported. But, the methods do not suit the preference and need of the resource poor farm units. Several species of *Lactobacillus* are reported for their ability to degrade cellulose or rice straw [13, 14]. Isolation of nitrogen fixing bacteria from various plant parts and their presence in cells and intercellular spaces in different parts of rice had been reported [15]. However, survival and function of the microorganisms remain to be a question as host defense mechanism was cited as a reason for declining colonies of *Herbaspirillum*

seropedicae Z67 in artificially inoculated rice seedlings [16]. Pre-harvest application of glyphosate, a non-selective herbicide, was reported to cause desiccation of wheat [17] and offers scope for use in rice stubbles which maintain green stems even after the crop harvest. Combined use of sugar cane molasses had been shown to provide the mineral matters to the applied microorganisms [18]. Accordingly, the present work was carried out to evaluate the efficacy of bacterial strains and commercial yogurt singly or with glyphosate or with glyphosate and sugar for *in situ* decomposition of rice stubbles under upland rice ecosystem.

Materials and Methods

Location and climate

The experiment was conducted during July to September, 2015 at Instructional cum Research (ICR) Farm of Assam Agricultural University (26°44'N, 94°10'E and 91 m above MSL), Jorhat, India. The soil of the experimental site had sandy loam texture with pH 5.8, and organic carbon 7.8 g/kg. The daily temperature of Jorhat during July to September remains high with an average maximum temperature of 32°C in August and with an average minimum temperature of 18°C in July. The monthly rainfall and number of rainy days during July and August are above 400 mm and 20 days, respectively, and in the month of September receives about little more than 300 mm rainfall in less than 20 rainy days. The average relative humidity in Jorhat during July to September remains at around 85%.

Treatments and experimental design

Twelve treatments were imposed to individual plots of size 5 m x 4 m after harvest (5th July 2015) of upland direct seeded rice (variety – Inglongkiri). Freshly prepared broth of an unidentified cellulose degrading microbe containing 10⁶ to 10⁷ effective colony

forming unit (cfu) per ml, and yogurt available in local market were used for spray. The cellulose degrading microbe (CDM) strain was isolated through Dubo's cellulose enrichment technique and morphological and functional characterization for carbohydrate utilization and enzyme activities were done following standard protocol [19]. The treatments comprised of spraying either 1 ml or 2 ml broth of CDM or 5 g or 10 g of yogurt per litre of water, or of water containing 2.05 g a.i. glyphosate [chemical name - N-(phosphonomethyl) glycine, chemical formula - $C_3H_8NO_5P$], or of water containing 2.05 g a.i. glyphosate and 5 g sugar. Two plots of identical area were also sprayed with either water or water containing 2.05 g a.i. glyphosate for comparison. The spray was done on 10-07-2015 using a manual operated knapsack sprayer fitted with hollow cone nozzle, with a spray volume of 600 litre per hectare. The treatments were allocated to plots in a randomized block design with three replications. Observations were recorded periodically in respective plots and the data were analyzed statistically for interpretation.

Rice stubble dry weight

A 1m x 1m quadrat was placed randomly in each plot and the stubbles were collected before imposing the treatments and at twenty five days interval after spray. The stubbles were cleared of weeds and soil particles and allowed to dry in a hot air oven at $55 \pm 1^\circ\text{C}$ till constant weight. The dry weight of the stubbles was recorded and expressed as gram per square metre.

Organic carbon content of rice stubble

After recording the dry weight of rice stubbles, grinding was done using an electrical grinding machine and the sample was stored in polythene bags for analysis of organic carbon content. The sample was homogenized by mixing several times before taking weight. Three sub samples of 500 mg each were drawn from respective sample and the organic carbon content was determined by wet digestion method [20]. Mean values of the three estimations was worked out and taken as the organic carbon content of rice stubble for that treatment under specific replication.

Diversity of weed species

The number of species under each category of grasses, broadleaved and sedges was counted for the whole plot at 75 days after treatment.

Results and Discussion

Rainfall and Temperature

The weekly rainfall and maximum and minimum temperature during the period of study are presented in [Fig-1]. The weekly rainfall increased from the 27th week (8th July 2015) up to 31st week (5th August 2015) with a total precipitation of 455.9 mm. Thereafter, the weekly rainfall decreased substantially during 6th August to 26th August 2015 (211.8 mm), and then slightly increased during 2nd September to 23rd September 2015 (239.2 mm). The average weekly maximum temperature during the period (9th July to 23rd September 2015) fluctuated between 30.2 to 34.4°C , and the minimum temperature was 24.6 to 25.9°C [Fig-1].

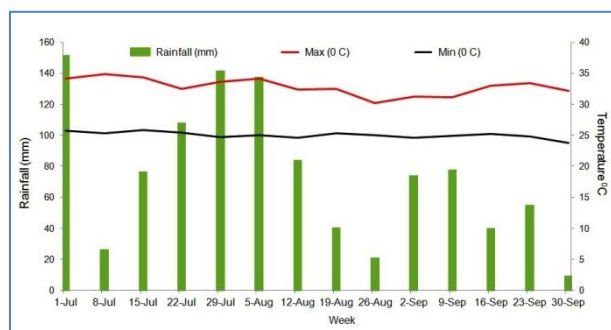


Fig-1 Weekly maximum and minimum temperature and rainfall during the study period

pH of the spray solution

The pH of the spray solution significantly decreased due to mixing with glyphosate 2.05 g a.i. per litre of water [Table-1]. Addition of CDM, or yogurt or sugar had no significant effect on pH of the spray solution. Acidification was reported to increase the

degradability of rice straw as ruminant feed [21]. Dilute acid pre-treatment causes some degree of depolymerization in cellulose [10], which might be reason for observed decomposition effect for spraying CDM or yogurt with glyphosate that reduced the pH of the spray solution. Different species of *Lactobacillus* isolated from yogurt showed growth ability in a wide range of pH from 4 to 8, with best results at pH 6 [22, 23] and low pH was not a critical factor dictating their survival [24].

Table-1 Spray solution pH just after mixing

Treatment	pH
Water	6.4
Glyphosate 2.05 g/litre	4.9
Water + CDM 1 ml/litre	6.4
Glyphosate 2.05 g + CDM 1 ml/litre	5.0
Glyphosate 2.05 g + CDM 1 ml + sugar 5 g/litre	5.0
Water + Yoghurt 5 g/litre	6.2
Glyphosate 2.05 g + Yoghurt 5 g/litre	4.9
Glyphosate 2.05 g + Yoghurt 5 g + sugar 5 g/litre	5.1
Water + CDM 2 ml/litre	6.4
Glyphosate 2.05 g + CDM 2 ml/litre	5.2
Glyphosate 2.05 g + CDM 2 ml + sugar 5 g/litre	5.1
Water + Yoghurt 10 g/litre	6.0
Glyphosate 2.05 g + Yoghurt 10 g/litre	5.2
Glyphosate 2.05 g + Yoghurt 10 g + sugar 5 g/litre	4.9
CD (P=0.05)	0.5
CV (%)	5.4

Dry weight and organic carbon content of rice stubble

The rice stubble dry weight significantly decreased due to spraying of glyphosate, and CDM or yogurt at 25, 50 and 75 days after treatment [Table-2]. Application of CDM or yogurt without herbicide had no effect on dry weight of the rice stubbles, irrespective of observation dates. Use of increased volume of broth (CDM) or mass of yogurt in the spray solution did not affect the stubble dry weight. The per cent decrease in stubble dry weight was highest during 25 to 50 days after spray in all the treatments [Table-3]. The relative decrease in stubble dry weight was significantly higher in glyphosate with CDM or, with yogurt with or without sugar compared to glyphosate or CDM or yogurt alone, or untreated plot at 0-25, 25-50, 50-75, and 0-75 days after spray. However, the difference in per cent decrease in stubble dry weight between CDM and yogurt with or without sugar was statistically not significant.

Table-2 Stubble dry weight (g/m^2) at different days after spray

Treatment	Days after spray			
	0	25	50	75
Untreated	255	238	213	192
Glyphosate 2.05 g/l	251	229	193	173
Glyphosate 2.05 g + CDM 1 ml/l	252	187	90	67
Glyphosate 2.05 g + CDM 1 ml + sugar 5 g/l	271	200	98	73
Glyphosate 2.05 g + yogurt 5 g/l	237	185	100	74
Glyphosate 2.05 g + yogurt 5 g + sugar 5 g/l	250	193	104	70
Glyphosate 2.05 g + CDM 2 ml/l	247	183	98	66
Glyphosate 2.05 g + CDM 2 ml + sugar 5 g/l	253	194	93	66
Glyphosate 2.05 g + yogurt 10 g/l	258	200	103	68
Glyphosate 2.05 g + yogurt 10 g + sugar 5 g/l	248	197	100	74
CDM broth 1 ml/l	274	247	210	187
Yogurt 5 g/l	256	239	213	182
CD (P=0.05)	NS	28	16	20
CV (%)	10	8	7	11

The organic carbon content of the stubble decreased up to 75 days after spray in all the treatments [Table-4]. The lowest organic carbon content in stubble (15.8%) was observed due to spraying of glyphosate with CDM broth 2 ml/l. the organic carbon content of the stubble significantly differed in treatments receiving spray of glyphosate with CDM or, with yogurt, irrespective of addition of sugar to the solution mixture. The decrease in rice stubble dry weight and corresponding reduction in carbon content may be attributed to the microbial decomposition following their effective inoculation through spraying with glyphosate-water solution, compared to untreated, or spraying with water alone. Rice stubble contains high content of cellulose and hemicellulose and served as a substrate for the CDM or bacteria in yogurt. However, the effect was not observed through spraying without glyphosate. The formation of complexes between

glyphosate and the Ca^{2+} ion [25, 26] reduced the polarity of the complex that enhanced its absorption through the plasmalemma [27]. While natural yogurt is a rich source of calcium [28, 29], sodium chloride was used for preparation of CDM broth and the presence of Na^+ might have brought about similar effect when microbes were sprayed with glyphosate solution than with water alone. The relatively lower reduction in stubble dry weight during 0 to 25 days after spray compared to 25 to 50 days after spray may be attributed to corresponding higher rainfall received. Faster decline in population of *Lactobacillus acidophilus* Ki in the first 60 days of storage at 22 °C followed by a much smoother pattern up to 180 days was reported [30], which was affected by an increase in humidity up to 45% [30, 31]. On the other hand, the difference between the relative reductions in stubble dry weight during 50 to 75 days after spray, compared to 25 to 50 days after spray, both periods receiving comparable rainfall, might be due to different reason.

Table-3 Relative decrease in stubble dry weight at different days after spray

Treatment	Days after spray			
	0-25%	25-50	50-75	0-75
Untreated	6.4	10.6	9.8	24.5
Glyphosate 2.05 g/l	25.6	51.7	25.6	73.3
Glyphosate 2.05 g + CDM 1 ml/l	26.0	50.8	24.9	72.6
Glyphosate 2.05 g + CDM 1 ml + sugar 5 g/l	21.9	45.7	26.0	68.6
Glyphosate 2.05 g + yogurt 5 g/l	22.5	46.3	32.2	71.8
Glyphosate 2.05 g + yogurt 5 g + sugar 5 g/l	8.7	15.7	10.5	31.1
Glyphosate 2.05 g + CDM 2 ml/l	25.1	46.4	32.8	73.1
Glyphosate 2.05 g + CDM 2 ml + sugar 5 g/l	23.0	52.1	29.4	74.0
Glyphosate 2.05 g + yogurt 10 g/l	22.2	48.4	33.9	73.3
Glyphosate 2.05 g + yogurt 10 g + sugar 5 g/l	20.6	49.3	25.5	70.0
CDM broth 1 ml/l	9.8	14.8	11.0	31.5
Yogurt 5 g/l	6.3	11.1	14.4	28.7
CD (P=0.05)	5.6	6.3	6.1	8.1
CV (%)	18.2	10.0	16.0	8.3

Table-4 Stubble organic carbon content (%) at different days after spray

Treatment	Days after spray			
	0	25	50	75
Untreated	46.2	45.6	44.8	43.2
Glyphosate 2.05 g/l	44.7	36.3	30.3	16.8
Glyphosate 2.05 g + CDM 1 ml/l	43.5	35.2	28.7	17.3
Glyphosate 2.05 g + CDM 1 ml + sugar 5 g/l	44.6	35.8	29.6	19.0
Glyphosate 2.05 g + yogurt 5 g/l	43.6	34.4	29.0	16.5
Glyphosate 2.05 g + yogurt 5 g + sugar 5 g/l	45.5	43.7	40.8	39.3
Glyphosate 2.05 g + CDM 2 ml/l	43.1	32.6	27.2	15.8
Glyphosate 2.05 g + CDM 2 ml + sugar 5 g/l	44.1	35.5	28.3	16.5
Glyphosate 2.05 g + yogurt 10 g/l	41.2	34.1	31.2	17.6
Glyphosate 2.05 g + yogurt 10 g + sugar 5 g/l	43.6	35.2	30.7	16.5
CDM broth 1 ml/l	43.0	42.9	41.0	40.3
Yogurt 5 g/l	45.6	44.1	43.0	42.5
CD	NS	6.4	4.0	4.0
CV (%)	8.2	10.0	7.1	9.3

C:N ratio of stubble

The C:N ratio of the stubble decreased up to 75 days after spray, except in untreated plot [Table-5], and that with application of spray solution mixture of glyphosate and bacteria with or without sugar ranged between 29 to 34 % at 75 days after spray. The relative decrease in C:N ratio of stubble was higher during 50 to 75 days after spray. During this period (50 to 75 days after spray), there was highest reduction in C:N ratio of the stubbles compared to 0 to 25 or 25 to 50 days after spray, which might be due to mineralization of simpler carbon compounds emanating from degradation of cellulose. Lower C:N ratio of manure enhanced greater reduction of organic carbon in rice straw in high speed composting process [32]. The decrease in stubble dry weight and decline in stubble organic carbon content was higher than that observed during winter experiment with CDM [33]. This might be due to relatively high temperature and rainfall recorded during the present study, compared to those in winter experiment. An initial substrate moisture content of about 72% was reported optimum for lactic acid production by *Lactobacillus casei* and *Lactobacillus delbrueckii* [34, 35, 36], beyond which a decrease was reported. Decrease in C:N ratio of rice straw inoculated with microbe was earlier reported and the effect was more pronounced in wet than dry season [37].

Table-5 Stubble C:N ratio at different days after spray

Treatment	Days after spray			
	0	25	50	75
Untreated	70.7	71.7	70.4	81.0
Glyphosate 2.05 g/l	69.9	58.0	48.5	29.2
Glyphosate 2.05 g + CDM 1 ml/l	71.4	57.1	46.6	29.9
Glyphosate 2.05 g + CDM 1 ml + sugar 5 g/l	71.2	58.6	49.7	34.5
Glyphosate 2.05 g + yogurt 5 g/l	66.3	56.1	47.6	30.1
Glyphosate 2.05 g + yogurt 5 g + sugar 5 g/l	73.8	72.1	67.7	71.7
Glyphosate 2.05 g + CDM 2 ml/l	70.6	51.6	43.1	27.3
Glyphosate 2.05 g + CDM 2 ml + sugar 5 g/l	71.1	57.4	45.3	28.9
Glyphosate 2.05 g + yogurt 10 g/l	65.4	56.4	51.7	32.0
Glyphosate 2.05 g + yogurt 10 g + sugar 5 g/l	69.0	56.5	49.3	30.3
CDM broth 1 ml/l	70.1	69.4	66.1	74.6
Yogurt 5 g/l	69.6	72.7	68.7	77.1
CD (P=0.05)	NS	10.6	9.0	11.3
CV (%)	12.5	10.2	9.7	14.7

Weed species diversity

The diversity of weeds in terms of grass, broadleaved weed (BLW) and sedge in different treatments is shown in [Table-6]. The number of BLW species and total number of weed species significantly increased at 75 days after spraying of glyphosate and bacteria (CDM or yogurt).

Table-6 Species diversity of weeds at 75 days after spray

Treatments	Grass	BLW	Sedge	Total
	Number* of species/plot			
Untreated	1.34 (1.3)	2.04(3.7)	1.05(0.7)	2.48(5.7)
Glyphosate 2.05 g/l	1.22(1.0)	1.95(3.3)	0.71(0.0)	2.20(4.3)
Glyphosate 2.05 g + CDM 1 ml/l	1.34(1.3)	2.48(5.7)	1.05(0.7)	2.86(7.7)
Glyphosate 2.05 g + CDM 1 ml + sugar 5 g/l	1.34(1.3)	2.41(5.3)	1.05(0.7)	2.79(7.3)
Glyphosate 2.05 g + yogurt 5 g/l	1.46(1.7)	2.41(5.3)	1.34(1.3)	2.96(8.3)
Glyphosate 2.05 g + yogurt 5 g + sugar 5 g/l	1.34(1.3)	2.34(5.0)	1.22(1.0)	2.80(7.3)
Glyphosate 2.05 g + CDM 2 ml/l	1.34(1.3)	2.48(5.7)	1.05(0.7)	2.86(7.7)
Glyphosate 2.05 g + CDM 2 ml + sugar 5 g/l	1.46(1.7)	2.41(5.3)	1.05(0.7)	2.85(7.7)
Glyphosate 2.05 g + yogurt 10 g/l	1.34(1.3)	2.35(5.0)	1.22(1.0)	2.80(7.3)
Glyphosate 2.05 g + yogurt 10 g + sugar 5 g/l	1.34(1.3)	1.87(5.0)	1.22(1.0)	2.80(7.3)
CDM broth 1 ml/l	1.34(1.3)	1.95(3.3)	1.05(0.7)	2.41(5.3)
Yogurt 5 g/l	1.34(1.3)	1.87(3.0)	1.05(0.7)	2.34(5.0)
CD	NS	0.26	NS	0.29
CV (%)	15.2	7.0	20.0	6.4

*Square root transformed data [$\sqrt{x+0.5}$], observed value (x)s are in parentheses

Application of glyphosate reduced weed growth during first one and half month of the spray in comparison to plots without treatment or with only water and microbial culture spray. The regeneration of weeds differed significantly in relation to broadleaved weed (BLW) species in glyphosate treated plots. In case of *Eunymus americanus* L. seeds, cellulase activity of cellulolytic ruminal bacterium *Clostridium cellobioparum* was indicated in the degradation of the testa of the seed, allowing imbibition and germination [38], while pre-treatment with cellulase increased seed germination in *Terminalia ivorensis* [39]. It is beyond the scope of this study to explain the observed differences and further study is required to examine the possible effects of decomposition and/or nutrient mineralization on weed seed germination.

Conclusion

Spraying of CDM culture or yogurt with glyphosate enhanced degradation of left over rice stubble under field condition, in terms of decrease in stubble dry weight and C:N ratio. The results need further verification through assessment and monitoring of additional parameters like cellulose content, cellulase and other relevant enzyme activity, and effects on succeeding crop.

Conflict of Interest: None declared

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