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EVALUATING SUITABILITY OF EXTRACTANTS IN ORGANIC NITROGEN ESTIMATION UNDER ORGANIC PRODUCTION SYSTEM IN NADIA DISTRICT, WEST BENGAL, INDIA

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ABSTRACT
With the progress of Soil Science, different extractants were developed to assess total organic nitrogen of soil. Sequential extraction, among them, has been using widely though it is tedious, time consuming having with potential errors and at times not very reproducible. To overcome this hurdle developing a novel extractant is an important alarming issue in present day agriculture. Soils were sampled from well-recognized organic farms and conventional farms of New Alluvial Zone in Nadia district of West Bengal. A set of extractants comprising graded strength of NaOH [0.125 to 1.0 (M)], with 0.05 (M) EDTA were used to extract different inorganic nitrogen fractions. On the other hand, popular KMnO4 oxidisable nitrogen (conventional method) was also done for all soil samples. Among the extractant 0.375M NaOH + 0.05 M EDTA and 0.5M NaOH + 0.05 M EDTA extracted highest amount of inorganic nitrogen recording 784 kg N/ha in 84 days after incubation in Phulia and Gayeshpur sites of Nadia district rather than conventional method. The modified method manifested better estimation of inorganic nitrogen status of soil. The outcome of the present exercise will help to develop novel soil tests method for routine nitrogen estimation to serve the need of organic farmers.

INTRODUCTION

Organic agriculture movement in commercial scale is a current annexure in Indian agriculture. Worldwide organic agricultural research network reaches in Indian farming society recently but with very rudimentary technology. Though India is a food surplus nation at present with about 231.5 million tonnes food grain production per annum, it will require about 4-5 million tonnes additional food grains each year if the trend in rising population persists (Anon, 2010). So, it is the time to polish the existing technology with current perspective. Biologically mediated available nitrogen in organic farming is not sufficient enough to meet up the crops demand (Areand, 2007). It has been hypothesized that extractants normally used in conventional soil testing methods for extracting dynamic nitrogen nitrogen fractions don't equally applicable for extraction of plant available nitrogen and for estimation of reserve and potentially mineralizable fractions of plant nutrients. So there is a desire to increase the reliability of such a suitable extractants which cut down the costs, save time and increase the efficiency of routine analysis of large number of soil samples (Jones, 1990).

Nitrogen (N) is a major macronutrient in Earth and plays a central role in regulating the composition, structure, and function of ecosystems (Galloway *et al.,* 2004; Fang *et al.,* 2009). In organically managed farms nitrogen remains in soil as a very complex structure. It is very difficult to predict nitrogen availability when organic manures are supplemented with nitrogen fertilizer (Bhat *et al.,* 2015). The solubility of nitrogen decreases due to the presence of strong chemical linkage and the complexes become highly resistance to microbial mineralization. Alkaline permanganate extraction procedure which was proposed by Subbiah and Asija (1956) does not provide satisfactory result to supply potentially available soil nitrogen for plant and it cannot break the complex chemical linkage which mineralizes the organic nitrogen. Moreover, many reports say that other conventional extraction method were also found the similar result (Elkarim and Usta, 2001).

There has been considerable discussion over whether alternative methods of chemical soil analysis are required for organic farming. Conventional soil analysis for advisory purposes relies on the interpretation of the chemical extraction of different soluble nutrient pools from the soil to predict nutrient availability to crops (Edwards *et al.,* 1997). It cannot extract all forms of nitrogen. Organic systems differ very significantly from conventional systems in that they depend very much more on the application of nutrients in insoluble or organic compounds. It is therefore critical to transfer nutrient from an unavailable to available form in organic systems.

Therefore it seems impossible from the outset to find a unique extractant which have the capacity to release the potential available soil nitrogen in Organic farms. To overcome the hurdle, basic EDTA was developed by Bowman and Moir (1993) for determination of total soil organic phosphorus which was also used here to extract inorganic nitrogen. As the solubility of different fractions of organic nitrogen pool is not identical due their structural integrity, it is hypothesized that same strength of base cannot extract all fractions from soil.

With this background graded strength of base starting from 0.1 (M) to 1 (M) with 0.05 (M) EDTA were used to extract different organic nitrogen fractions.

It has also been hypothesized that organic soils have strict biologically regulated N-pools and potentially mineralizable N pools cannot be characterized by conventionally used extractants. The development of noble extractant is essential which have the capacity to increase the mineralizable nitrogen to crop in organic farming system.

The objective of this study is to evaluate such promising chemical extractant which have the capacity to mineralize nitrogen for crop utilization. In addition the study was also carried out to compare with popular alkaline permanganate method to assess the suitability.

MATERIALS AND METHODS

With a view to understand the soil samples were collected from different organic plots of Strawberry and Rice in *Sahadev Jaib Krishi Farm* at Phulia in West Bengal, which is situated at 23.23ºN latitude and 88.49ºE longitude at an elevation of 10.25 m above mean sea level. Soils in conventional plot of Rice of this region were collected and understand the edge of organic husbandry. Soil samples were also collected from organic and inorganic plots of Rice in Central Research Farm, B.C.K.V. Gayeshpur, Nadia, W.B. which is situated at 23ºN latitude and 89ºE longitude at an elevation of 9.75m above mean sea level. A set of extractants comprising graded strength of NaOH [0.125M to 1M], with 0.05 (M) EDTA were used to extract different fractions of soil nitrogen.

In initial case we have studied various soil parameters like pH, EC which were measured with the help of systronics glass electrode pH-meter (Jackson, 1973). The organic carbon (%) was estimated by wet digestion method of Walkey and Black (1934). Available Phosphorus content of the soil sample was determined by extracting the soil with Olsen's extractant (0.5 M $NaHCO_3$ solution at pH 8.5). Estimation of Available Phosphorus was done by Spectrophotometer as described by Jackson in 1973. The K concentration of standard and samples were estimated by Flame Photometer. (Jackson, 1973). In the physical parameter, particle size analysis of soil was done by hydrometer method based on Stokes law (Black, 1965). The percent of moisture was calculated from the loss in weight and the results were expressed in oven dry weight basis. (Blake and Hartge, 1986)

Analysis of Soil Sample

The available nitrogen (N) of the soils was estimated through the hot alkaline potassium permanganate method (Subbiah and Asija, 1956). Extraction of potentially mineralizable phosphorus pools is done by Alkaline Extraction method used by Bowman and Moir, (1993) which was also used here for estimation of available nitrogen. With this background, following alkali extractants were tested to track the mineralizable organic N-pools and their sizes in soils of organic production systems. There are 5 extractants as follow-

E_1 = 0.1 (M) NaOH + 0.05 (M) EDTA

E_2 = 0.25 (M) NaOH + 0.05 (M) EDTA

E_3 = 0.375 (M) NaOH + 0.05 (M) EDTA

E_4 = 0.25(M) NaOH + 0.05 (M) EDTA

E_5 = 1 (M) NaOH + 0.05(M) EDTA.

In this method, 1 g of finely ground soil sample(approx 0.1 mm) was weighted into a 50 ml polypropylene screw-cap centrifuge tube and 25 ml of mixed solutions of different concentration of NaOH plus 0.05 M EDTA were added to the soil. The tubes were loosely capped and heated in water bath for 15 minutes. The screw caps were tightened firmly and the tubes were heated for 2 hr. The tubes were allowed to cool, centrifuged at 1500 rpm for 1 hour and filtered through a Whatman no. 42 filter paper. Total nitrogen in the filtrate was determined from a 2 ml aliquot placed into 50 ml conical flask and digested with 1 g $K_2S_2O_4$ on a hot plate at 150° C for 30 minutes. Digestion was completed when vigorous boiling subsided. Inorganic nitrogen was determined by placing a 10 ml aliquot of the extract into a 50 mL centrifuge tube with 5 ml 1.8 M H_2SO_4 centrifuging at 1500 rpm for 1 hour and filtering the supernatant through a Whatman no. 42 filter paper and distillate the content with Potassium permanganate. (Chakraborty, 2015)

Statistical analysis of the soil

The observed data was subjected for standard CRD analysis [using SPSS software, Version 20] and testing for the significance of treatments is made. The multiple comparisons are made using DMRT [Duncan Multiple Range Comparison] technique. The treatment means with similar tagged alphabets was found not significantly different among themselves.

RESULTS AND DISCUSSION

Physical and Chemical properties of soil

The results showed that soil is alkaline with medium to high organic carbon content, low available Nitrogen content, low to medium available phosphorus and potassium content (Table 1). The particle size distribution of soil has been ranged from

Table 1: Physical and chemical properties of the soils used in the experiment-

Sites	Treatment	pH	EC(μ Sm^{-1})	OC (%)	Av.N (kg/ha)	Av. P (kg/ha)	Av. K (kg/ha)	Textural class	Moisture content (%)
Sahadev Jaib Krishi farm(Strawberry)	Vermicompost+ Edible oilcake	7.92	152.9	0.916	219.5	25.85	274.19	Loam	25
Sahadev Jaib Krishi farm(Rice)	Vermicompost+ organic manure	8.19	197.4	0.861	165.8	21.73	133.39	Silt loam	21.5
Sahadev Jaib Krishi farm(Rice)	Conventional	8.11	105.5	0.653	134.8	13.94	92.4	Silt loam	21.8
Central research farm(Gayeshpur)	Vermicompost	8.08	177.4	0.902	213.6	24.57	102.48	Loam	27.38
Central research farm(Gayeshpur)	Conventional	8.32	124.9	0.51	112.8	8.96	145.34	Silt loam	27.07

Table 2: Available nitrogen (kg/ha) of different organic and conventionally managed plots by potassium permanganate method

Site	Status	0 days	21 days	42 days	63 days	84 days
Sahadev Jaib Krishi farm(Strawberry)	Vermicompost+ Edible oilcake	188.16^{b}	172.48^{c}	188.16^{b}	125.44^{b}	141.12^{bc}
Sahadev Jaib Krishi farm(Rice)	Vermicompost+ organic manure	125.44^{d}	156.8^{b}	125.44^{d}	156.8^{ab}	156.8^{b}
Sahadev Jaib Krishi farm(Rice)	Conventional	250.88^{a}	219.52^{a}	203.84^{a}	172.48^{a}	156.8^{b}
Central research farm(Gayeshpur)	Vermicompost	156.8^{c}	125.44^{d}	156.8^{c}	156.8^{ab}	156.8^{b}
Central research farm(Gayeshpur)	Conventional	172.48^{bc}	156.8^{b}	188.16^{b}	156.8^{ab}	172.48^{a}

Table 3: Change in Basic EDTA extractable Inorganic N (kg N/ha of soil during incubation) of organically and conventionally managed Strawberry and Rice plot in *Sahadev Jaib Krishi Farm* in Phulia and Organically and conventionally managed Rice plot in Gayeshpur in West Bengal

Treatment	Phulia Organic-Strawberry					Phulia-Organic rice				
	day0	day21	day42	day63	day84	day0	day21	day42	day63	day84
E1	125.4^{c}	156.8^{d}	235.2^{d}	270.4^{c}	313.6^{c}	156.8^{c}	156.8^{d}	235.2^{d}	235.2^{d}	313.6^{d}
E2	156.8^{b}	235.2^{c}	313.6^{c}	470.4^{b}	548.8^{b}	313.6^{b}	313.6^{c}	470.4^{c}	470.4^{c}	470.4^{c}
E3	156.8^{b}	313.6^{b}	627.2^{a}	627.2^{a}	784^{a}	156.8^{c}	392^{c}	470.4^{c}	470.4^{c}	627.2^{b}
E4	313.6^{a}	470.4^{a}	627.2^{a}	627.2^{a}	784^{a}	313.6^{b}	470.4^{b}	548.8^{b}	627.2^{b}	627.2^{b}
E5	156.8^{b}	313.6^{b}	470.4^{b}	627.2^{a}	784^{a}	470.4^{a}	548.8^{a}	627.2^{a}	784^{a}	784^{a}

Table 3: Cont.......

Treatment	Phulia-Inorganic rice					Gayeshpur- Organic rice					Gayeshpur- InOrganic rice				
	day0	day21	day42	day63	day84	day0	day21	day42	day63	day84	day0	day21	day42	day63	day84
E1	156.8^{c}	345^{d}	235.2^{d}	156.8^{d}	156.8^{c}	313.6^{c}	313.6^{b}	156.8^{d}	156.8^{c}	125.4^{c}	470.4^{a}	392^{c}	313.6^{c}	156.8^{c}	470.4^{c}
E2	156.8^{c}	313.6^{d}	392^{c}	470.4^{c}	627.2^{b}	156.8^{d}	313.6^{b}	313.6^{c}	627.2^{a}	784^{a}	156.8^{c}	470.4^{b}	627.2^{b}	784^{a}	784^{a}
E3	156.8^{c}	470.4^{b}	470.4^{b}	627.2^{b}	784^{a}	392^{b}	470.4^{a}	548.8^{a}	627.2^{a}	627.2^{b}	470.4^{a}	627.2^{a}	627.2^{b}	627.2^{b}	784^{a}
E4	313.6^{b}	392^{c}	470.4^{b}	627.2^{b}	627.2^{b}	313.6^{c}	313.6^{b}	313.6^{c}	470.4^{b}	627.2^{b}	313.6^{b}	470.4^{b}	627.2^{b}	627.2^{b}	548.8^{b}
E5	470.4^{a}	627.2^{a}	627.2^{a}	784^{a}	784^{a}	470.4^{a}	470.4^{a}	470.4^{b}	627.2^{a}	784^{a}	470.4^{a}	235.2^{d}	784^{a}	784^{a}	784^{a}

Mean followed by common letter are not significantly different ($P < 0.05$) by Duncan's Multiple Range Test and different letter indicates significant difference ($P < 0.05$).

loam to silty loam and adequate amount of moisture percentage. Continuous assessment of soil physico-chemical properties is essential to make sound fertilizer management for optimizing crop productivity (Nayak *et al.,* 2015).

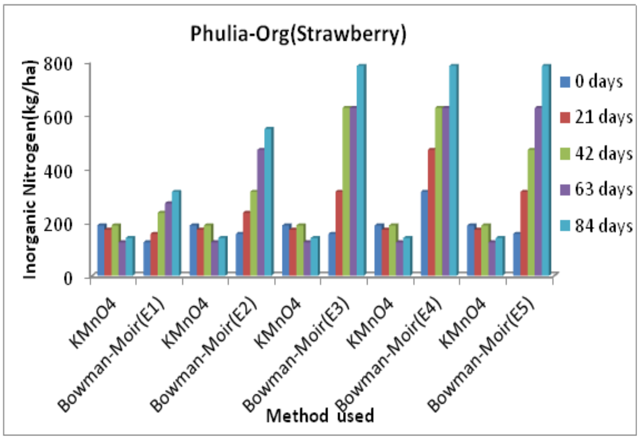
Available Nitrogen Content

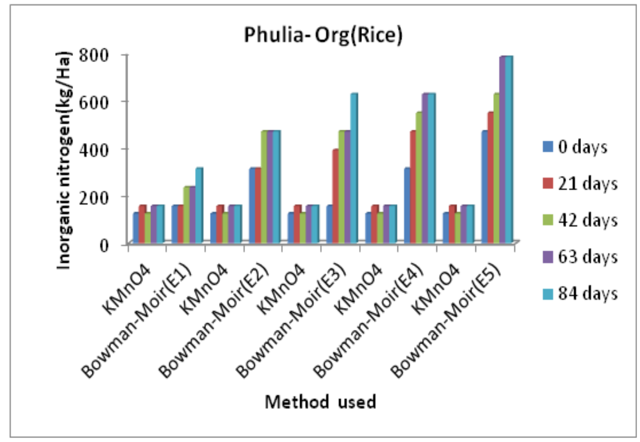
The result shows that soils of conventional farming irrespective to location and management practice retained higher magnitude of $KMnO_4$ oxidisable nitrogen as compared to that of organic farming soils. Routine use of chemical fertilizers in conventional farming accumulated higher amount of nitrogen in soils. Many studies revealed that, they yield of organic farming is 25% lower than conventional yield and according to a recent meta-analysis this varies with crop types and species and depends on the comparability of farming systems (Gabriel, Salt, Kunin and Benton, 2013). Among the organically managed farms in Phulia, Strawberry cultivated soils retained higher amount available nitrogen because of its higher organic matter content (Table 2). Addition of organic manure such as vermicompost, green leaf manure which is an important source of nitrogen has resulted higher amount of organic carbon content. These results are similar with the findings of Bhuyan and Gaur (2009). In most of the cases we see that the available nitrogen content is deteriorating during the incubation period because of readily available sources of nutrient is decreased day by day. In organic farming system the nutrient present in insoluble form and for this case the traditional KMnO4 method cannot extracts significant amount of inorganic nitrogen.

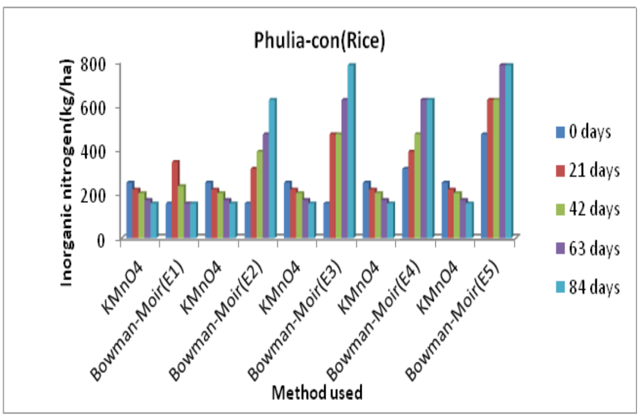
Basic EDTA Extractable inorganic nitrogen

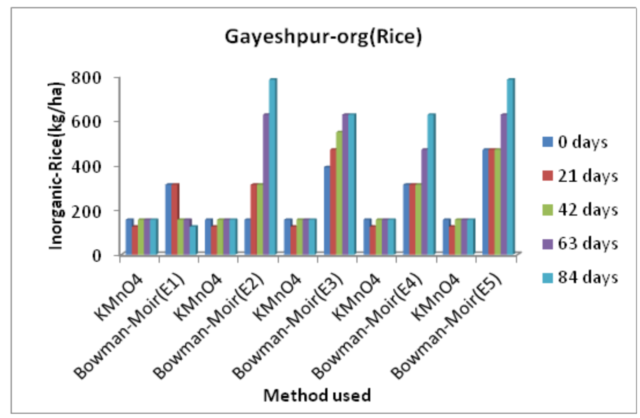
During whole scale of incubation, soils were extracted with different strength of basic EDTA for estimation inorganic N content which was developed by Bowman and Moir (1993) for organic P estimation. Results show that, in both cases of organic and conventional managed farms retained diverse range of inorganic Nitrogen pools. It indicates that differential capacity of extractants extracts different amount of inorganic nitrogen from soil. Among the extractants 0.375 M NaOH + 0.05 M EDTA extracted significantly the highest magnitude(784 kg N/ha) of inorganic nitrogen in Phulia site of Nadia district and 0.5 M NaOH+ 0.05 M EDTA extracted significantly the highest magnitude of inorganic nitrogen (784 kg N/ha) in Central Research Farm in Gayeshpur sites (Table 3). On the other hand traditional method extracts 141.12 kg N/ha and 156.8 kg N/ha in Phulia sites and 156.8 kg N/ha and 172.48 kg N/ha (Table 2) in Gayeshpur sites. It may be noted that, 0.375 M NaOH + 0.05 M EDTA extractable inorganic N recorded the highest changes contributing 80% during 84 days of incubation. This argues that bulk organic N is not a sole important factor for speedy decomposition, rather structural simplicity and liability are the factors influencing the disappearance of organic N pools in soil and increasing the rate of mineralizable nitrogen. Results shows that stock of different inorganic N fractions varies in different farms even in same agro ecological region. One garden while use vermicompost; others use other organic manures of different nature of substrates. These are due to management approaches adopted by different farms.

This confirms that capacity of the extractants lies with the









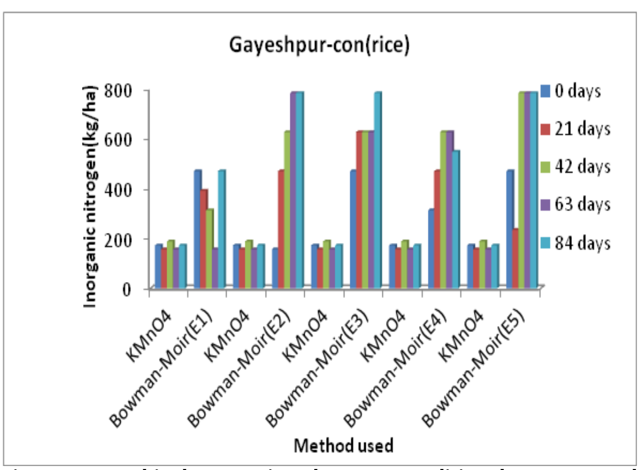


Figure 1: Graphical comparison between Traditional ($KMnO_4$) and modified method (Bowman and Moir) of inorganic nitrogen (kg/ha) in org and conventional plot in Phulia and Gayeshpur

strength of base used as well as the nature of organic N fractions. However, amount of organic N extracted by 0.375 M NaOH and 0.1 M NaOH did not differ significantly. This phenomenon argues that soil of these farms contains more or less similar volume of those fractions. On the other way, it may be explained that organic fraction extracted by least strength of base was simple in their structure and consequently higher solubility. 1 M NaOH +0.05 M EDTA as it is relatively harsh; it extracted comparatively resistant organic nitrogen fractions and thus gave less initial stock.

Suitability of Basic EDTA Extractable inorganic nitrogen over traditional method

In the graphical representation (Fig 1) we see that with the progress of incubation the concentration of different fractions of inorganic nitrogen extracted by different strength of basic EDTA increased gradually irrespective of location and management adopted. This interprets the continual mineralization of remained organic N in subsequent incubation period. Dynamics of organic N fraction mineralization across the incubation period varied even ignoring the initial stock of organic nitrogen. The highest stock of organic N registered the lower change of organic N. Whereas, the less amount of initial organic nitrogen fraction recorded the highest. This indicates that not the volume of substrate *per se* is important for mineralization, the liability and structural simplicity rather more congenial factor for microbial access for mineralization. But in conventional $KMnO_4$ method, the value inorganic nitrogen is diminutive rather than modified Bowman and Moir method (Fig 1) during whole scale of incubation. It fails to extract the recalcitrant form of nitrogen. So the value of inorganic nitrogen obtained from traditional KMnO4 method is relatively less than modified method. This indicates that the nature of organic N pools under traditional method is relatively recalcitrant in nature, thus, less vulnerable to microbial attack. Beside this, conventional soil test for N estimates the soil labile N supply available for plant uptake. It is the snapshot of the most dynamic fraction N in soil. The dynamic fraction of N considered in conventional soil testing, cannot explain the N status of soils of organic farming (Saha and Mandal, 2011). But basic EDTA can chelate metal cations to eliminate the metal organic nitrogen bridge to increase the efficiency of SOM associated organic nitrogen solubilisation. Chelating agents are involved in metal complexation by providing N (nitrogen) and O (oxygen) atoms as donors. The number of these atoms may determine the chelating strength. As a strong chelating reagent, EDTA could partly remove organically bound metals; carbonate bound metals, and parts of metals occluded in oxides and secondary clay minerals (Gupta and Sinha, 2007; Anju and Banerjee, 2011). It also be noted that EDTA might extract more metals than the plant is able to take up and overestimate phytoavailability (McLaughlin *et al.*, 2000; Menzies *et al.,* 2007). When comparing farming systems, measurement method may affect the results; routine soil testing may not be able to predict available soil N in a biodynamic system due to the interaction of crop and soil factors in controlling mobility of N ions (Oberson *et al*., 2003).

In conclusion it can be stated that basic EDTA method of extracting inorganic nitrogen can be putatively selected as a suitable extractant as compared to conventional method. Among all this extractant 0.375 (M) NaOH + 0.05 (M) EDTA and 0.5 (M) NaOH + 0.05 (M) EDTA are most promising which can easily extract the recalcitratant form of inorganic nitrogen easily. Considerable efforts will also be done to apply this modified method in practical field for increasing the availability of all forms of nitrogen to crops.

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