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

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**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; Prosekov 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. Mycorrhizae 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 I).

**Table 1: Influence of organic amendments and mycorrhizae on agronomic attributes of spinach.**

| Treatments | Plant height (cm) | SFW (g plant$^{-1}$) | SDW (g plant$^{-1}$) | RFW (g plant$^{-1}$) | Leaf area (cm$^2$ plant$^{-1}$) |
|---|---|---|---|---|---|
| Control | 14.24 g | 6.24 g | 0.99 g | 1.05 f | 148.24 g |
| Compost | 15.65 f | 7.10 e | 1.45 e | 1.51 e | 157.48 f |
| FYM | 16.47 e | 6.68 f | 1.28 f | 1.49 e | 162.58 e |
| BGS | 16.25 e | 6.89 f | 1.31 f | 1.54 e | 164.89 e |
| Mycorrhizae | 17.24 d | 7.25 d | 1.62 d | 1.62 d | 171.58 d |
| Mycorrhizae + Compost | 19.25 b | 7.89 c | 1.87 c | 2.12 b | 187.98 c |
| Mycorrhizae + FYM | 18.89 c | 8.25 b | 2.12 b | 1.99 c | 199.67 b |
| Mycorrhizae + BGS | 20.45 a | 8.45 a | 2.45 a | 2.54 a | 205.77 a |

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

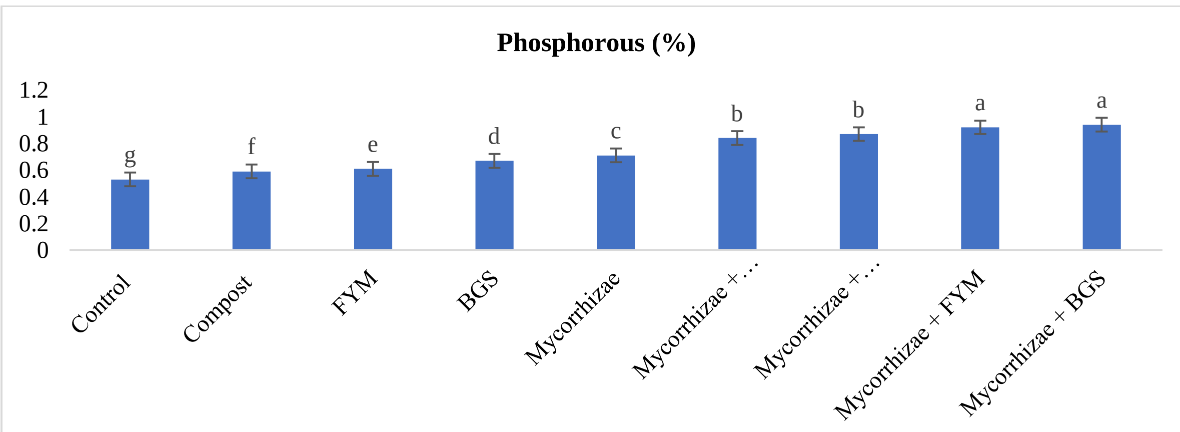


**Figure I: Influence of organic amendments and mycorrhizae on phosphorous concentration (%) of spinach**

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.

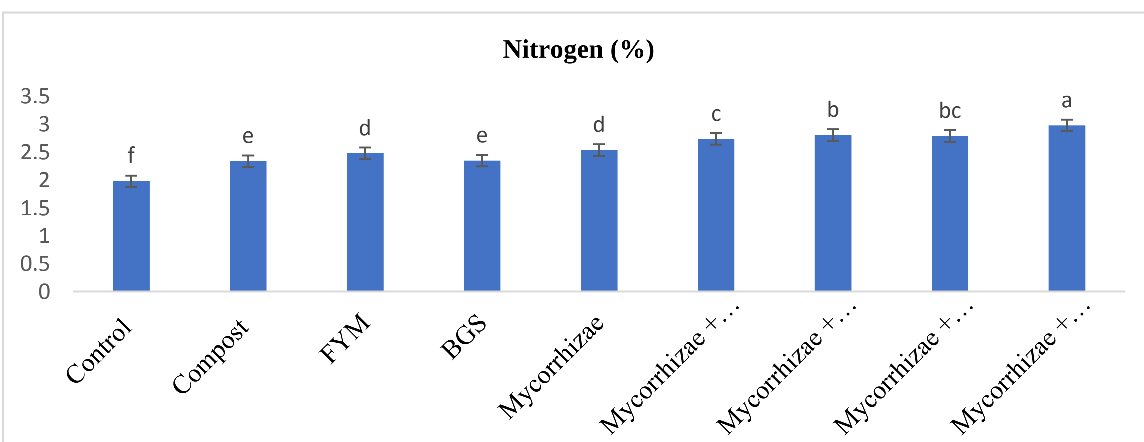


**Figure II: Influence of organic amendments and mycorrhizae on nitrogen concentration (%) of spinach**

## 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 in improvements in plant 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.

## REFERENCES


Babalola, O.O., Glick, B., 2012. Indigenous African agriculture and plant associated microbes: current practice and future transgenic prospects. Sci. Res. Essays 7, 2431-2439.

Begum, N., Qin, C., Ahanger, M.A., Raza, S., Khan, M.I., Ashraf, M., Ahmed, N. and Zhang, L., 2019. Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. *Frontiers in plant science*, *10*, p.1068.

Bhattacharyya, P.N., Jha, D.K., 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World Journal of Microbiology Biotechnology 28, 1327-1350.

Bücking, H., Liepold, E., Ambilwade, P., 2012. The role of the mycorrhizal symbiosis in nutrient uptake of plants and the regulatory mechanisms underlying these transport processes. Plant Sci 4, 108-132.

Celestina, C., Hunt, J.R., Sale, P.W., Franks, A.E., 2019. Attribution of crop yield responses to application of organic amendments: A critical review. Soil Tillage Research 186, 135-145.

Kang, J., Amoozegar, A., Hesterberg, D., Osmond, D., 2011. Phosphorus leaching in a sandy soil as affected by organic and incomposted cattle manure. Geoderma 161, 194-201.

Khan, M.S., Zaidi, A., Ahemad, M., Oves, M., Wani, P.A., 2010. Plant growth promotion by phosphate solubilizing fungi–current perspective. Archives of Agronomy Soil Science 56, 73-98.

Muhammad, S., Müller, T., Joergensen, R., 2008. Relationships between soil biological and other soil properties in saline and alkaline arable soils from the Pakistani Punjab. Journal of Arid Environments 72, 448-457.

Prosekov, A.Y. and Ivanova, S.A., 2018. Food security: The challenge of the present. *Geoforum*, *91*, pp.73-77.

Sanwal, S., Laxminarayana, K., Yadav, R., Rai, N., Yadav, D., Bhuyan, M., 2007. Effect of organic manures on soil fertility, growth, physiology, yield and quality of turmeric. Indian Journal of Horticulture 64, 444-449.

Sarker, A., Kashem, M.A. and Osman, K.T., 2014. Influence of lime and phosphorus on growth performance and nutrient uptake by indian spinach (Basella alba L.) grown in soil. *Open Journal of Soil Science*, *2014*.

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., Gobi, T.A., 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springer plus 2, 587.

Smith, S.E., Read, D., 2010. Mycorrhizal symbiosis. Academic press.

Srinivasan, R., Yandigeri, M.S., Kashyap, S., Alagawadi, A.R., 2012. Effect of salt on survival and P-solubilization potential of phosphate solubilizing microorganisms from salt affected soils. Saudi journal of biological sciences 19, 427-434.

Thao, H.T.B., Yamakawa, T., Myint, A.K. and Sarr, P.S., 2008. Effects of phosphite, a reduced form of phosphate, on the growth and phosphorus nutrition of spinach (Spinacia oleracea L.). *Soil Science and Plant Nutrition*, *54*(5), pp.761-768.

Zahedifar, M., Karimian, N., Ronaghi, A., Yasrebi, J. and Emam, Y., 2011. Soil–plant nutrient relationship at different growth stages of spinach as affected by phosphorus and manure applications. *Communications in soil science and plant analysis*, *42*(15), pp.1765-1781.

Zhu, F., Qu, L., Hong, X., Sun, X., 2011. Isolation and characterization of a phosphate-solubilizing halophilic bacterium Kushneria sp. YCWA18 from Daqiao Saltern on the coast of Yellow Sea of China. Evidence-Based Complementary Alternative Medicine 2011.