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Effect of Phosphate Solubilizing Microorganisms (PSM) on growth of *Mentha spicata* L.

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ABSTRACT

The use of phosphate solubilizing microorganisms (PSM) as live microbial biofertilizers provides a promising alternative to chemical fertilizers. In the present study the effect of various doses of PSM culture on growth of spearmint (*Mentha spicata* L.) plants were observed.A series of experiments were conducted in pot cultures in randomised block design (RBD). A commercial consortium of *Bacillus megaterium, Pseudomonas putida* and *Aspergillus awamori* was used as the PSM culture. The various growth parameters such as Fresh and dry weight, chlorophyll content, Nitrogen content, Phosphate Phosphorous content and potassium content of spearmint were tested after 60, 90 and 120 DAS. The results were encouraging and demonstrated significantly positive effect of increasing doses of PSM culture on various growth parameters of spearmint plants throughout the experiment. Amongst the various treatments, the treatment T_3(0.03gm PSM culture per Kg sterilized soil) proved to be better for harvest at 90 DAS than the remaining treatments.

Key words : *Biofertilizers, PSM, Spearmint (Mentha spicata L.), Pot cultures, Plant growth, Harvest.*

Introduction

Phosphorus (P) is one of the essential plant nutrients that is necessary for plant development and growth (Alori *et al*., 2017). Phosphorus is a vital element as it performs functions in the plant metabolism, structure, and reproduction. However, Phosphorus, both native in soil and applied in inorganic fertilizers becomes unavailable to crops because of its low levels of mobility, solubility and its tendency to become fixed in the soil and hence cannot be utilized by the plants (Vassileva *et al*., 1998). The soil microorganisms that colonize the rhizosphere, and solubilize the unavailable forms of inorganic phosphorous into soluble forms by releasing a variety of organic acids, lowering the pH of rhizosphere soil (Sharma *et al*., 2013; Shankar *et al*., 2013), also by producing enzymes like phosphatases and phytases (Richardson *et al*., 2000; Tarafdar *et al*., 2003; Aseri *et al*., 2009). The micro-organisms with a capacity to solubilize inorganic phosphates and make it available to plants are together known as Phosphate Solubilizing microorganisms (PSM) (El-Tantawy *et al.,* 2009). Species of *Aspergillus* and *Penicillium* are amongst fungal isolates identified to have phosphate solubilizing capabilities. Among the bacterial genera, *Pseudomonas*, *Azospirillum*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Arthrobacter*, *Serratia*, *Enterobacter*, *Acinetobacter*, *Erwinia*, etc. possess phosphate solubilizing ability (Sharma *et al*., 2013).

Mentha spicata L., commonly known as spearmint, is an annual herb of the family Lamiaceae (Labiatae). Spearmint is grown for its aromatic leaves. It is chiefly employed in cooking as a condiment to flavor the curries, soups, chutneys, sauces, etc. Spearmint is a useful source of essential oil and

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has been used for a long time in the perfumery, cosmetic, food and pharmaceutical industry. Being stimulant, antiseptic, restorative, carminative and antispasmodic, spearmint oil is used in various medicines. Due to low menthol (0.5 per cent compared to 40 per cent in peppermint) and menthone content, these medicines can be safely given to children and old people. Spearmint has a great export potential (Kumar *et al*., 1997). Thus, there is a need to focus on the cultivation of spearmint plant to enhance its production.

Due to public awareness programs and various government policies, the farmers have realized the hazardous effect of chemical fertilizers and hence, they are increasingly shifting to sustainable agricultural practices which include use of natural resources and organic inputs to get maximum yield without harming the ecosystem.In the past few decades, PSM have emerged as potential biofertilizers, a cheap, environmentally friendly alternative to expensive chemical fertilizers (Kalayu, 2019).

Against this backdrop, the present study was undertaken to observe **the effect of PSM culture on growth of spearmint (*Mentha spicata* L.)** plants and also to determine the optimum dose of PSM culture in order to harness the maximum benefit.

Materials and Methods

The soil used for the experiment, was procured from Pathare Nursery, Kalyan, India having pH 6.75, electric conductivity (EC) 0.816 mS, water holding capacity 112%, organic carbon 1.031%, nitrogen 0.761 mg/g soil, phosphate phosphorous 1.763 mg/g soil and potassium 0.336 mg/gm soil. A commercial consortium of *Bacillus megaterium, Pseudomonas putida* and *Aspergillus awamori* manufactured by Niranjan Biotech, Pune, Maharashtra, India, was used as the PSM culture.

Four doses of PSM culture viz., 0.01g PSM culture per Kg sterilized soil (T_1), 0.02 g PSM culture per Kg sterilized soil (T_2), 0.03 g PSM culture per Kg sterilized soil (T_3) and 0.04 g PSM culture per Kg sterilized soil (T_4) were tested separately. Sterilized soil was used as a control. Four replications of control pots and four replications of each treatment pot were maintained. 10 pieces of spearmint suckers having 1 – 2 inches length were sown in each pot. Pots were watered on alternate days.

The effect of various concentrations of PSM culture on the growth of spearmint plants was studied separately after 60, 90 and 120 days of sowing (DAS) with respect to various parameters viz., Fresh and Dry weight, Chlorophyll content (Arnon, 1949), Nitrogen by micro-kjeldahl (Sadasivam and Manickam, 2008), Phosphate phosphorous by colorimetry (Bhargava and Raghupathi, 1993) and Potassium by flame photometry (Bhargava and Raghupathi, 1993).

The experiment was laid in randomized block design (RBD) with four replicates of control pots and four replicates of each treatment pot. Data were expressed as mean value of these four replicates. The mean values were subjected to statistical analysis and the one way Analysis of Variance (ANOVA) was constructed. The test was carried out by referring the 'F' value obtained to the standard 'F' value at 5% level of significance. The standard error and critical differences were also calculated. All the calculations were made by using data analysis tool pack for Microsoft Excel 2007 and Windows 7.

Results and Discussion

Fresh weight and Dry weight

Though all the PSM treated plants show higher fresh weight and dry weight than that of control plant, the significant results are only obtained after 90DAS (Table 1). The treatment T_3 gave significant results at 90 DAS whereas all the treatments were significant over the control at 120 DAS.The increased weight in the spearmint plant is attributed to the greater availability of nutrients to the plant. Sokhangoy *et al*. (2012) reported a significant increase in fresh weight of dill plants treated with PSM (*Pseudomonas florescence*). Similar trends of observations were reported by Iwuagwu (2013) and Shankar *et al*. (2013), Sandhya *et al*. (2013) in *Marsdenia volubilis*, Madani *et al*. (2014) in alfalfa plants and by Manoharachary*et al*. (2008) in *Casuarinaequisitifolia.*

Total Chlorophyll content

The application of PSM treatments was found to have beneficial effect on chlorophyll content of spearmint leaves; however, the treatments did not exhibit any specific trend. The treatment T_3 gave consistent significant readings for total chlorophyll content all over the study period.The enhancing effect of PSM on chlorophyll content may be due to the increased nutrient status of the spearmint plants

Table 1. Effect of various levels of PSM culture on Fresh and Dry weight, and chlorophyll content of spearmint plants

Treatments	Fresh weight (g)			Dry Weight (g)			Total Chlorophyll content (mg per g fresh leaves)		
	60 DAS	90 DAS	120 DAS	60 DAS	90 DAS	120DAS	60 DAS	90 DAS	120DAS
C	0.696	0.311	0.405	0.088	0.034	0.054	1.548	2.253	1.612
T_1	0.801	0.322	0.771	0.098	0.046	0.093	1.910	2.405	2.327
T_2	1.007	0.633	0.782	0.122	0.073	0.098	2.623	2.629	1.808
T_3	0.843	0.980	0.770	0.093	0.121	0.123	1.858	3.171	2.731
T_4	0.793	0.337	0.682	0.111	0.038	0.088	1.909	2.707	1.761
F Test	Non-Significant	Significant	Significant	Non-Significant	Significant	Significant	Non-Significant	Significant	Significant
S.E.	-	±0.183	±0.093	-	±0.023	±0.011	±0.090	±0.182	±0.298
C.D.	-	0.389	0.199	-	0.049	0.024	0.0192	0.388	0.636

inoculated with PSM culture. The microorganisms involved in phosphorous solubilization can enhance plant growth by enhancing the availability of other elements (Ngoc *et al*., 2006). Han *et al*. (2006) found out that the integrated treatment of phosphate solubilizers and application of rock phosphate significantly increased leaf photosynthesis. The enhanced production of chlorophyll content under PSM treatments was also reported in *Marsdenia volubilis* plants by Sandhya *et al*. (2013) and in rice by Ravikumar *et a*l. (2013).

Nitrogen

Nitrogen is an essential nutrient for all forms of life on earth as it is a key component of proteins, nucleic acids and other cell constituents (Lenin and Ravimycin, 2013). All the PSM treated plants showed higher nitrogen content than that of control plants.At 90 DAS, all the treatments showed significantly higher nitrogen content than that of control plants whereas at 120 DAS, the treatment T_3 continued to produce significant effect on nitrogen content. Increased nitrogen content in PSM treated plants may be due to some of the members especially *Bacillus* sp. might have the capacity to fix atmospheric nitrogen (Han *et al*., 2006) which was absorbed by the spearmint plants. Besides, it is also known that phosphorus availability in soil is important for the uptake of nitrogen from soil and its utilization in plant (Kim *et al*., 2003). Moreover, phosphate solubilizers are reported to enhance root growth which in turn helps to increase the nitrogen uptake (Iwuagwu*et al.,* 2013).

Phosphorus

PSM treated plants consistently furnished with higher phosphate phosphorous content than that in control plants throughout the study. Statistically, no significant effect of PSM on phosphate phosphorus content was observed at 60 DAS. However, all the treatments were significantly superior over control at 90 DAS. At 120 DAS, the phosphate phosphorous content was decreased may be due to dilution effect (Malik *et al*., 2012). In spite of low values, the treatments T_1 and T_2 were continued to produce significant result over control after 120 DAS. The higher

Table 2. Effect of various levels of PSM culture on NPK content of spearmint plants

Treatments	Nitrogen content (mg per g dry plant material)			Phosphate phosphorous content (mg per g dry plant material)			Potassium content (mg per g dry plant material)		
	60 DAS	90 DAS	120 DAS	60 DAS	90 DAS	120 DAS	60 DAS	90 DAS	120 DAS
C	17.561	19.222	12.499	0.182	0.237	0.128	1.105	3.689	2.290
T_1	25.898	27.573	18.201	0.293	0.781	0.268	1.815	5.396	4.154
T_2	28.116	30.302	21.575	0.232	0.622	0.253	1.547	5.150	3.482
T_3	19.508	33.908	22.892	0.324	0.467	0.215	3.359	5.425	3.879
T_4	24.703	30.818	19.445	0.258	0.487	0.201	3.106	4.770	3.421
F Test	Non-Significant	Significant	Significant	Non-Significant	Significant	Significant	Significant	Significant	Significant
S.E.	-	±3.294	±4.645	-	±0.104	±0.044	± 0.454	± 0.420	±0.430
C.D.	-	7.017	9.894	-	0.222	0.043	0.967	0.865	0.917

phosphate phosphorous content in PSM treated plants may be due to greater availability of phosphorousdue to the solubilization of insoluble forms of phosphorous by PSM. Experiments with seed inoculation with black gram (Tomar *et al*., 1993) and sorghum (Salih *et al*., 1989) showed that inoculation of PSM significantly increased the available amount of phosphate phosphorous for absorption.

Potassium

Potassium is a macronutrient that plays an important role in many physiological processes (Fenn, 1940). The PSM treatments were proved to be excellent in significantly raising the potassium content of spearmint plants throughout the experiment. The PSM treatments T_3 and T_4 gave consistent significant results for potassium content throughout the experiment whereas treatments T_1 and T_2 started presenting significant increase in potassium content 90 days onwards. The significant increase in potassium content is ascribed to increased availability of potassiumin PSM inoculated soil. The role of PSM in dissolution of potassium from soil was also reported by Anthoni Raj (2004). The potassium solubilizing ability of *Bacillus* and *Pseudomonas* sp. was reported by Archana *et al.* (2012). Taipodia and Yubbey (2013) found application of PSB either alone or in combination with FYM gave higher potassium content as well as its uptake in Maize plants.

Determination of Optimum dose

From the present study, it is apparent that the PSM culture had a stimulating effect on growth, productivity and quality of spearmint plants throughout the course of study. 90 DAS stage is considered as very vital from harvest point of view (Sud and Kumar, 2004), hence, to determine the optimum dose of PSM culture, the results obtained at 90 DAS were analyzed, compared and significant results were counted (Table 3).

Table 3. Determination of optimum dose of PSM culture

Parameters Tested		AMF			
		T_1	T_2	T_3	T_4
1.	Fresh Weight	-	-	S	-
2.	Dry Weight	-	-	S	-
3.	Total Chlorophyll	-	-	S	-
4.	Nitrogen	S	S	S	S
5.	Phosphorous	S	S	S	S
6.	Potassium	S	S	S	S
	Total	03	03	06	03
	Best Treatments	T_3			

Note: 'S' : Significant, '–' : Non-significant

From Table 3, it is clear that treatment T_3 (0.03gm PSM culture per Kg sterilized soil) should be considered as the best treatment amongst the remaining ones, as it gave significant results for all growth parameters tested which are considered as very crucial for enhancing the healthy plant growth.

Conclusion

From the present study, it was observed that the PSM culture had a stimulating effect on growth, productivity and quality of spearmint plants throughout the study. It was also observed that to obtain better growth and yield of spearmint plants, high inputs of biological fertilizerswere not needed. PSM treatment T_3were proved to be the better than the remaining treatments from harvest point of view. The application of PSM for enhancing the growth of spearmint plants has promising future. This will not only reduce the dependence of spearmint plants on hazardous chemical fertilizers but will also ensure the maintenance of soil health, owing to eco-friendly nature of PSM.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Alori, E.T., Glick, B.R. and Babalola, O. O. 2017. Microbial Phosphorus Solubilization and Its Potential for Use in Sustainable Agriculture. *Front. Microbiol.* 8: 971. doi: 10.3389/fmicb.2017.00971

Anthoni Raj, S. 2004. Solubilization of silicate and concurrent release of phosphorous and potassium in rice ecosystem. In: *Biofertilizers Technology for Rice based cropping system.* Kannaiyan, S., Kumar, K. and Govindarajan, K.(Ed.) Scientific Publishers (Jodhpur), Pages 372-378.

Archana, D. S., Nandish, M. S., Savalagi, V. P. and Alagawadi, A. R. 2012. Screening of potassium solubilizing bacteria (KSB) for plant growth promotional activity. *Bioinfolet*. 9(4A): 627-630.

Arnon, D. I. 1949. In: Sadasivam S. and A. Manickam (ed). 2008. *Biochemical Methods.* New Age International Publishers, Third edition. Pages 201-202.

Aseri, G. K., Jain, N. and Tarafdar, J. C. 2009. Hydrolysis of organic phosphate forms by Phosphatases and Phytase producing fungi of arid and semi-arid soils of India. *American-Eurasian Journal of Agriculture and Environment Science*. 5(4): 564-570.

Bhargava, B. S. and Raghupathi, H. B. 1993. In: Methods of Analysis of soils, plants, water and fertilizers. Ed. HLS Tandon. *Fertilizer Development and Consultation Organization (New Delhi).* Pages 49-82.

Bolan, N. S. 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorous by plants. *Plant and Soil*. 134: 189-207.

El-Tantawy, M.E. and Mohamed, M.A.N. 2009. Effect of Inoculation with Phosphate Solubilizing Bacteria on the Tomato Rhizosphere Colonization Process, Plant Growth and Yield under Organic and Inorganic Fertilization. *Journal of Applied Sciences Research.* 5(9): 1117-1131.

Fenn, W. O. 1940. In: Lenin, M. and Ravimycin, T. 2013. The effects of different levels of Vermicompost (VC) on the nutrient contents of (*Arachis hypogaea* L.) under arbuscular mycorrizha fungi (AMF) (*Gloumusintraradices*) application. *Indian Journal of Science Research and Technology*. 1(2): 37-45.

Han, H. S., Supanjani and Lee, K. D. 2006. Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. *Plant, Soil and Environment.* 52(3): 130-136.

Hariprasad, P. and Niranjana, S.R. 2009. Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. Plant *and Soil.* 316: 13-24.

Iwuagwu, M.K. S. Chukwuka, U.N. Uka and Amandianeze, M.C. 2013. Effects of Biofertilizers on the Growth of *Zea mays*. Asian Journal of Microbiology. *Biotechnology and Environmental Science.* 15(2): 235-240.

Kalayu, G. 2019. Phosphate Solubilizing Microorganisms: Promising Approach as Biofertilizers. *International Journal of Agronomy.* 2019, Article ID 4917256, 7 pages. Cited at: https://doi.org/10.1155/2019/4917256

Kim, T., Jung, W., Lee, B., Yoneyama, T., Kim, H. and Kim, K. 2003. P effects on N uptake and remobilization during regrowth of Italian ryegrass (*Lolium mutiflorum*). *Environmental and Experimental.* Botany 50: 233-242.

Kumar, N., JBM Md. Abdul Khader, Rangaswami, P. and Irulappan, I. 1997. *Introduction to Spices, Plantation crops, Medicinal and Aromatic plants.* Oxford and IBH Publishing Co. Pvt. Ltd. Pages 22-23.

Lenin, M. and Ravimycin, T. 2013. The effects of different levels of Vermicompost (VC) on the nutrient contents of (*Arachis hypogaea* L.) under arbuscular mycorrhizal fungi (AMF) (*Glomusintraradices*) application. *Indian Journal of Science Research and Technology.* 1(2): 37-45.

Madani, H., Stoklosa, A., Zarei, J. and Usefi, Z. 2014. Alfalfa (*Medicago sativa* L.) forage yield responses to triple superphosphate, phosphate solubilizing Bacteria and Gibberllic acid foliar application. *Scientific papers Series A*. Vol. LVII: 346-349.

Malik, A., Zargar, M. Y., Lone, S., Lone, F. A. and Sheikh, F. A. 2012. Influence of microbial inoculants on nutrient uptake of Himalayan Cypress (*Cupressus torulosa* Don.) seedlings under temperate nursery conditions. *International Journal of Recent Scientific Research*. 3(12): 1013-1018.

Manoharachary, C., Mohan, K. C., Kunwar, I. K. and Reddy, S.V. 2008. Phosphate Solubilizing Fungi associated with *Casuarina equisetifolia*. *Journal of Mycology and Plant Pathology*. 38(3): 507-513.

Ngoc, S.T.T., Ngoc, D.C.M. and Giang, T.T. 2006. In: Mohammadi, K. and Sohrabi, U. 2012. Bacterial biofertilizers for sustainable crop production: a review. *ARPN Journal of Agricultural and Biological Science.* 7(5): 307-316.

Ravikumar, S., Shanthy, S., Kalaiarasi, A. and Sumaya, S. 2013. Halophilic phosphobacteria for raising vigorous growth improvement in Rice (*Oryza sativa*). *African Journal of Agricultural Research.* 8(18): 1872-1876.

Richardson, A. E., Hadobas, P. A. and Hayes, J. E. 2000. Acid phosphomonoesterase and phytase activities of Wheat (*Triticum aestivum* L.) roots and utilization of organic phosphorous substrates by seedlings grown in sterile culture. *Plant, Cell and Environment.* 23(4): 397- 405.

Sadasivam, S. and Manikam, A. 2008. *Biochemical Methods.* New Age International Publishers, Third edition. Pages 32-35.

Salih, H. M., Al Yahya, Abdul Rahem,A. M. and Munam, B.H. 1989. Availability of phosphorous in a calcareous soil treated with rock phosphate and super phosphate as affected by phosphate dissolving fungi. *Plant Soil.* 120: 181-185.

Sandhya, A., Vijaya, T., Sridevi, A. and Narasimha, G. 2013. Influence of vesicular arbuscular mycorrhiza (VAM) and phosphate solubilizing bacteria (PSB) on growth and biochemical constituents of *Marsdeniavolubilis*. *African Journal of Biotechnology.* 12(38): 5648-5654.

Shankar, T., Sivakumar, T., Asha, G., Sankaralingam,S. and Sundaram, V. M. 2013. Effect of PSB on Growth and Development of Chilli and Maize Plants. *World Applied Sciences Journal.* 26(5): 610-617.

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

Sokhangoy, S. H., Ansari, K. and Eradatmand Asli, D. 2012. Effect of bio-fertilizers on performance of dill (*Anethum graveolens*). *Iranian Journal of Plant Physiology*. 2(4): 547-552.

Sud, R. K. and Kumar, S. 2004. *Herbs: Culinary, Medicinal, Aromatic (Secretes and Human Happiness)*. Scientific Publishers (India). Pages 107-115.

Taipodia, R. and Yubbey, D. 2013. Application of Phosphate Solubilizing Bacteria and its ecological effect on Growth and Yield of Winter Maize (*Zea mays* L.). *IOSR Journal of Agriculture and Veterinary Science.* 4(1): 71-75.

Tarafdar, J. C., Bareja, M. and Panwar, J. 2003. Efficiency of some Phosphatase producing soil fungi. *Indian Journal of Microbiology*. 43(1): 27-32.

Tomar, S. S., Pathan, M. A., Gupta, K. P. and Khandkar, U. R. 1993. Effect of phosphate solubilizing bacteria at different levels of phosphate by black gram (*Phaselous mungo*). *Indian Journal of Agronomy.* 38: 131-133.

Vassileva, M., Vassilev, N. and Azcon, R. 1998. Rock phosphate solubilization by *Aspergillus niger* on olive cake-based medium and its further application in a soil–plant system. *World J. Microbiol. Biotech.* 14: 281-284.