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RESEARCH ARTICLE

INFLUENCE OF CAP-34 AND *Pseudomonas putida* strain P1F ON GERMINATION AND GROWTH ENHANCEMENT IN *Pisum sativum*

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ABSTRACT

This study evaluated the growth-promoting efficiency of CAP-34 and *Pseudomonas putida* strain P1f on seed germination and overall performance of *Pisum sativum*, an economically important crop in India. CAP-34 is a 34 kDa basic protein purified from *Clerodendrum aculeatum* leaves known to induce resistance in plants, whereas *P. putida* P1f is a plant growth–promoting rhizobacterium (PGPR) isolated from the rhizosphere of healthy plants. PGPR enhance plant growth directly through auxin production, phosphate solubilization, and siderophore secretion, and indirectly by inducing systemic resistance and suppressing pathogens. Seeds treated with CAP-34 showed the highest germination percentage (76.63%), followed by P1f (68.88%) and control (66.66%). However, the vigour index was highest in P1f-treated seeds (1183), compared to CAP-34 (1113.87) and control (837.91), indicating stronger seedling development under P1f treatment. For growth assessment, six treatment combinations involving foliar spray and root inoculation were evaluated for plant height, flower number, fruit number, fresh weight of root and shoot, and fruit weight after three months. The combined treatment of CAP-34 (foliar) and P1f (root) resulted in maximum plant height, while root inoculation with P1f alone produced the highest fruit number. Foliar application of P1f enhanced flowering, CAP-34 foliar treatment increased root fresh weight, and dual application of P1f (foliar + root) yielded the highest shoot fresh weight. ANOVA showed significant differences for plant height ($P = 0.026$) and fresh weight ($P = 0.006$). Overall, both agents effectively promoted growth from germination to fruit yield, with treatment-specific advantages depending on application method.

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INTRODUCTION

The pea plant, *Pisum 6sativum*, is an important leguminous crop cultivated worldwide for its nutritional value, soil-enriching properties, and economic significance. As a rich source of proteins, vitamins, and minerals, pea contributes substantially to global food and nutritional security. Being a legume, it enhances soil fertility through symbiotic nitrogen fixation in association with Rhizobium spp., thereby reducing dependence on synthetic nitrogen fertilizers (Ćujić *et al.*, 2025.). In sustainable agriculture, plant growth–promoting rhizobacteria (PGPR) have emerged as eco-friendly alternatives to chemical fertilizers and growth regulators. Among these beneficial microbes, *Pseudomonas putida* is well documented for its plant growth–promoting properties. It enhances crop performance through mechanisms such as indole-3-acetic acid (IAA) production, phosphate solubilization, siderophore secretion, ACC deaminase activity, and induced systemic resistance (Pérez-Montaño *et al.*, 2025.). Several studies have demonstrated that *P. putida* strains significantly improve seed germination, root elongation, biomass accumulation, flowering, and yield parameters in legumes and other crops (Kloepper *et al.*, 2004; Vessey, 2003,Raupach *et al.*, 1996; Zehnder *et al.*, 2000).

Seed germination percentage and vigour index are widely accepted indicators of seed quality and early seedling establishment. The vigour index, calculated as the product of germination percentage and seedling length, provides an integrated assessment of physiological seed performance (Abdul-Baki& Anderson, 1973). Improved seed vigour is directly associated with better field establishment, enhanced vegetative growth, and higher productivity. In addition to rhizobacterial inoculation, foliar application of microbial formulations has gained attention as an effective strategy to promote plant growth and improve nutrient uptake efficiency. Combined application methods, such as foliar spray along with root inoculation, may result in synergistic effects, leading to enhanced morphological and yield parameters. However, comparative studies evaluating different modes of application and their relative efficiency remain limited. CAP-34, a microbial growth-promoting formulation, has shown potential in enhancing plant development (Verma *et al.*, 1996; Srivastava *et al.*, 2015), yet its comparative efficiency with *Pseudomonas putida* strain P1f under different application strategies has not been extensively studied in pea. Morphological parameters such as plant height, number of flowers, fruit number, and fresh weight of root and shoot are reliable indicators of plant performance and productivity under various treatments. Therefore, the present study was undertaken to comparatively evaluate the efficiency of CAP-34 and *Pseudomonas putida* strain P1f on seed germination, vigour index, and growth promotion of *Pisum sativum*. The study further assesses the influence of foliar and root inoculation treatments on key morphological and yield attributes, aiming to contribute toward the development of sustainable microbial-based strategies for enhancing pea productivity.

MATERIALS AND METHODS

Effect of CAP-34 and *P. Putida* strain P1f treatments on *Pisum sativum* seeds: *Pisum sativum* seeds were soaked for 5 minutes in 0.2 % (w/v) $HgCl_2$ for surface sterilization. Sterilized seeds were rinsed 2-3 times in sterile DW and then soaked for 30 minutes in a suspension of 24 hours old broth culture of *Pseudomonas putida* strain P1f or purified CAP-34 (20µg/ml).Seeds were sown in the pot and leave for 2 days. The% germination of seeds were analysed by the following formula by counting the number of seeds sown and number of seeds germinated.

% germination = Seeds germinated/ total seeds ×100

The seeded pots were left for seven days and observation done for growth parameters such as length of root and shoot after 7day of germination. After the germination of seeds, the seedlings were transferred to the clay pots for further development. The vigour index calculated by the following formula-

Vigour index= (mean root length + mean shoot length) × % germination.

Effect of different treatment ofCAP-34 and *P. putida* strain P1f strain on Pea plant growth: Total basic protein fraction from *C. aculeatum* leaf extract, which majorly consisted of CAP-34 and 24 h. old broth culture of*Pseudomonas putida* strain P1f treated the Pea plants at leaf, root and on both root and leaf. At 3-4 leaf stage pea plants, contain 10 plants in a set were treated with CAP-34 as foliar spray, P1f strain treated in root,P1f strain sprayed on leaf and inoculated in root, P1fstrain sprayed only on leaf, and CAP-34 sprayed on leaf and P1f strain inoculated in root for the enhancement of plant growth. Total six set of plants treated with differently with CAP-34 and P1f strain. The treatment was repeated thrice at a 3 day interval and plant growth was measured after different time intervals. The control set of plants were sprayed with DW only.

Statistical analysis: Data were analyzed using one-way analysis of variance (ANOVA), using IBM SPSS Statistics version 20. Statistical significance was assessed at a 95% confidence level ($P< 0.05$). Growth promotion data were further analyzed using the Least Significant Difference (LSD) multiple comparison test, with differences considered statistically significant at $P \leq 0.05$.

RESULTS

The comparative efficiency of CAP-34 and *Pseudomonas putida*s train P1f treatment on *Pisum sativum* seeds were evaluated for seed germination. The % germination of CAP-34 is 76.63% higher than P1f strain (68.88%) and DW (66.66%).The vigour index calculated by the formula given above and result of vigour index of *P. putida* strain P1f treated seed is 1183, is better than CAP-34 treated seeds (1113.87) and control treated with DW seeds had 837.91 vigour index. (Table 1, Fig.1).

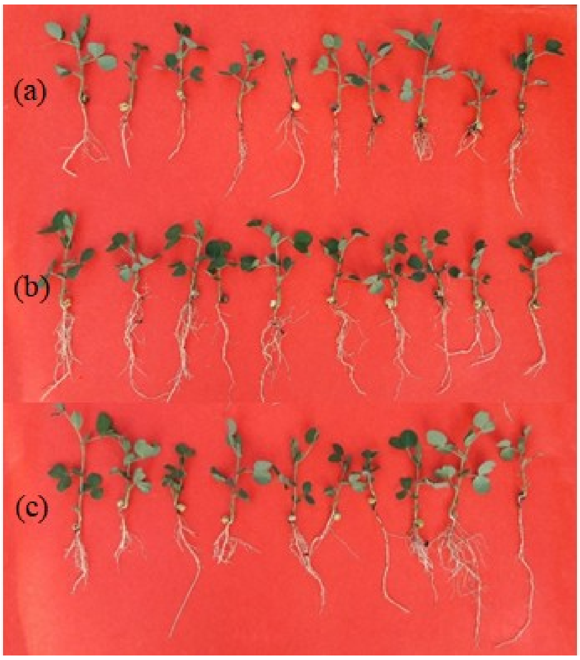

Fig. 1. Germination of seeds after treatment with DW, CAP-34 and P1f. Seeds were treated with DW (a), CAP-34 (b) and *Pseudomonas putida* strain P1f (c). Enhanced growth in CAP-34 and P1f treated seeds can be observed, compared to DW treated set of seeds

Table 1. % germination and vigour index of treated seeds of Pea with DW, *P.putida* strain P1f and CAP-34

Treatment	% germination	Vigour index
Control (DW)	66.66 ± 1.92^{a}	837.91^{a}
P. putida strain P1f	68.88 ± 1.11^{a}	1183^{b}
CAP-34	76.63 ± 1.92 ^{c}	1113.87^{c}

The effect of different treatments on growth and yield parameters of *Pisum sativum* is presented in Fig. 2. Significant variations were observed among treatments for plant height, flowering, fruiting, and biomass accumulation. Plant height was highest in plants treated with CAP-34 (foliar) + P1f (root-dip) (51.8 ± 2.51 cm), followed closely by CAP-34 (foliar) and P1f (foliar), both recording 51.1 cm. The lowest plant height was observed in control plants treated with distilled water (42.4 ± 2.61 cm).Flower production was maximum in P1f (foliar) treatment (1.8 ± 0.29), followed by CAP-34 (foliar) and CAP-34 (foliar) + P1f (root-dip) (1.7 ± 0.26). Control and P1f (root-dip) treatments recorded the lowest flower numbers (1.2).Fruit number was highest in P1f (root-dip) treatment (2.5 ± 0.26), followed by P1f (foliar + root-dip) (2.3 ± 0.39). Control plants produced the lowest fruit number (1.7 ± 0.21).Fresh root weight was markedly higher in CAP-34 (foliar) treatment (3.16 ± 1.76 g), whereas the control showed the lowest root biomass (0.55 ± 0.03 g). In contrast, the highest shoot fresh weight was recorded in P1f (foliar + root-dip) treatment (8.67 ± 0.53 g), substantially higher than control (5.6 ± 0.58 g).Fruit weight was maximum in P1f (root-dip) treatment (4.7 ± 0.27 g), followed by P1f (foliar) (4.62 ± 0.25 g), while control plants showed the lowest fruit weight

Table 2. Effect of different treatments on plant growth and yield parameters

S.N.	Treatment	Height(mean)	Flower	Fruit	Fresh weight of Root	Fresh weight of Shoot	Fruit weight
1.	DW	42.4 ±2.61	1.2±0.24	1.7±0.21	0.55±0.03	5.6±0.58	3.8±0.51
2.	CAP-34 (foliar)	51.1±1.31	1.7±0.26	2.2±0.2	3.16±1.76	5.9±0.37	4.41±0.29
3.	P1f (foliar)	51.1±2.21	1.8±0.29	2±0.14	1.99±0.26	5.72±0.37	4.62±0.25
4.	CAP-34 (foliar) + P1f (root-dip)	51.8±2.51	1.7±0.26	2.2±0.2	1.29±0.12	6.05±0.57	4.52±0.15
5.	P1f (root-dip)	47.1±2.25	1.2±0.2	2.5±0.26	1.11±0.11	6.07±1.03	4.7±0.27
6.	P1f (foliar + root-dip)	50.4±1.5	1.6±0.26	2.3±0.39	1.42±0.1	8.67±0.53	4.54±0.35

(3.8 ± 0.51 g). Overall, microbial treatments significantly enhanced vegetative growth and yield parameters compared to control, with treatment-specific effects observed for different growth attributes. Six sets of plants, with ten plants in each set, were treated with DW, CAP-34 on leaf, P1f on leaf, CAP-34 on leaf and P1f strain in root, P1f in root and P1f strain in root and leaf. DW-treated plants served as a control and all treatments were administered thrice, at an interval of three days each. The plant height was measured at the end of three months and the data analysed statistically. $P \leq 0.05$ value calculated through the ANOVA.

Fig. 2. Effect of different treatment ofCAP-34 and *Pseudomonas putida* strain P1fon growth promotion of Pea plants, (1) Control treated with DW, (2) treatment of CAP-34 on leaf (3) P1f strain on leaf (4) treatment of CAP-34 on leaf and root inoculation with strain P1f, (5)Strain P1f inoculated to root (6) treatment of P1f on leaf and root also

DISCUSSION

The present investigation demonstrates that both CAP-34 and *Pseudomonas putida* strain P1f significantly enhanced seed germination, vigour index, and growth attributes of *Pisum sativum*, although their effects varied depending on the parameter measured and mode of application. CAP-34 treatment resulted in the highest germination percentage, indicating its positive role in early seed metabolic activation. Improved germination in microbial-treated seeds has been widely attributed to phytohormone production (IAA, gibberellins), enhanced enzymatic activity, and better nutrient mobilization (Abdul-Baki& Anderson, 1973). Although P1f showed slightly lower germination percentage, it recorded the highest vigour index, suggesting superior seedling growth. Increased vigour index reflects enhanced root and shoot elongation, which may be linked to ACC deaminase activity, siderophore production, and phosphate solubilization by *Pseudomonas* spp. (Lugtenberg & Kamilova, 2009; Ahmad *et al*., 2008). Improved early seedling growth often results in better field establishment and long-term productivity (Vessey, 2003). Morphological observations revealed treatment-specific responses. The combined application of CAP-34 (foliar) and P1f (root inoculation) produced maximum plant height, suggesting synergistic interaction between foliar-mediated physiological stimulation and rhizospheric colonization. Root inoculation with P1f resulted in the highest fruit number, highlighting the importance of rhizosphere competence in reproductive development. Previous studies have shown that PGPR-mediated hormonal modulation enhances flowering and fruiting through improved nutrient uptake and auxin–cytokinin balance (Kloepper *et al*., 2004; Hayat *et al*., 2010). Foliar application of P1f enhanced flower number, indicating possible systemic signaling or metabolite-mediated responses. CAP-34 foliar treatment significantly improved root fresh weight, which may be associated with induced systemic effects promoting root proliferation. The highest shoot fresh weight under dual P1f application (foliar + root) suggests that multi-site inoculation enhances colonization efficiency and metabolic stimulation. Similar improvements in biomass and yield attributes due to PGPR application have been reported in legumes and other crops (Bhattacharyya &Jha, 2012; Lucy *et al*., 2004). Statistical analysis confirmed significant differences among treatments for plant height ($P = 0.026$) and fresh weight ($P = 0.006$), reinforcing the biological significance of microbial inoculation. Overall, both CAP-34 and *P. putida* strain P1f positively influenced pea growth, with P1f showing stronger effects on seedling vigour and yield-related traits, while CAP-34 effectively enhanced germination and root biomass. These findings support the integration of microbial bioinoculants into sustainable agricultural practices to improve productivity while reducing chemical inputs (Backer *et al*., 2018; Babalola, 2010).

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Declarations

Conflict of interest the authors declare that they have no conflict of interest in the publication.

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