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SHORT COMMUNICATION

Microbial decomposer assisted *in situ* degradation of surface retained paddy straw

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Abstract

Purpose Paddy (rice) straw burning is an alarming issue all over India because of its huge volume generated every year. Combine harvesting results in generation of about 5-7 ton/hectare paddy straw residue that needs to be managed with in a small period (3 weeks) before sowing of wheat crop.

Method Six microbial decomposer preparations including four commercial (C-1 to C-4) and two in house culture (*Delftia*, Microbial consortium) have been evaluated at four different sites of Central Plain Zone of Punjab during 2018-19 and 2019-20. The microbial formulations were sprayed on the surface retained paddy straw and straw samples were evaluated after 30 days of spray for percent decrease in lignin and C/N ratio.

Result A location and decomposer specific decrease in lignin (%) and C/N ratio during paddy straw surface retention trials was observed, without any significant change in wheat yield. In control (without spray), percent decrease in lignin was in the range of 2.11 to 13.57. Whereas, with microbial sprays, it varies from 7.51 to 35.79. At Ludhiana, maximum decrease in C/N ratio was obtained by C-1 and C-2 (55%), While, at Kapurthala maximum decrease in C/N ratio was obtained by *Delftia* (71.25%) followed by C-3 (62.62%).

Conclusion The variable trend in delignification as well as C/N ratio during surface retained paddy straw trials, highlights that with microbial decomposer application the surface retained paddy straw is not appreciably decomposed in the provided window period of 3 weeks between paddy harvesting and wheat sowing.

Keywords C/N ratio, *In situ* degradation, Lignin, Decomposer microbes, Paddy straw

Introduction

Paddy straw burning is adopted by several farmers to manage its huge volumes as a very short window period of 2-3 weeks is available between paddy harvesting and sowing of wheatin the country.However, it leads to severe air pollution affecting climate and human health. Burning of oneton of rice straw leads to loss of 5.5 Kg N, 2.3 Kg P and 25 Kg K alongwith 9-11 Kg S, 100 g Zn, 777 g Fe and 745 g Mn (Gadi et al. 2003; Singh and

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Sidhu 2014). Some studies have reported enhancement in organic carbon and nutrient content of the soil due to *in situ* treatment of rice straw with straw degrading bacterial and fungal inoculations (Dobermann and Fairhurst 2002, Sannathimmappa et al. 2015; Jin et al. 2020; Kumar et al. 2022). The C/N ratio is an important factor to determine the decomposition of straw and the appropriate C/N ratio for soil microorganisms is 25-30:1 (Kochsick and Knops 2013). The fresh rice straw has high C/N ratio (Approx 75:1) and hence an appropriate fertilizer application is required for soil microbes to act on straw (Jin et al. 2020).

Thus, by carrying out the *in-situ* degradation, the organic matter content per hectare can be increased with simultaneous increase in mineral content of the soil.In fact, soil microorganisms form a consortium of bacteria (70-90%), fungi (3-5%) and actinomycetes (5-7%) that act coherently to degrade residual organic matter like straw whereby bacteria mostly degrade cellulose, fungi are responsible for overall straw decomposition and actinomycetes hydrolyze lignin (Jin et al. 2020).

Paddy straw is a type of lignocellulosic material which contains 29-35% cellulose, 23-29% hemicellulose and 17-24% lignin (Lim et al. 2012; Isikgor et al. 2015). Degradation of straw by the plethora of lignocellulolytic enzymes released by such microorganisms can release nutrients from straw and in turn make the straw amorphous to ease the farm operations after the intervening window period.Surface retained straw as a mulch also has added advantages like retaining moisture and reducing the population of weed in the field. Unfortunately, after paddyharvesting around October in North India, there is a fall in temperature that slows down the rate of microbial degradation of straw. Therefore,it is imminent to develop a consortium of efficient lignocellulolyticpsychrotrophic microbes (*Eupenicillium crustaceum*, *Paceliomyces* sp., *Bacillus atropheus* and *Bacillus* sp.) and commercial fungal consortia (*Aspergillus awamori*, *Aspergillus nidulans*, *Trichoderma viride* and *Phanerochaete chrysosporium*) to achieve timely degradation of straw (Shukla et al. 2016).

Keeping this in view, research trials were initiated in the year 2015-2016 at Punjab Agricultural University Campus, Ludhiana by using different decomposer bacterial and fungal cultures in axenic and co-culture conditions.One of the bacterial culture identified as *Delftia* has been tried along with fungal cultures like *Aspergillusterreus*and *Trichoderma harzianum* and other in-house potential cultures as well as commercial formulations. Earlier , six microbial preparations including four commercial (C-1 to C-4) and two in house cultures have been tried at four different locations of Central Plain Zone of Punjab viz. Soil Research Farm of P.A.U-Ludhiana, Seed Farm Ladhowal, KVK Sangrur, and KVK Kapurthala. After the initial standardization of inoculum preparation, spraying and sampling schedule during the initial three years of trials during 2015-2018, the present study reports the results of trials conducted in the year 2018-19 and 2019-20 in terms of influence of microbial decomposition approach on delignification and C/N ratio of surface retained paddy straw as well as on wheat grain yield.

Materials and methods

Location of trials

The work on microbial degradation of paddy straw was initiated in the year 2015, the number of locations at which experimental trials were laid and number of different microbial formulations tested are given in Table 1. Attempts were made to carry out the trials at sites which differ w.r.t. agro-climatic conditions.

Table 1 Trials conducted on microbial decomposer assisted *in situ* degradation of surface retained paddy straw during 2015-17

S. No.	Period	Number of Locations	Microbial Decomposers tested	Spray Schedule**	Important Inference	
					Degradation (%)	Yield increase (%)
1	2015-16	2 (Ludhiana and Bathinda)	2	Three sprays at weekly interval	20.0	3
2	2016-17	4 (Ludhiana, Kapurthala, Sangrur and Bathinda)	2	Three sprays at weekly interval	13.2	Nil
3	2017-18	1 (Ludhiana)	4 (2*)	Spray on 0 and 5 days post Harvest	17.8	Nil

* Commercial Decomposers ** Supplementation of urea @ 1 Kg/Acre

Punjab is divided into five agro-climatic zones as shown in Fig 1, first year trial was carried out in Ludhiana (Zone 3) and Bathinda (Zone 4) as these are the main paddy growing areas of Punjab. In 2016-17, because of high amount of paddy straw generated, three areas of Zone 3 (Ludhiana, Kapurthala and Sangrur) and one area (Bathinda) of Zone 4were selected. While in 2017-18, instead of using different locations, more number of microbial formulations was tried at single location (Ludhiana of Zone 3).

On the basis of trials conducted initially, the selected consortia were used in the year 2018-2019 and 2019-2020. These includefour commercial microbial decomposer preparations (C-1 to C-4) along with two in-house preparations (*Delftia* and Microbial Consortium).

Microbial suspension was sprayed using mechanical sprayer after use of cutter cum spreader in the field. Three sprays at aweekly interval were carried out and samples were collected for 30 days. Field trials were conducted in triplicate (Randomized Block design) using paddy variety PR 121 and the date of sowing during 2018-2019 and 2019-2020 was 29.11.2018 and 11.12.2019, respectively. To get a better idea of degradation potential of cultures, the collected samples were analyzed for change in lignin and C/N ratio after 30 days of treatment. Themultilocation trials were conducted at the aforementionedfour stations. Each microbial decomposer culture was grown in 1% jaggery solution (2 Kg jaggery dissolved in 200 L water) for 7-10 days at room temperature.

Determination of paddy straw degradation

Proximate analysis of paddy straw was carried out using Van Soest scheme for fibre analysis (Van Soest 1963). For measuring Neutral detergent fibre (NDF) and Acid detergent fibre (ADF), dried sample was homogenously ground to powder and 0.5 g of it was reflexed with 50 ml of neutral detergent solution and acid detergent solution, respectively. Then, the contents were kept at 80ºC for one hour followed by filtration using G-1 crucible. The residue was then washed with hot distilled water followed by acetone and was kept in hot air oven till the constant weight was achieved.Thus, hemicellulose content was calculated from the difference betweenNDF and ADF. Similarly, from the difference between ADF and 72% H_2SO_4 treated residues, cellulose content was calculated. For estimation of Acid detergent lignin (ADL), crucibles containing 72% H_2SO_4 treated residue were ignited in the muffle furnace at 600 ˚C for 3 hrs.

Analysis of C/N ratio: Straw samples were analyzed for C: N ratio using a CHNS Elemental analyzer (model Vario EL III) at Department of Soil Science, Punjab Agricultural University, Ludhiana.

Grain Yield: Grain yield (ton per hectare) was calculated after manual threshing of harvested plants of one square meter of each plot, isolating the straw from the grain and purifying them. The grains were weighed, weight converted from gm^{-2} to $tonha^{-1}$ and grain weights were corrected according to the safe moisture content (12%).

Statistical Analysis: To study the effect of different treatments on percent decrease in lignin and its C/N ratio, twoway analysis of variance (ANOVA) was carried out using the online software WASP version 2.0.

Fig. 1 Five agroclimatic zones of Punjab and location of trials conducted in different year are underlined

Results and discussion

Results presented in Table 2 revealed that percent decrease in lignin is significantly less in control (without microbial spray) fields as compared to different microbial formulations which is an important indication of the role played by microbial formulations in lignin hydrolysis. Compared to control, lignin degradation was high in all treatments (microbial spray) in both the years. Lignin is the most recalcitrant component of straw that lengthens the period required for straw decomposition. The biochemical decomposition of rice straw is a sequential process that initially involves the loss of less recalcitrant components (i.e., oligosaccharides, organic acids, hemicellulose and cellulose) followed by the degradation of the remaining highly recalcitrant compounds i.e., lignin or suberin (Guo et al. 2018). Therefore, during the initial decomposition period, the level of lignin either remains stable or increases under natural decomposition by intrinsic soil flora. As shown in Table 2, in control (without spray) percent decrease in lignin was in the range of 2.11 to 13.57whereas, with different microbial spray, it varies from 7.51 to 35.79. However, there was significant variation among different locations.During 2018-19 at PAU Ludhiana maximum decrease in lignin percent occurred with C-1 (24%) and C-3 (22.4%), followed by C-4 and C-2 each with a 20% decrease (Table 2). However, at Kapurthala, it decreased to a maximum extent with *Delftia* (24.8%), followed by C-3 and microbial consortium, respectively. At Sangrur and Ladhowal, maximum decrease was observed with C-3 and C-1that were at par. In the following year's trial, again C-1 led to maximum percent reduction in lignin (irrespective of the location) followed by C-2 at Ludhiana and Ladhowal. Maximum degradation was achieved with C-1 and was followed by C-3 and microbial consortium at Kapurthala and Sangrur, respectively. These location specific variations can be due to different field conditions such as soil type, moisture content, straw load etc. (Wu et al. 2019; Jin et al. 2020). Rice residues often have high C/N ratios (Nwite et al. 2012; Adubasim et al. 2018). Thus during paddy straw composting, C/N ratio is an important parameter to be observed.Since composting is a microbial degradation process, C/N ratio is the most important factor to initiate the composting process. C/N ratio must be around 50:1 at the initiation stage for the composting process to proceed at a faster pace. During composting this ratio narrows down further and the more the decrease in the C/N ratio, the greater the efficacy of microbial formulation in straw decomposition. The results presented in Table 3 revealed that C/N ratio decreased significantly within 30 days in all treatments including control. The treatment wise decrease in C/N ratio however reflected a mixed trend in both the years of trial. In 2018-19, three locations (except Sangrur) depicted significant percent decrease in C/N ratio with all decomposers besides C-3 application at Kapurthala. Literature reports that though a continuous and appropriate straw returning is beneficial for accumulation of soil organic carbon, in actual agricultural production process, the straw returning should be reduced with consecutive years (Jin et al. 2020). It may be the reason of variable behavior of C/N ratio at different locations. In some cases, the percent decrease in C/N ratio was more in the control than in microbial treatments (Sangrur trials in both the years). This can be due to high microbial growth that demands more N content from the soil (Alvaro-Fuentes et al. 2013). At Ludhiana, maximum decrease in C/N ratio (Table 3) was obtained by C-1and C-2 (55%),while at Kapurthala maximum decrease in C/N ratio was obtained by *Delftia* (71.25%) followed by C-3 (62.62%). At Sangrurand Ladhowal, maximum decrease in C/N ratio was obtained byC-2 and microbial consortium (61.1%) that differed non-significantly from *Delftia* (56.29%). Jayabalanand Neelanarayanan (2015), who studied the effect of combination of three lignocellulolytic fungi viz. *Rhizopus oryzae, Aspergillus oryzae* and *A. fumigatus* on paddy straw decomposition reported a decrease in C/N ratio from 70:1 to 10:1 with a significant increase in macro nutrients of the compost.Thus, in the present study, lignin and C/N ratio decrease was significant with the use of microbial decomposer, yet it was not sufficient to the extent that paddy straw may be rated as practically decomposed. Earlier studies on paddy straw decomposition have been conducted under controlled conditions viz. Litter bag method (Guo et al. 2018) and pots (Dash et al. 2021) or pits (Sharma et al. 2014), whereby paddy straw was chopped and moisture level was kept consistent unlike the present study which was carried out under actual environmental conditions.

Table 2 Percent decrease in lignin content of paddy straw after 30days of treatment during 2018-19 and 2019-20

Treatments	**Percent decrease w.r.t initial lignin content**							
	2018-19				**2019-20**			
	Ludhiana	**Kapurthala**	**Sangrur**	**Ladhowal**	**Ludhiana**	**Kapurthala**	**Sangrur**	**Ladhowal**
Control (without microbial spray)	12.0	9.9	9.2	13.6	10.7	10.2	8.9	2.1
Delftia	20.0	24.8	15.2	21.5	17.7	9.5	16.1	8.2
Microbial Consortium	14.4	19.2	20.0	22.6	15.7	12.1	17.1	11.7
C-1	24.0	26.0	28.0	25.3	35.8	27.9	27.8	25.1
C-2	20.0	11.1	10.4	12.4	19.5	9.5	16.3	16.1
C-3	22.4	22.8	29.6	28.7	14.5	13.4	15.1	11.9
C-4	20.0	15.2	17.3	23.4	17.6	11.4	13.2	7.5
Initial lignin content	25.0	25.3	24.9	26.5	15.9	15.7	16.9	16.1
	Two factor Factorial (CD%) Factor A (Location) – 1.957 Factor B (Microbial spray) – 3.094 Treatment (A X B) – 6.188				Two factor Factorial (CD%) Factor A (Location) – 1.767 Factor B (Microbial spray) – 2.651 Treatment (A X B) – 5.302			

A comparison of two years wheat grain yield data revealed non-significant effect of microbial application at all the four locations (Table 4). Thus, it may be stated that the retention of paddy straw in fields does not affect the yield of wheat grains. Therefore, more in depth studies are required for the screening of the lignocellulolytic bacteria/fungi for improvement in the decomposition of returned straw, extensive investigation on the allelopathic effects of straw incorporation (if any) and suggesting a comprehensive straw management technology. A combination of microbial-assisted and mechanical approach is needed to develop new technologies and equipment for proper straw management to improve the utilization efficiency and benefit of straw.

Table 3 Percent decrease in C: N ratio after 30days of treatment during 2018-19 and 2019-20

Treatments	**Percent decrease w.r.t initial C: N ratio**							
	2018-19				**2019-20**			
	Ludhiana	**Kapurthala**	**Sangrur**	**Ladhowal**	**Ludhiana**	**Kapurthala**	**Sangrur**	**Ladhowal**
Control (without microbial spray)	36.0	38.5	38.3	31.0	36.5	34.4	34.3	33.1
Delftia	47.1	71.3	26.6	56.3	44.5	26.6	24.8	30.8
Microbial Consortium	41.5	54.9	25.3	61.1	45.5	58.5	32.6	31.0
C-1	55.0	53.1	36.2	47.0	40.9	10.3	19.5	36.4
C-2	55.0	55.9	53.2	53.3	40.5	22.9	21.6	28.6
C-3	51.2	62.6	19.9	47.2	43.2	42.5	27.5	25.9
C-4	41.8	56.1	39.2	53.8	44.9	17.1	28.6	34.3
Initial C: N ratio	78:1	80:1	76:1	81:1	86:1	91:1	82:1	88:1
	Two factor Factorial (CD%) Factor A (Location) – 3.726 Factor B (Microbial spray) – 5.892 Treatment (A X B) – 11.784				Two factor Factorial (CD%) Factor A (Location) – 2.351 Factor B (Microbial spray) – 3.526 Treatment (A X B) – 7.052			

Conclusion

Keeping in view the variable trend in delignification as well as C/N ratio during surface retained paddy straw trials, it may be concluded that with microbial decomposer application the surface retained paddy straw is not appreciably decomposed in the provided window period of 3 weeks between paddy harvesting and wheat sowing. But simultaneously, the surface retained paddy straw does not affect wheat grain yield. It can be suggested that mixing / incorporation of straw into the soil can be adopted to increase the contact between microbial application and straw that can lead to better degradation of strawas literature also indicates that conventional mixed-rotation straw has better contact with soil leading to faster straw decomposition as well as further accumulation of soil organic carbon.

Table 4 Effect of microbial treatment on wheat grain yield during 2018-19 and 2019-20

Treatments	**wheat grain yield (tons / hectare)**									
	2018-19					**2019-20**				
	Ludhi-ana	**Kapurthala**	**San-grur**	**Ladhowal**	**Aver-age**	**Ludhi-ana**	**Kapurthala**	**San-grur**	**Ladhowal**	**Aver-age**
Control (without microbial spray)	5.0	5.4	5.1	4.7	5.1	4.8	5.1	5.1	4.4	4.8
Delftia	4.4	5.5	5.1	4.8	4.9	4.8	5.3	5.1	4.3	4.9
Microbial Consortium	4.7	5.5	5.1	4.9	5.0	4.6	5.2	5.1	4.3	4.8
C-1	4.8	5.3	5.1	4.8	5.0	4.9	5.0	5.1	4.3	4.8
C-2	4.9	5.5	5.0	4.9	5.1	4.8	5.1	5.1	4.6	4.9
C-3	4.8	5.2	5.1	4.8	5.0	4.8	5.1	5.0	4.5	4.9
C-4	4.8	5.2	5.1	4.7	5.0	4.7	5.1	5.1	4.5	4.9
CD (5%)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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