Impact of Organic Fertilizers with and without Chemical Fertilizers on Soil Chemical Properties and the Establishment of Nitrogen-Fixing Bacteria in the Rhizosphere

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Effects of organic fertilizers with and without the application of chemical fertilizers for seven years as part of a wheat-pearl millet cropping sequence on soil chemical properties and the establishment of nitrogen fixing bacteria in the rhizosphere were examined. The application of farmyard manure, poultry manure, and sugarcane filter cake alone or in combination with chemical fertilizers improved the soil organic C, total N, P, and K status. Larger populations of Azotobacter chroococcum and Rhizobium leguminosarum bivar trifoli in the rhizosphere of wheat and Egyptian clover respectively, were maintained in soils receiving organic fertilizers either alone or in combination with chemical fertilizers than in soils given chemical fertilizers alone.

Key words: genetically marked strains, nitrogen fixing bacteria, organic fertilizers, rhizosphere soil

The maintenance of soil organic content is important for the long-term productivity of soil. The level of organic matter depends on climate, soil type and land management practices.13,18 Changes in soil organic matter are related to organic amendments as well as turnover rates of native soil organic matter. Additionally, the application of chemical fertilizers can increase soil organic matter by increasing the input of root biomass to the soil. The integrated use of chemical fertilizers and organic amendments is being advocated to maintain soil organic matter and crop production.41

Microorganisms play an important role in soil fertility management. Microorganisms have the ability to carry out nutrient transformations and serve as a source and sink of nutrients.14 The use of nitrogen-fixing bacteria is increasingly being advocated to maintain agricultural production by reducing the input of fertilizer N.12 The survival, persistence and competitive ability of the inoculated strains limit their success in the soil. Various methods have been used to identify the introduced bacteria using molecular markers. Nitrogen-fixing bacteria tagged with lacZ and gusA genes have been identified as colored colonies on chromogenic substrates.20,21 Appropriate management practices need to be followed to ensure maximum contribution by a particular microbe. The present study was conducted to examine the effect of organic fertilizers with and without chemical fertilizers on soil chemical properties and the persistence of genetically marked nitrogen-fixing bacteria in the rhizosphere.

Materials and Methods

Field site

The experiment was started at the research farm of the CCS Haryana Agricultural University, Hisar, India with a wheat (Triticum aestivum L.)-pearl millet (Pennisetum glaucum (L.) R. Br.) cropping sequence using the treatments listed in Table 1. Wheat was grown during winter (November–April) and pearl millet was grown in summer (July–October). The experimental site is located at 29°02′ N latitude and 75°05′ E longitude, at an altitude of 215 m above mean sea level. The climate of the area is semi-arid with a mean annual rainfall of about 400 mm varying between 170 and 700 mm and a mean annual temperature of 28.3°C with a minimum of 2°C and maximum of 47°C. The soil of the study site was sandy loam (70% sand, 16% silt and 14% clay) classified as typic Ustochrept. The soil initially contained 0.41% organic C, 0.03% total N, 0.02% total P, and 0.35% total K and had a pH of 7.5 and electrical conductivity of 0.25 dS m−1.

Fertilizer N was supplied through urea and P, through single superphosphate. Chemical fertilizers were drilled into the soil with a seed cum fertilizer drill. Organic fertilizers were added once a year to the wheat crop only. The pearl millet crop was supplied only with the chemical fertilizers. The organic fertilizers were uniformly spread on the soil surface and then incorporated in the soil by a cultivator. The farmyard manure (FYM), poultry manure (PM) and sugarcane filter cake (SFC) contained on average: 24.3, 35.1 and 39.6% organic C; 0.76, 1.38 and 1.93% total N; 0.39, 1.03 and 1.46% total P; and 2.53, 1.11 and 0.51% total K, respectively. The sugarcane filter cake is an organic waste produced during the clarification of sugarcane juice in the manufacture of sugar.

Soil sampling

Soils (0–15 cm) from each treatment were sampled after seven years of ten different locations in each plot, and pooled. The fresh soil samples were sieved through a 2 mm sieve, adjusted to a maximum water holding capacity of 60% and preincubated at 30°C for 10 days to permit uniform rewetting and to allow microbial activity to settle down after the initial disturbance. Sub-samples of each soil were air-dried and used for chemical analysis.

Soil analysis

Soil pH and electrical conductivity were measured in 1:2.5 (soil:water) ratio. The amount of mineral N was measured by the steam distillation method.22 The total N content was measured by the Kjeldahl method and the amount of organic C, by dichromate oxidation.23 The total P content was measured colorimetrically after wet digestion with perchloric-nitric acid (1:4 v/v).24 The total K content in the wet digest was measured with a flame photometer.

Bacterial culture

Azotobacter chroococcum Mac 27 lacZ was procured from the

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Table 1. Chemical properties of soils as affected by organic and chemical fertilizers

<table>
<thead>
<tr>
<th>Treatments*</th>
<th>Wheat</th>
<th>Pearl millet</th>
<th>pH</th>
<th>Organic C (g kg⁻¹ soil)</th>
<th>Total N (g kg⁻¹ soil)</th>
<th>C:N ratio</th>
<th>Mineral N (mg kg⁻¹ soil)</th>
<th>Total P (mg kg⁻¹ soil)</th>
<th>Total K (mg kg⁻¹ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FYM (15 t ha⁻¹)</td>
<td>N120P60</td>
<td>7.65</td>
<td>9.9</td>
<td>1.38</td>
<td>7.2</td>
<td>3.8</td>
<td>20.8</td>
<td>24.6</td>
<td>887</td>
</tr>
<tr>
<td>PM (5 t ha⁻¹)</td>
<td>N120P60</td>
<td>7.60</td>
<td>8.5</td>
<td>1.28</td>
<td>6.6</td>
<td>6.3</td>
<td>33.0</td>
<td>39.3</td>
<td>945</td>
</tr>
<tr>
<td>SFC (7.5 t ha⁻¹)</td>
<td>N120P60</td>
<td>7.58</td>
<td>9.7</td>
<td>1.17</td>
<td>6.7</td>
<td>5.6</td>
<td>25.5</td>
<td>31.1</td>
<td>807</td>
</tr>
<tr>
<td>PM (5 t ha⁻¹)+N60</td>
<td>N120P60</td>
<td>7.59</td>
<td>8.6</td>
<td>1.15</td>
<td>7.5</td>
<td>7.7</td>
<td>34.7</td>
<td>42.4</td>
<td>1114</td>
</tr>
<tr>
<td>SFC (7.5 t ha⁻¹)+N60P30</td>
<td>N120P60</td>
<td>7.60</td>
<td>8.0</td>
<td>1.10</td>
<td>7.3</td>
<td>5.8</td>
<td>28.5</td>
<td>34.3</td>
<td>894</td>
</tr>
<tr>
<td>N60P30</td>
<td>N120P60</td>
<td>7.68</td>
<td>5.7</td>
<td>0.62</td>
<td>9.2</td>
<td>4.1</td>
<td>18.6</td>
<td>22.7</td>
<td>760</td>
</tr>
<tr>
<td>N120P60</td>
<td>N120P60</td>
<td>7.64</td>
<td>6.0</td>
<td>0.65</td>
<td>9.2</td>
<td>4.5</td>
<td>21.3</td>
<td>25.8</td>
<td>796</td>
</tr>
<tr>
<td>LSD (P&lt;0.05)</td>
<td>NS</td>
<td>0.9</td>
<td>0.13</td>
<td>0.2</td>
<td>0.6</td>
<td>0.7</td>
<td>1.20</td>
<td>0.89</td>
<td>89</td>
</tr>
</tbody>
</table>

* N120 and N60 represent 120 and 60 kg N ha⁻¹ through chemical fertilizers, respectively. P60 and P30 represent 60 and 30 kg P₂O₅ ha⁻¹ through chemical fertilizers, respectively. FYM=Farmyard manure; PM=Poultry manure; SFC=Sugarcane filter cake.

Results

The chemical properties of the soils as affected by organic and chemical fertilizers are shown in Table 1. The soil pH varied from 7.58 to 7.68. There was no significant difference in the pH of soils that received organic fertilizers, a combination of organic and chemical fertilizers, and only chemical fertilizers. The organic C content was significantly higher in the soils treated with organic fertilizers and a combination of chemical and organic fertilizers, than in the soils treated with chemical fertilizers alone. The amount of total N was about 110% higher in organically amended soils compared to soils that received chemical fertilizers, while there was no significant difference between total N in soils given a combination of organic and chemical fertilizers and only organic amendments. Similarly, the amount of mineral N was 40–85% higher in soils treated with both organic and chemical fertilizers compared to soils which received either organic or chemical fertilizers only. The C:N ratio ranged from 6.6 to 7.2 in soils treated with organic fertilizers only and from 7.1 to 7.5 in soils that received a combination of organic and chemical fertilizers. The soils given chemical fertilizers had a significantly higher C:N ratio than those fertilized with organic manures and a combination of organic manures and chemical fertilizers. The amount of total P was highest in soil given a combination of poultry manure and chemical fertilizers followed by soil that received poultry manure only. The lowest amount of total P was observed in soils given chemical fertilizers. The amount of total K was highest in FYM-amended soil compared to poultry manure and sugarcane filter cake-amended soils. The total K content was higher in chemically fertilized soils compared to soils that received a combination of organic and chemical fertilizers.

In the rhizosphere of wheat, the population of inoculated A. chroococcum was significantly larger on treatment with organic fertilizers alone as well as treatment with a combination of organic and chemical fertilizers compared to treatment with chemical fertilizers only (Table 2). At 40 days, the population of A. chroococcum decreased significantly in the soils treated with FYM and sugarcane filter cake compared to the rhizosphere population at 20 days. Nevertheless, the number of A. chroococcum remained the same on treatment with poultry manure.

In the rhizosphere at 20 days, the log number of lacZ⁺ rhizobial cells ranged from 5.89 to 6.10 on treatment with organic fertilizers only and from 5.72 to 6.01 on treatment with a combination of organic and chemical fertilizers (Table 3). The smallest lacZ⁺ rhizobial population was observed in the soils treated with chemical fertilizers. At 40 days, the number of rhizobial cells decreased for all treatments, com-

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* N120 and N60 represent 120 and 60 kg N ha⁻¹ through chemical fertilizers, respectively. P60 and P30 represent 60 and 30 kg P₂O₅ ha⁻¹ through chemical fertilizers, respectively. FYM=Farmyard manure; PM=Poultry manure; SFC=Sugarcane filter cake.
Table 2. Population of inoculated *Azobacter chroococcum* in the rhizosphere of wheat as affected by organic and chemical fertilizers

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Log number of <em>lacZ</em></th>
<th>20 day</th>
<th>40 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Azobacter chroococcum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FYM (15 t ha⁻¹)</td>
<td>N120P60</td>
<td>5.78</td>
<td>5.61</td>
</tr>
<tr>
<td>PM (5 t ha⁻¹)</td>
<td>N120P60</td>
<td>5.72</td>
<td>5.72</td>
</tr>
<tr>
<td>SFC (7.5 t ha⁻¹)</td>
<td>N120P60</td>
<td>5.74</td>
<td>5.61</td>
</tr>
<tr>
<td>FYM (15 t ha⁻¹)+N120P30</td>
<td>N120P60</td>
<td>5.62</td>
<td>5.60</td>
</tr>
<tr>
<td>PM (5 t ha⁻¹)+N60</td>
<td>N120P60</td>
<td>5.61</td>
<td>5.60</td>
</tr>
<tr>
<td>SFC (7.5 t ha⁻¹)+N60P30</td>
<td>N120P60</td>
<td>5.71</td>
<td>5.70</td>
</tr>
<tr>
<td>N60P30</td>
<td>N60P30</td>
<td>5.33</td>
<td>5.40</td>
</tr>
<tr>
<td>N120P60</td>
<td>N120P60</td>
<td>5.30</td>
<td>5.40</td>
</tr>
</tbody>
</table>

* Log initial *Azobacter chroococcum* soil population=4.73 g⁻¹. LSD (P=0.05) Treatments (T)=0.27; Sampling time (ST)=0.12; Interaction (TxST)=NS.

Table 3. Population of *Rhizobium leguminosarum* bv. *trifolii* in the rhizosphere of Egyptian clover as affected by organic and chemical fertilizers

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Log number of <em>lacZ</em></th>
<th>20 day</th>
<th>40 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Rhizobium leguminosarum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FYM (15 t ha⁻¹)</td>
<td>N120P60</td>
<td>6.10</td>
<td>5.91</td>
</tr>
<tr>
<td>PM (5 t ha⁻¹)</td>
<td>N120P60</td>
<td>6.02</td>
<td>5.93</td>
</tr>
<tr>
<td>SFC (7.5 t ha⁻¹)</td>
<td>N120P60</td>
<td>5.89</td>
<td>5.80</td>
</tr>
<tr>
<td>FYM (15 t ha⁻¹)+N120P30</td>
<td>N120P60</td>
<td>5.91</td>
<td>5.81</td>
</tr>
<tr>
<td>PM (5 t ha⁻¹)+N60</td>
<td>N120P60</td>
<td>6.01</td>
<td>5.80</td>
</tr>
<tr>
<td>SFC (7.5 t ha⁻¹)+N60P30</td>
<td>N120P60</td>
<td>5.72</td>
<td>5.60</td>
</tr>
<tr>
<td>N60P30</td>
<td>N60P30</td>
<td>5.56</td>
<td>5.43</td>
</tr>
<tr>
<td>N120P60</td>
<td>N120P60</td>
<td>5.52</td>
<td>5.41</td>
</tr>
</tbody>
</table>

* Log initial soil population of *Rhizobium leguminosarum* bv *trifolii* =4.56 g⁻¹. LSD (P=0.05) Treatments (T)=0.15; Sampling time (ST)=0.10; Interaction (TxST)=NS.

pared to 20 days. The decrease was significant for all the fertilizers except the poultry manure and sugarcane filter cake alone and FYM along with chemical fertilizers.

### Discussion

Managing the supply of nutrients to plants is an important aspect of crop productivity. The use of organic fertilizers either alone or in combination with chemical fertilizers plays a key role in sequestering C and building up soil fertility. There was no significant change in the pH of soils upon various treatments due to the buffering capacity of these soils. The maximum increase in organic C with the application of FYM is attributed to more carbon going to the soil. The C present in poultry manure and sugarcane filter cake gets readily mineralized compared to FYM. Increases in organic C and total N have been observed in organically managed soils compared to conventionally managed soils receiving chemical fertilizers only. The C:N ratio provides information on the capacity of the soil to store and recycle nutrients. A lower C:N ratio with the application of organic fertilizers indicates a build up of N in the soil, which is a potential source of N on mineralization. Phosphorus in organic fertilizers is released after mineralization and is available to the crops. The build up of total K in soil was higher with only FYM. Bulluck III et al. observed that the K concentration in soil amended with organic wastes increased compared to that in soils fertilized with chemicals where K content decreased with time. Potassium was not supplied through chemical fertilizers in the present experiment as the soil at the experimental site is rich in K and so crops do not respond to the application of K. The build up in the soil is due to a net balance between plant uptake and additions through organic fertilizers and root biomass as crop residues were not added to the soil.

The survival and persistence of introduced microorganisms in the root zone is very important to the harnessing of their potential. The persistence of beneficial microorganisms in soil is dependent on soil and environmental conditions. Soil management practices have an indirect influence by creating conditions conducive for proliferation as well as protection from adverse environmental impacts. A variety of microorganisms have been used to inoculate soil and improve the supply of nutrients to crops. The *R. leguminosarum* biovar *trifolii* and *A. chroococcum* increased in number up to 20 days after the inicial inoculation and then declined. This may be due to the limited availability of substrate in the rhizosphere. Ellis et al. observed the survival of *Rhizobium japonicum* under field conditions up to 56 weeks. The numbers of inoculant species were increased within the first seven weeks and thereafter decreased to background levels. Decline in numbers has been observed for typical soil bacteria such as fluorescent *Pseudomonas*, *Flavobacterium* and *Rhizobium* and may be due to a scarcity of nutrients available to microbes in soil and the hostility of the soil environment to incoming microbe due to a myriad of adverse abiotic and biotic factors. It is generally accepted that organic substances such as organic acids, sugars and amino acids exuded by plant roots result in the proliferation of microbes in the rhizosphere. Thompson et al. observed that the survival of *Flavobacterium* and *Arthrobacter* was greater in rhizosphere soils than in unplanted soils. Bashan et al. observed the survival of *Azospirillum brasilense* in the rhizosphere of 23 soil types. Large numbers of *A. brasilense* were detected in all the rhizospheres tested, regardless of soil type and origin. Number of *R. leguminosarum* biovar *trifolii* and *A. chroococcum* were larger in soils receiving organic fertilizers alone or in combination with inorganic fertilizers compared to soils receiving only inorganic fertilizers. This may be because different soil management practices affect soil’s physical and chemical properties, which in turn affects the survival of microorganisms. Continuous use of organic manures improves a soil’s physicochemical properties and may lead to increased survival of microorganisms.

### Conclusions

Organic amendments can be helpful in increasing the survival and persistence of inoculated nitrogen fixing bacteria in the rhizosphere. This is mainly due to improvements in the soil’s chemical properties, particularly organic matter. Changes in physical properties such as bulk density and...
water holding capacity in response to organic fertilizers can
not be ruled out either. Such physicochemical changes are
beneficial to low input agriculture; particularly organic farming
where the potential of nitrogen-fixing bacteria and other organic
soil microbes can be harnessed to meet some of the require-
ments of crops.

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