

On-Farm Production & Bio-Efficacy Study Of 'Baif Elixer' Biofertilizer On Momordica Charantia

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Abstract

Today's agriculture sector requires reducing dependency on chemical fertilizers and increasing the use of bio-based formulations for implementing organic farming practices. In organic farming, the role of bio-fertilizers, bio-pesticides, and growth stimulants is crucial, but the availability of bio-based products in larger quantities always remains a constraint. Hence the purpose of this study is to formulate a microbial-based bio-fertilizer formulation 'BAIF ELIXER' and evaluate its bio-efficacy in field trials. Six types of microbial cultures, potential as N₂ fixers, phosphate solubilizers, and bio-stimulants from BAIF Development Research Foundation were cultivated in a 200 L fermenter and used to develop a BAIF ELIXER bio-fertilizer formulation. The final count of 1X10⁹ CFU/ml was used to develop an on-farm liquid bio-fertilizer BAIF ELIXER formulation. To study its bio-efficacy, seed, and on-farm treatment were conducted on Momordica charantia on 900 plants planted in a 0.13 hector area for each treatment in Research Farm, Pune. During the field experiment, plant growth and yield were recorded for 7 consecutive harvests at four days intervals. BAIF ELIXER on-farm treatment produces the highest yield of 997 kg at the 7th harvest. Both seed and on-farm treatment give higher yields as compared to control plots. As a result, the crops can be produced organically with the combined application of biofertilizers for sustainable crop development. The study will help to understand the role of beneficial microbes in agriculture in improving organic farming practices.

Keywords: Organic Farming, Biofertilizer, Momordica Charantia, Bio-Efficacy.

1. Introduction

Biofertilizers are natural fertilizers that are living microbial inoculants of bacteria, algae, and fungi that boost the availability of nutrients to plants individually or in combination. Biofertilizers are products that contain one or more species of microorganisms that have the ability to mobilize nutritionally important elements to plant-available form [1]. By nitrogen fixation, phosphate, and potassium solubilization or mineralization, the release of plant growth regulating substances, the production of antibiotics, and the biodegradation of organic matter in the soil, biofertilizers maintain a soil ecosystem rich in micro and macronutrients [2]. They are live strains of bacterial microorganisms or a mix of algae, fungi, and other microbes found all over the earth's surface, with elevated concentrations at the root and performing important functions. When applied as seed or soil inoculants, biofertilizers multiply and participate in the nutrient cycle, enhancing crop productivity [3].

Normal treatments of biofertilizer consist of Water treatment or control, seed treatment, set /cutting treatment, seedling root treatment, soil treatment, and application on standing crop treatment. Seed treatment is the process of

soaking seeds in our liquid fertilizer overnight before planting them in the soil [4, 5].

The production of biofertilizers depends not only on understanding plant physiology and soil microbes, but also on other technological obstacles such as formulation type, microflora populations, and their interaction system. As a result, designing an effective bioformulation is possible by combining plant physiology knowledge with microbiological and technical aspects [6]. Additionally, changes in edaphic qualities influence the composition of soil microbes and functional metrics; thus, adequate agricultural management such as irrigation, tillage, crop rotation, and a fertilizer regime can enable soil microorganisms to perform their various ecological roles [7].

On-farm technique or treatment that is employed during this study is the utilization of pure substance up to 10%, farmers grow BAIF ELIXER up to two hundred liters. and apply through the various systems such as drip, spray pumps, rain guns, flood irrigation, and any other irrigation system to a one-acre area. Farmers can use this technique for the production of microorganisms with starter cultures. Pseudomonas Fluorescents, Bacillus subtilis, Azotobacter, Trichoderma

viride, KSB, and PSB stains are included in the BAIF ELIXER. This technique necessitates the use of readily available, low-cost, and high-energy ingredients such as Jaggery, chickpea flour, and rock or raw salt. This is the basic formula, and it has incredible effects on the growth of beneficial bacteria and soil health. The prevalence of helpful bacteria is completely dependent on soil health. Farmers can multiply microorganisms two hundred times with this technology and the original mother culture.

The foundations of the imminent green revolution are now laid by several bacterial and fungal inoculants, as well as by genetically modified organisms. The effectiveness and activity of these microbes will indeed influence the development of sustainable agriculture [8].

2. Materials and Methods

Cultures for the experiment were provided by the BAIF Development Research Foundations microbiology department, which included strains of *Pseudomonas Fluorescens*, *Bacillus subtilis*, *Azotobacter*, *Trichoderma viride*, KSB, and PSB in each culture grown in different media like *Pseudomonas* media, *Bacillus* media, *Azotobacter* media, PDCA (Potato dextrose chloramphenicol media), *Pikovskaya* 's media, and Nutrient Agar medium.

2.1. Preparation of Primary Liquid Inoculum

The primary liquid inoculum for making the biofertilizer brew was prepared. In each 5-liter flask, 2.5 liters of *Pseudomonas* broth, *Bacillus* broth, *Azotobacter* broth, PDCA (Potato dextrose chloramphenicol media), *Pikovskaya* broth, and Nutrient Broth were made. The broths were autoclaved for 15 minutes at 15 psi and 121°C. After cooling, a loopful pure culture of all the microorganisms was inoculated in each flask. The flasks were kept on the rotary shaker for four days at room temperature at 180 rpm after inoculation.

2.2. Production of Biofertilizers

Starter culture is the culture obtained in the flask. For large-scale production, the inoculum from the starter culture is transferred to the large fermenter and cultivated until the desired cell count is reached. In the fermenter, the first culture media was prepared by sterilizing the media components at 121 °C for 15 minutes. After sterilization, the basic media was cooled at 30°C before adding the starter culture. The fermentation procedure was carried out according to the optimal incubation period. The temperature was maintained at 30°C to 32°C. The aeration and agitation were provided with the help of a sparger and agitator. The same steps are performed in all cultures.

2.3. Total Viable Count (Tvc) Of Biofertilizer Brew

Bacterial viable cell numbers, bacterial genus and strains, and plant growth stimulating abilities such as strains' ability to solubilize insoluble phosphate, auxin, and siderophore synthesis, as well as potassium releasing ability, were all examined in this Quality control work. The TVC was performed to check cell count i.e. viable cells present in the biofertilizer brew.

2.4. Production of Baif Elixer

The technique used in the production of BAIF ELIXER is composed of 5 grams of jaggery, 5 grams of chickpea flour, and 8 grams of NaCl, mixed with one liter of water. This is the standard proportion or the formula for the large-scale production of BAIF ELIXER. BAIF culture has been used as a mother culture or starter inoculum. With the use of pure inoculum up to 10%, farmers grow BAIF ELIXER up to 200 liters. This is the final product, which contains millions of beneficial microorganisms.

2.5. Pot Experiment and Field Experiment

The pot experiment was carried out with the fenugreek plant. The experiment was set up in a Complete Randomised Design with three treatments and ten replications, resulting in 30 pots, one with water treatment, one with On-farm treatment BAIF ELIXER, and one with seed treatment of ELIXER.

In BAIF Development Research Foundation Uruli Kanchan, field trials of *Momordica charantia* were undertaken in 15 Guntha (15000 square feet) of the field for each treatment. Control, seed treatment of BAIF ELIXER, and on-farm treatment of BAIF ELIXER were used on all of the crops. There were 2700 total plantations, with 900 for each treatment.

3. Results and Discussion

This study aimed to investigate the effectiveness of a biofertilizer made from six different microorganisms on the growth and yield of two crops, fenugreek, and bitter melon. The results indicated that the biofertilizer was highly effective in promoting plant growth and increasing yield, particularly when applied through on-farm production. Specifically, the crops treated with the on-farm production of the biofertilizer showed a significant increase in plant height, leaf count, and fruit yield, when compared to those treated with water or seed treatment. These findings demonstrate the potential of biofertilizers as a sustainable and viable alternative to chemical fertilizers in agriculture.

To produce the biofertilizer, primary cultures of *Pseudomonas fluorescens*, *Bacillus subtilis*, *Azotobacter*, *Trichoderma viride*, KSB, and PSB were cultured in 2.5 liters of respective media for a duration of four days. The purity of the cultures was confirmed through morphological observations. Following the fermentation process, the biofertilizer was prepared on the fourth day, with a consistent temperature of 30°C and pH maintained at 6.8 to 7.0 using 0.1N NaOH. The prepared biofertilizer brews of the six microorganisms were cultured in specific media, including *Pseudomonas*, *Bacillus*, *Azotobacter*, PDCA, *Pikovskaya*, and Nutrient Broth. The growth of the microbes in all media was significant and assessed through a total viable count analysis. The high CFU/ml count obtained for all six microorganisms, with a dilution of 10-10, indicates the potential agricultural applications of the biofertilizer.

In the pot experiment, both on-farm and seed treatments showed superior plant growth in terms of height and leaf count compared to the control or water treatments. These findings are consistent with previous studies that have

demonstrated that treating fenugreek with microbial consortia including multiple microorganisms can boost plant growth and yield [9, 10]. Among the treatments, the on-farm treatment resulted in the most plant growth, with the high-

est plant height and leaf count. Similar results have been reported in other studies where seed treatments were used [11, 12].

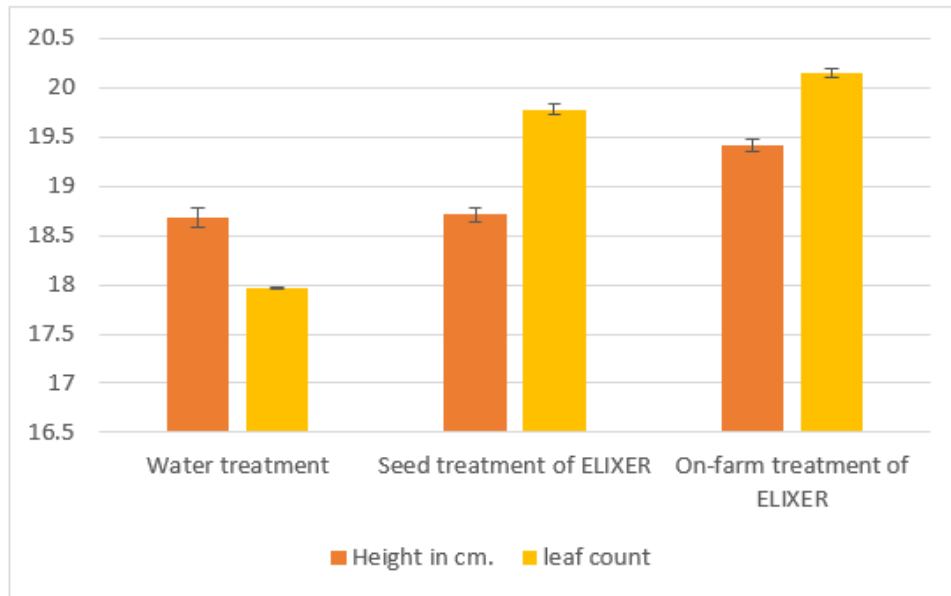


Figure 1: A visual depiction illustrating the average values of plant height and leaf count of Fenugreek grown under three treatments, including seed treatment, on-farm treatment, and control treatment.

Experiments were carried out for *Momordica Charantia* in an area of 15000 square feet (15 Guntha) located at BAIF Development Research Foundation, Pune for the purpose of this study. Three treatments, including control (water treat-

ment), seed treatment, and on-farm production treatment, were applied to all the crops. Each treatment had 900 plantations out of a total of 2700. Table 1 displays the results of the statistical analysis.

Table 1: Field and statistical analysis data

		Control (Water treatment)	Treated with Seed Treatment	Treated with On-farm Production
I.	Area	15000 sq. Ft	15000 sq. Ft	15000 sq. Ft
II.	Sowing Date	05.02.2022	05.02.2022	05.02.2022
III.	Germination Days	4 to 7 Days	2 to 7 Days	4 to 5 Days
IV.	Lines	18	18	18
V.	Total Number of Plantation	900	900	900
VI.	Starting for Yield	60 Days	45 Days	45 Days

The seeds germinated in 4-7 days for water treatment, 2-7 days for seed treatment, and 2-5 days for on-farm treatment. The on-farm treatment led to faster germination compared to the other two treatments. The crops with seed treatment and on-farm started yielding at 45 days, while water-treated crops started yielding at 60 days. These findings line up with previous studies that have indicated that microbial consortia used as biofertilizer treatments can improve *Momordica charantia* germination rates [13-15]. The seed germination rate and starting yield of water-treatment crops were the lowest compared to seed treatment and on-farm treatment.

Bitter gourds were harvested 45-60 days after seeding. The harvesting season lasted at least six weeks, and the fruit was harvested when it was neither too small and young nor too hard and fibrous. The fruits that were collected had a dark green color and were club-shaped with 7-8 continuous ribs per fruit, weighing between 100-120 grams. The total fruit yield per plant depended on factors such as fruit length, diameter, weight, and number.

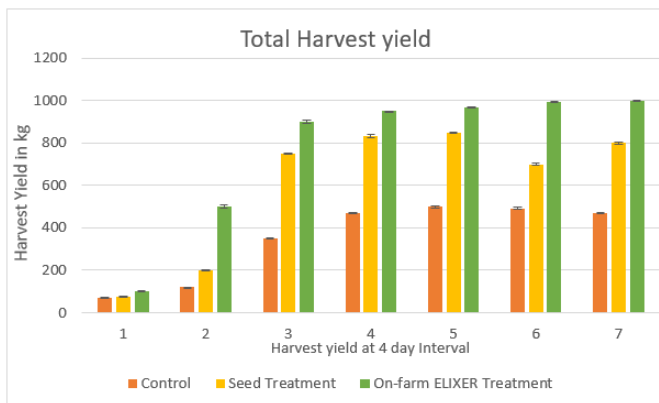


Figure 2: The graph displays the average yield of *Momordica charantia* obtained from different treatments, including the Seed treatment, On-farm treatment, and control.

Figure 2 shows that crops treated with on-farm production or BAIF ELIXER resulted in the highest yields, up to 997 kg at the seventh harvest. The yield of water treatment was the least, with 472 kg, while seed treatment yielded 800 kg. The distinctions among the treatments exhibited statistical significance ($P < 0.05$). Similar results have been reported in other studies where microbial consortia were used as bio-fertilizer treatments that can improve *Momordica charantia* germination rates [16]. Treatment 2, which is the on-farm treatment of BAIF ELIXER, displayed the highest mean yield when compared to the other treatments.

The current findings in bitter melon are also consistent with those described by Prasad, Param Hans [17, 18]. The data obtained from the experiment will be able to formulate a nutrition programme based on plant growth and fruit yield [17, 18].

The utilization of biofertilizers not only amplified plant growth and yield but also had a positive impact on soil health and microbial population. The biofertilizer application induced an augmentation in the population of advantageous microorganisms such as rhizobacteria, mycorrhizal fungi, and actinomycetes, which are renowned for improving soil fertility and nutrient cycling [19, 20]. Additionally, the bio-fertilizer enhanced soil pH and nutrient availability, resulting in a more propitious growing milieu for the crops [21]. The escalation in plant growth and yield observed in biofertilizer-treated plants can be ascribed to improved nutrient uptake, substantiated by elevated levels of nitrogen, phosphorus, and potassium in the plants [22]. In essence, these findings propose that the use of biofertilizers can augment crop yield, encourage sustainable agriculture by advancing soil health, and curtail the dependence on chemical fertilizers [23].

4. Conclusion

The study encloses the high efficiency of biofertilizers to ensure high yields and sustainability of agricultural practices. But it is important to understand that not all biofertilizers treatment are supportive or efficient. Competition between microorganisms and less compatibility with soil or crop or

weather can lead such combinations to underperform. While in the case of a BAIF EIXER six different types of biofertilizers, it can boost a few specific growth parameters. Based on the data and observations, it was determined that biofertilizer treatments outperformed the control or water treatment in terms of crop performance efficiency. The Fenugreek pot experiments and Bitter Guard field studies suggest that the eco-friendly on-farm treatment of biofertilizers demonstrated significant efficacy when compared to water treatment. This article's information can assist us understand the role of microorganisms in agriculture and techniques to formulate these microorganisms as biofertilizers for sustainable crop development.

Ethical Approval

Not applicable

Consent to Participate

Not applicable

Consent to Publish

Not applicable

References

- Mitter, E. K., Tosi, M., Obregón, D., Dunfield, K. E., & Germida, J. J. (2021). Rethinking crop nutrition in times of modern microbiology: innovative biofertilizer technologies. *Frontiers in Sustainable Food Systems*, 5, 606815.
- Kumar, S., Sindhu, S. S., & Kumar, R. (2022). Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. *Current Research in Microbial Sciences*, 3, 100094.
- Bhardwaj, D., Ansari, M. W., Sahoo, R. K., & Tuteja, N. (2014). Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microbial cell factories*, 13, 1-10.
- Sawane, R., Shinde, S., & Shinde, M. (2023). On-farm Production & Bio-efficacy Study of 'Baif Elixer' Biofertilizer on *Momordica Charantia*.
- Gautam, K., Sirohi, C., Singh, N. R., Thakur, Y., Jatav, S. S., Rana, K., ... & Parihar, M. (2021). Microbial biofertilizer: Types, applications, and current challenges for sustainable agricultural production. In *Biofertilizers* (pp. 3-19). Woodhead Publishing.
- Pirttilä, A. M., Mohammad Parast Tabas, H., Baruah, N., & Koskimäki, J. J. (2021). Biofertilizers and biocontrol agents for agriculture: How to identify and develop new potent microbial strains and traits. *Microorganisms*, 9(4), 817.
- Rao, D., Meng, F., Yan, X., Zhang, M., Yao, X., Kim, K. S., ... & Zhang, W. (2021). Changes in soil microbial activity, bacterial community composition and function in a long-term continuous soybean cropping system after corn insertion and fertilization. *Frontiers in Microbiology*, 12, 638326.
- Arora, N. K., Fatima, T., Mishra, I., & Verma, S. (2020). Microbe-based inoculants: role in next green revolution. *Environmental Concerns and Sustainable Development: Volume 2: Biodiversity, Soil and Waste Management*, 191-246.

9. Mehta, R. S., Anwer, M. M., Aishwath, O. P., & Meena, R. S. (2012). Growth, yield and quality of fenugreek (*Trigonella foenum-graecum* L.) as influenced by nitrogen, phosphorus and bio-fertilizers.
10. Saxena, A. K., & Singh, S. (2019). Growth and Yield of fenugreek (*Trigonella foenum-graecum* L.) as influenced by liquid and solid biofertilizers (Rhizobium, PSB and KSB). *Res J Chem. Environ Sci*, 7(3), 52-55.
11. Soyam, S. R., Wagh, A. P., Dod, V. N., Nagre, P. K., & Gade, R. M. (2012). Effect of different biofertilizers on growth, yield and quality of fenugreek. *Asian Journal of Horticulture*, 7(1), 28-30.
12. Patel, R., Shroff, J. C., & andSN Shah, P. P. (2022). Enhancement of yield and quality of fenugreek (*Trigonella foenum-graecum* L.) through fertilizer level and bio NP.
13. Mulani, T. G., Musmade, A. M., Kadu, P. P., & Mangave, K. K. (2007). Effect of organic manures and biofertilizers on growth, yield and quality of bitter gourd (*Momordica charantia* L.) cv. Phule Green Gold. *Journal of Soils and Crops*, 17(2), 258-261.
14. Kumar, S. M., Chowdappa, P., & Krishna, V. (2015). Development of seed coating formulation using consortium of *Bacillus subtilis* OTPB1 and *Trichoderma harzianum* OTPB3 for plant growth promotion and induction of systemic resistance in field and horticultural crops. *Indian Phytopath*, 68(1), 25-31.
15. Habiba, M., Khatun, K., Mostarin, T., Samad, M. A., Tania, M., Malo, K., & Akter, S. (2021). Influence of Bio-fertilizer Application Method with Organic and In-organic Fertilizer on Growth and Yield of Bitter-gourd in Winter Season (*Momordica charantia* L.). *Asian Journal of Advances in Agricultural Research*, 17(1), 1-15.
16. Patel, A. R., Patel, M. V., & Chandni, V. (2020). Effect of integrated nutrient management on growth and quality of bitter gourd (*Momordica charantia* L.). *International Journal of Chemical Studies*, 8(3), 2575-2576.
17. Prasad, P. H., Mandal, A. R., Sarkar, A., Thapa, U. M. E. S. H., & Maity, T. K. (2009). Effect of biofertilizers and nitrogen on growth and yield attributes of bitter gourd (*Momordica charantia* L.). In *Proceedings, International Conference on Horticulture* (pp. 738-739).
18. Masih, D., Prasad, V. M., Bahadur, V., & Yadav, N. P. (2020). Influence of organic, inorganic and bio-fertilizers on growth, flowering, yield and quality attributes of bitter gourd (*Momordica charantia* L.) var. green long. *International Journal of Chemical Studies*, 8(6), 1240-1244.
19. Seenivasagan, R., & Babalola, O. O. (2021). Utilization of microbial consortia as biofertilizers and biopesticides for the production of feasible agricultural product. *Biology*, 10(11), 1111.
20. Alori, E. T., Dare, M. O., & Babalola, O. O. (2017). Microbial inoculants for soil quality and plant health. *Sustainable agriculture reviews*, 281-307.
21. Khan, M. Y., Haque, M. M., Molla, A. H., Rahman, M. M., & Alam, M. Z. (2017). Antioxidant compounds and minerals in tomatoes by *Trichoderma*-enriched biofertilizer and their relationship with the soil environments. *Journal of Integrative Agriculture*, 16(3), 691-703.
22. Mukhongo, R. W., Tumuhairwe, J. B., Ebanyat, P., Abdel-Gadir, A. H., Thuita, M., & Masso, C. (2017). Combined application of biofertilizers and inorganic nutrients improves sweet potato yields. *Frontiers in plant science*, 8, 219.
23. Jin, N., Jin, L., Wang, S., Li, J., Liu, F., Liu, Z., ... & Yu, J. (2022). Reduced chemical fertilizer combined with bio-organic fertilizer affects the soil microbial community and yield and quality of lettuce. *Frontiers in Microbiology*, 13, 863325.