INTEGRATED APPLICATION OF ORGANIC AMENDMENTS AND MYCORRHIZAE IMPLEMENTATION FOR SPINACH (SPINACIA OLERACEA) GROWTH AND DEVELOPMENT

F. Ahmad1, Z. Mushtaq2*, N. Itrat3, A. Nazir3, A. Hussain4, A. Akhtar4, M. Khurshid5, I. Saeed1, S. Rahid1, A. Çiğ6, F. Çiğ1, A.R. Siddiqui8

1School of Food Sciences and Technology, Minhaj University, Lahore, Pakistan
2Department of Soil Science, Faculty of Agricultural Sciences, University of the Punjab, Lahore Pakistan,
3Department of Nutrition and Dietetics, The University of Faisalabad, Pakistan
4Department of Plant Pathology, University of the Punjab, Lahore, Pakistan
5Scholl of Biochemistry and Biotechnology, Quaid-i-Azam Campus, P.O Box 54590, University of Punjab, Lahore
6Department of Horticulture, 7Department of Field Crops, Faculty of Agriculture, Siirt University, Siirt, Türkiye
8Department of Agriculture, Government College University Lahore 54000, Punjab

Corresponding email: zmushtaq60@gmail.com

ABSTRACT: For plants and other species alike, phosphorus (P) is the second most essential macronutrient. However, it becomes unavailable to plants because of its fixation with soil collisions, and hence cannot enter the food chain. In a pot trial, the impact of mycorrhizae and various organic amendments on P uptake and plant growth of spinach (Spinacia oleracea) was assessed. Farmyard manure (FYM), biogas slurry (BGS), and compost were used as organic amendments at a rate of 1.5% w/w in each treatment, along with mycorrhizae. Mycorrhizae and organic amendments applied together were found to boost plant growth and increase P uptake. FYM + mycorrhizae and BGS + mycorrhizae combinations resulted in increased root length, shoot height, shoot fresh weight, root fresh weight and leaf area. BGS + mycorrhizae combinations also showed increased P absorption, leading to higher photosynthetic activity and biomass. Mycorrhizae and organic amendments were shown to increase P uptake by as much as 42.25% and plant growth by 57.39%. We concluded that using this strategy in the field can be an economically viable option while also lowering the potential for negative environmental effects caused by the overuse of chemical fertilizers.

Keywords: mycorrhizae, biogas, compost, farmyard manure, plant growth.

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INTRODUCTION

The soil's health declines, and food security is threatened when people overuse and misuse it without regard for the long term (Sanwal et al., 2007; Prosekrov and Ivanova, 2018). Environmentalists, social scientists, and economists are all worried about the impact this might have on food supplies and the economy. It is challenging work to keep soils in cultivation in optimal chemical, biological, and physical condition so that they continue to produce abundant crops (Prosekov and Ivanova, 2018).

Phosphorus is second only to nitrogen in importance to plant life (Thao et al., 2008). It plays a part in photosynthesis, signal transduction, respiration, energy transfer, and macromolecule synthesis, to name a few of the primary biosynthetic activities in plants. Only a tiny fraction of the soil's phosphorus (P) (0.1%) is used by plants. Soil phosphorus is said to be relatively insoluble and so unavailable to plants. It has long been believed that adding phosphorus fertilizer was the sole way to correct soil P shortage. Since most of the P fertilizer applied to plants is inaccessible to them, it poses environmental risks contaminants in the groundwater and eutrophication are two examples (Thao et al., 2008; Kang et al., 2011).

Increased plant nutrient absorption is facilitated by soil microbes. Some of these bacteria can convert insoluble nutrients into plant-available forms through a process called solubilization and mineralization (Zahedifar et al., 2011; Babalola and Glick, 2012). Only by microbial solubilization and mineralization can plant-available P be increased beyond what can be achieved with chemical fertilization. Soil phosphorus concentrations can be increased by a number of rhizospheric bacteria through a variety of processes (Zahedifar et al., 2011). Numerous microbial taxa, includiseveral and fungi, have been shown to be capable of solubilizing P in vitro (Zhu et al., 2011).

Fungi generate and secrete a wider variety of acids than bacteria, including tartaric, citric, gluconic, 2-ketogluconic, acetic, and lactic acid. In addition, fungi can easily traverse greater distances within the soil than bacteria (Sharma et al., 2013; Srinivasan et al., 2012).
Nearly 80% of plant species on Earth have a mycorrhizal relationship with soil fungus. Mycorrhizae are just one example of the many fungal relationships that benefit both the plant and the fungus in the long run (Bücking et al., 2012). Mycorrhizal plants have been shown to increase their P uptake via several different methods. Root-soil mycorrhizal interactions are one of numerous adaptive methods plants have developed to resist P stress (Begum et al., 2019; Smith and Read, 2010).

Semiarid soils typically have low plant-available nutrients, especially P, and low organic matter (1%) rather than high total soil P concentration (Sarker et al., 2014; Muhammad et al., 2008). Organic matter has been shown to improve the soil's biological, chemical, and physical qualities, all of which are essential for fostering plant growth. Compost and manure are two examples of organic amendments that can be used to boost yields and soil organic matter. Better seed germination and stronger root systems are only two of the many benefits. Celestina et al. (2019) found that organic fertilizers also promote the growth of foreign microorganisms. Therefore, the purpose of this research was to examine how mycorrhizae and organic amendments work together to boost spinach's growth and phosphorus intake.

**METHODOLOGY**

The University of Agriculture in Faisalabad conducted an experiment in a wire greenhouse. Ten kilograms of soil with the same chemical makeup was used to fill each pot. Soil was amended with organic matter and mycorrhizae before planting. Mycorrhiza of a single strain were administered alongside four different organic amendments (1.5% w/w basis): FYM, biogas slurry, compost, and chicken manure. All the fertilizers DAP, urea, and MOP were used at recommended rates. The pots were filled with soil, and the seedlings were put in them and given water as needed. One treatment was kept control along with different combination of compost, farmyard manure, biogas slurry and mycorrhizae. The treatments were repeated three times each. The experiment was conducted using a completely randomized approach. All treatments produced comparable growth and yield results. Biomass was calculated from root and shoot samples, and phosphorus levels in fruit were determined. Wet digestion was performed on fruit samples. Overnight, 10 ml of sulphuric acid was left in a digestion tube with 1 gram of oven-dried, finely crushed fruit. For 30 minutes at 300°C, samples were heated and then digested on a hot plate. Hydrogen peroxide was added very slowly until the filtrate was completely clear. The necessary P-determining reagents have been assembled. The solution of ammonium heptamolybdate (22.5 g in 400 ml DI water) and ammonium metavanadate (1.25 g in 300 ml DI water) was combined and cooled in a 1 L flask. The flask was filled with 250 ml of nitric acid, and the rest of the volume was made up with DI water. A 1 L solution of oven-dried potassium dihydrogen phosphate was used to make 0.2197 g of standard stock solution. This solution had a concentration of 50 ppm. Sub-standards were prepared by adding standard stock solution (1, 2, 3, 4, and 5 ml) to a 100 ml flask and then filling it to the appropriate level with DI water. A volumetric flask of 100 ml was filled with distilled water to the appropriate level, a 10-milliliter transparent filter was added, and 1 milliliter of ammonium-vanadomolybdate solution was made. After 30 minutes, the spectrophotometer was calibrated to 410 nm wavelengths (Olsen, 1954) and the absorbance of the samples, standards, and blank were recorded.

**RESULTS**

**Agronomic attributes:** Plants that were inoculated grew more leaves and looked healthier than their control counterparts because mycorrhizae had reached deeper into the soil and brought up more nutrients. Table 1 shows that when mycorrhizae and organic amendments were used together, the plants flourished because the mycorrhizae released nutrients from the organic matter more quickly. All of these combinations resulted in a doubling of leaf production compared to controls and non-inoculated plants, but biogas slurry combined with mycorrhizae produced the most growth.

Roots on plants that had been inoculated with mycorrhizae and modified with organic matter were noticeably longer than those on plants that hadn't been inoculated. Table 1 displays the superior root growth achieved by plants treated with a combination of mycorrhizae and farmyard waste. Longer roots and more total root surface area are the result of mycorrhizae infecting plant roots and growing as they seek water and nutrients. However, the nutrients in the soil were released by the organic matter, and the mycorrhizae in the soil assisted the roots in absorbing the nutrients. Inoculated plants grown in soil modified with organic matter, particularly biogas slurry and mycorrhizae combinations, had taller shoots than plants grown in soil without amendments or as a control (Table 1).

**Phosphorus and nitrogen concentration in spinach:** The amount of phosphorus in plant fruits varied widely. Inoculated plants had greater phosphorus concentrations than non-inoculated plants, even in the absence of organic matter, because mycorrhizae created acids to maintain a pH that helped plants absorb phosphorus. Non-inoculated plants had lower phosphorus concentrations. The plant that had been treated with biogas slurry and mycorrhizae had the highest phosphorus level (Figure 1).
Table 1: Influence of organic amendments and mycorrhizae on agronomic attributes of spinach.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>SFW (g plant⁻¹)</th>
<th>SDW (g plant⁻¹)</th>
<th>RFW (g plant⁻¹)</th>
<th>Leaf area (cm² plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.24 g</td>
<td>6.24 g</td>
<td>0.99 g</td>
<td>1.05 f</td>
<td>148.24 g</td>
</tr>
<tr>
<td>Compost</td>
<td>15.65 f</td>
<td>7.10 e</td>
<td>1.45 e</td>
<td>1.51 e</td>
<td>157.48 f</td>
</tr>
<tr>
<td>FYM</td>
<td>16.47 e</td>
<td>6.68 f</td>
<td>1.28 f</td>
<td>1.49 e</td>
<td>162.58 e</td>
</tr>
<tr>
<td>BGS</td>
<td>16.25 e</td>
<td>6.89 f</td>
<td>1.31 f</td>
<td>1.54 e</td>
<td>164.89 e</td>
</tr>
<tr>
<td>Mycorrhizae</td>
<td>17.24 d</td>
<td>7.25 d</td>
<td>1.62 d</td>
<td>1.62 d</td>
<td>171.58 d</td>
</tr>
<tr>
<td>Mycorrhizae + Compost</td>
<td>19.25 b</td>
<td>7.89 e</td>
<td>1.87 c</td>
<td>2.12 b</td>
<td>187.98 c</td>
</tr>
<tr>
<td>Mycorrhizae + FYM</td>
<td>18.89 c</td>
<td>8.25 b</td>
<td>2.12 b</td>
<td>1.99 c</td>
<td>199.67 b</td>
</tr>
<tr>
<td>Mycorrhizae + BGS</td>
<td>20.45 a</td>
<td>8.45 a</td>
<td>2.45 a</td>
<td>2.54 a</td>
<td>205.77 a</td>
</tr>
</tbody>
</table>

SFW: Shoot fresh weight  
SDW: Shoot dry weight  
RFW: Root fresh weight  
FYM: Farmyard manure  
BGS: Biogas slurry

Figure I: Influence of organic amendments and mycorrhizae on phosphorous concentration (%) of spinach

Figure II showed the impact of organic amendments and mycorrhizae on nitrogen concentration of spinach. Organic amendments along with mycorrhizae had a significant impact on nitrogen concentration of spinach. Maximum nitrogen was recorded in treatment where mycorrhizae was applied along with biogas slurry.

Figure II: Influence of organic amendments and mycorrhizae on nitrogen concentration (%) of spinach
DISCUSSION

Constant environmental challenges, of both biotic and abiotic origin, must always be surmounted by plants in order to survive. Specifically, dryness is one of the most prevalent environmental stressors that soil plants undergo (Begum et al., 2019; Shinozaki et al., 2003). The effects of drought stress on plant–water interactions, as well as specific and nonspecific physiological responses (Beck et al., 2007), have a significant negative impact on plant growth and nutrition, and as a result, crop output is reduced. According to Vinocur and Altman (2005), drought is often regarded as the primary factor responsible for falling agricultural yield across the world. There is widespread agreement that our planet's climate is changing, and that these harmful consequences are likely to become much more severe in the years to come. As a result, the growth of plants and crops in many parts of the world will face substantial challenges because of these changes. According to Denby and Gehring (2005), these challenges will be especially significant in areas that are now semi-arid in agricultural terms.

Both the individual effects of PGPR and AMF as well as their combined effects were analyzed. Data were collected and analyzed regarding the fresh and dry weight of plants, the circumference of onion bulbs, the fresh and dry weight of bulbs, chlorophyll a and b, carotenoids, and the concentration of P in plants at the time of harvesting. A combined inoculation of endomycorrhiza and rhizobacteria led to improvements in foliage fresh and dry weight, onion bulb circumference, bulb fresh and dry weight, chlorophyll a and b, and carotenoids, as evidenced by the findings. The application of endomycorrhiza and rhizobacteria resulted in a 77% improvement in the plant's ability to absorb phosphorus, in contrast to the control treatment.

Conclusion: The results obtained showed that mycorrhizal application alone and in combination with different organic amendments significantly enhanced the agronomic attributes, phosphorous and nitrogen concentration in spinach compared with control treatments. The plants that were amended with farmyard manure and biogas slurry showed better growth, P and N contents than control. On the other hand, the plants that were inoculated with mycorrhizae and amended with biogas slurry uptake maximum nitrogen, phosphorus and showed better agronomic growth as compared to other treatments.

Conflicts of interest: The authors declared that they have no know competing interest.

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