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Influence of Potassium and Phosphate Solubilizing Bacteria on Growth and Development of Davana (*Artemisia Pallens* Bees)

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***Abstract*:** The study assessed the impact of KSB and PSB application on *Artemisia pallens* growth, yield, and its impact on endophytic and rhizosphere bacterial populations. The plant height and flower diameter of davana were increased due to the application of T_7 100% RDF+PSB+KSB over control and Recommended Dose of Fertilizer. Significantly higher plant height and flower diameter was 100% RDF+PSB+KSB compared to RDF alone. Treatments 75% RDF+PSB+KSB exhibited higher fresh plant weight than RDF alone. Soil samples from T_7 showed the highest bacterial population (49×104 Cfu/ml), enhancing plant yield and essential oil quality. Endophytic bacteria were predominantly Gram-negative with rod and cocci shapes. RDF with PSB and KSB increased respiration rate, indicating faster nutrient mineralization. Overall, 75% RDF with PSB and KSB benefits davana performance, bacterial population, and soil nutrient mineralization, reducing reliance on synthetic fertilizers and environmental risks.

***Keywords*:** Davana, Essential oil, PSB, KSB, Endophytes

Artemisia pallens Bees (Davana) is an aromatic annual herb, belonging to the Compositae family, indigenous to India and cultivated in Karnataka, Tamil Nadu, and Andhra Pradesh. The genus Artemisia contains more than 480 species around the world, out of which about 45 species are found in India (Singh et al 2021). It is a short-season crop from November to March, prefers red-loamy and well-drained soil performs better during moderate winter conditions; however, it cannot withstand heavy rain. Davana has high economic value due to its aromatic essential oil, commercially used to replace artificial flavoring agents with natural flavoring in food products like cakes, candy, chewing gum, and ice creams (Trendafilova et al 2020). The essential oils are also used widely in the aroma industry for making perfumes on the other side davana extract is used in the beverage industry. Monoterpenes, sesquiterpenes, and derivatives of fatty acids make up the essential oil constituents. More than ninety components were identified, with *cis*-davanone, bicyclo germacrene, *trans*-ethyl cinnamate, davana ether isomer, and spathulenol being the main ones. These constituents have also been shown to be effective against certain pathogenic microbes (Singh et al 2021).

Chemical fertilizers are widely employed in large-scale commercial davana production to boost output and meet customer demand, which lowers the amount of nutrients that are readily available in the soil and microflora (Kumar et al 2019). Every nutrient that the plant absorbs from various sources plays a specific role that none of the others can provide. The three main nutrients-nitrogen (N), phosphorus (P), and potassium (K) are needed in high concentrations for the majority of crops and are essential to plant metabolism. The majority of primary minerals are insoluble due to their complex structure, which renders them unavailable for plant uptake. Potassium is designated as a macronutrient because plants absorb substantial amounts of it. Little amount of potash is primarily obtained in India from bedded marine evaporite deposits, glauconite, polyhalite, and sylvite, as well as surface and subsurface potash-rich brines. In addition to minerals, many other microorganisms coexist in symbiotic relationships with plants, including endophytic and rhizosphere microbes. Because of the reported advantages of these microbes, scientists have been interested in them. These microbes have an innate connection to their host plant and are capable of developing endogenously. Within the rhizosphere, bacteria identified as plant growth-promoting bacteria (PGPB) have the capacity to directly enhance plant growth by increasing the absorption of macronutrients and minerals, along with elevating essential hormone concentrations. Additionally, these bacteria can indirectly support plant growth by mitigating the harmful impacts of pathogens. Numerous plant tissues, such as leaves, stems, roots, and flowers, have been found to have endophytic bacteria (Kobayashi and Palumbo 2000). These tissues are a potential but not utilized source of phyto-constituents. Bacterial strains found in association with roots can contribute to the improvement of plant health and growth through mechanisms such as phyto-stimulation, biofertilization, and biocontrol. *Pseudomans, Pantoea*, and

Bacillus (Andreolli et al 2016), are advantageous for plant growth. This pot experiment uncovered a significant gap in research regarding the effects of applying potassium-solubilizing bacteria (KSB) and phosphate-solubilizing bacteria (PSB) on the economically important davana crop.

MATERIAL AND METHODS

Pot experiments followed by analytical lab procedures were conducted to evaluate the effect of phosphate and potassium solubilizing bacteria on the growth, development, and essential oil yield of *Artemisia pallens* followed by the identification and characterization of endophytes and various microorganisms in a soil sample from the rhizosphere of davana.

Study area: The pot experiment was conducted at CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre Bengaluru, Karnataka during 2022-23. Geologically the experiment site was located at 77º33′30″ East longitude and 13º05′06″ North latitude with a mean sea level of about 920 meters under the southern plateau and hilly zone.

Experimental design: The pot experiment consisted of eight treatments including T_1 (control), T_2 (RDF), T_3 (100% RDF+KSB), T_4 (75% RDF+KSB), T_5 (100% RDF+PSB), T_6 (75% RDF+PSB), T_7 (100% RDF+PSB+KSB) and T_8 (75% RDF+PSB+KSB) which were replicated thrice in randomized block design. The weather parameter did not affect the growth due to the pots were kept under a glass house to maintain a stable environment.

Crop husbandry: The experiment was performed in pots capable of filling 5 kg of soil using davana as a test crop. The 35 days old seedlings of davana raised in the nursery have been transplanted into pots containing sand, soil, and manure in equal proportions (1:1:1). The required quantity of microbial inoculation was applied at the rate of 10 kg/ha on hectare soil weight basis to respective pots along with RDF. Regular watering was done to maintain the soil moisture as and when required. No signs of pest or disease incidence were noted throughout the crop growth period; however, the precautionary spray of pesticide was taken accordingly. The crop was harvested at physiological maturity followed by drying and extraction of essential oil.

Extraction of essential oil: The herbage from each treatment was extracted individually, and using a Clevenger-type apparatus (Soxhlet extraction unit manufactured by Super Scientific Company Bengaluru), the herbage was hydro-distilled for six hours in a 2000 ml flask (Kumara et al 2023). The Triplicate of each treatment sample was obtained and the mean essential oil yield values were taken for the computation. The extracted essential oil was collected over glass vial contains anhydrous sodium sulphate and kept in cold and dark place until further analysis. To identify various components, gas chromatography analysis was also performed on essential oils.

Microbial Analysis of Plant and Soil Samples

Soil and plant sample collection: The soil samples for determination of the microbial population were collected at a depth of 10–15 cm below the surface from each treatment. The isolation of PSB was carried out from the rhizosphere soil. The well-grown plants are identified and plucked by gentle pulling. It was possible to isolate endophytic bacteria from the davana plant's leaves, stems, buds, and roots. Therefore, the mentioned samples were all taken straight to the lab and stored in labelled sterile polythene bags. We ran an hour-long analysis on these samples to get better findings.

Bacterial population count and isolation of bacteria: The bacterial population present in the rhizosphere soil was counted at the beginning, and after harvest. Phosphate-solubilizing bacteria were isolated and the bacterial count was estimated in each sample by using the serial dilution technique in nutrient agar media and incubated at 30 °C for 24 hours. The microbial growth was recorded by the colony count parameter. Each colony that appeared on the plate was considered one colony-forming unit (CFU) (Creach et al 2003). The different colonies according to color, shape, and size were further subjected to the identification of genera using staining techniques. As per morphological and biochemical features, the isolates were characterized and classified up to the genus level on Pikovskaya's and Aleksandrov's agar media respectively.

$$CFU = \frac{\text{Number of colonies counted on plate}}{\text{Volume of the sample x Dilution factor}}$$

Isolation of phosphate solubilizing bacteria: 1g of davana crop rhizospheres soil sample was collected and serially diluted and poured on pikovskaya medium agar (Himedia M520-100G), and incubated at 30˚C until halazone appeared. Halazone appearance indicates the presence of phosphate solubilization, and it was recorded as a phosphate solubilization index (SI) (Pande et al 2017).

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

Isolation of endophytic bacteria: Davana plant sample was dissected into roots, stems, and leaves and then thoroughly washed. Surface-sterilized tissues were inoculated on nutrient media (NA) and incubated for 24 hours at 30˚C. Plates were sub cultured to gain pure culture isolate and to revive bacteria. Each issue sample's colonies were selected (Based on morphological appearance) for further genera identification by biochemical analysis (Duhan et al 2020).

Microbial respiration: The 250 g of soil mixed with the respective solubilizers with and without RDF were prepared. A 50 ml centrifugal tube containing 25 ml of 0.5N NaOH suspended inside the conical flask was incubated at 32ºC for 24 hours and carbon dioxide emitted due to the respiration of bacteria was trapped in NaOH which converted to sodium carbonate. Unused NaOH was back titrated against standard sulfuric acid (H_2SO_4 0.5M) in the presence of phenolphthalein indicator. After every 24-hour cycle, fresh NaOH was filled and repeated the above procedure for 10 days. The respiration rate was calculated (Dehsheikh et al (2020).

$$MR = \frac{(N \times (V1 - V2) \times 22}{(T \times DW)}$$

Where, MR represents the microbial respiration of the soil, expressed in terms of mg of emitted CO_2 per gram per day. N denotes the normality of the acid, V1 is the volume of H_2SO_4 used for titrating the remaining NaOH in the standard control solution (without inoculants), V2 is the volume of H_2SO_4 used for titrating the remaining NaOH in the sample, T represents time in days, and DW stands for the weight of the inoculants.

Statistical analysis: Statistical analyses were performed using SPSS, version 16.0 for the Duncan's Multiple Range Test (DMRT), and data visualization was conducted using GraphPad Prism, version 8.0.2.

RESULTS AND DISCUSSION

Effect on Growth, Development, and Yield of Davana

Growth attributes: The RDF along with biofertilizers alone or in combination have a significant effect on the growth and yield of davana as compared to control the statistical analysis of different growth attributes such as plant height and numbers of branches (Table 1). Application of 100% RDF+PSB+KSB increase significant plant height (47.40 cm) over control treatment (35.08 cm) and RDF (39.38cm) which was on par with treatment of 100% RDF+PSB+KSB. The sole application of 75% RDF+KSB, 100% RDF+KSB, 100% RDF+PSB, and 75% RDF+KSB was significant in increasing plant height over RDF and control. Biofertilizers like VAM (Vesicular Arbuscular Mycorrhizae) and Azospirillum significantly enhanced the growth and development of Kadam seedlings. These biofertilizers notably increased as shoot length, collar diameter, number of leaves per plant, total leaf area per plant, root length, as well as fresh and dry biomass (Chauhan 2023). The number of branches of davana did not vary significantly due to the application of different solubilizers with recommended doses of fertilizers. The better availability and assimilation of nutrients from the soil by plants enhanced the better photosynthetic activity, build-up of cells, and their elongation where the better availability was aided by the nutrient solubilizing action of PSB and KSB application to the soil (Olaniyan 2022) probably caused the better growth of davana plant.

Yield attributes: An increment of 22.8 and 21.40% enlargement in flower diameter in treatment 75% RDF+PSB+KSB and 100% RDF+PSB+KSB respectively over the untreated plants (Table 1), which was statistically significant over untreated (2.76 mm) and RDF (3.24 mm). Where the different proportions (75 and 100%) of chemical fertilizer with the sole application of PSB and KSB significantly influenced the flower diameter of davana over RDF alone treatment. The least yield was recorded in the control treatment. Kumari et al (2019) also reported a significantly higher flower diameter of marigolds on application of PSB with RDF. The significant rise in davana single plant weight was observed due to the application of RDF, increasing from 33.27 g (control) to 39.43 g. This further increased to 48.14 g and 46.78 g with 100% and 75% RDF+PSB+KSB, respectively. Comparatively, 75% RDF with PSB (42.38 g) or KSB (43.53 g) was similar to 100% RDF with

Table 1. Effect of PSB and KSB inoculation on growth, development, and yield of davana in a pot experiment

Treatment	Plant height (cm)	No. of branches	Flower diameter (mm)	Single plant fresh weight (g)	Oil recovery (%)
T_1	35.08 e	18.67 a	2.76 d	33.27 d	0.21 c
T_2	39.38 d	21.00 ab	3.24 c	39.43 c	0.22 bc
T_3	43.78 bc	22.67 b	3.63 b	43.93 b	0.23 a
T_4	42.49 c	22.33 b	3.56 b	43.53 b	0.24 a
T_5	44.90 abc	21.47 ab	3.62 b	43.65 b	0.22 a
T_6	45.02 abc	22.17 b	3.47 bc	42.38 b	0.22 ab
T_7	47.40 a	21.33 ab	4.14 a	48.14 a	0.23 a
T_8	46.21 ab	21.00 ab	4.20 a	46.78 a	0.23 a
CD (1%)	2.85	NS	0.28	2.47	NS

The common letter(s) in a column shows no difference at the level of 5% probability as per DMRT; T_1: Control, T_2: RDF, T_3: 100% RDF+KSB, T_4: 75% RDF+KSB, T_5: 100% RDF+PSB, T_6: 75% RDF+PSB, T_7: 100% RDF+PSB+ KSB, T_8: 75% RDF+PSB+ KSB

PSB (43.65 g) or KSB (43.93 g), all significantly higher than control and RDF. The improved nutrient uptake, growth, photosynthesis, and biomass accumulation in davana were due to the mobilization of sparingly soluble P and K minerals or chelating ions, aligning with findings in geranium (Prasad et al 2012) and Sabja (Cheena et al 2021).

Essential oil recovery: The chemical composition of davana essential oil showed little variation among different treatments (Table 2). The major constituent, *cis*-davanone, ranged from 48.82% to 51.25%, with the highest content in the RDF treatment, followed by 75% RDF+PSB. The combined application of 100 and 75% RDF+PSB+KSB recorded *cis*-davanone levels of 50.88% and 49.78%, respectively. Davana ether (Isomer-1) was the second major constituent, ranging from 11.15% to 13.25%, with the highest levels in 75% followed by 100% RDF+KSB. Bicyclogermacrene content was highest in 75% RDF+PSB+KSB (5.32%) and lowest in the control treatment (4.05%). Other constituents, such as trans-davanone + trans-nerolidol, humulene epoxide-II, epi-alpha-cadinol, cis-methyl jasmonate, davanol acetate, and cis-hydroxy davanone, showed minimal variation among treatments.

Effect Microbiological Parameters of Soil and Plant Sample

Isolation of phosphate and potassium solubilizing bacteria from rhizosphere soil: A single phosphate-solubilizing bacterial colony was isolated, characterized, and identified. The organism was Gram-negative, white-coloured, and irregular in the shape of the colony; whereas the cell was in rod shape and rough surface with flat elevation. The colony exhibited clear halo zones around the bacterial growth; this isolate had a high phosphate solubilization index (PSI) of 2.5 mm. The isolates might be attributed to rhizosphere colonization, and better nutrient availability reported by Rafique et al (2012). The ability of PSB to solubilize insoluble phosphorus in Pikovaskya's agar medium by observing the halo zone thus concluding that bacteria tend to solubilize the insoluble phosphorous (Yaghoubi et al 2018).

Isolation of endophytic bacteria from leaf, bud, stem, and root of davana: Bacterial species were isolated based on the distinctive features of the colonies. Based on the findings, a variety of bacteria were identified in the leaf, stem, and root. In terms of morphological characterization, the endophytic bacterial isolates showcased a range of colony attributes, including diverse shapes (circular and irregular), colours (orange, white, and yellowish), margins (regular and wavy), and textures (Fig. 1). Concerning gram staining, 6 isolates demonstrated Gram-negative characteristics, while 2 exhibited Gram-positive features. In the biochemical and physiological characterization, all isolates underwent various tests including catalase, IMViC, H2S production, methyl red, and oxidase tests. Positive results were observed in certain isolates, (Table 3). Majority of bacterial endophytes might belong to the genus *Bacillus* and *Streptococcus*. Davana possesses a diverse taxonomic range of endophytes, with numerous studies (Suryanarayanan et al 2009) primarily concentrating on fungal endophytes, and there has been limited exploration of endophytic bacteria in this context.

Impact on bacterial population: The initial soil sample of the pot experiment count of bacteria was 25×10^4 Cfu/ml and varied significantly in soil sampled before harvest of the crop in each treatment. The highest bacterial count was in the combined application of 100% RDF+ PSB+KSB treatment T_7 (49×10^4 Cfu/ml), which was followed by 75% RDF+ PSB+KSB T_8 (49×10^4 Cfu/ml). The lowest bacterial count was in RDF alone T_2 (7×10^4 Cfu/ml) and control (7×10^4 Cfu/ml). Similarly, the impact of the sole application of KSB and PSB along with a recommended dose of inorganic fertilizer (75% and 100% RDF) on bacterial count was obtained after the application of T_4 (34×10^4 Cfu/ml) was similar to T_6, T_3 and T_5 (Fig. 2). The synergic effect of combined application in improving microbial population. Based on observation of both the combination and sole application of PSB and KSB

Table 2. Effect of PSB and KSB on essential oil constituents of *Artemisia pallens*

Treatment	Bicyclo germacne	Davana ether (Isomer -1)	*Cis*-davanone	Davanol acetate	*Cis*-hydroxy davanone	Davana ether (Isomer -2)	*Trans*-davanone + *trans*-nerolidol	*Cis*-methyl jasmonate
T_1	4.05	11.15	48.86	2.6	2.54	2.98	1.78	2.5
T_2	4.1	11.25	52.56	2.86	2.85	2.99	1.83	3
T_3	4.65	12.65	48.82	2.57	2.6	4.85	1.78	2.28
T_4	4.85	13.25	49.85	2.78	2.47	3.97	1.86	2.57
T_5	4.25	12.45	50.65	2.72	2.38	4.23	1.09	2.74
T_6	4.98	12.83	51.25	3.43	2.43	3.02	1.09	2.3
T_7	5.1	11.35	50.88	3.94	2.44	3.15	1.86	2.76
T_8	5.32	11.35	49.48	2.63	2	3.92	1.76	2.57

See Table 1 for details

along with inorganic fertilizer, positive synergistic effects with soil bacteria were observed, which promoted each other's growth. However, the combined application exhibited an overall greater bacterial population as compared to the initial RDF alone and controlled the bacterial population. Aswathy et al (2017) observed that the rapid growth in rhizosphere microbial population, which may be attributed to the multiplication of the strains in the rhizosphere, uses root exudates produced by the plants and synergistic interactions between introduced microbial inoculants and also the microorganisms in the root zone of the crop. In their study on rose-scented geranium, Negi et al (2022) noted that the presence of plant growth-promoting bacteria contributes to maintaining soil ecological balance, simultaneously improving both yield and essential oil quality. The use of microbial inoculants has the potential to mitigate the negative effects of chemical fertilizers and as a result, promote both the quantity and the quality of plant yield. Microbial inoculants offer a more sustainable way of delivering plant nutrients, and their utilization is environmentally beneficial too.

Microbial respiration: Among all the treatments RDF+PSB+KSB was the first to attain the highest peak of respiration i.e., on 3rd day whereas the sole application of RDF+PSB and RDF+KSB was on the 4th day. Among sole applications of PSB and KSB without RDF, inflated emission was recorded in KSB than in PSB with peak respiration on the 6th and 5th day respectively; whereas with RDF it was 4th day in both RDF+PSB and RDF+KSB treatments (Fig. 3). On the 10th day, RDF+PSB+KSB have a lower amount of carbon evolution than all other treatments indicating quick recession of respiration and more mineralization of substrate. The high

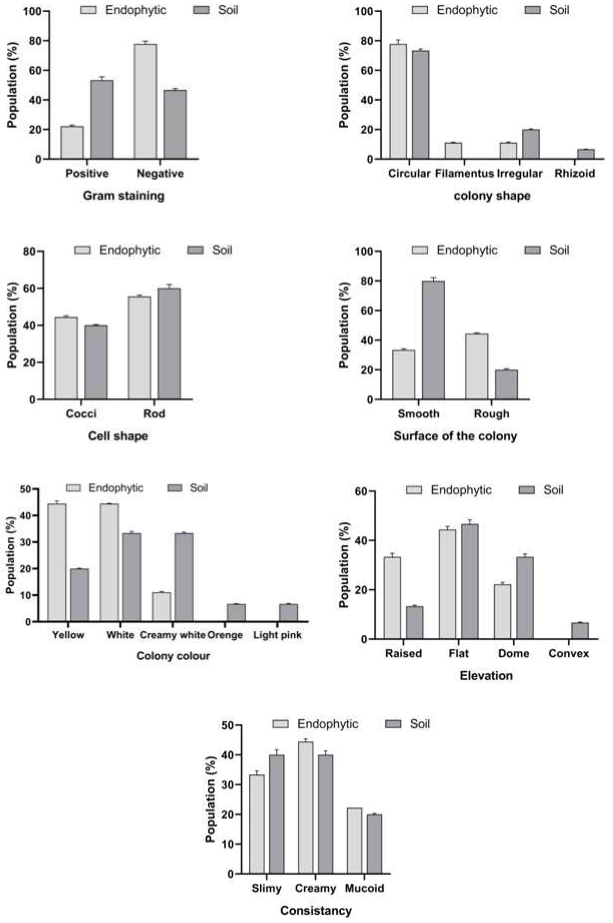

Fig. 1. Morphological characterization of endophytic bacteria

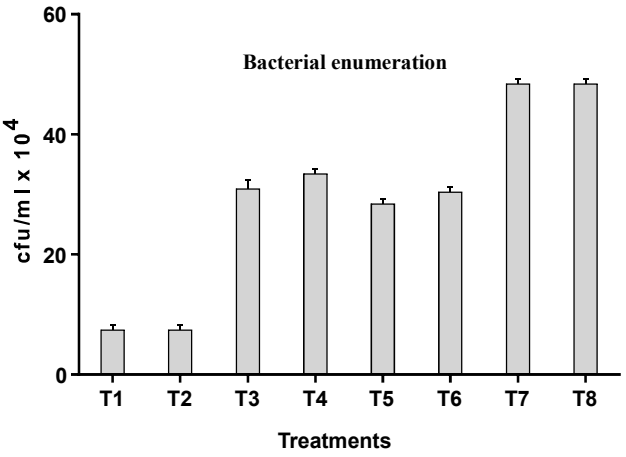

Fig. 2. Effect of PSB and KSB on Bacterial enumeration

Table 3. Biochemical characterization of endophytes and PSB in soil

Biochemical test	Endophytes		Soil	
	G-Positive	G-Negative	G-Positive	G-Negative
Catalase	100.00	0.00	46.67	53.33
Soxidase	50.00	50.00	40.00	60.00
Starch hydrolysis	62.50	37.50	53.33	46.67
Phenol red	62.50	37.50	46.67	53.33
Urease test	50.00	50.00	46.67	53.33
Indole test	37.50	62.50	66.67	33.33
Methyl red test	50.00	50.00	53.33	46.67

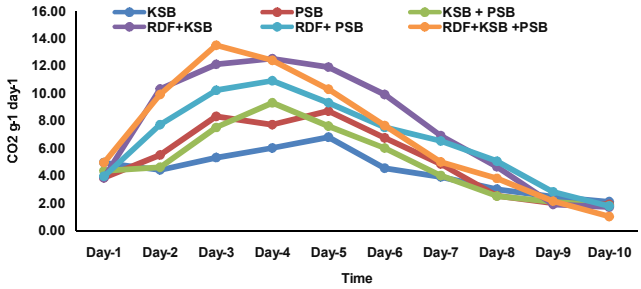

Fig. 3. Respiration study of different solubilizers effect

rate of respiration represents the more activity of microbes intern the rapid mineralization of nutrients (Jacoby et al 2017). The application of RDF showed more rapid respiration in both organisms indicating the significance of bio-fertilizers instead of using chemical fertilizers for the cultivation of crops. The study describes KSB as having a quicker mineralization capacity than PSB.

CONCLUSION

Application of 75% of recommended dose of fertilizer (RDF) with KSB and PSB had saved 25% chemical fertilizer compared to 100% RDF which may increase the annual income by improving the growth parameters, yield, and essential oil content of davana by the symbiotic bacterial effect. The symbiotic bacterial effect increases the crop productivity by the solubilization ability which reduce the fertilizer utility up to 25% might be valuable to economic and environmental viewpoint while using biofertilizers along with chemical fertilizer.

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