



Influence of phosphate and potassium solubilizing bacteria on performance of crop, endophytic and rhizosphere microbial population in chamomile

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ABSTRACT

Phosphate (*Bacillus megaterium*) and potassium (*Frateuria aurantia*) solubilizing bacteria (PSB and KSB) were assessed for their agronomic impact on chamomile performance in a pot experiment at CSIR-CIMAPRC, Bengaluru. Results demonstrated that 75% recommended dose of fertilizer (RDF) combined with PSB and KSB significantly improved chamomile performance, enhancing plant height (41.4 cm), stem diameter (1.10 mm), number of flowers (348.67), flower: leaf ratio (1.24), and flower dry weight (4.28 g plant⁻¹) compared to control, RDF alone, and sole solubilizer application. The combined application with 100% RDF synergistically increased rhizosphere bacterial population from 25 × 10⁴ CfU g⁻¹ to 57 × 10⁴ cfu g⁻¹, while 75% RDF + PSB + KSB resulted in 53 × 10⁴ cfu g⁻¹. Morphological and biochemical analyses revealed diverse variations in chamomile endophytes and rhizosphere soil bacterial populations, mainly *Bacillus* and *Streptococcus* genera. PSB and KSB, whether applied individually or combined, significantly influenced chamomile growth and yield, with microbial dynamics enhancing nutrient solubilization and availability.

Key words: *Matricaria recutita*, *Bacillus megaterium*, *Frateuria aurantia*, endophytes.

INTRODUCTION

Chamomile (*Matricaria recutita* L.), often known as German chamomile, is an annual aromatic herb in the Asteraceae family. The crop is native to Eastern and Southern Europe, can be found in North Africa, Asia, North and South America, and Australia, and is also grown in India and Hungary (Singh *et al.*, 18). In India, the plant has been cultivated to produce dried herbs, known as “Babuna”, and flowers, known as Gule Babuna in trade. The flower heads and their extracts are used in several herbal remedies, tea, cosmetics, food flavors, dyes, and pest repellent. The blue-colored essential oil obtained from flower contains approximately 120 secondary metabolites where bisabolol (20.9%), bisabolol-oxides A (21.6%), and chamazulene (19.9%) (Ghasemi *et al.*, 5) are considered marker compounds for quality parameters.

Currently, the unscientific use of chemical fertilizers for crop production, not only impairs the soil flora and fauna but also makes the crop plants harbour pests and diseases and also causes contamination of the earth's ecosystem. Additionally, these fertilizers contribute to the underutilization of the native nutrient pool and disrupt the nutrient equilibrium in the soil, despite the soil's inherent

richness in various nutrient concentrations. Thus, the reduction in the application of these fertilizers is a crucial measure that enables alternative technologies such as organic nutrient sources, and mobilization of native immobile nutrients through the utilization of microorganisms. The exploitation of nutrient solubilizers and mobilizers is a novel method to reduce the chemical fertilizer input and effective utilization of native nutrients (Suman *et al.*, 19). Harish *et al.* (6), reported that the combined application of phosphate solubilizing bacteria (PSB) and arbuscular mycorrhiza (AM) significantly improved the growth and productivity of holy basil compared to control and sole applications. Similarly, Kumar *et al.* (9) revealed that the application of 60% RDF with PSB and potassium solubilizing bacteria (KSB) significantly improved the fruit yield of cape gooseberry. The application of nutrient solubilizers has been extensively studied in cereals pulsed, fruits, and vegetables, yet remains relatively limited in the context of medicinal and aromatic plants. These plants are predominantly cultivated in marginal land where nutrient availability is constrained by various edaphic and environmental factors rendering the native nutrient pool largely inaccessible to plants. This creates an opportunity to employ bio-fertilizers to effectively harness bound nutrients, thereby improving crop outcomes. Consequently, the experiment was conducted to know the efficacy of nutrient solubilizers in improving the growth and yield of chamomile.

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MATERIALS AND METHODS

The experiment was conducted at the CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP) Research Centre in Bengaluru in 2023-24. The pot experiment was laid out using a completely randomized design with 8 treatments replicated thrice by using treatments *viz.*, T₁: Control, T₂: Recommended dose of fertilizer (RDF), T₃: 100% RDF + KSB, T₄: 75% RDF + KSB, T₅: 100% RDF + PSB, T₆: 75% RDF + PSB, T₇: 100% RDF + PSB + KSB, T₈: 75% RDF + PSB + KSB. The 35-day-old seedlings of chamomile were transplanted into the pot containing the growing medium of sand and soil in the ratio of 1:1. The RDF was computed on a soil weight basis using an application rate of 100-50-50 kg NPK per hectare. Phosphate (*Bacillus megaterium*) and potassium (*Frateruria aurantia*) solubilizing bacteria were inoculated to pots of respective treatments before transplanting at the rate of 10 kg ha⁻¹. The crop management was carried out as per the package of practice for chamomile given by CSIR-CIMAP. The harvested flowers were dried in the shade until moisture reached 12-15% followed by hydro-distillation using cleveger for 4-5 h and essential oil was collected over anhydrous sodium sulphate to remove the moisture content. The chemical constituents of the essential oils were determined through gas chromatography (GC) (Thermo Fischer Trace GC-1300) and gas chromatography- mass spectrometry (GC-MS) (PerkinElmer Clarus 680 GC coupled with an SQ 8C MS).

The serially diluted rhizosphere of the soil of chamomile and surface sterilized root, stem and leaves of chamomile were inoculated into nutrient agar media and incubated at 30°C for 24 h. The microbial growth was recorded by the colony count parameter. Each colony that appeared on the plate was considered one colony-forming unit (cfu). Plates were sub-cultured to gain pure culture isolate and to revive bacteria. The different colonies according to color, shape, and size were further subjected to the identification of genera using staining techniques and biochemical analysis. (Duhan *et al.*, 4).

$$CFU = \frac{\text{No. of colonies counted on plate}}{\text{Vol. of the sample} \times \text{Dilution factor}}$$

Similarly, 1 g of chamomile rhizosphere soil sample was collected, serially diluted and poured on pikovskaya medium agar (Himedia M520-100G), and then incubated at 30°C until halo zone appeared. Halo zone appearance indicates the presence of phosphate solubilization, and it was measured as a phosphate solubilization index (PSI) (Pande *et al.*, 14).

$$PSI = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$

The data on plant growth and yield parameters was documented at the time of harvest and subjected to statistical analysis using a completely randomized design as specified by Gomez and Gomez (7). The analysis of variance (ANOVA) was executed and the F test was performed at a 5% level of significance.

RESULTS AND DISCUSSION

Phosphorus and potassium solubilizer strains significantly improved chamomile growth attributes (Table 1). Combined application of 100% RDF + PSB + KSB and 75% RDF + PSB + KSB showed significantly superior plant height, number of branches, and stem diameter (43.60 cm, 25.21, 1.15 cm, and 41.41 cm, 24.40, 1.10 cm, respectively) compared to control, RDF, and sole applications. Further, combined applications with 100 and 75% RDF exhibited comparable outcomes. Sole applications of PSB and KSB, either with 100 or 75% RDF, were significant over control but comparable to RDF alone and insignificant against the combined application. The mineralization of native unavailable nutrients by PSB and KSB by releasing organic acids which chelate cations bound to phosphate and solubilization of potassium-bearing minerals followed by converting them into a soluble form (Cheng *et al.*, 3; Itelima *et al.*, 7) facilitating their subsequent availability during the crop's growing period caused the notable enhancement in growth of chamomile. The present results are concurrent with the findings of Singh *et al.* (17) in china aster and Meena *et al.* (11) in pomegranate who reported the improvement of growth attributes on the application of biofertilizers along with RDF.

ANOVA for yield attributes (Table 2) showed that the number of flowers per plant increased significantly

Table 1. Effect of PSB and KSB on growth attributes of chamomile.

Treatment	Plant height (cm)	Stem dia (mm)	No. of branches
Control	30.58±1.01	0.65±0.05	12.00±0.41
RDF	35.01±0.79	0.80±0.05	14.00±0.42
100% RDP + PSB	36.48±2.25	0.99±0.08	19.19±0.56
75% RDP + PSB	36.50±1.61	0.95±0.06	18.53±0.99
100% RDK + KSB	37.38±1.08	0.97±0.08	22.47±1.08
75% RDK + KSB	36.95±1.59	0.93±0.03	22.07±0.37
100% RDF + PSB + KSB	43.60±1.78	1.15±0.05	25.21±0.65
75% RDF + PSB + KSB	41.41±1.08	1.10±0.09	24.40±1.06
SE.m (±)	0.85	0.04	0.43
CD _(p<0.05)	2.55	0.11	1.30

Table 2. Effect of PSB and KSB on yield attributes and flower yield of chamomile.

Treatment	No. of flowers	Flower dia (mm)	F:L ratio	Dry wt. of flower (g plant ⁻¹)	Oil (%)
Control	224.00 ± 11.79	1.77 ± 0.10	0.92 ± 0.04	2.12 ± 0.06	0.71 ± 0.04
RDF	296.67 ± 12.50	1.89 ± 0.02	1.02 ± 0.08	2.76 ± 0.05	0.73 ± 0.02
100% RDP + PSB	306.00 ± 11.79	2.06 ± 0.05	1.21 ± 0.07	3.29 ± 0.28	0.74 ± 0.02
75% RDP + PSB	315.00 ± 11.79	2.11 ± 0.03	1.19 ± 0.04	3.25 ± 0.12	0.75 ± 0.06
100% RDK + KSB	319.00 ± 8.89	2.08 ± 0.03	1.18 ± 0.03	3.34 ± 0.12	0.73 ± 0.03
75% RDK + KSB	327.33 ± 10.69	2.09 ± 0.09	1.15 ± 0.03	3.31 ± 0.15	0.76 ± 0.01
100% RDF + PSB + KSB	359.00 ± 06.93	2.29 ± 0.05	1.27 ± 0.06	4.34 ± 0.61	0.74 ± 0.03
75% RDF + PSB + KSB	348.67 ± 15.18	2.27 ± 0.09	1.24 ± 0.05	4.28 ± 0.09	0.75 ± 0.01
SE.m (±)	6.60	0.04	0.03	0.15	0.02
CD _(p<0.05)	19.78	0.11	0.09	0.44	NS

from 296.67 in RDF to 359 with 100% RDF + PSB + KSB, closely comparable to 75% RDF + PSB + KSB. Flower diameter and flower-to-leaf ratio (F:L ratio) were statistically superior with 100% RDF + PSB + KSB (2.29 mm and 1.27) and on par with 75% RDF + PSB + KSB (2.27 mm and 1.24) compared to RDF (1.89 mm and 1.02), control (1.77 mm and 0.92), and all sole applications. Sole applications of PSB or KSB with 75% RDF had significantly more flowers, Flower diameter, and F:L ratio than RDF and were on par with 100% RDF with sole applications but not significantly different from combined solubilizer application. Effective metabolic performance, on essential nutrients availability throughout growth stages due to solubilizers, improved chamomile yield attributes (Silva *et al.*, 16). Phosphorus-solubilizing bacteria may also contribute to plant growth by stimulating biological nitrogen fixation, producing phytohormones, and improving trace element availability like zinc and iron (Wani *et al.*, 21). Current results align with Kumar *et al.* (9) illustrating that the simultaneous application of PSB and KSB alongside chemical fertilizers substantially improved the yield parameters of cape gooseberry.

Flower yield significantly varied with nutrient solubilizer application (Table 2). KSB showed a 1.68% higher yield potential than sole PSB. The chamomile flower dry weight was 4.34 g plant⁻¹ with 100% RDF + PSB + KSB which was significant over RDF, control, and all sole applications, with comparable results for 75% RDF + PSB + KSB (4.28 g plant⁻¹). Sole PSB or KSB applications with 100% RDF (3.29 and 3.34 g plant⁻¹, respectively) also significantly surpassed RDF (2.76 g plant⁻¹) and control (2.12 g plant⁻¹), with on-par results for 75% RDF (3.25 and 3.31 g plant⁻¹, respectively). Primarily improved growth and yield attributes (Tables 1 & 2) by action

of solubilizers, led to superior chamomile flower yield. Increased phosphorous and potassium levels, facilitated by microorganism solubilization, enhanced photosynthesis rates and nutrient translocation, contributing to optimal development and increased yield (Chauhan and Raghav, 2; Kumar *et al.*, 9). Similar results were also reported by Yadegari *et al.* (22) in thyme Paramanik and Chikkaswamy (15) in *Ocimum basilicum* and Mahala *et al.* (10) in onion.

Essential oil recovery and chemical constituents did not significantly vary with solubilizer application (Table 2 and 3) however recovery ranged from 0.71% to 0.76%. The combined use of 75% RDF + PSB + KSB exhibited peak concentrations of α -bisabolol oxide-A and bisabolol oxide-B, while α -bisabolol peaked in RDF alone. Chamuzelene, contributing to the oil's blue colour, reached its highest level in sole PSB application, followed by combined solubilizer use. Similar results were reported in thyme and sage crops by Nadjafi *et al.* (12), where biofertilizer application had an insignificant effect on oil recovery and constituents.

The utilization of various solubilizers demonstrated a noteworthy impact on the rhizosphere bacterial population (Fig. 1). The initial bacterial count of 25×10^4 CfU g⁻¹ showed a significant increase to 57×10^4 CfU g⁻¹ with the application of 100% RDF + PSB + KSB, followed by 53×10^4 CfU g⁻¹ with 75% RDF + PSB + KSB. Intriguingly, the sole application of PSB or KSB with 75% RDF resulted in a higher population count than the 100% RDF combinations. The lowest population was observed in the RDF and control treatments.

A solitary colony of PSB was isolated, characterized, and identified. The bacterium appeared gram-negative, displaying a white hue and irregular colony morphology, while its cells were

Table 3. Chemical constituents of chamomile essential oil.

Compound	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈
Limonene	0.10	0.10	0.10	0.10	0.20	0.10	0.10	0.10
(E)-β-ocimene	0.20	0.30	0.20	0.10	0.40	0.20	0.20	0.30
Artemisia ketone	1.60	1.60	1.10	1.30	2.50	1.80	1.70	2.50
Camphor	0.70	0.60	0.40	0.30	0.90	0.50	0.60	0.50
Isoborneol	0.60	0.30	0.30	0.30	0.40	0.40	0.30	0.40
β-elemene	0.20	0.10	0.30	0.10	0.20	0.10	0.10	0.20
β-caryophyllene	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
(E)-β-farnesene	4.80	4.40	5.10	3.50	5.70	6.00	5.70	5.60
Germacrene-D	0.70	0.50	0.40	0.30	0.40	0.70	0.50	0.40
Bicyclogermacrene	0.20	0.10	0.30	0.10	0.10	0.10	0.10	0.10
10-epi-γ-eudesmol	0.80	0.90	0.70	1.70	0.90	1.00	1.90	1.10
α-bisabolol oxide B	21.50	23.70	24.36	22.50	20.50	21.34	23.45	25.90
α-bisabolol	23.64	26.00	22.70	20.60	26.65	22.45	23.80	25.65
Chamazulene	1.40	1.70	3.20	2.80	3.00	3.20	2.10	2.60
α-bisabolol oxide A	16.78	15.00	16.80	20.90	18.45	18.65	16.54	18.86
(Z)-spiro ether	8.20	10.90	11.80	9.20	8.10	11.70	10.90	5.80
(E)-spiro ether	0.30	0.60	0.70	0.10	0.40	0.60	0.60	0.30

Note: (T1: Control, T2: Recommended dose of fertilizer (RDF), T3: 100% RDF + KSB, T4: 75% RDF + KSB, T5: 100% RDF + PSB, T6: 75% RDF + PSB, T7: 100% RDF + PSB + KSB, T8: 75% RDF + PSB + KSB).

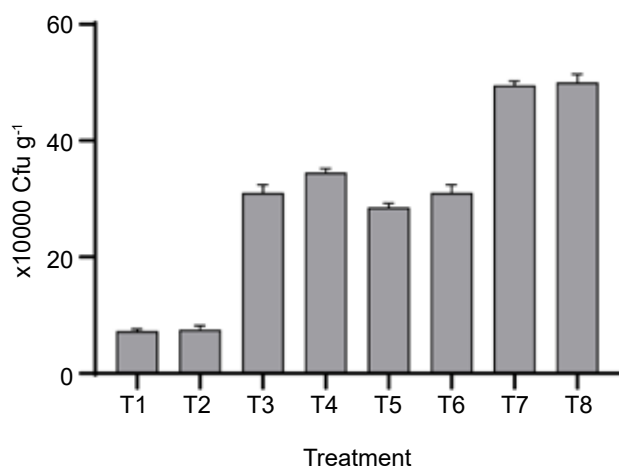


Fig. 1. Soil microbial population as influenced by application of PSB and KSB.

rod-shaped with rough surfaces and flat elevations. Surrounding the bacterial growth, distinct halo zones were observed. This isolate demonstrated a notable phosphate solubilization index of 2.45 ± 0.5 mm.

A varied assortment of bacterial populations in both endophytes and soil was identified by examining morphological characteristics and biochemical tests (Fig. 2 and 3). Gram-positive bacteria constituted 70.00% of endophytes and 58.33% in soil, with gram-negative bacteria accounting for 30% and 41.67%,

respectively. The cell morphology in both populations predominantly featured rod-shaped cells, with a lesser occurrence of cocci-shaped cells. Circular-shaped colonies were predominant in both soil bacteria and endophytes (75 and 90% respectively) followed by irregular shapes. Surface morphology of the colony was found smooth in both categories (83.33 and 90%, respectively) followed by minimal occurrence on rough surfaces. An equal share of raised and flat elevation was observed in colonies with varied colours and consistency in isolates of bacteria from soil and chamomile plants as represented in Fig. 3. Most soil bacterial populations and endophytes of chamomile have given positive test results for different biochemical tests (Fig. 4). The outcomes

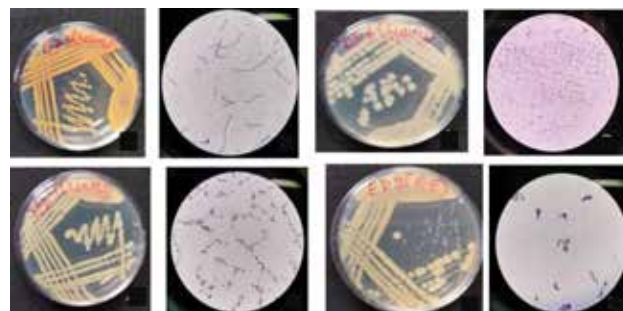


Fig. 2. Isolation of endophytic and rhizosphere bacteria of chamomile.

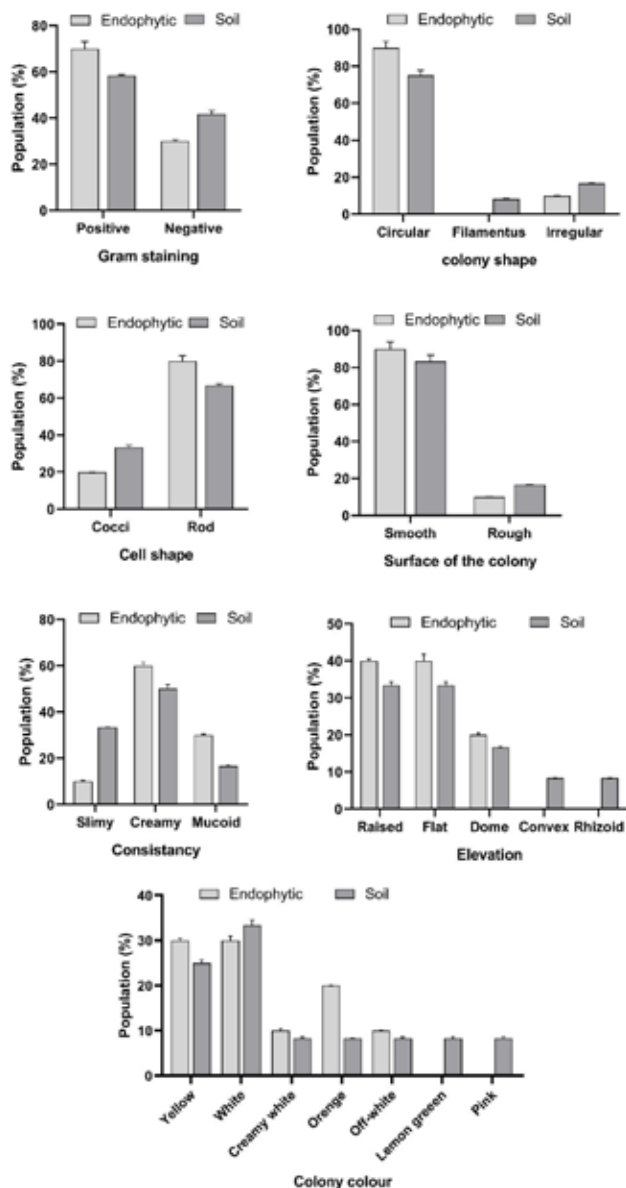


Fig. 3. Morphological characterization of endophytes and rhizosphere soil bacteria.

from morphological and biochemical tests suggest the likely presence of bacteria belonging to the *Bacillus* and *Streptomyces* genera. The increase in the microbial population of samples inoculated with PSB and KSB elucidates the synergetic impact of these inoculants on the proliferation of other bacterial groups (Aswathy *et al.*, 1). The present synergic effect of PSB and KSB in proliferating rhizosphere bacterial population was also reported by Wang *et al.* (20) and Negi *et al.* (13) who documented the enhancement in the bacterial population through the application of biofertilizers. Another study by Kujur *et al.* (8) also demonstrated the improvement in the population density of PSB on the application of biofertilizers. These improvements in beneficial microbes ensure the solubilization of bound nutrients and promote the significant economic outcome of the chamomile.

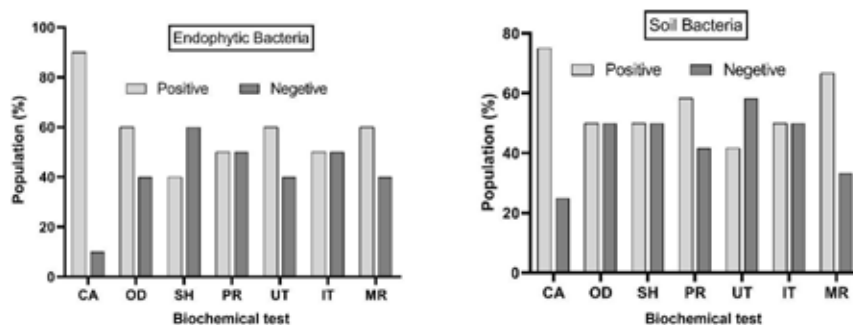
In conclusion, 75% RDF with PSB and KSB enhanced chamomile performance compared to RDF or solubilizers alone. A 25% reduced RDF with PSB and KSB, individually or combined, improved growth, yield, and microbial dynamics in chamomile while mitigating the negative impacts of chemical fertilizers on soil and the environment. These insights are valuable for optimizing nutrient management in chamomile cultivation.

AUTHORS' CONTRIBUTION

Conceptualization, supervision and correction of the original draft (YND), conducting field research, lab analysis, and manuscript writing (PM), assisted in conducting field research, data analysis, and manuscript writing (PKM), assisted in conducting field research and lab analysis (CH), correction of the original draft (DK), GC and GC-MS analysis of essential oil, manuscript correction (PRC).

DECLARATION

The authors declare that they have no competing interest.



(CA = Catalase; OD = Oxidase; SH = Start Hydrolysis; PR = Phenol red; UT = Urease test; IT = Indole test; MR = Methyl red test)

Fig. 4. Biochemical test of endophytes and rhizosphere soil bacterial population of chamomile.

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