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Compatibility of Plant Growth-promoting Rhizobacterial Strains to the Integrated Specific Nutrient Mixture Formulated for Lateritic Soils of Goa

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Abstract

The experiment was conducted from January to August, 2017 at ICAR-Central Coastal Agricultural Research Institute, Old Goa, Goa to study the compatibility of the plant growth-promoting rhizobacterial strain to the integrated specific nutrient mixture (ISN mix) prepared to alleviate the multinutrient deficiency of the laterite soils of Goa. The mixture was composed of organic manures and inorganic secondary and micronutrients. The ISN mix had a neutral to slightly alkaline pH of 7.78 with a particle density of 0.48 mg m^{-3}. The mixture contained 0.806% N, 0.163% P, 1.09% K, 0.226% S, 1111 mg kg^{-1} Ca, 139.3 mg kg^{-1} Mg, 5394 mg kg^{-1} Fe, 66.68 mg kg^{-1} Mn, 6480 mg kg^{-1} Zn, 57.36 mg kg^{-1} Cu and 477 mg kg^{-1} B. This mixture was mixed with the locally isolated and well-characterized plant growth-promoting rhizobacteria Bacillus methylotrophicus strains STC-4 and Rch6-2b. The compatibility of these strains with the native microbial population already existing in the ISN mixture was tested, and positive results were observed. Further, its compatibility was tested with the ISN mixture containing organic and inorganic components in 2%, 5%, and 10% talc formulations. Both strains were compatible with the ISN mix with a 9.01×10^9 log CFU g^{-1} population for up to 6 months in all the concentrations. For the fortification of the ISN mix, the salt-tolerant strain, STC-4 was selected at a 5% concentration. The effect of ISN mix on plant growth, yield and quality need to be studied further.

Keywords: Compatibility, PGPR, INM, nutrient deficiency, lateritic soil, Goa

1. Introduction

Lateritic soils are acidic, compact, and infertile with low nutrient content (Goel and Kalamdhad, 2017a; Bodian et al., 2018, Oladoja et al., 2019; Saing and Djainal, 2018). In India, 70 mha is under red soil and 40 mha is under lateritic soil (Ghosh et al., 2019). Goa state is located in the west coast region of the country and has a mean annual rainfall of 3000 mm, and a 30°C mean temperature (Panandiker et al., 2020). Shukla et al. (2021a; 2021b) reported severe secondary and micronutrient deficiency in Goan soils. According to the GIS and GPS-based district map of Goa state by Anonymous (2025), 99.50% of the geographical area of Goa comes under acidic soils (pH <6.5). Around 3% of the area is slightly saline (EC:1.0–2.0 dS m^{-1}) and more than 97% area is fertile with high organic carbon (>0.75) content. Nitrogen content was low (< 280 kg ha^{-1}) in 88% of the area. 76.50% of the area is under the medium phosphorus (11–25 kg ha^{-1}) Category, and 60.50% of the area is under medium potassium (120–280 kg ha^{-1}) category. In the case of micronutrients, 93.50% of the area is under zinc deficiency (<0.6 mg kg^{-1}); however, most of the area is sufficient in Iron (>0.4 mg kg^{-1}), copper (>0.2 mg kg^{-1}), and manganese (>0.2 mg kg^{-1}).

Many times, the recommended dose of chemical fertilizers may not be sufficient to meet the crop requirements in such a problematic soil conditions as suggested by Kalhoro et al. (2015) and subsequently, the insensitive use of chemical fertilizers lead to damage to the soil microflora, and low productivity in the long run (Xie et al., 2018). Organic manures enhance soil conditions to a great extent, though their bulk requirement, low nutrient content, uneasy handling, and slow release of nutrients (Timsina, 2018), and the risk of spreading pests, disease, and weeds are the limitations. Biofertilizers containing plant growth-promoting rhizobacteria (PGPRs) enhance plant growth by the production of phytohormones and enhancing soil conditions (Mahmud et al., 2021; Kapadia et al., 2022a). They help in biological nitrogen fixation, phosphate solubilization, rhizosphere engineering, siderophore output, 1-Aminocyclopropane-1-carboxylate deaminase (ACC) output, quorum sensing (QS) signal intervention, production of phytohormone and inhibition of biofilm production (Kapadia et al., 2022a, 2022b). The role of PGPRs in crop production and soil reformation was well portrayed by Aloo et al., 2022; Hasan et al., 2024 and Iqbal et al., 2024.

Integrated nutrient management (INM) practices with PGPRs ameliorate soil acidity and help in sustainable crop production and soil health management in red and lateritic soils (Pattanayak and Rao, 2014; Srivastava et al., 2021). The use of synthetic fertilizers can be reduced, and sustainable crop production can be ensured by the use of Rhizobacterial-based technologies. According to Nunes et al. (2024), *Bacillus* is considered a widely used beneficial microorganism for consortia formulations. The biofertilizer potential of *Bacilli* strains was depicted by Zhao et al., 2018; Bhutani et al., 2018. For a soil microbial consortium, the carrier material can be either organic or inorganic (Bamdad et al., 2021). However, while designing the integrated specific nutrient mixture, the compatibility of the biological components with the inorganic components and the naturally existing microbial population of the organic components was critical, as in the case of microbial consortium (Xu and Yu, 2021). This study aims to test the compatibility of locally isolated *Bacillus methylotrophicus* strains in an integrated specific nutrient mixture that may serve as a convenient solution to fight multi-nutrient deficiencies and enhance the growth and production of crops in acidic red and lateritic soils of Goa and other West coast agroclimatic regions.

2. Materials and Methods

2.1. Preparation of the integrated specific nutrient (ISN) mix

The experiment was conducted in 2018 for eight months at ICAR-Central Coastal Agricultural Research Institute, Old Goa. The ISN mix had 80% organic components, 15% inorganic components, and 5% biological components. The organic raw ingredients such as cow manure, goat manure, poultry manure, and ash were collected from the Animal Science units of ICAR- Central Coastal Agricultural Research Institute, Old Goa. They were mixed well after drying and powdering in predefined proportions. Mineral components of the supplement include rock phosphate (18% P_2O_5), zinc sulfate (33% Zn, 15% S), granubor (15% B), and dolomite (54.35% $CaCO_3$, 45.65% $MgCO_3$) were procured from the local market and mixed with the organic manure mixture in the desired concentration. The physical properties like particle density of the ISN mix were determined by pycnometer and pH and EC were estimated in 1:5 wv^{-1} water suspensions. The nutrient content of the ISN mix and its raw components were estimated (N- Kjeldahl method, P-Molybdenum blue spectroscopic method, K, Ca, Mg, S, Fe, Mn, Zn, Cu & B- Atomic Absorption Spectrophotometry).

2.2. Compatibility of rhizobacterial strains with the nutrient mixture

The native microbial population in the nutrient mixture and its components were analyzed by plate culture by the streak method. The compatibility of two well-characterized plant growth-promoting rhizobacteria (PGPR) *Bacillus methylotrophicus* strains (STC-4 as Goa Bio 1 and Rch6-2b as Goa Bio 2) (Ramesh et al., 2016a, 2016b) tested in a sterile and non-sterile integrated mixture @2%, 5%, and 10% (ww^{-1}). Talc formulations (9 Log CFU g^{-1}) were thoroughly mixed and the ISN mixture was stored in zip-lock polythene packets at room temperature. The population of the inoculated bacterial strains and the total bacterial population of the mixture were assessed for up to six months at monthly intervals by serial dilution and plating.

3. Results and Discussion

3.1. Properties of the ISN mix

The nutrient composition and properties of the individual components and the overall composition of the integrated nutrient mixture are given in Table 1. The colour of the ISN mixture was brown and more than 90% of the materials passed through the 2 mm sieve. The mixture had a neutral to slightly alkaline pH of 7.78 with a particle density of 0.48 Mg m^3. It had 0.806% N, 0.163% P, 1.09% K, 0.226% S, 1111 mg kg^{-1} Ca, 139.3 mg kg^{-1} Mg, 5394 mg kg^{-1} Fe, 66.68 mg kg^{-1} Mn, 6480 mg kg^{-1} Zn, 57.36 mg kg^{-1} Cu and 477 mg kg^{-1} B. The pH of the ISN mix was neutral to alkaline pH, which helps to

Table 1: Nutrient concentration of ISN mixture and its raw components

Sample	pH 1:5	EC 1:5 (dS m^{-1})	N (%)	P (%)	K (%)	Ca (ppm)	Mg (ppm)	S (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)
Ash	8.02	0.011	0.109	0.257	4.392	1250	250.1	0.307	8123	107.71	5904	41.8	466.1
Cow	8.99	0.009	0.801	0.124	0.691	18.77	2.848	0.427	102.5	58.335	24.03	47.67	74.1
Poultry	6.83	0.011	1.266	0.293	1.667	64.13	14.16	0.166	65.68	43.375	44.02	68.47	21.4
Goat	7.32	0.005	1.383	0.318	1.056	652.4	29.08	0.244	7522	76.39	448.8	92.69	48.7
ISN mixture	7.78	0.006	0.806	0.163	1.09	1111	139.3	0.226	5394	66.68	6480	57.36	477

balance soil acidity at the site of application. It helps in the absorption of mineral nutrients pH has a great influence on nutrient availability, mobility, and soil biological processes. The organic manure base material will act as a perfect medium for holding the secondary and micronutrients and help to release them into the soil. The secondary and micronutrients help in crop production in less fertile sub-Saharan Africa (Kihara et al., 2017).

3.2. Compatibility of rhizobacterial strains with the native microbes of the organic components

The highest microbial count was recorded in cow manure (6.15×10^9 log CFU g^{-1}) followed by goat manure (4.85×10^9 log CFU g^{-1}) (Figure 1). Ash had the least bacterial colonies (1.19×10^9 log CFU g^{-1}). The organic mixture without the addition of the inorganic components had 1.51×10^9 log CFU g^{-1}) colonies 48 hours after inoculation. Prasad and Babu (2017) reported poor root growth by plant growth-promoting bacteria, Azospirillum, in the presence of Pseudomonas in a mixture containing both organisms. The experiment was conducted in sterile and non-sterile conditions to identify the antagonistic effect of the native microbial species and found that it is compatible with the microbial population of the raw components of the ISN mixture. The compatibility of microbial populations in the integrated nutrient mixture is essential for the symbiotic or non-negative interaction between them.

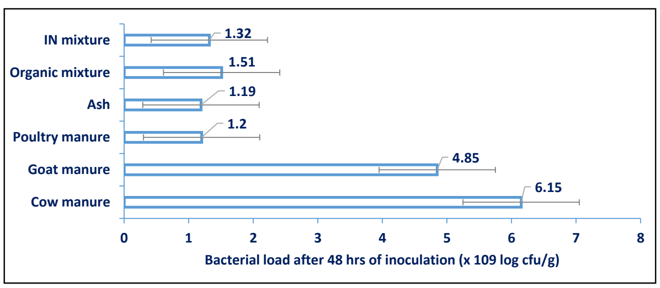

Figure 1: Native bacterial load in the non-sterile integrated nutrient mixture and its components

3.3. *Compatibility of rhizobacterial strains with the inorganic components of the ISN mix*

The bio-fortified ISN mixture was tested to confirm the presence of inoculated PGPR strains and their compatibility with other components of the mixture. Plate culture was performed with two strains and found that the STC- 4 strain had better performance than RCh6-2b (Figure 2). Among all the combinations, the 10% talc formulation of STC-4 has the highest population even after 6 months (9.01×10^9 log CFU g^{-1}). A monthly plate culture study showed that the population of both strains was good enough to be incorporated into the IN mixture. The talc formulation of 5% talc formulation STC- 4 was finally selected to incorporate in the ISN mixture as a biological component. The compatibility of growth-promoting bacteria with inorganic fertilizers were also reported previously by Vipitha and Geethakumari (2014).

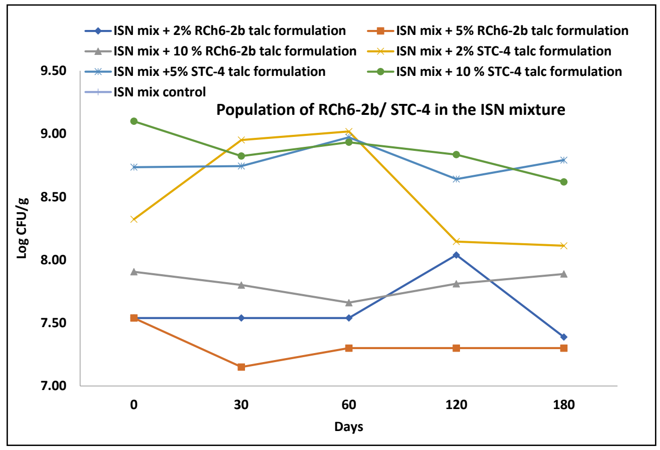

Figure 2: The population of *Bacillus methylotrophicus* strains in the ISN mix

The combined application of organic manures along with mineral fertilizers is essential to maintain soil health and crop productivity (Bandyopadhyay et al., 2010). Trichoderma-enriched bio-organic fertilizer enhanced the yield and quality of tomatoes and reduced fertilizer requirements (Ye et al., 2020).

4. Conclusion

A site-specific integrated specific nutrient mix (ISN) containing organic manures (80%), secondary and micronutrients (15%) and PGPR (5%) was prepared for the lateritic soil conditions of Goa. The mixture was fortified with *Bacillus methylotrophicus* salt-tolerant strain, STC-4, and found compatible for up to 6 months with a population of 9.01×10^9 log CFU g^{-1}. The effect of the integrated specific nutrient mix in the crop yield, and production economics needs to be studied further.

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