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Bio-priming with enhanced soaking agents: a precision approach to augment seed emergence and yield in Ajwain (*Trachyspermum ammi*)

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Abstract

Bio-priming, a novel seed treatment technique, has garnered attention for its capacity to enhance seed germination, crop growth, and yield. This study aimed to assess the efficacy of *Trichoderma* (commercial strains) and *Bacillus* and *Pseudomonas* (newly isolated strains) as sole inoculants and co-inoculants with soaking agents during seed bio-priming to enhance seed germination and initial seedling growth of ajwain. A randomized block design (RBD) was employed, comprising seven treatments replicated three times each. The highest seed yield (2510.247 kgha^{-1}) and associated attributes were observed in treatment T5 (Chitosan + *Trichoderma viride*) compared to the control (2068.51 kg ha^{-1}). Treatment T3 (Chitosan + *Bacillus paramycoides + Pseudomonas aeruginosa*) improved all parameters, including final germination, shoot length, root length, plant height, primary and secondary branches, and test weight, compared to the control. The correlation analysis revealed positive associations between yield and plant height, umbellateumbelkgha^{-1}, seed emergence, secondary branch, and umbelplantkgha^{-1}. Consequently, *Trichoderma* strains followed by *Bacillus paramycoides + Pseudomonas aeruginosa* combined with chitosan exhibit potential as both single inoculants and co-inoculants for ajwain bio-priming, pending further validation through farmer field evaluations.

Keywords: bio-priming, ajwain, soaking agent, seed and seedling quality, yield.

Introduction

In the evolving landscape of seed treatment, the innovative practice of seed priming has garnered considerable attention, finding widespread use for purposes ranging from optimizing germination to fostering robust plant growth. This paper focuses on the intricacies of biopriming, a distinctive subset of seed priming that utilizes biological compounds for seed hydration, creating an ideal environment for bacterial inoculation and colonization. Positioned as a pivotal facet of biological seed treatment, biopriming integrates seed hydration with the introduction of beneficial microorganisms, promoting environmentally sustainable practices by enhancing nutrient uptake, generating growth-promoting substances, and providing protection against pathogens (Carrozzi *et al*., 2012; Chakraborty *et al*., 2011; Gholami *et al*., 2009).

Plant growth-promoting rhizobacteria (PGPR) is one of the microbial cultures that, when combined with other strains such as *Trichoderma* and *Vesicular Arbuscular Mycorrhiza* (VAM), can function in concert to increase the germination and growth of seed spice crops. The advantages of microbial treatments are widely established; yet, there is a noteworthy lack of information regarding the biopriming of seed spice crops, especially in India. Thus, the goal of the current study is to add to the body of knowledge by examining the impact of specific PGPR strains on biopriming. The main goal is to create a method for biopriming spice seeds in order to close the current knowledge gap and possibly provide long-term solutions for better seedling growth and germination in these crops (Ashraf and Foolad, 2005; Bennett *et al*., 2009).

Soaking agents play a crucial role in biopriming because they act as a bioplastic, increasing bio efficacy and making bio inoculate sticky. The deacetylation of chitin from crustacean waste produces chitosan. Chitosan is ecologically beneficial since it can be spontaneously destroyed by the environment and contains an aminegroup, which gives it antibacterial qualities that are useful as a bioplastic manufacturing ingredient. Carrageenan increases ionic and chemical cross-linking, which improves the mechanical characteristics of bioplastics (Favian and Nugraheni, 2023).

The Apiaceae family of seed spices, which are known for their tiny seeds, have difficulties with delayed germination and uneven seedling growth, which are especially noticeable in crops such as ajwain. These problems are a major barrier to the best possible crop establishment, particularly in cases where weather-related delays cause sowing to be postponed. In light of these conditions, it is posited that ajwain seeds may experience a bio-priming impact upon the application of advantageous bacteria. In order to solve the issues related to delayed germination and ensure more uniform growth in these seed spices, it is expected that this postulated bio-priming effect will result in early seed germination. The hypothesis of this paper to discern the most effective seed priming treatment among several priming treatments, which provide early and maximum germination, prosperous growth, and prodigious yield.

Materials and Methods

Treatment details

The field experiment was executed at Indian Council of Agricultural Research- National Research Centre on Seed Spices (ICAR-NRCSS), India, spanning the Rabi season of 2022-2023. Building upon the promising outcomes derived from the laboratory experimentation, two bacterial consortia coupled with specific soaking agents, which demonstrated notable enhancement in seedling growth, were selected for further validation under field conditions. To this end, a designed randomized block design (RBD) was implemented, comprising seven distinct treatments replicated threetimes each. These treatments included T1 (Chitosan + *Pseudomonas fluorescens* + *Pseudomonas aeruginosa*), T2 (Carrageenan + *Pseudomonas fluorescens* + *Pseudomonas aeruginosa*), T3 (Chitosan + *Bacillus paramycoides* + *Pseudomonas aeruginosa*), T4 (Carrageenan + *Bacillus paramycoides* + *Pseudomonas aeruginosa*), T5 (Chitosan + *Trichoderma viride*), T6 (Compost extract + *Trichoderma viride*) and T7 (Control), selected to elucidate the nuanced impact of each treatment on seedling growth and vigor within the field setting. Compost was extracted from the field and ready for the 100 parts per millilitre seed biopriming solution. Comparably, 500 ppm of chitosan and 100 ppm of caragennen were utilised.

Field experimental site

A two-year laboratory experiment (examined for germination indices) was carried out (2020-2022), after promising strain selected carried out in two-year (2021-2023) field experiment (examined for yield and growth indices) at ICAR-NRCSS, Ajmer, Rajasthan, India (74° 35' 39" E to 74° 36' 01" E longitude and 26° 22' 12" N to 26° 22' 31" N latitude at an altitude of 460.17 m above mean sea level). The soil was sandy loam with EC of 0.31 dSm^{-1} and a pH of 8.00. Initial soil properties of the study site were: 0.31% soil organic carbon, 156 kg ha^{-1} available N, 10.43 kg ha^{-1} available P and 267.34 kg ha^{-1} available K.

Field observation

For the evaluation of growth parameters in ajwain seedlings, emergence at 12 days after sowing, as well as root and shoot lengths at 30 days after sowing, were meticulously quantified using a precision measuring scale. Additionally, the ajwain seed yield, expressed in kilograms per hectare (kg ha^{-1}), along with its associated attributes, was systematically measured.

Statistical Analysis

The field research was carried out for validation using seven treatments organised in a randomised block design (RBD) with three replications. If the f-value was significant, the least significant difference (LSD) test with a significance threshold of $p < 0.05$ was used to compare the means. The data were statistically processed by R 4.1.2 (Developer: Posit and Joseph J. Allaire & organization located in Vienna, Austria).

Result and discussion

Yield and their attributes

The results of this extensive investigation show that the microbial consortium × soaking agent interaction has a significant impact on ajwain seed yield and related characteristics, which are carefully described in Table 1. The investigation also revealed a significant interaction impact between soaking agent treatments and microbial consortia, emphasizing the beneficial role of soaking agents in augmenting microbial consortia's efficacy on seed yield and yield components. In comparison to treatment T7 (control), significantly higher values were seen in seed yield and yield components, such as seed umbellate^{-1}, umbelplant^{-1}, and umbellate umbel^{-1}, in all treatments. Even though there were no appreciable variations in the number of seeds per umbellate or umbellate per umbel between T1, T2, T3, T4, T5, and T6, the sum of these findings highlights the usefulness of soaking agents in conjunction with microbial consortia to increase ajwain seed yield. Finally, the highest seed yield of 2510.247 kg ha^{-1} was obtained by using treatment T5 (Chitosan + *Trichoderma viride*) in the best possible combination. This is a significant percentage increase of 21.37% over treatment T7 (2068.51 kg ha^{-1}).Co-inoculation, frequently, increased growth and yield, compared to single inoculation, provided the plants with more balanced nutrition, and improved absorption of nitrogen, phosphorus, and mineral nutrients (Bashan, 1998).In comparison to uninoculated control plants, rhizobia strains and *Trichoderma* were able to enhance total dry matter, nitrogen fixation, weight of seed, weight of seeds per plant, number of pods per plant, number of seeds per pod, seed yield, and total dry matter (Ahmad *et al.*, 2008).

Growth attributes

No statistically significant interaction was discerned between microbial consortia and soaking agent treatments concerning the test weight, primary branch, secondary branch, seedemergenceofajwain, as depicted in Table 2. The results consistently revealed that seed biopriming with a soaking agent enhances seedling emergence compared to the control treatment. Particularly noteworthy is treatment T4, which exhibited the highest Plant height at harvest was statistically at par with treatments T2, T3 and T5 except the T1, T6 and control (T7). Impressively, treatment T4 recorded a remarkable 112.0 cmplant height at harvest, reflecting a 44.31% increment compared to the control (77.61 cm). Notably, the data underscore that seed emergence is more robust with microbial consortia combined with chitosan exhibiting superior effectiveness compared to carrageenan.The pivotal role of priming agents, particularly biostimulants, in augmenting seed germination underscores their significance in advancing food security and sustainability objectives. Timely and uniform germination, facilitated by such agents, contributes significantly to seedling vigour and subsequent developmental processes in numerous plant species valued for their nutritional and medicinal properties (Makhaye *et al.*, 2021).

Table 1: Mean seed yield and their attributes of ajwain affected by microbial inoculants + soaking agent. Means with the same letter are not significantly different according to Tukey test at P = 0.05

Treatment	Yield	Seed umbellate^{-1}	Umbel plant^{-1}	Umbellate umbel^{-1}
T1	2389.136 a	16.333 bc	139.111 ab	18.889 a
T2	2386.667 a	17.111 ab	148.889 a	17 ab
T3	2272.593 a	17.667 a	135.222 ab	17.222 ab
T4	2329.259 a	18 a	163.889 a	17.778 ab
T5	2510.247 a	15.778 c	135 ab	17.778 ab
T6	2475.556 a	17.778 a	156.222 a	18 ab
T7	2068.519 b	16.444 bc	117.333 b	15.778 b
CV(%)	12.186	5.985	13.955	1.1
p-value	p<0.001	p<0.001	0.003	0.061

Table 2: Mean seed yield and their attributes of ajwain affected by microbial inoculants + soaking agent. Means (± SD) with the same letter are not significantly different according to Tukey test at P = 0.05

Treatment	Test weight	Primary branch	Secondary branch	Seed emergence	Plant height at harvest
T1	2.568 a	15.111 a	65.444 a	80 a	109.167 b
T2	2.55 a	14.556 a	66 a	83.333 a	110.167 ab
T3	1.835 a	14.111 a	60 a	85 a	109.667 ab
T4	1.842 a	15.556 a	69 a	85 a	112.056 a
T5	2.08 a	14.778 a	62.222 a	85 a	110.611 ab
T6	1.979 a	14.667 a	67.778 a	80 a	109.389 b
T7	1.811 a	14.556 a	53.778 a	71.667 a	77.611 c
CV(%)	78.872	5.913	12.465	2.321	1.731
p-value	0.59	0.567	0.092	0.109	p<0.001

Root and shoot length

No statistically significant interaction was discerned between microbial consortia and soaking agent treatments concerning the shoot and root length of ajwain, as depicted in Figure 1. Treatment T3 exhibited the highest shoot length at 2.47 cm; however, this measure did not significantly differ from the other treatments. In contrast, the control treatment (T7) demonstrated a lowest shoot length of 2.18 cm, which did not significantly differ from the other treatments. Conversely, treatment T3 recorded the highest root length at 1.22 cm, yet this measure did not exhibit a significant difference from the other treatments. These findings elucidate the nuanced response of ajwain shoot and root length to microbial consortia and soaking agent treatments, with specific treatments manifesting notable effects on shoot and root development without significant differences among them.In addition to improving plant development and grain output of different crops, Pseudomonas and Bacillus quickly and aggressively colonise the root system and reduce a number of harmful bacteria (Dey *et al.*, 2004).

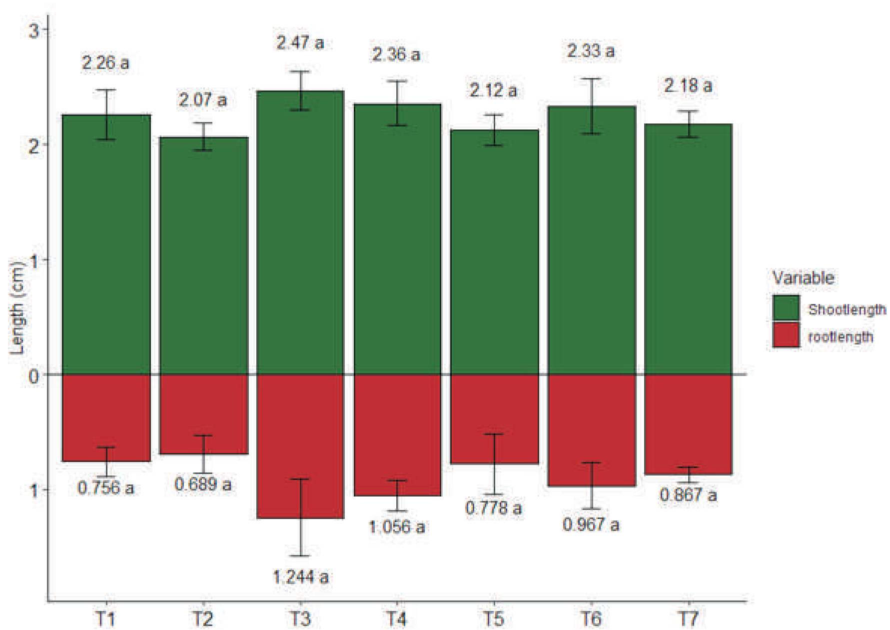

Figure 1. Interaction of root and shoot length of ajwain affected by microbial inoculants + soaking agent. Means with the same letter are not significantly different according to Tukey test.

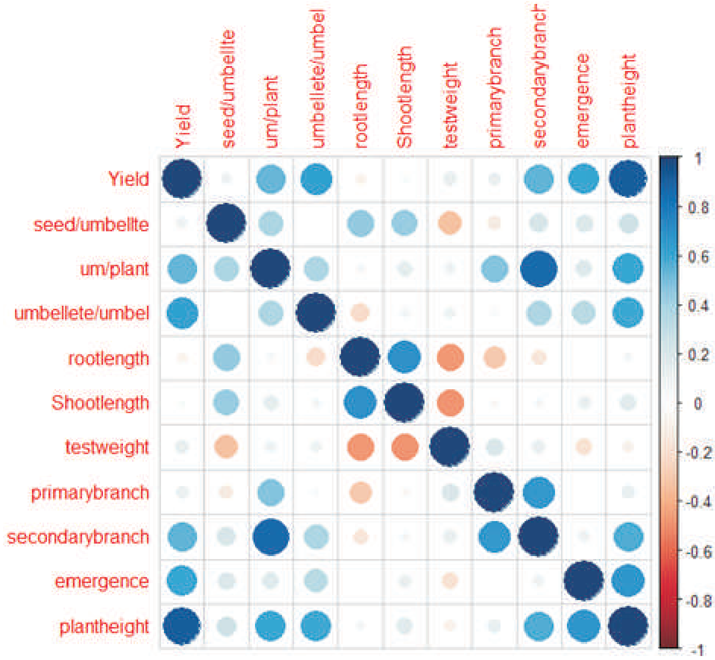

Figure 2: Pearson's correlation matrix analysis among yield and studied morphological parameters. Red circles indicate negative correlations, while blue are positive correlations. In the correlogram scale from - 1 to +1, Pearson's correlation coefficient for variables is on the vertical and horizontal axis. × indicates values that are not statistically different at $p < 0.01$.

Correlation matrix among Yield and Morphological Parameter

There were positive correlations between yield and plant height, umbellate umbel^{-1}, seed emergence, secondary branch and umbel plant^{-1} as shown in Figure 2. Negative and non-signi□cant differences were observed among tested yield and root and shoot length. The higher the yield value is, the greater the values obtained for plant height and umbellate umbel^{-1} as well as for umbel plant^{-1}.

Conclusion

Overall, *Bacillus, pseudomonas* and co-inoculation improved the most of the growth and yield contributing characters of ajwain compared to *Trichoderma*. The greatest improvement was obtained for the shoot length, root length, and seedling emergence in T3 (Chitosan + *Bacillus paramycoides + Pseudomonas aeruginosa*), followed by T4 (Carrageenan + *Bacillus paramycoides + Pseudomonas aeruginosa*). Bio-priming treatments led to the highest increase in seed yield and their attributes in T5 (Chitosan + *Trichoderma viride*), whereas the effect was lower in control. The present study represents the first experimental evidence of using, T5 (Chitosan + *Trichoderma viride*), for seed bio-priming of ajwain under different conditions of field tests. This technique can be recommended for priming seeds prior to field planting as an eco-friendly strategy to improve seed germination and initial seedling growth.

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of the data; in the writing of the manuscript, or in the decision to publish the results.

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