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Comparative study of organic matter and humicacid on N mineralization in rice-mustard cropping sequence

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Abstract
The influence of organic matter vis-a-vis humic acids on soil nitrogen status and its availability and economic impact, during the cultivation of rice (variety MTU 1010) followed by mustard (variety B-9), was studied in the soil of Typic *Fluvaquents* situated in old alluvial soil of West Bengal, India. The status of soil texture, bulk density, oxidizable organic carbon, pH, total nitrogen, available nitrogen and microbial biomass nitrogen were sandy clay loam, 1.34mg m^{-3}, 1.16 g $100g^{-1}$, 6.34, 0.14 g 100 g^{-1}, 231.36 kg ha^{-1}, 11.86 µg g^{-1} respectively. The C: N ratio of the added FYM, commercial and FYM extracted humic acid were 32.11, 32.61, 13.53 respectively. Along with recommended dose of fertilizers, basal soil of paddy ($N:P_2O_5:K_2O$:: 60:30:30) followed by mustard ($N:P_2O_5:K_2O$:: 80:40:40) was treated with FYM (@ 5.0 and 2.5 t ha^{-1}), commercial humic acid (@ 0.5, 0.25 kg ha^{-1}) and FYM extracted humic acid (@ 0.5, 0.25 kg ha^{-1}) respectively. The experiment was undertaken with three replications with plot size of (3 × 4) =12 sq.m. Following the randomized block design (RBD). Rhizosphere (0-15 cm) soil-plant samples were collected and analysed for total nitrogen, available nitrogen, microbial biomass nitrogen, plant uptake and their overall effect on economic yield of crops. As panicle initiation and branching stages of paddy and mustard highest content of total nitrogen was recorded which gradually decline towards harvesting stage. FYM extracted humic acid increased N-availability, MBN in soil which signified N-uptake within plants resulted significant yield of paddy. Residual effect of FYM along with additional dose to mustard resulted highest significant yield of plant biomass whereas irrespective of treatments the yield of mustard is similar.

Keywords: FYM, commercial humic acid, FYM extracted humic acid, available N, MBN

Introduction
Nitrogen plays an important role in increasing agricultural production and since it is part of protein, its use may increase the food value of crops. Nitrogen has been one of the most extensively investigated element with regards to source, availability, transformation and cycling in soil-plant-water-atmosphere system. The liberation pattern of N from native as well as applied organic sources is not only controlled by the environmental conditions but also by management practices.
It is well known that soil organic matter (SOM) is the main source of indigenous soil N supply and a number of studies have documented a positive relationship between SOM concentration and soil N-supplying capacity [1]. On an elemental basis, organic substances contain 42% carbon, 42% oxygen, 8% hydrogen and 8% ash on a dry weight basis. The resistant portion of the soil organic matter is humus which plays an important role in soil- forming processes and soil fertility. Because of the complexity of the soil-humus, a comprehensive study of this subject is of utmost importance. Nitrogen present in humus can serve as a slow-release source of nitrogen [2, 3]. The N content of humic acid varies from 1 to 5% [4] depending upon its origin and sources of organic matter.
Addition of organic matter as a source of nitrogen is well recognized [5] but direct application of humic acid as source of nitrogen to a soil has not been much studied in detail. It is now well established that N transformation in soil differs due to addition of either organic matter or humic acid [6]. Therefore, it is of utmost importance to study N transformation in soils with respect to added organic matters. Addition of organic matter to soil before a cropping season is

helpful in maintaining N pool in the field. Time of application of organic matter also influences the release of N from the organic source to a soil system. The role of humic acid in this regard is not clear and well known.

Keeping the above information in view, the present research work was undertaken to study the effect of time of application of organic matter or humic acid on N-transformation in soil.

Materials and Methods

A laboratory experiment was conducted with the soil collected from the 0-to-15 cm depth of rice (variety MTU 1010) followed by mustard (variety B-9) from cultivated field located in Pundibari block in the district of Cooch Behar, West Bengal, India. Along with recommended dose of fertilizers, basal soil of paddy ($N:P_2O_5: K_2O$: 60:30:30) followed by mustard ($N:P_2O_5:K_2O$: 80:40:40) was treated with FYM (@ 5.0 and 2.5 t ha^{-1}), commercial humic acid (@ 0.5, 0.25 kg ha^{-1}) and FYM extracted humic acid (@ 0.5, 0.25 kg ha^{-1}) respectively. The experiment was undertaken with three replications with plot size of (3×4) =12 sq.m. Following the randomized block design (RBD). The soil was classified as Aquic ustifluvent [7]. The physical and chemical properties of the soil are given in Table 1.

The experiment was conducted in a completely randomized factorial design with three replications. The applications consist of farm yard manure (FYM) at 5 tons ha^{-1}, humic acid (HA) equivalent to FYM on the basis of N content and nitrogen as urea at 150 kg ha^{-1}. Humic acid was extracted from the FYM with 0.5 N NaOH following the method of Stevenson [8].

Table 1: Some physical and chemical properties of the soil used in the research

Properties	Quantities	Methods adapted
Mechanical Separates		**Hydrometer method [28]**
Sand (%)	54.6	
Silt (%)	42.0	
Clay (%)	3.4	
Textural Class	Sandy loam	
USDA Classification	Aquic ustifluvent	USDA [7].
pH	7.35	Glass Electrode pH meter
EC (mS cm^{-1})	0.114	conductivity meter
Oxidizable Organic Carbon (%)	0.91	Walkley and Black [29].
Available P_2O_5 (kg ha^{-1})	132.82	Jackson [30].
Available K_2O (kg ha^{-1})	114	Jackson [30].
Exchangeable NH_4^+ (mg kg^{-1})	77.89	Bremner [31].
Soluble NO_3^- (mg kg^{-1})	47.93	Bremner [31].
Hydrolysable NH_4^+-N (mg kg^{-1})	176.96	Bremner [32].
Amino acid N (mg kg^{-1})	199.08	Bremner [32].
Total hydrolysable organic N (mg kg^{-1})	530.88	Bremner [32].
Total nitrogen (mg kg^{-1})	982.34	Bremner [32]

Six Treatments were employed as follows

T_1 = Soil, T_2 = Soil + N at 75 mg kg^{-1}, T_3 = Soil + FYM at 2.5gkg^{-1}, T_4 = Soil +FYM at 2.5gkg^{-1} + N at 75 mg kg^{-1}, T_5= Soil + HA equivalent to FYM on N basis, T_6 = Soil + HA equivalent to FYM on N basis+ N at 75 mg kg^{-1}.

There were two sets of same treatments for two different times of application of organic matters. In the 1st set organic matter either FYM or humic acid was added 21 days before the start of experiment. In the 2nd set FYM or humic acid was added on the day of start of the experiment. Nitrogen as urea at 75 mg kg^{-1} was added to all the N-treated systems as basal dose. Separate sets were maintained for identical collection of soil samples on 15th, 45th, 75th and 120th day of the experiment to analyze different forms of inorganic [9] and organic [10] N. Soils were maintained at 60% of water holding capacity and kept at room temperature (26±2 ºC) throughout the experimentation period. The loss of water due to evaporation was replenished by periodic addition of sterile distilled water on every alternate day by difference in weight.

Data of inorganic and organic N as well as total N were statistically analyzed and critical difference were calculated at 5% level of significance to test the significance of means for the treatment difference [11].

Results and Discussion

Irrespective of treatments and stages of incubation, comparatively higher amount of exchangeable NH_4^+ is accumulated in soil treated either with FYM or humic acid 21 days before than on the day of start of experiment (Table 2). Furthermore, irrespective of treatments, exchangeable NH_4^+ tended to decrease up to 45th day, then increased up to 75th day and again decreased on 120th day in soils treated with either forms of organic matter 21 days before the start of incubation. However, application of FYM or humic acid on 0th day did not show similar trend of results. Addition of FYM or humic acid 21 days before the actual cropping season significantly increased the exchangeable NH_4^+ in soil because of the time period received by the microorganisms for decomposing the organic matter and releasing N from it [12, 13, 14, 15]. The decrease and increase in exchangeable NH_4^+ in soil during the whole incubation period under different stages of organic matter addition is perhaps due to immobilization of exchangeable NH_4^+ [16, 17] or loss of N due to denitrification [18] and or volatilization [19] as well as mineralization of dead microorganisms and organic matter [20]. Results in Table 2 further revealed that irrespective of treatments and stages of incubation, comparatively higher amount of exchangeable NH_4^+ is accumulated in N-treated over untreated systems. The effect of added inorganic N on accumulation of exchangeable NH_4^+ in soil has been mentioned earlier also by Onikura *et al.* [21]. Data in Table 2 also pointed out that humic acid is more prone to mineralization [22] leading to accumulation of higher amount of exchangeable NH_4^+ in humic acid than FYM treated systems. This trend of results in general, is particularly true at the earlier stages of incubation.

Results in Table 3 reveal that irrespective of treatments, comparatively higher amount of NO_3^- is accumulated in soil treated with organic matters 21 days before than that of the start of experiment upto 15 days of incubation. However, a significant reverse trend of results is observed from 15th onwards to 75th day of the incubation. Again, at the last stage of incubation, the amount of soluble NO_3^- increased in soil treated with FYM or humic acid 21 days before over that of the soil treated on 0th day of the experiment. Like exchangeable NH_4^+, NO_3^- is transformed differently during the incubation period depending upon the time of addition of organic matter to a soil system. Addition of organic matter either as FYM or humic acid 21 days before the start of experiment encourages nitrification which is reflected by the accumulation of NO_3^- up to 15th day of incubation. The increased NO_3^- content on 45th and 75th day in soil treated with organic matter on 0th day of the experiment also is due to enhanced nitrification of exchangeable NH_4^+. At the last stage of incubation no statistical difference in the accumulation of NO_3^- content is observed in soil treated either with FYM or humic acid 21 days before or on the day of start of the experiment. Data in Table 3 further revealed that addition of either FYM or humic acid did not show any significant difference in accumulation of soluble NO_3 in soil. It is also interesting to note that a similarity is maintained in accumulation of soluble NO_3^- with that of exchangeable NH_4^+ in regard to time of application of organic matter throughout the period of incubation. Irrespective of treatments, the effect of added N is not observed at the earlier stage of the experiment. However, the effect is observed clearly at every sampling stage at the later period of the incubation [21].

Results of changes in the amount of hydrolysable organic NH_4^+ in soil treated either with FYM or humic acid 21 days before or on 0th day of the experiment are presented in the figures 1a and 1b respectively. Comparatively higher amount of NH_4^+ is accumulated in soil treated either with FYM or humic acid 21 days before the start of the experiment upto 15th day of the incubation study. However, a reverse trend of results was observed on 45th day onwards to last stage of the experiment. Results further revealed that irrespective of treatments, exchangeable NH_4^+ progressively increased to 15th day of the experiment. However, on 45th day NH_4^+ decreased and thereafter increased to last stage of incubation. This trend of results was observed in soil treated either with FYM or humic acid 21 days before the start of experiment. Soils received FYM or humic acid on the start of experiment showed different trend of results. Accumulation of NH_4^+- N progressively increased upto 45th then decreased on 75th but again increased on 120th day i.e., last stage of experiment. The difference in accumulation of hydrolysable NH_4^+-N is due to treatment of soil with organic matter at different times of the experiment. The increase or decrease in accumulation of NH_4^+ in soil during the incubation period is due to mineralization and immobilization of N. Critical analysis of the results reveal that in general, irrespective of treatments, NH_4^+ increased over 120 day period of incubation and the amount of increase is more in soil treated either with FYM or humic acid on the day of start of the experiment. Thus it is clear that hydrolysable NH_4^+ is mineralized at a faster rate in soil treated with organic matters 21 days before than that on the day of start of the experiment [11]. Closer examination of the figures 1a and 1b further reveal that in general, comparatively higher amount of hydrolysable NH_4^+ is accumulated in soil treated with humic acid than that of FYM particularly at the early stage of incubation. The effect of added inorganic N on accumulation of higher amount of hydrolysable NH_4^+ in soil is prominent upto 15th day of incubation. However, at the last stage of incubation, no definite trend of results is observed with regard to effect of added inorganic N. The variation in accumulation of NH_4^+ in N treated and untreated systems is due to addition of organic matter at different stages of the incubation study [23].

Data in Table 4 reveal that irrespective of treatments amino acid N increased and decreased again with the stages of incubation. This increase and or decrease in the amount of amino acid N in soil clearly pointed out that amino acid N is subjected to mineralization and immobilization during a cropping season [24, 25]. The time of application of organic matter did not show any significant effect on mineralization and immobilization pattern of amino acid N in soil (Table 4). In general, comparatively higher amount of amino acid N is accumulated in soil treated either with FYM or humic acid 21 days before than on the day of start of the experiment. This trend of results is, in general, observed throughout the period of investigation. Data in Table 4 further showed that irrespective of the time of application of organic matters, comparatively higher amount of amino acid is mineralized from humic acid than FYM treated soils particularly at the later stages of incubation. Irrespective of treatments, effect of added inorganic N is prominent through the experimentation period. The results of N effect on amino acid accumulation are in agreement with earlier works of Xu *et al.*, 2003 [25].

Results presented in figures 2a and 2b revealed that comparatively higher amount of total hydrolysable organic N is accumulated in soil treated with organic matter 21 days before the start of experiment upto 15th day of the incubation study. However, a reverse trend of results is observed after 15th day onwards to last stage of the experiment. During this period comparatively higher amount total hydrolysable organic N is accumulated in soil treated either with FYM or humic acid on the day of start of experiment. It is clear from the results that mineralization of total hydrolysable organic N differs depending upon the time of addition of organic matter to a soil system. Closer examination of the results in fig. 2a and 2b further revealed that irrespective of N-treatment and time of addition of organic matters, humic acid is more prone to mineralization than FYM particularly at the later period of incubation [22]. The effect of added inorganic N on accumulation of total hydrolysable organic N is noticeable throughout the investigation period.

Results presented in figures 3a and 3b revealed that the amount of total N in soil progressively decreased upto 45th day of the incubation. However, the amount of total N increased on75th but again decreased at the last stage of experiment. Soils treated with organic matter on the day of start of experiment did not show similar trend of results. The amount of total N increased on 45th, decreased on 75th and then increased on 120th day of the incubation study. The increase and decrease in total N in soils under both the treatments over the whole incubation period is due to release and fixation of non-exchangeable NH_4^+ in soils [26, 27]. It is interesting to note that total N decreased sharply from 15th to 120th day of incubation when soils are treated with either types of organic matter 21 days before the start of experiment. However, on the other hand, total N increased slightly from 15th to 120th day of incubation when soils are treated either with FYM or humic acid on the day of start of experiment. Results thus clearly showed that organic matters added 21 days before the start of experiment mineralized at a faster rate than FYM or humic acid applied on the start of experiment [12, 14]. The effect of added inorganic N is reflected on the data of N-treated systems at all the stages of incubation [25].

Table 2: Effect of time of application of FYM or humic acid on changes in the amount (mg kg^{-1}) of exchangeable NH_4^+ in soil

Treatment	Incubation period (Days)											
	15			45			75			120		
	D_1	D_2	Mean	D_1	D_2	Mean	D_1	D_2	Mean	D_1	D_2	Mean
T_1	68.86	31.96	50.41	55.92	71.90	63.91	167.85	48.08	107.97	52.07	48.08	50.08
T_2	99.86	51.93	75.90	69.90	77.89	73.90	85.88	51.09	68.48	60.10	48.08	54.09
T_3	91.88	33.95	62.92	43.94	89.88	66.91	69.90	39.07	54.49	54.09	36.77	45.43
T_4	199.73	59.92	129.82	43.94	121.84	82.89	97.86	62.07	79.97	60.10	57.10	58.60
T_5	203.72	59.91	131.82	53.92	103.86	78.89	71.90	51.09	61.49	48.08	48.08	48.08
T_6	211.70	75.88	143.79	59.92	114.06	86.99	73.90	57.10	65.50	51.09	48.08	49.59
Mean	145.96	52.26		54.59	96.57		94.55	51.42		54.26	47.70	
	T	D	TXD	T	D	TXD	T	D	TXD	T	D	TXD
S.Em (+)	4.984	2.878	7.049	3.403	1.965	4.813	3.918	2.262	5.541	3.257	1.881	4.607
CD at 5%	14.619	8.440	20.674	9.981	5.763	14.116	11.491	6.634	16.250	NS	5.516	NS

D_1: FYM/ Humic acid applied 21 days before the start of experiment, D_2: FYM/ Humic acid applied on 0^{th} day of the experiment.

T_1: Soil, T_2: Soil + Nitrogen (Urea @75 mg kg^{-1}), T_3: Soil + Farm Yard Manure @ 2.5g kg^{-1}, T_4: Soil+ Farm Yard Manure @ 2.5g kg^{-1}+ Nitrogen (Urea @75 mg kg^{-1}), T_5: Soil + Humic Acid, T_6: Soil+ Humic Acid+ Nitrogen (Urea @75 mg kg^{-1}),T= Treatments, D= Time of application of FYM/humic acid, NS= Not Significant.

Table 3: Effect of time of application of FYM or humic acid on changes in the amount (mg kg^{-1}) of soluble NO_3^- in soil

Treatment	Incubation period (Days)											
	15			45			75			120		
	D_1	D_2	Mean	D_1	D_2	Mean	D_1	D_2	Mean	D_1	D_2	Mean
T_1	83.88	63.91	73.90	83.88	159.78	121.83	193.74	105.18	149.46	102.18	123.21	112.70
T_2	119.83	81.88	100.86	137.81	219.70	178.76	251.67	156.27	203.97	141.25	165.29	153.27
T_3	85.88	65.91	75.90	119.84	163.78	141.81	175.76	96.17	135.97	120.21	126.22	123.21
T_4	115.84	99.86	107.85	175.76	259.65	217.71	303.62	129.22	216.42	162.28	165.29	163.79
T_5	85.88	45.92	65.90	129.82	195.74	162.78	205.72	93.16	149.44	102.18	105.18	103.68
T_6	112.83	83.57	98.20	153.79	203.72	178.76	237.68	135.24	186.46	150.26	150.26	150.26
Mean	100.69	73.51		133.48	200.40		228.03	119.21		129.73	139.24	
	T	D	TXD	T	D	TXD	T	D	TXD	T	D	TXD
S.Em (+)	4.670	2.696	6.605	6.468	3.734	9.147	5.099	2.944	7.211	2.793	1.613	3.950
CD at 5%	13.697	7.908	NS	18.969	10.952	NS	14.954	8.634	21.148	8.193	4.730	11.586

D_1: FYM/Humic acid applied 21 days before the start of experiment, D_2: FYM/ Humic acid applied on 0^{th} day of the experiment.

T_1: Soil, T_2: Soil + Nitrogen (Urea @75 mg kg^{-1}), T_3: Soil + Farm Yard Manure@ 2.5g kg^{-1}, T_4: Soil+ Farm Yard Manure @ 2.5g kg^{-1}+ Nitrogen (Urea @75 mg kg^{-1})T_5: Soil + Humic Acid, T_6: Soil+ Humic Acid+ Nitrogen (Urea @75 mg kg^{-1}), T= Treatments, D=Time of application of FYM/humic acid, NS= Not Significant.

Table 4: Effect of time of application of FYM or humic acid on changes in the amount (mg kg^{-1}) of amino acid nitrogen in soil

Treatment	Incubation period (Days)											
	15			45			75			120		
	D_1	D_2	Mean	D_1	D_2	Mean	D_1	D_2	Mean	D_1	D_2	Mean
T_1	420.75	339.17	379.96	239.68	309.68	274.68	360.64	347.76	354.20	329.54	241.92	285.73
T_2	480.85	383.41	432.13	339.55	383.41	361.48	450.80	390.69	420.75	383.41	302.40	342.91
T_3	450.80	412.91	431.85	324.43	279.63	302.03	450.80	302.40	376.60	300.53	257.04	278.79
T_4	519.20	417.89	468.55	459.44	294.93	377.19	480.85	360.64	420.75	360.64	287.28	323.96
T_5	379.49	235.95	307.72	332.64	221.20	276.92	450.80	272.16	361.48	333.86	181.44	257.65
T_6	507.30	324.43	415.86	450.80	241.92	346.36	460.64	302.40	381.52	360.64	272.16	316.40
Mean	459.73	352.29		357.76	288.46		442.42	329.34		344.77	257.04	
	T	D	TXD	T	D	TXD	T	D	TXD	T	D	TXD
3	15.119	8.729	21.381	10.215	5.898	14.447	9.205	5.314	13.017	10.288	5.940	14.550
CD at 5%	44.342	25.601	62.709	29.961	17.298	42.371	26.996	15.586	38.178	30.174	17.421	42.673

D_1: FYM/ Humic acid applied 21 days before the start of experiment, D_2: FYM/ Humic acid applied on 0^{th} day of the experiment.

T_1: Soil, T_2: Soil + Nitrogen (Urea @75 mg kg^{-1}), T_3: Soil + Farm Yard Manure@ 2.5 g kg^{-1}, T_4: Soil+ Farm Yard Manure @ 2.5 g kg^{-1}+ Nitrogen (Urea @75 mg kg^{-1}), T_5: Soil + Humic Acid, T_6: Soil+ Humic Acid+ Nitrogen (Urea @75 mg kg^{-1}), T= Treatments, D= Time of application of FYM/humic acid, NS= Not Significant.

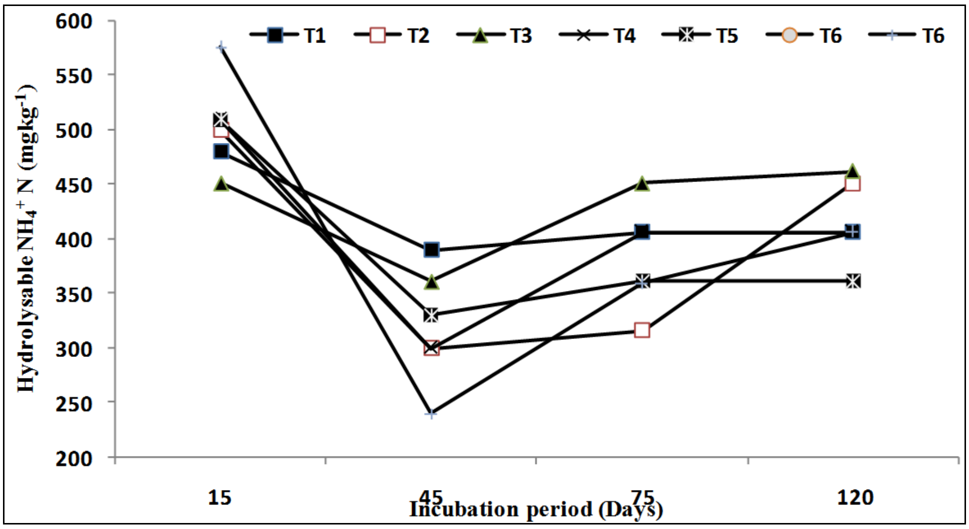

Fig 1a: Changes in the amount of Hydrolysable ammonium (NH_4^+) in soil treated with FYM or humic acid 21 days before the start of experiment

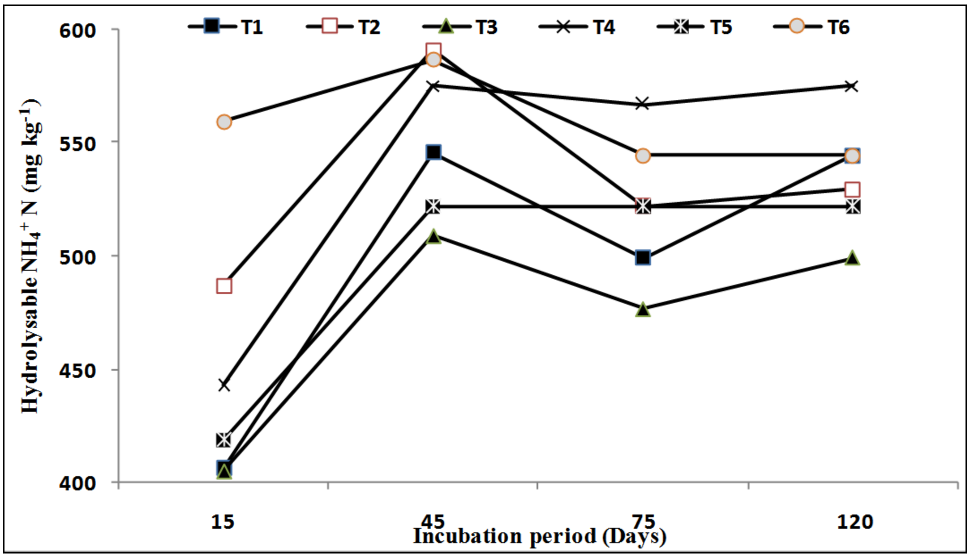

Fig 1b: Changes in the amount of Hydrolysable ammonium (NH_4^+) in soil treated with FYM or humic acid on 0^{th} day of the experiment

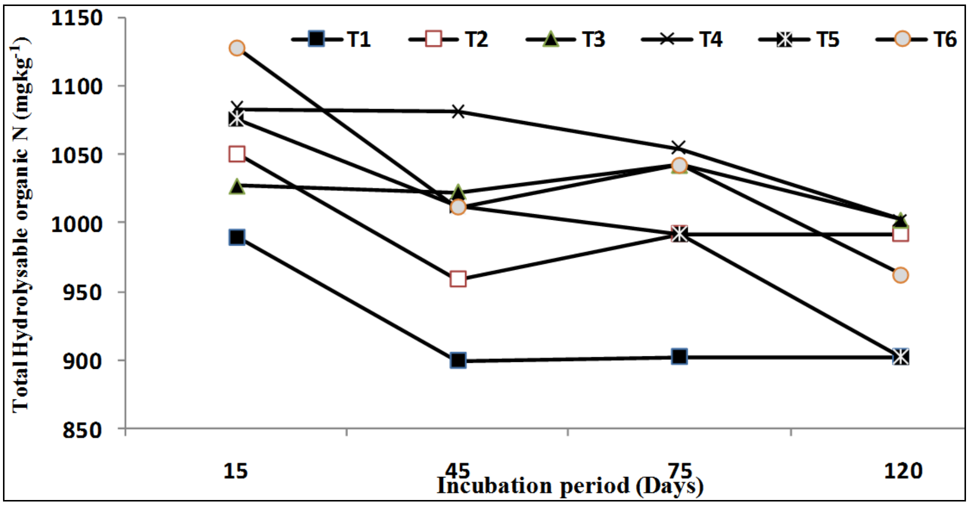

Fig 2a: Changes in the amount of total hydrolysable organic N in soil treated with FYM or humic acid 21 days before the start of experiment

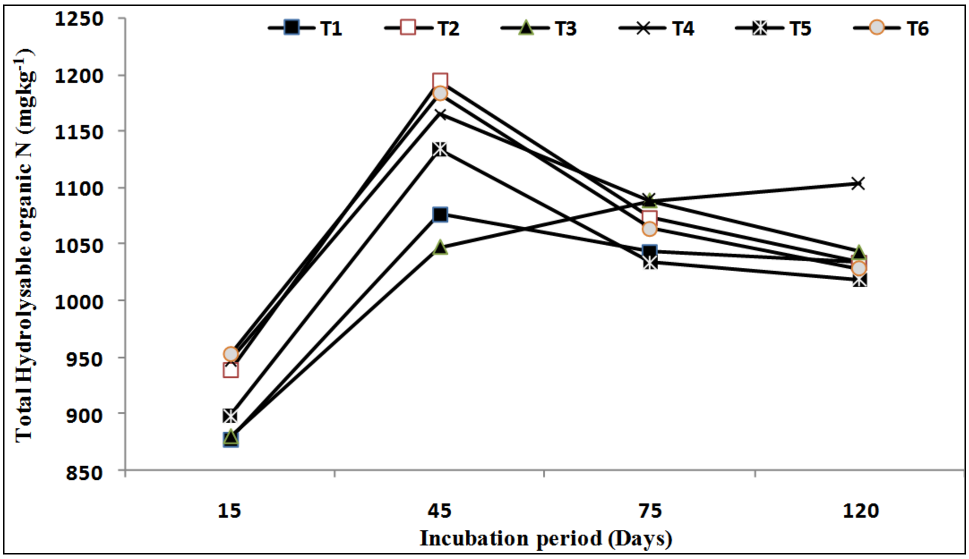

Fig 2b: Changes in the amount of total hydrolysable organic N in soil treated with FYM or humic acid on 0th day of the experiment

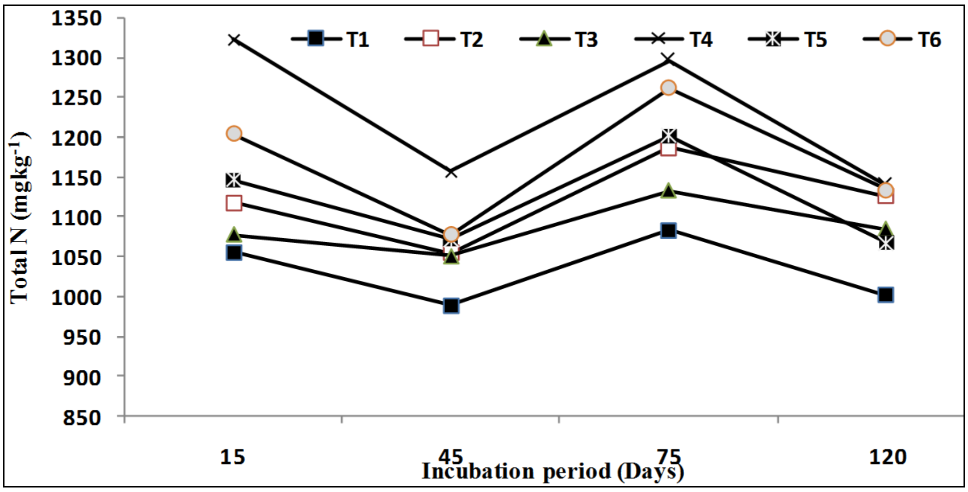

Fig 3a: Changes in the amount of Total N in soil treated with FYM or humic acid 21 days before the start of experiment

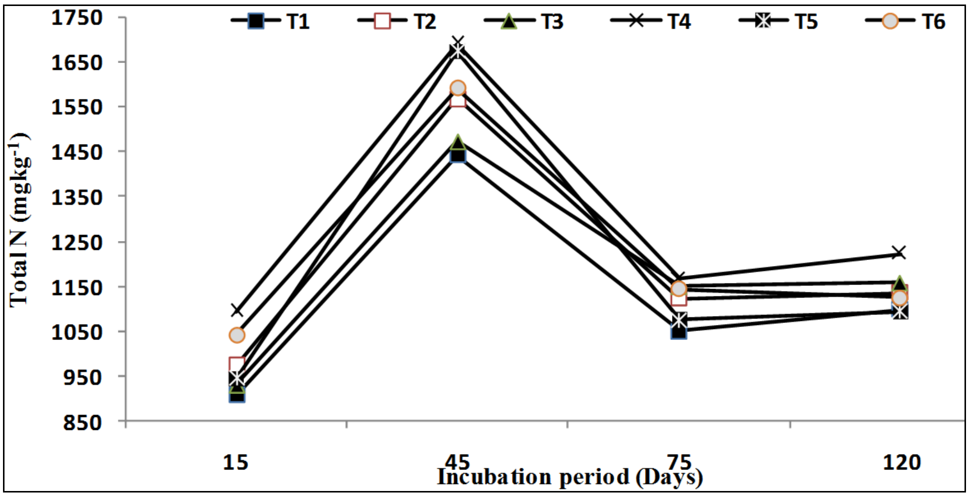

Fig 3b: Changes in the amount of Total N in soil treated with FYM or humic acid on 0th day of the experiment

Conclusion

Addition of FYM or humic acid showed more or less similar trend of results on changes in different forms of inorganic and organic N in soil. FYM or humic acid treatment 21 days before the start of experiment leads to accumulate higher amount of available N in soil. Furthermore, humic acid is more prone to N-mineralization than that of FYM. Therefore, bulky FYM can be replaced by humic acid, particularly with respect to release of N from organic sources.

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