International Journal of Agriculture and Plant Science
www.agriculturejournal.in
Online ISSN: 2664-7664, Print ISSN: 2664-7656
Received: 07-11-2022, Accepted: 23-11-2022, Published: 09-12-2022
Volume 4, Issue 2, 2022, Page No. 93-97

Influence co-inoculation of phosphobacteria and potash solubilizing bacteria on growth, yield attributes, and nutrient uptake in lettuce (*Lactuca sativa* L.) under greenhouse conditions

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Abstract
Biofertilizers are obtaining significant importance in sustainable agricultural trends because of their environment-friendly nature, ability to increase soil fertility and health, producing chemical-free products. The hazardous effect of synthetic fertilizers on human health and the environment, high cost, etc. are leading to choose alternative options of farming. Administration of biofertilizers enhances crop productivity, improves soil fertility and maintains an eco-friendly environment as well. The present study is focused to evaluate the effect of the application of liquid biofertilizers *viz.*, PSB, KMB, and KSB isolate on growth, yield, and nutrient content uptake in lettuce under greenhouse conditions. Individual and co-inoculation of PSB, KMB, and KSB isolates have highly influenced the biomass, number of leaves as well as nutrient content of lettuce. The experiment was conducted in randomized block design containing six treatments both individual and in consortia. The growth parameters evaluated were root length, shoot length, number of leaves, fresh weight, and dry weight. Among the six treatments, co-inoculation with PSB+KMB+KSB (T_4) recorded the highest root length, shoot length, number of leaves, fresh weight, and dry weight. All the other treatments were par with each other, whereas, control (uninoculated) recorded the least.

Keywords: biofertilizers, lettuce, consortia, growth parameters, greenhouse

Introduction

Lettuce (*Lactuca sativa*), a leafy salad vegetable belongs to the family Asteraceae considered to be highly beneficial and healthy due to its different nutritional properties. Lettuce plays a crucial role in improving body metabolism. It contains potassium, magnesium, and iron which stimulates body metabolism; in one word it is a rich source of energy. It is also a very good source of several vitamins and β-carotene which lowers the cholesterol quantity in the blood and also lowers the risk of heart diseases due to the presence of fiber & vitamin C. It possesses anti-inflammation properties and antioxidants activities. In India, lettuce is not grown on a larger scale like other vegetables. But due to the health benefits of lettuce, the demand for growing lettuce also has increased. The growth of lettuce is affected by different factors like fertilization, nutrients, etc.

Potassium is the third essential macronutrient that influences most of the biochemical and physiological processes and plays a vital role in plant growth and metabolism. Inadequate supply of potassium leads to stunted growth, increased susceptibility to diseases, and reduced crop yield. potassium is abundant in Indian soil but is mostly found in rock mineral forms like feldspar, illite, muscovite mica, biotite (Sparks & Huang, 1985). Depending on soil type, approximately 90-98 % of total soil K is found in non-exchangeable forms (silicate minerals) such as feldspars and micas which can't be utilized by plants and only 1-2% can be directly utilized by plants (Zhang and Kong, 2014) [22]. In nature, the soil has limited potassium and expensive synthetic fertilizers along with its adverse effects lead to the substitution of potassium (Parmar and Sindhu, 2013) [11]. Microorganisms possess properties that play a vital role in soil withering by dissolving nutrients from insoluble minerals (Hu, *et.al*, 2018) [7]. Among these microorganisms, Potassium solubilizing bacteria (KSB) can liberate potassium (K) from feldspar and aluminosilicate minerals by acidolysis, chelation, exchange reaction, and complexolysis (Meena, *et.al*, 2015, Sattar, *et al.*, 2019) [10] as well as decomposition of organic matter and crop residues (Etesami, *et.al.*, 2017). Therefore, the importance of Potassium solubilizing bacteria are growing in sustainable agriculture due to their ability to release potassium from insoluble potash minerals and plant growth-promoting properties.

Phosphorus is an important key nutrient element next to nitrogen. It is involved in the different metabolic processes including photosynthesis, signal transduction, energy transfer, macromolecular biosynthesis, signal transduction, etc., Khan, *et al*., 2010 [8]. Like potassium, phosphorus is also abundant in Indian soil but most of the soil phosphorus is found in insoluble forms. Only 1% of soil is found in soluble forms for uptake of plants. Phosphate fertilizers are expensive because the efficiency of the added P fertilizer is as low as about 10% (Werft Van Der & Dekkers, 1996) [20]. This has led to the search for environment-friendly and economically feasible alternative strategies for improving crop production in low or P deficient soils. Microorganisms endued with P

solubilizing (PS) activity, may provide a viable substitute to chemical P fertilizers. Phosphorus can be released in soil by phosphorus solubilizing bacteria which are a group of beneficial rhizospheric microorganisms that are very efficient in solubilizing the organic P into an inorganic form. Phosphorus solubilizing microorganisms not only solubilize the insoluble P but also promotes plant growth and crop yield. Phosphorus solubilizing microorganisms produce growth promoting substances like phytohormones (auxin, cytokinins, and gibberellins) (Ahmad, *et.al.*, 2008, Wani, *et.al*., 2007a) [1, 18], Besides P solubilization and phytohormone production, these microorganisms can influence nitrogen fixation (Chung, *et.al*, 2005, Wani, *et.al*, 2007c,) [5, 19] siderophore production, etc (Zaidi & Khan, 2007) [21].

Constant use of synthetic inputs and chemical fertilizers causes decline in soil nutrients which results in decreasing soil microflora, environmental threats by contaminating ground and surface water, and finally escalating risk to farmers economically. So, there is a growing concern to rejuvenate soil health as well as increase crop yield. This can be reached through biofertilizer application as microorganisms play a vital role in fixing, solubilizing, mobilizing, and nutrient recycling. Therefore, the present study has been taken up to evaluate the effect of individual and co-inoculation application of potassium and phosphorus microorganisms on the growth and nutrient uptake of lettuce.

Materials and Methods

The experiments were carried out in the Department of Agril. Microbiology, UAS, Bangalore, GKVK Campus, under greenhouse conditions.

Preparation of broth culture

Phosphorus solubilizing bacterial (PSB) cultures were grown on Pikovskaya media and sub-cultured in regular intervals, Potassium mobilizing bacteria (KMB) were grown and maintained on GYCA (Glucose yeast extract calcium carbonate agar) media. Potassium solubilizing bacterial (KSB) isolates were isolated from two different potassium-bearing minerals *viz.*, muscovite mica and orthoclase feldspar, and grown on Aleksandrov medium.

All the bacterial cultures were maintained regularly and used for application in the seedling trays under greenhouse conditions. PSB, KMB, and KSB bacterial isolates were applied individually and in consortia.

Four replications were kept for each treatment. Control has been kept as uninoculated.

A pot experiment was carried out under greenhouse conditions at the Department of Agricultural Microbiology, UAS, GKVK, Bengaluru. The experiment consisted of 7 treatments in single and consortia and one uninoculated kept as control. Three replications were kept for each treatment and the experiment was designed in randomized block design.

The pots containing 10 kg of soil were selected for the experiment. Red soil, vermicompost, and sand were mixed into a 2:1:1 ratio and pots were filled.

Leaf lettuce variety "Grand Rapids" was selected for the pot trial experiments under greenhouse conditions.

Seeds of lettuce were soaked overnight in a petri plate containing filter paper before sowing. 4-to 5 seeds were placed in each pot. During sowing, broth cultures of PSB, KMB, and KSB isolates were applied @ 30ml per pot. After 4-5 days of sowing, seeds were found sprouted with the two-leaf stage. Seedlings were watered in regular intervals depending upon the moisture content in the pot. Once the seedlings were in four to five-leaf stages, thinning was done in each pot.

Treatment details

Experiments were carried out in replicated randomized block design with three replications for each treatment. PSB, KMB, and KSB bacterial isolates were applied individually and in consortia. Control has been kept as uninoculated. Total six treatments were applied to the plants.

The treatment details are followed:

T_1: Red Soil +Sand + Vermicompost +SBF (KSB isolates)
T_2: Red Soil +Sand + Vermicompost +SDM (KSB isolates)
T_3: Red Soil +Sand + Vermicompost +SBF+SDM
T_4: Red Soil +Sand + Vermicompost +SBF+SDM+PSB+KMB
T_5: Red Soil +Sand + Vermicompost +PSB (*Bacillus megaterium*)
T_6: Red Soil +Sand + Vermicompost +KMB (*Frateuria aurentia*)
Control: Red Soil +Sand + Vermicompost

Treatments were given during sowing and on the 15th day of plant growth and the plants were harvested on the 45th day.

Growth parameters studied

The following parameters were recorded after harvest. Plant growth and biomass were recorded for each replication of the treatments. Growth parameters *viz*., shoot length, root length, number of leaves fresh weight and dry weight were recorded.

Nutrient content

Nutrient content *viz.*, macronutrients nitrogen, phosphorus, potassium, secondary nutrients calcium and magnesium and tertiary nutrients iron, copper, zinc were recorded.

Results

Growth parameters

Application of different biofertilizers exhibited significant leaf yield and other growth parameters of lettuce. Co-inoculation with PSB, KMB, and KSB (T_4) profusely influenced the leaf yield. Whereas, the effect of individual application of T_6 (KMB) and T_5 (PSB) recorded an adequate amount of growth. All the other treatments are on par with each other and control recorded the lowest. Similar results were reported by Chatterjee, 2015. The study showed that higher leaf yield was recorded with the combined application of *Azotobacter* and PSB. Shams, *et.al,* 2013, also studied the effect of biofertilizers on lettuce production under a drip irrigation system. Their results reveal that the combined application of minerals, organic manures, and biofertilizers recorded the highest leaf yield.

Table 1: Growth parameters of lettuce influenced by the application of biofertilizers

Treatments	Root length (cm)	Shoot length (cm)	No. of leaves	Fresh wt (g)	Dry weight
T_1	13.00^{bcd}	33.33^{ab}	6.66^{ab}	23.62^{a}	1.98^{d}
T_2	12.90^{bcd}	27.00^{b}	6.66^{ab}	24.82^{a}	2.11^{cd}
T_3	11.90^{cd}	35.00^{a}	7.66^{ab}	25.73^{a}	1.77^{d}
T_4	17.66^{a}	36.66^{a}	8.66^{a}	26.43^{a}	3.58^{a}
T_5	14.16^{bcd}	33.66^{ab}	8.33^{a}	25.96^{a}	2.49^{c}
T_6	15.00^{ab}	34.33^{a}	8.00^{a}	21.77^{a}	3.03^{b}
Control	10.66^{d}	14.16^{c}	3.66^{b}	11.64^{b}	0.41^{e}
SEM	0.65	1.60	0.65	1.77	0.11
LSD ($p< 0.05$)	0.93	4.86	1.99	5.39	0.34

Chamangasht *et.al* 2012, also studied the effect of biofertilizers on the growth of lettuce. Their study showed the highest number of leaf yields was recorded in the plants treated with *Azospirillum*.

The highest shoot length and root length were observed in T_4 (co-inoculation with PSB, KMB, and KSB). Both phosphorus solubilizing bacteria and Potassium solubilizing bacteria possess growth-promoting attributes. The production of IAA leads to the elongation of roots in plants which directly or indirectly helps in the increase of nutrient uptake for better growth and development. KSB isolates selected for this present study showed the production of phytohormones like IAA, cytokinin, and Gibberellic acid (Biswas *et.al.,* 2018) which might have enhanced the root growth and eventually accelerated the nutrient uptake resulting in the highest yield attributes. Similar results were reported by Rather *et.al*., 2018. Their study reported that the application of biofertilizers *viz., Azotobacter,* PSB, and KSB significantly increased the growth and yield parameters.

Nutrient Uptake

Nutrient uptake was also highly influenced by the application of biofertilizers in lettuce. Application of consortia of KSB isolates, KMB, and PSB (T_4) recorded the highest nutrient uptake. Macronutrients N, P, K, secondary nutrients (Ca, Mg) and tertiary nutrients (Fe, Zn) uptake was visibly highest in T_4. This is maybe due to the dissolution of minerals by the activity of these rhizospheric microorganisms.

Table 2: Nutrient uptake of lettuce influenced by biofertilizers

Treatments	Nitrogen (N %)	Phosphorus (P) (ppm)	Potassium (K) (ppm)	Calcium (Ca) (ppm)	Magnesium (Mg) (ppm)	Iron (Fe) (ppm)	Zinc (Zn) (ppm)
T_1	316.95d	120.82d	2215.45d	21.14e	10.30c	3.14c	5.41b
T_2	238.22e	107.96e	2055.09e	24.64d	7.13e	2.11d	4.32c
T_3	320.53d	114.16de	1869.22f	20.60e	8.62d	2.50d	3.50d
T_4	839.70a	203.05a	3503.08a	31.13a	13.28a	4.32a	6.05a
T_5	642.60c	159.17c	2528.62c	27.34c	11.71b	3.50bc	5.22b
T_6	715.91b	194.72b	3133.69b	30.22b	11.22b	3.84b	5.61ab
Control	107.72f	51.45f	638.18g	10.3f	2.18f	1.06e	1.54e
SEM	5.82	2.15	20.99	0.22	0.15	0.10	0.11
LSD ($p< 0.05$)	17.67	6.52	63.69	0.68	0.47	0.33	0.34

Nutrient availability through the mineralization by these rhizospheric microorganisms leads in resulting the chelation of the soil nutrients might have helped in the absorption of secondary and tertiary nutrients. During the mineralization process, both KSB and PSB solubilize the insoluble form of potassium and phosphorus minerals by the production of organic acids leading to the availability of more minerals and the macro and micronutrients for the ultimate growth. The adequate translocation of nutrients promotes all the physiological processes in plants thereby resulting in superior growth. Similar reports were observed by Tosic *et al,* 2016 [17].

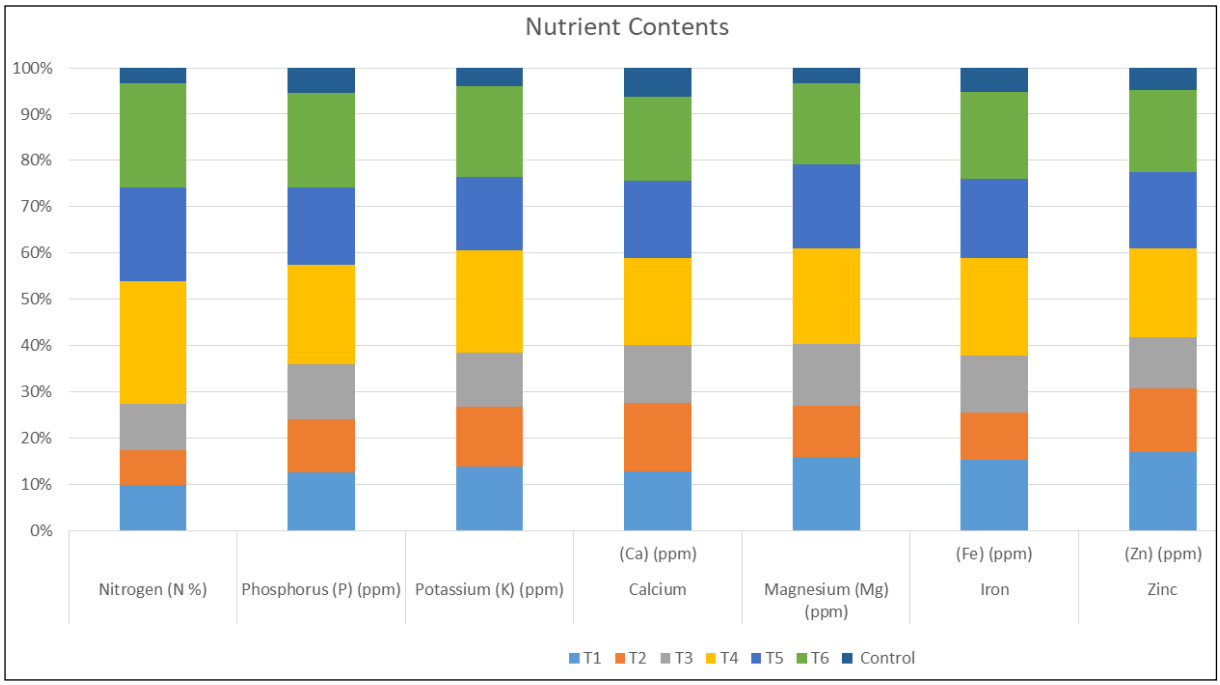

Fig 1

Fig 2: Pot Experiment of lettuce under greenhouse condition

Discussion

The present study emphasized the efficiency of PSB, KMB, and KSB on the growth and yield of lettuce grown under greenhouse conditions. The growth and yield were highly influenced by the application of consortia as well as individual applications of biofertilizers. The significantly highest yield was recorded in T_4 (co-inoculation with PSB, KMB, and KSB Individual application of PSB recorded a notable amount of vegetative growth. Potassium mobilizing bacteria also showed equally good yield. All other treatments were on par with each other. In the case of nutrient uptake, the effect of biofertilizers was found to be very beneficial. Similar reports were found by Stamford *et.al,* 2019 [16]. Their study revealed that microbial fertilizers increased growth characteristics in lettuce compared to conventional fertilizer. The enhancement in nutrient uptake was observed in lettuce was also highly influenced by the application of consortia of PSB, KMB, and KSB isolates followed by the application of KMB and PSB. This is maybe due to the effect of microbial activities of biofertilizers applied and solubilization of soil nutrients by the consortia which were readily available to the plants for their nutrient requirements. Rhizospheric microorganisms provide nutrient contents to the plants by contributing significantly to the solubilization of fixed forms of soil minerals both primary, secondary, and tertiary resulting in higher crop performances.

Conclusion

The results obtained from the present study indicate that the application of biofertilizers has significantly influenced the growth parameters as well as nutrient uptake in lettuce. Co-inoculation with PSB, KMB, and KSB isolates influenced the growth and yield profusely compared to the individual application. All the biofertilizers exhibited the potential ability to promote growth by increasing the availability of nutrients. These rhizospheric microorganisms can be an alternate option in sustainable agriculture.

Acknowledgments

The Post Doctoral Fellow is grateful to UGC, New Delhi for providing financial assistance as Post Doctoral Fellowship for SC/ST Candidates to carry out the research work.

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