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ORIGINAL ARTICLE

Effect of potassium solubilizing bacterial consortium on the watermelon (*Citrullus lanatus*) cultivation

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ABSTRACT
Potassium is the major nutrient essential for the growth and development of plants. However, the usage of chemical potash fertilizers is uneconomical for the farmers, especially in developing countries. Hence the experiment was designed to evaluate the effect of potassium solubilizing bacteria on watermelon cultivation. In the study conducted, the two potassium solubilizing bacterial strains Enterobacter hormaechei 1110BP and Klebsiella variicola as consortium have proved as a promising biofertilizer for watermelon. This is the first report showing the usage of potassium solubilizing bacteria in watermelon cultivation. The in vivo studies on the germination of watermelon seeds in presence of Enterobacter hormaechei 1110BP and Klebsiella variicola have noticed earlier germination, an increase in germination percentage (68%), as well as a significant increase in the radical length and plumule length as compared to un-inoculated seeds. The field experiment also revealed a 31.34% increase in the weight of fruits, a 24.55% increase in total sugar content, and a 10% increase in the yield of fruits.
Keywords: Potassium solubilizing bacteria, Watermelon, Chemical fertilizers, Biofertilizer
Abbreviations:
KSB = Potassium solubilizing bacteria; OD_{600nm} *= Optical density at 600nm*

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INTRODUCTION
Watermelon (*Citrullus lanatus*) is a warm-season crop. It is a popular fruit for fresh consumption and agro-processing. It contains about 6% sugar and 91% water. It is low in fat and sodium [1], hence it is a favorite fruit of diet-conscious people. The fruit has medicinal importance due to the presence of several bioactive components like lycopene, citrulline, vitamin C, vitamin A, etc. Lycopene which constitutes about, 40% compared to raw tomatoes, is of great interest to researchers due to its anti-cancer and other medicinal properties [2, 3].
The cultivation of watermelon demands the application of a huge quantity of potassium [4]. Several physiological and metabolic processes occurring in plants need potassium [5]. It is absorbed in the form of cation by plants [6]. It plays an important role in photosynthesis and it regulates water, oxygen, and carbon dioxide concentration in tissues [7]. Potassium activates nearly sixty different enzymes in plants. It is also essential for the production of energy (ATP). Simultaneously, it is involved in the formation and translocation of starch and sugar [8]. A very huge quantity of potassium is present in the soil but nearly 98% potassium is present in an unavailable form [9]. Hence the requirement of potassium is fulfilled through application in the form of chemical fertilizers. However, chemical potash fertilizers are expensive in countries like India as India is dependent on the import of potash fertilizers [8]. Moreover, chemical fertilizers exhibit several negative consequences on the environment and human health [10]. A very large number of soil microorganisms like *Burkholderia* spp., *Bacillus* spp., *Panebacillus* spp. [11, 12], *Aspergillus* spp. [13] etc. can convert insoluble potassium to soluble form which is easily absorbed by the plants. The application of such microorganisms as biofertilizers is an economical and eco-friendly alternative to chemical potash fertilizers.

MATERIAL AND METHODS

Potassium solubilizing bacterial strains

The two potassium solubilizing bacterial strains previously isolated from rhizospheric soil on Aleksandrov's medium [14] were used in the study. The strains were identified based on morphological, biochemical, and 16S rRNA gene sequence analysis.
1) *Enterobacter hormaechei* 1110BP (NCBI GenBank accession number - MH057393) 2) *Klebsiella variicola* (NCBI GenBank accession number - MN515153)

In vivo seed germination plate assay

Fresh culture of *Enterobacter hormaechei* 1110BP and *Klebsiella variicola* was suspended separately in the sterile saline and cell density was adjusted to a specific value (OD_{600nm}=0.16). Both cell suspensions were mixed in equal quantity (1:1). Watermelon seeds were surface sterilized and treated with saline suspension for 24 hrs. Further, seeds were kept on moist cotton in a Petri plate, and the plate was incubated in dark at room temperature. The germination of seeds was monitored daily and radicle (root) length and plumule (shoot) length [15] of seeds was measured after 8 days of incubation. Seeds dipped in sterile saline were served as 'control'.

Field trial

The effect of *Enterobacter hormaechei* 1110BP and *Klebsiella variicola* consortium on the watermelon cultivation was studied on a private farm at Tirpan (16.7576174° N, 74.0623244° E), Panhala, Maharashtra, India. Watermelon: var. NS 750 (Namdhari) was used in the study. It is a high-yielding hybrid variety with a maturity of 80 - 85 days depending on the season. Fruit is with excellent bright red flesh having good juicy granular texture and sweetness of 12-13% total soluble solids (TSS).

Trials were started in December 2020. The field was divided into equal parts as inoculated 'Test' plot and uninoculated 'Control' plot. Holes were prepared in the field 60 cm apart from each other. The distance between the rows was kept at approximately 180 cm. Two watermelon seeds were sown in each hole. The seeds treated with *Enterobacter hormaechei* 1110BP and *Klebsiella variicola* were sown in the 'Test' plot. Untreated seeds were sown in the 'Control' plot. On the 30th day of sowing, seedlings of the 'Test' plot were again inoculated with *Enterobacter hormaechei* 1110BP and *Klebsiella variicola* by drenching technique.

Growth and yield analysis

Watermelons were harvested on the 82nd day of sowing the seeds when tendrils closest to the fruit started drying out and turned brown. Five fruits were randomly collected from each plot and different parameters of fruits like weight, length, width, the weight of red pulp, the weight of seeds, etc. were recorded. The yield was determined based on the weight of all matured fruits in the plot.

Chemical analysis of fruit juice

The juice of inoculated and un-inoculated fruits was obtained and quantification of the total sugar content of juice was done by the phenol sulphuric acid method. The watermelon juice was diluted with water to prepare a sample. It was added with 1 ml 5% phenol, quickly followed by 5 ml concentrated H_2SO_4. The absorbance of the sample tube was recorded at 490 nm after 15 minutes of incubation. Finally, the total sugar concentration in the juice was determined with the help of the standard curve.

RESULTS AND DISCUSSION

Seed germination assay

The germination study revealed that the watermelon seeds in presence of *Enterobacter hormaechei* 1110BP and *Klebsiella variicola* (Fig. 1) have an early and vigorous germination process as compared to uninoculated seeds (Fig. 2).

The treated seeds were seen to start germination on the 2nd day of incubation while untreated seeds started germination on the 5th day of incubation. The germination was only 4 % in untreated seeds and it was 28 % in treated seeds on the 5th day. On the 8th day, 68% of treated seeds recorded germination however, in the case of untreated seeds germination was only 20%. Simultaneously the plumule length in inoculated seeds was significantly higher in comparison with uninoculated seeds. Further, all uninoculated seeds were failed to develop embryonic roots however, 36% of inoculated seeds recorded root development on the 8th day of incubation (Table 1).

Fig 1. Germination analysis of watermelon seeds inoculated with *Enterobacter hormaechei* 1110BP and *Klebsiella variicola*

Fig. 2 Germination of watermelon seeds in absence of *Enterobacter hormaechei* 1110BP and *Klebsiella variicola*

Table 1 Evaluation of effects of *Enterobacter hormaechei* 1110BP and *Klebsiella variicola* on the germination of watermelon seeds on 8th day

Parameter	% of germination	Radicle length (cm)	Plumule length (cm)
EK	68	2.0±0.5	6.30±3.01
Control	20	-	0.42±0.5

EK: Watermelon seeds treated with *Enterobacter hormaechei* 1110BP and *Klebsiella variicola*; Control: Untreated watermelon seeds

Table 2 Different parameters of watermelon fruit at the time of harvest

Treatment	Weight of fruit (kg)	Pulp weight (kg)	Weight of seeds (g)	Rind weight (g)	Length of fruit (cm)	Width of fruit at the center (cm)	Total sugar (%)
EK	10.05 ±5.02	9.68 ±1.32	49.05 ±0.42	313.66 ±0.78	58.0 ±1.12	24.5 ±0.23	10.02 ±0.34
CONTROL	6.90 ±6.22	6.56 ±2.22	47.79 ±0.56	302.21 ±0.55	47.5 ±1.37	17.5 ±0.12	7.56 ±0.53

EK: *Enterobacter hormaechei* 1110BP and *Klebsiella variicola* inoculated seedlings; Control: Un-inoculated seedlings. The values represent the average values ± SD

Watermelon field trials

Application of *Enterobacter hormaechei* 1110BP and *Klebsiella variicola* to watermelon have shown promising effects on the growth of the watermelon plant along with the enhancement in yield and quality of fruits (Table 2). The length of vines was seen to increase in inoculated watermelons as compared to uninoculated plants. Simultaneously a significant increase in the size and weight of fruits in the inoculated plants were recorded. The weight of inoculated fruits ranged from 8.5 kg to 11.6 kg while the weight of uninoculated fruits ranged from 6.2 to 7.6 kg. The overall yield of watermelon was seen to increase by 10% in *Enterobacter hormaechei* 1110BP and *Klebsiella variicola* inoculated field in comparison to the un-inoculated plot.

The watermelons of inoculated plants were identified a white powdery deposit on the surface which is an indication of increased sugar levels in the fruits. The chemical analysis of the total sugar content of fresh juice confirmed the enhancement of the sweetness of the fruit. The inoculated fruits recorded 24.55% increased sugar content over uninoculated fruits.

Thus the overall increase in the size, yield, and quality of watermelon fruits could be attributed to the supply of potassium through potassium solubilization activity of *Enterobacter hormaechei* 1110BP and *Klebsiella variicola* consortium.

According to previous research, potassium is seen to be present in the highest concentration in watermelon fruit as compared to nitrogen and phosphorous [16]. This indicates the importance of potassium among the three macronutrients N, P, and K for watermelon production. Thus, the powerful potassium solubilization capacity of *Enterobacter hormaechei* 1110BP and *Klebsiella variicola* consortium may have contributed to the significant increase in the weight of fruits. Several research studies have documented the increase in the weight, width, number, and quality of watermelon fruits after adequate potassium fertilization [4, 17, 18]. The NPK requirement of watermelon is high. However long term and heavy application of inorganic fertilizers decreases fruit number and delays the maturation of fruits [19, 20]. Moreover, the application of inorganic fertilizers increases the cost of watermelon production. In this context, the application of potassium solubilizing *Enterobacter hormaechei* 1110BP and *Klebsiella variicola* is an economical solution for acquiring profit in watermelon farming. The previous research studies have documented the positive effect of *Azotobacter* [21] and *Bacillus* **s**pp. [22] on the growth of watermelon.

CONCLUSION

In today's world, indiscriminate usage of chemical fertilizers to increase crop yield has emerged as a serious issue in agriculture due to their hazardous effects on the ecosystem. The application of plant beneficial microorganisms in the soil could help to save the ecosystem. Potassium is an essential nutrient in the growth and development of all types of plants. The huge cost of chemical potash fertilizers makes it unaffordable for Indian farmers. The present research work proves the beneficial effects of potassium solubilizing bacteria *Enterobacter hormaechei* 1110BP and *Klebsiella variicola* on the cultivation of watermelon. Thus, the exploitation of these bacteria as potash biofertilizers to increase crop productivity is a cheap alternative to chemical potash fertilizers for sustainable agriculture.

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CONFLICT OF INTEREST

The authors have declared that no competing interest exists.

REFERENCES

1. Toluwase, S. O. W., & Owoeye, R. S. (2017). Cost-benefit Analysis of Watermelon Production in Ekiti State, Nigeria. Russian Journal of Agricultural and Socio-Economic Sciences, 66(6).
2. Guner, N., & Wehner, T. C. (2004). The genes of watermelon. HortScience, 39(6), 1175-1182.
3. Zamuz, S., Munekata, P. E., Gullón, B., Rocchetti, G., Montesano, D., & Lorenzo, J. M. (2021). Citrullus lanatus as source of bioactive components: An up-to-date review. Trends in Food Science & Technology.
4. Okur, B., & Yagmur, B. (2004). Effects on enhanced potassium doses on yield, quality and nutrient uptake of watermelon. In IPI regional workshop on potassium and fertigation development in West Asia and North Africa.
5. Zhao, F., Sheng, X. F., Huang, Z., & He, L. (2008). Isolation of mineral potassium-solubilizing bacterial strains from agricultural soils in Shandong Province. Biodivers Sci, 16(6), 593-600.
6. Sedaghathoor, S., Torkashvand, A. M., Hashemabadi, D., & Kaviani, B. (2009). Yield and quality response of tea plant to fertilizers. African Journal of Agricultural Research, 4(6), 568-570.

7. Wang, N., Hua, H., Eneji, A. E., Li, Z., Duan, L., & Tian, X. (2012). Genotypic variations in photosynthetic and physiological adjustment to potassium deficiency in cotton (*Gossypium hirsutum*). Journal of Photochemistry and Photobiology B: Biology, 110, 1-8.
8. Kinekar, B. K. (2011). Potassium fertilizer situation in India: Current use and perspectives. Karnataka Journal of agricultural sciences, 24(1).
9. McAfee, J. (2008). Potassium, a key nutrient for plant growth. Department of Soil and Crop Sciences.
10. Adesemoye, A. O., & Kloepper, J. W. (2009). Plant–microbes interactions in enhanced fertilizer-use efficiency. Applied microbiology and biotechnology, 85(1), 1-12.
11. Sheng, X. F. (2005). Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of Bacillus edaphicus. Soil Biology and Biochemistry, 37(10), 1918-1922.
12. Sangeeth, K. P., Bhai, R. S. & Srinivasan, V. (2012). Paenibacillus glucanolyticus, a promising potassium solubilizing bacterium isolated from black pepper (Piper nigrum L.) rhizosphere. Journal of Spices and Aromatic Crops, 21(2), 118-124.
13. Prajapati K., Sharma M. C., & Modi H. A. (2012). Isolation of two potassium solubilizing fungi from ceramic industry soils. Life Sci Leaflets, 5, 71-75.
14. Hu, X., Chen, J., & Guo, J. (2006). Two phosphate-and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. World journal of Microbiology and Biotechnology, 22(9), 983-990.
15. Demissie, S., Muleta D., & Berecha, G. (2013). Effect of phosphate solubilizing bacteria on seed germination and seedling growth of faba bean (Vicia faba L.). Int. J. Agric. Res, 8(3), 123-136.
16. Kang, F., Wang, Z., Xiong, H., Li, Y., Wang, Y., Fan, Z., & Zhang, Y. (2020). Estimation of Watermelon Nutrient Requirements Based on the QUEFTS Model. Agronomy, 10(11), 1776.
17. Song, Q. S., Chen, G., Wu, L. S., YI, Y. R., DING, M., LI, Y. H., & SUN, Y. H. (2007). Effect of different potassium supply levels on yield and quality of watermelon. Hubei Agricultural Sciences, 46(5), 732-734.
18. Du, S. P., Ma, Z. M., & Xue, L. (2011). Effect of Boron and Potassium on Yield and Quality of Watermelon in Gravel Mulched Fields. China Vegetables, 8.109-113
19. John, L. W., Jamer, D. B., Samuel, L. T., & Warner, L. W. (2004). Soil fertility and fertilizers: An introduction to nutrient management. Person Education, Delhi, 106-153.
20. Eifediyi, E. K., Remison, S. U., Ahamefule, H. E., Azeez, K. O., & Fesobi, P. O. (2017). Performance of watermelon (Citrullus lanatus L.) in response to organic and NPK fertilizers. Acta Universitatis Sapientiae, Agriculture and Environment, 9(1), 5-17.
21. Kharat, B. M., Shinde, S. J., & Jadhav, S. D. (2021). Effect of different organic sources on yield and yield attributing character of watermelon (Citrullus lanatus Thunb.). Journal of Pharmacognosy and Phytochemistry, 10(1), 827-832.
22. Kokalis-Burelle, N., Vavrina, C. S., Reddy, M. S., & Kloepper, J. W. (2003). Amendment of muskmelon and watermelon transplant media with plant growth-promoting rhizobacteria: Effects on seedling quality, disease, and nematode resistance. HortTechnology, 13(3), 476-482.