



Research Article

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Consequences of Different Organic Resources on Microbial Community in Rice-Mustard Cropping Sequence

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ABSTRACT

In Typic *Fluvaquent* soils of the Old Alluvial zone in West Bengal, India, an investigation was carried out to study the significance of organic matter, particularly humic acid, on the microbial community throughout the paddy cultivation (Variety MTU 1010), followed by mustard (Variety B-9). The soil has been allocated with fertilizers having recommended doses for rice field (N:P₂O₅:K₂O::60:30:30) and mustard (N:P₂O₅:K₂O::80:40:40), following the application of farmyard manure (FYM) @ 5.0 and 2.5 t ha⁻¹, respectively. Additionally, commercial humic acid and FYM-extracted humic acid were applied @ 0.5 and 0.25 kg ha⁻¹, respectively, according to the treatment arrangements. The experiment was carried out using a Randomized Block Design (RBD). Surface soil (0-15 cm) samples were gathered in definite interval to analyse organic carbon, microbial biomass carbon and microbial population. The application of extracted humic acid (EHA) demonstrated a significant positive correlation with increased organic carbon and microbial biomass carbon during cultivation of paddy, followed mustard. Hormonal behaviour of EHA, in combination to root exudation process, enhanced the microbial population at rhizosphere soil, regardless of the crop grown.

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INTRODUCTION

Carbon is the fundamental constituent of all life and its cycle represents the story of life on this planet. The presence of carbon in organic and microbial biomass form is the major prerequisite of soil organic substance and biosphere serves as the source

of organic matter of soil. Through photosynthesis, plants assimilate carbon, oxygen and hydrogen and as a part of ecological niche animals acquire carbon directly or indirectly from plants. After death, decomposition and/or burning of living body return

these elements in the form of CO₂ and H₂O to nature. The intermediate C-based products of decomposition are actual precursors of the carbon present in the soil. Microbial biomass carbon (MBC) is an index for comparing natural and degraded system of microbial activity in soil. This is actually microbial body carbon, developed with microbial decomposition in soil and act as major source of energy to rhizosphere required for aggregate formation and nutrient conservation (Fahramand *et al.*, 2014).

In soil different organic ameliorates, especially compost, FYM, green manure, synthetic polymers, *viz.* polyacrylamides and polyvinyl alcohols are disposing to microbial degradation (Nyssola and Ahlgren, 2019). Many rhizosphere soil microbes of different taxonomic and functional groups respond on application of humic colloids in soil (Vallini *et al.*, 1993). During composting, by aerobic biological decomposition, all easily decomposable, semi-resistant and resistant materials are gradually converts to a stable mature compost (Goyal *et al.*, 2005), recommended for direct application in cultivated soil to enhance soil fertility (Suarez-Estrella *et al.*, 2008).

Various workers study importance of humic acid (HA) for enhancing crop productivity and soil fertility. The functional groups (both aromatic and aliphatic) and amines cause HA to be biologically active as well as increase the CEC of the soil (Fahramand *et al.*, 2014). HA additionally has positive influence on microbial population ensuing in plant enlargement and biomass production (Suarez-Estrella *et al.*, 2008).

Present study compares FYM and HA (both commercial and extracted) on the changes in the status of SOC, MBC, soil microbial population using field experiments carried out with rice mustard cropping pattern.

MATERIALS AND METHODS

Site of Study

In rice-mustard cropping sequence, two field experiments were conducted in succession (*Kharif* followed by *Rabi*) at Adaptive Research Farm, India (23.95° N and 88.03° E), to examine the effect of different organic sources on microbial community. Throughout the experiment (Figure 1), the climate was humid subtropical, with a rainfall of 1481 mm

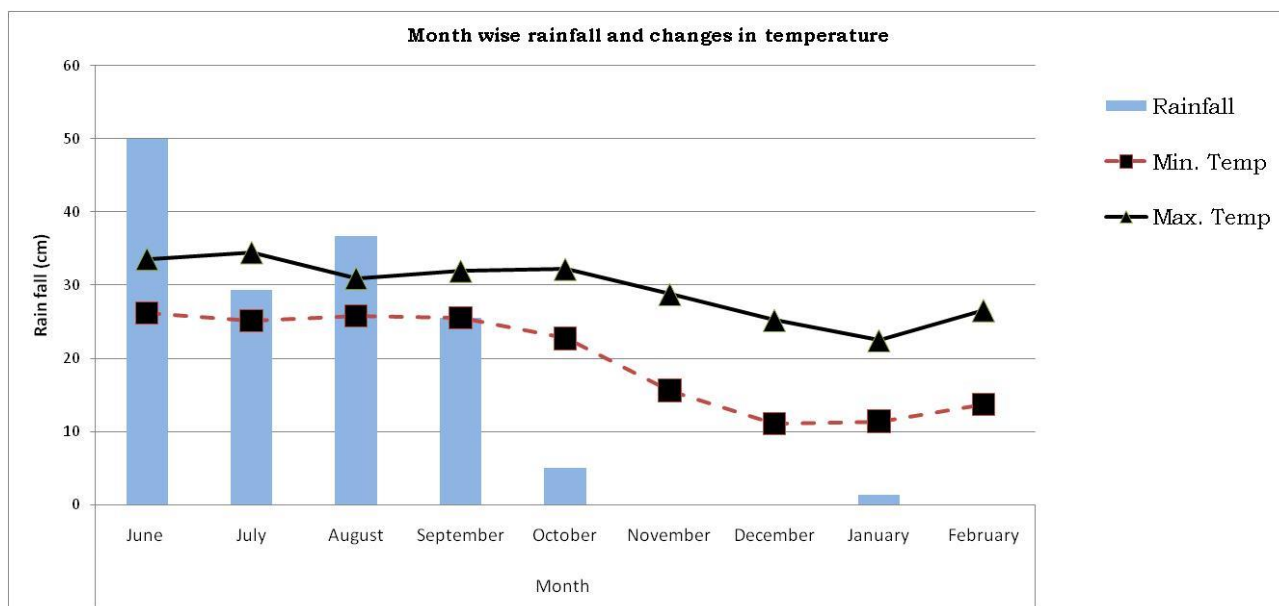


Figure 1: Meteorological changes during the experimental seasons

and temperature ranging from 34.4 °C (maximum) and 11.0 °C (minimum). Table 1 represents the

physical and chemical characteristics of the soil (Typic *Fluvaquent*).

Table 1: Physico-chemical characteristics of the soils the study location

Sl. No.	Properties	Unit	Surface soil
1	Category of the soil	-	Typic <i>Fluvaquent</i>
2	Texture of the soil	-	Sandy Clay Loam
3	Oxidizable organic carbon	g per 100 g	1.16
4	pH	Soil:water = 1:2.5	6.34
5	Microbial biomass carbon	$\mu\text{g g}^{-1}$	99.85
6	Bacterial population	(cfu $\times 10^6$)	23.04
7	Actinomycetes population	(cfu $\times 10^5$)	95.02
8	Fungal population	(cfu $\times 10^4$)	26.12

Description of Treatments

Humic acid was extracted from farmyard manure (FYM) using the process of Kononova and Belchikova (1961) and purchased from open market.

The commercial humic acid was characterised with an 8% ash content. The properties of the FYM and humic acid used in the study are illustrated in table 2.

Table 2: Properties of farmyard manure and humic acids from various sources

Sl. No.	Properties	FYM	Extracted Humic acid from FYM (EHA)	Purchased humic acid (CHA)
1	Oxidizable organic carbon (%)	32.56	29.77	43.36
2	Aggregate nitrogen (%)	1.014	2.20	1.29
3	C:N ratio	32.016	13.53	33.61
4	Viscosity (Ubelhode Viscometer)	-	133.10	139.00
5	E ₄ :E ₆	-	3.193	3.41
6	Functional group (Dragunova, 1958) (meq Ba)	-	6.803	6.803
7	Ash-free carboxylic group (Kononova, 1966) (meq)	-	628.3	415.9

Urea, SSP and MOP were applied at the recommended doses (N:P₂O₅:K₂O :: 60:30:30) for paddy (Variety MTU-1010) during the *kharif* season, irrespective to treatments. Half of the external nitrogen was used as a basal dose, while the remaining nitrogen was split into two applications, viz., tillering stage and flowering stage of rice. A Randomized Block Design (RBD) was followed, with 3 replications. Mustard (B-9) was cultivated as a *rabi* crop after paddy, with the following nutrient doses (N:P₂O₅:K₂O :: 80:40:40 kg ha⁻¹).

These treatments were applied in the first experiment, followed by the second experiment consecutively.

Treatments adopted in the first experiment with rice:

T₁ = Control

T₂ = Basal application of FYM @ 5 tons ha⁻¹

T₃ = Basal application of CHA @ 0.5 kg ha⁻¹.

T₄ = Basal application of EHA @ 0.5 kg ha⁻¹.

Treatments adopted in the second experiment with mustard:



- T₁' = Control
T₂' = Basal application of FYM @ 2.5 tons ha⁻¹.
T₃' = Basal application of CHA @ 0.25 kg ha⁻¹.
T₄' = Basal application of EHA @ 0.25 kg ha⁻¹.

Collection and Assessment of Soil and Plant Samples

Surface soil samples (0-15 cm) were gathered from each experimental site at key growth stages: tillering, panicle initiation, flowering and harvesting of rice, followed by branching, flowering and harvesting stages of mustard.

Oxidizable organic carbon and MBC were determined using the Walkley and Black (1934) method and the chloroform fumigation direct extraction (CFDE) method (Joergensen, 1995). The biomass of microorganisms in the rhizosphere soil were estimated using Thornton's method (Thornton, 1922), Jensen's method (Jensen, 1930) and Martin's

rose bengal streptomycin agar medium (Martin, 1950).

Statistical Assessment

The findings of the trials were statistically evaluated using analysis of variance (ANOVA), and the critical differences were calculated at a 5% level of significance to assess the significance of treatment methods. This evaluation followed the guidelines established by Jackson (1973) and Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Organic Carbon

The effects of farmyard manure (FYM) applied at 5.0 and 2.5 t ha⁻¹, as well as commercial and extracted humic acid applied at 0.5 and 0.25 kg ha⁻¹, respectively, on the organic carbon (OC) content in the soil, following the paddy cultivation and the mustard cultivation, are presented in table 3.

Table 3: Changes in the content of organic carbon (g/ 100 g) in soil treated with FYM and humic acid in rice-mustard cropping sequence

Treatments	Rice				Treatments	Mustard		
	Days after transplanting of rice					Days after sowing of mustard		
	Tillering	Panicle initiation	Flowering	Harvesting		Branching	Flowering	Harvesting
T ₁	11.07	10.47	10.20	9.90	T ₁ '	10.38	9.55	8.78
T ₂	12.07	11.90	10.57	10.37	T ₂ '	11.42	10.74	10.61
T ₃	11.13	10.83	10.23	9.63	T ₃ '	11.53	10.48	7.86
T ₄	12.00	11.30	11.20	11.00	T ₄ '	12.97	10.48	9.56
SEm (±)	0.2093	0.1846	0.1808	0.1244	SEm (±)	0.0856	0.0649	0.2843
CD (5%)	0.7241	0.6387	0.6255	0.4302	CD (5%)	0.2962	0.2245	0.9836
CV%	3.1338	2.8739	2.9681	2.1064	CV%	1.2809	1.0896	5.3501
SEm (±)		0.1387			SEm (±)		0.4568	
CD (5%)		0.4437			CD (5%)		1.5806	

[SEm = Standard Error of mean, CD = Critical Difference, CV = Coefficient of Variation]

In paddy, FYM serves as the richest source of organic carbon, resulting in increases of 9% and 13.7% at tillering and panicle initiation (PI) stages, respectively, followed by EHA (8.4% and 8.0%). This enhancement might be due to the synergistic effect of applied fertilizers and soil organic matter

(SOM) (Table 1) (Korschens, 2002). In soil EHA gripped the highest position through raising the content of organic carbon at flowering (9.8%) and harvesting stages (11.1%) as equated to that of control. EHA affects the root exudation status of the soil (Trevisan *et al.*, 2010) which might be helpful



for improving the biochemical and microbial activity in root zone (da Silva Lima *et al.*, 2014).

The effect of EHA was further reflected in mustard, cultivated followed by paddy and jointly with applied EHA at mustard increased the content of OC by 24.9% followed by CHA (11.0%) and FYM (10.0%) at branching stage as compared with control in soil. This result was further justified by Chouriya (2016). On the contrary at harvesting stage of mustard FYM take lead role in increasing the content of OC (20.8%) than EHA (8.8%) as compared with control in rhizosphere soil.

Higher microbial proliferation on easily decomposed followed by semi-resistant and resistant organic substances might be the reason of the availability of organic carbon (Michel Jr. *et al.*, 1996). The declining trend of OC was much higher with CHA

than EHA and FYM might be rise in aerobic microbial inhabitants and their activity on added organic sources (Kumari *et al.*, 2011).

Microbial Biomass Carbon

Application of FYM, CHA and EHA on paddy followed by mustard field brought different influence on the microbes' body carbon, derived from organic sources in soil by the chemo-heterotrophic microorganisms (Table 4). Similar observation was also reported by Sellamuthu and Govindaswamy (2003). Among all the treatments in soil, EHA imparted significant increase from 51.4% (tillering) to 78.2% (PI) and 15.2% (flowering) to 34.6% (harvesting phase) of rice in comparison to that respective control. Addition of humic acid enhanced the microbial incubation period under semi-aerobic conditions of paddy cultivation.

Table 4: Changes in the content of microbial biomass carbon ($\mu\text{g g}^{-1}$) in soil treated with FYM and humic acid in rice-mustard cropping sequence

Treatments	Rice				Treatments	Mustard		
	Days after transplanting of rice					Days after sowing of mustard		
	Tillering	Panicle initiation	Flowering	Harvesting		Branching	Flowering	Harvesting
T ₁	101.41	145.02	189.27	159.98	T ₁ '	142.20	177.97	153.79
T ₂	151.00	169.25	221.05	198.34	T ₂ '	189.60	205.03	206.91
T ₃	140.29	252.52	229.50	161.49	T ₃ '	166.00	222.43	205.03
T ₄	153.55	258.42	218.01	215.31	T ₄ '	284.40	358.80	335.89
SEm (\pm)	1.3760	3.7970	3.8847	3.0891	SEm (\pm)	4.3871	5.5321	1.4255
CD (5%)	4.7610	13.1372	13.4409	10.6881	CD (5%)	15.1790	19.1406	4.9321
CV%	1.7452	3.1878	3.1375	2.9114	CV%	3.8858	3.9749	1.0954
SEm (\pm)		13.3920			SEm (\pm)		7.9732	
CD (5%)		42.8370			CD (5%)		27.5868	

[SEm = Standard Error of mean, CD = Critical Difference, CV = Coefficient of Variation]

Additionally EHA raised the content of MBC from 100 to 118.4% during the period between branching phase and harvesting stage of mustard.

This result substantiate the findings of Filip and Kubat (2004) manifesting the application of EHA serves as a provider of nitrogen and carbon for enhancing the metabolic activity of micro-organisms in rhizosphere soil (Suarez-Estrella *et al.*, 2008). Moreover, dead cells of susceptible organisms might

be the good source of MBC in soil (Alexander, 1977). The residual effect of added EHA during paddy cultivation was also reflected in mustard justified the findings of Demkina and Zolotareva (1997).

Jones *et al.* (2007) reported that CHA brought about declining trend from PI (74.1%) to harvesting (0.9%) stage in the content of MBC as compared with control in soil. On the contrary, CHA exhibited

similar significant increasing trend as like EHA from branching (16.7%) to harvesting (33.3%) during mustard cultivation as compared with control in soil (Costa *et al.*, 2008). Fate of FYM have significant effect on MBC status resulting in significant declination from tillering (48.9%) and branching (33.3%) to flowering (16.8% and 15.2%) of paddy and mustard respectively. This might be due to positive correlation between soil organic carbon and MBC, as reported by Singh *et al.* (1989). The level of MBC was increased at harvesting stage of both the crops; and this might be due to highest root exudation during flowering stage (Islam and Borthakur, 2016).

Micro-Organism

Humic substances persuade the expansion curve of soil microbial population. Soil micro-entities of diverse taxonomic and functional groups exhibit positive reactions to the presence of humic acid in *in-vivo* experiments (Suarez-Estrella *et al.*, 2008). Changes in microbial population at rhizosphere with the application of FYM, CHA and EHA during cultivation of paddy followed by mustard were tabulated in table 5, 6 and 7 for bacteria, actinomycetes and fungi, respectively. The pH (6.34) and climatic condition (Figure 1) of the

experimental field also develop favourable climatic conditions of microbial growth.

EHA inclusion in soil resulted highest significant boost in population of bacteria (36.7%) followed by CHA (22.2%) and FYM (17.3%) at tillering stage of paddy as compared to control. A parallel pattern was also observed at harvesting stage of paddy and throughout the entire incubation period of mustard. FYM established highest increase of 60.6% in bacteria population at flowering stage of paddy, followed by EHA (57.6%) and CHA (34.7%) respectively in comparison with control.

During paddy cultivation stagnant water and or swampy condition in rhizosphere soil was observed which might be responsible for lower bacterial growth. Aerobic climatic condition with optimum moisture percent is favourable for bacterial growth and this might be the actual reason of more than one fold increase in bacterial population during mustard cultivation across all treatments compared to the control in soil, as well as cultivation of paddy. EHA was responsible for increasing the activity and population of nitrogen fixing bacteria (Suarez-Estrella *et al.*, 2008) and this might be the reason of highest bacterial growth with EHA after that CHA and FYM as equated to that of control in soil.

Table 5: Changes in number of total bacteria (CFU×10⁶) in soils treated with FYM and humic acid in rice-mustard cropping sequence

Treatments	Rice				Treatments	Mustard		
	Days after transplanting of rice					Days after sowing of mustard		
	Tillering	Panicle initiation	Flowering	Harvesting		Branching	Flowering	Harvesting
T ₁	23.34	30.58	23.30	19.84	T ₁ '	24.60	30.95	28.16
T ₂	27.37	31.40	37.42	30.23	T ₂ '	49.20	71.32	60.75
T ₃	28.51	35.97	31.38	27.03	T ₃ '	55.92	75.36	67.25
T ₄	31.90	37.05	36.71	30.76	T ₄ '	62.62	87.46	75.93
SEm (±)	0.3464	0.7773	0.4006	0.7605	SEm (±)	2.3570	1.4436	1.5218
CD (5%)	1.1984	2.6892	1.3861	2.6313	CD (5%)	8.1552	4.9947	5.2652
CV%	2.1595	3.9890	2.1549	4.8853	CV%	8.4904	3.7730	4.5428
SEm (±)		1.3040			SEm (±)		2.3724	
CD (5%)		4.1711			CD (5%)		8.2084	

[SEm = Standard Error of mean, CD = Critical Difference, CV = Coefficient of Variation]

Table 6: Changes in number of actinomycetes (CFU×10⁵) in soils treated with FYM and humic acid in rice-mustard cropping sequence

Treatments	Rice				Treatments	Mustard		
	Days after transplanting of rice					Days after sowing of mustard		
	Tillering	Panicle initiation	Flowering	Harvesting		Branching	Flowering	Harvesting
T ₁	96.57	104.55	105.90	63.88	T ₁ '	98.40	113.02	99.75
T ₂	144.85	185.15	211.80	186.34	T ₂ '	219.18	226.05	216.94
T ₃	127.10	152.90	136.85	95.83	T ₃ '	174.42	204.52	177.89
T ₄	173.80	273.70	217.33	179.87	T ₄ '	223.64	247.58	221.28
SEm (±)	2.8900	6.0651	0.3296	0.3279	SEm (±)	0.2897	1.8102	0.7957
CD (5%)	9.9991	20.9847	1.1405	1.1344	CD (5%)	1.0024	6.2633	2.7530
CV%	3.6920	5.8663	0.3399	0.4319	CV%	0.2805	1.5852	0.7701
SEm (±)		11.5913			SEm (±)		3.2783	
CD (5%)		37.0768			CD (5%)		11.3427	

[SEm = Standard Error of mean, CD = Critical Difference, CV = Coefficient of Variation]

Table 7: Changes in number of fungi (CFU×10⁴) in soils treated with FYM and humic acid in rice-mustard cropping sequence

Treatments	Rice				Treatments	Mustard		
	Days after transplanting of rice					Days after sowing of mustard		
	Tillering	Panicle initiation	Flowering	Harvesting		Branching	Flowering	Harvesting
T ₁	26.62	40.25	60.37	63.88	T ₁ '	33.55	40.37	27.12
T ₂	40.25	80.50	120.75	207.64	T ₂ '	111.82	139.94	119.32
T ₃	106.37	111.12	88.25	155.31	T ₃ '	78.27	94.19	92.20
T ₄	120.80	161.00	152.93	196.98	T ₄ '	162.14	181.65	178.98
SEm (±)	0.1060	0.1094	0.1884	3.1569	SEm (±)	1.7913	1.9202	1.2740
CD (5%)	0.3666	0.3784	0.6518	10.9227	CD (5%)	6.1978	6.6437	4.4079
CV%	0.2497	0.1929	0.3091	3.5061	CV%	3.2170	2.9165	2.1135
SEm (±)		10.6577			SEm (±)		3.8389	
CD (5%)		34.0908			CD (5%)		13.2825	

[SEm = Standard Error of mean, CD = Critical Difference, CV = Coefficient of Variation]

Total population of actinomycetes in soil was significantly affected with EHA and FYM in paddy followed by mustard. Highest increase in population was recorded with EHA throughout the entire growth cycle of paddy and mustard except harvesting of paddy. Fungal population with EHA was significantly and gradually increased upto more than four and a half fold and five and half fold at

harvesting stages of paddy and mustard, respectively, compared to the respective control in the soil. This increment was supplementary followed by FYM (more than 3 fold) in both the crop as compared with control.

Humic substances in soil have a very good nutritive value (Potter and Meyer, 1990) and have enzymatic activation of nutrient uptake (Tejada *et al.*, 2006).

Spectrometric study established hormonal effect of humic substances and usually higher in rhizosphere soil, probably because of acceleration in microbial population or of a speed up metabolism that results from the presence of root exudates (Trevisan *et al.*, 2010).

CONCLUSION

Irrespective of chemical fertilizers in their respective recommended doses, imputation of extracted humic acid (EHA) and commercial humic acid (CHA) increase the availability of organic carbon and microbial body carbon into utilizable form for soil microorganisms. In spite of higher competition among bacteria, actinomycetes and fungi for the utilizable form of organic carbon, presence of humic substances increases the metabolic rate as well as populace of these living entities especially during physical growing period of paddy followed by entire life cycle of mustard.

Conflict of Interest

The authors declare no conflict of interest.

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