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Impact of Seed Coating with Prebiotics on the Physiological Performance and Soil Microbial Activity of Blackgram (*Vigna mungo* L.) during Storage

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ABSTRACT

Background: Soil microbial abundance is necessary for soil health, influencing plant growth and resilience. This study examines the effects of prebiotic seed coating on the physiological performance and soil microbial activity of blackgram (*Vigna mungo* L.) over six months of storage.

Methods: In storage studies, blackgram seeds were coated with a prebiotic formulation at two doses: 6 g/kg and 8 g/kg with uncoated seeds as control. The coated seeds were stored in cloth bag at ambient conditions and assessed monthly for germination percentage, seedling vigor index (SVI), root and shoot length, dry matter production and microbial activity in the spermosphere and rhizosphere, including dehydrogenase activity.

Result: Results revealed that seeds coated with prebiotics, particularly at the 8 g/kg dose maintained higher germination rates, vigor and microbial activity over six months compared to uncoated seeds. The 8 g/kg achieved the highest germination (91%) and SVI (3464) at the end of storage, along with increased microbial populations and enhanced dehydrogenase activity (17.93 µg TPF/g soil/day), suggesting sustained microbial and biochemical activity in the soil. The stability of the formulation was assessed by storing 6 months old prepared formulation. Newly prepared formulation performed better in terms of physiological attributes and soil microbial activity. The findings indicate that seed coating with prebiotics at higher doses significantly improve seed viability, growth parameters and soil microbial health over extended storage, demonstrating their potential as a sustainable approach to enhance plant productivity and soil quality.

Key words: Blackgram, Prebiotics, Seed coating, Stability, Storage.

INTRODUCTION

Blackgram (*Vigna mungo* L.) is the major pulse crop grown over 4.63 million hectares with a total (Agricultural Statistics Division, DES, MoAF and W, 2022) production of 2.78 million tonnes and a productivity rate of 600 kg per hectare. In Tamil Nadu, it is cultivated on 407,000 hectares, yielding a total production of 269,000 tonnes and a productivity of 660 kg per hectare (AICRP PC Report, 2023). Significant improvements in crop yields due to high-yielding crop varieties, innovative farming practices and the application of chemical fertilizers, has also sparked worries regarding its impact on environmental quality and soil health (Pramanik *et al.,* 2023) after Green revolution era.

Soil microbial diversity and abundance are key indicators of soil fertility. Enhancing the population of beneficial microorganisms in soil can significantly support plant growth and development (Wei *et al.,* 2024) through nutrients and carbon. Under biotic and abiotic stresses, microorganisms can stimulate the production of indole-3-acetic acid (IAA) and osmolytes such as glycine betaine and proline. Additionally, microorganisms' play a major role in stress tolerance by solubilizing mineral phosphates, fixing nitrogen, producing organic acids and synthesizing enzymes like ACC-deaminase, chitinase and glucanase, thereby enhancing plant growth (Nanda *et al.,* 2019). Plant growth-promoting rhizobia (PGPR) enhance nutrient uptake from the rhizosphere and produce beneficial substances. They indirectly protect plants by suppressing soil-borne pathogens and directly stimulate plant growth by producing growth regulators like gibberellins, auxins and cytokinin's (Nagargade *et al.,* 2018).

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In response, prebiotics are substances that enhance microbial diversity and soil health by fostering the growth of microorganisms that naturally exist within the soil-plant ecosystem. These are natural obtained from agro-industrial wastes such as sewage sludge, biochar, humus, animal manure and compost. These substances improve soil structure, enhance biochemical activity and boost microbial populations and diversity, particularly in degraded soils (Vassileva *et al.,* 2020; Alahmad *et al.,* 2023).

Seed coating is a process of seed enhancement technique which can deliver essential micro and macronutrients or bio stimulants, ultimately results in boosting germination, seedling vigour and establishment (Amirkhani *et al*., 2016; Shinde *et al.,* 2017). Film coating is the process of directly applying a thin layer of a beneficial coating agent to the seed coat's surface, often up to 5% of the seed weight (Pedrini *et al.,* 2020). Film seed coating mediated by chitosan and polyethylene glycol (PEG) was successful in promoting castor germination and vigorous plant growth (Chandrika *et al.,* 2024). Maize seeds coated with slow release nutrients led to better emergence potential and enhanced yield attributes (Dong *et al.,* 2016). With this background in mind, this research aims to evaluate the effects of prebiotic seed coating formulations on the physiological and soil microbiological properties of black gram over six months and to assess the stability of the formulation after storage.

Materials and Methods

The seed coating experiment was conducted on blackgram cultivar 'VBN 11' in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during January 2024 - July 2024. The initial quality of blackgram seeds was with 92% germination and 8% seed moisture content.

Seed coating procedure

Blackgram seeds were coated with Rotary Seed Coater. The seed coating formulation was prepared using various nutrient substances combined at appropriate ratio. The nutrient used was Murashige and Skoog media (17%), chitosan (3%), glycerol (34%), Beef extract (34%), gelatin (7%), Carboxy methyl cellulose (2.5%), potassium dihydrogen phosphate (1.25%) and magnesium sulphate (1.25%). These substances were mixed with white polymer. The coating formulation obtained was self-sticky nature. The seeds were coated with formulation at 2 different doses such as T_1: 6g kg^{-1} and T_2: 8g kg^{-1}. Uncoated seeds served as control (To). After coating, seeds were shade dried (25°C) for one h.

Experiment 1: Storage of coated seeds

Coated and uncoated seeds were stored for six months in cloth bags under storage conditions (temperature 30°C and 45% relative humidity). Then, stored seeds were sown under prescribed germination conditions (temperature 25°C and 65% relative humidity) recommended by ISTA to determine the potential of seeds at monthly intervals. The experiment was conducted in a completely randomized design with seven replications. During storage, seed germination, seedling vigour, spermosphere and rhizosphere microbial activity was recorded at monthly up to six months of storage. Germination percentage was determined by sand and soil method (1:1). Each treatment replicated four times. At the end of germination test, ten normal seedlings were randomly selected and the shoot and root length were measured in centimeters. Further, the seedlings were kept in an oven at 80±20°C for 72 h for measuring seedling dry weight. The seedling vigour index (SVI) was calculated as the product of the germination percentage and seedling length as suggested by Abdul-Baki and Anderson (1973).

One day after sowing, the seeds were carefully excavated from the germination tray and the soil attached to the seeds was carefully collected for spermosphere activity. Subsequently, at 7 days after sowing (DAS), the seedlings were removed from the trays, ensuring the roots remained intact. The soil adhered with the roots was then collected for rhizosphere activity. The bacterial and fungal population in both the spermosphere and rhizosphere were determined using serial dilution and plating methods (Parkinson *et al.,* 1971). Additionally, the dehydrogenase enzyme activity in the rhizosphere soil was measured following the procedure outlined by Casida *et al*. (1964).

Experiment 2: Evaluation of formulation stability

In a parallel experiment, the same seed coating formulation was prepared and stored for six months at refrigerated conditions (5°C). After this storage period, a fresh batch of blackgram seeds was coated with the stored formulation, using the same doses (T_1: 6 g kg^{-1} and T_2: 8 g kg^{-1}) as in the first experiment. Freshly prepared formulation was also coated on blackgram seeds at 2 doses (T_3: 6 g kg^{-1} and T_4: 8 g kg^{-1}) and T_0 serves as control (uncoated seeds). Observations on seed germination, seedling vigor and spermosphere and rhizosphere microbial activity and dehydrogenase activity were recorded with the same methodologies as described earlier.

Statistical analysis

The laboratory experiment was laid out in a completely randomized block design (CRD). All data were analyzed using GRAPES software. ANOVA was performed at $P<0.05$ and treatment means were compared with Duncan's multiple range test (DMRT). Graphical analyses of seedling growth, soil microbial activity, enzyme levels were carried out using Origin software.

Results and Discussion

Coated seeds maintain their properties during long-term storage. Seed coatings deliver essential substances like microorganisms, nutrients and growth regulators, while also containing protective chemicals, reducing the need for spraying or fertilizing (Torre-Roche *et al*., 2020). Seed

coating with prebiotics produced significant effect evaluated in the laboratory in all physiological and microbial attributes irrespective of the treatment dosage. Over the 6-month storage period, the speed of germination showed a gradual decline in all treatments. The initial values at 0 month after storage (MAS) indicated 8 g kg^{-1} had the highest germination speed (8.23) compared to control (7.40). After 6 MAS, 8 g kg^{-1} maintained a relatively higher speed of germination (6.95) over control (6.20). Germination percentage also decreased over time, with 8 g kg^{-1} maintaining higher germination rates (91%) at 6 MAS over control (85%). The prebiotic coating at 8 g kg^{-1} dose helped in preserving a higher germination rate throughout the storage period (Fig 1). Over time, both root and shoot lengths declined across treatments, with 8g kg^{-1} consistently having higher root lengths than 6 g kg^{-1} and control. At 6 MAS, 8 g kg^{-1} exhibited a root length of 12.33 cm, followed by 6 g kg^{-1} (12.36 cm) and control (11.90 cm). The shoot length for 8 g kg^{-1} remained slightly higher than the other treatments at each MAS, with a final value of 17.1 cm compared to 17.4 cm in 6g kg^{-1} and 16.7 cm in control at 6 MAS (Fig 2). There was a decrease in dry matter production for all treatments over the storage period. Initially, 8 g kg^{-1} showed the highest dry matter production (0.403 g/10 seedlings), while at 6 MAS, the values declined, with 8 g kg^{-1} at 0.196 g/10 seedlings compared to 6 g kg^{-1} (0.193 g/10 seedlings) and control (0.194 g/10 seedlings). Vigour index values also followed a similar trend, with 8 g kg^{-1} achieving the highest values initially and maintaining a slight edge at the end of the storage period (Table 1). At 0 MAS, 8g kg^{-1} showed the highest bacterial population in the spermosphere (77.0×10u CFU/g soil) and even after 6 MAS, the population remained relatively higher at 17.0 compared to T0 (11.0) (Fig 3). Similarly, the rhizosphere bacterial population was highest in 8 g kg^{-1} at 6 MAS. Prebiotic seed coating formulation at 8 g kg^{-1} had consistently resulted in fungal populations over control (Table 2). Increased microbial activity resulted in increased soil enzyme activity which has been proved in our study. Dehydrogenase activity, an indicator of soil microbial activity, was initially highest in 8 g kg^{-1} (24.56 µg TPF/g soil/day) and remained significantly elevated across the storage period. At 6 MAS, 8 g kg^{-1} recorded highest dehydrogenase activity (17.93 µg TPF/g soil/day) (Fig 4). Soil microorganisms play a vital role in improving seed germination by synthesizing plant growth-promoting hormones (Wu *et al.,* 2016). They improve soil structure by

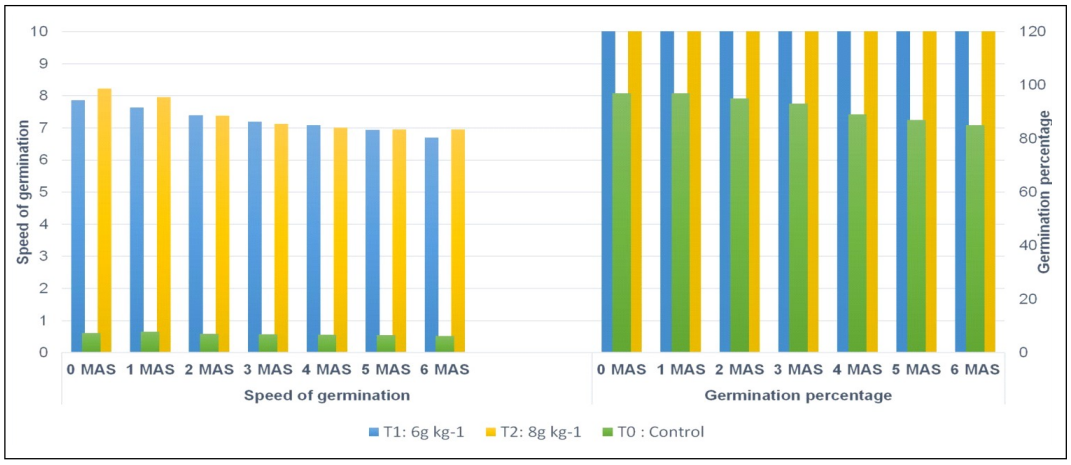


Fig 1: Influence of seed coating with prebiotics on speed of germination and germination percentage of blackgram during storage.

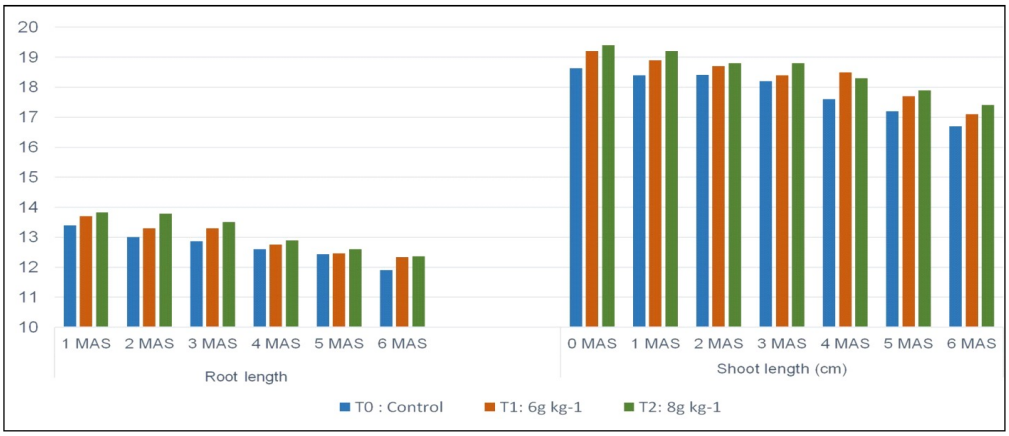


Fig 2: Influence of seed coating with prebiotics on root length and shoot length of blackgram seeds over a 6-month storage period.

Table 1: Influence of seed coating with prebiotics on dry matter production and seedling vigour index of blackgram during storage.

Treatments	Dry matter production (g 10 seedlings^{-1})							Seedling Vigour index						
	Month after storage							Month after storage						
	0	1	2	3	4	5	6	0	1	2	3	4	5	6
T_0 : Control	0.343	0.239	0.213	0.210	0.210	0.190	0.194	3155	3103	2976	2897	2695	2572	2412
T_1: 6 g kg^{-1}	0.353	0.354	0.227	0.213	0.214	0.204	0.193	3239	3172	3030	2963	2880	2732	2657
T_2: 8 g kg^{-1}	0.403	0.365	0.250	0.233	0.223	0.194	0.196	3300	3254	3093	2970	2880	2771	2665
Mean	0.366	0.319	0.230	0.219	0.216	0.196	0.194	3231	3176	3033	2943	2818	2692	2578
	T			M		T × M		T			M		T × M	
SE (d)	0.005			0.008		0.014		36.85			56.29		97.50	
CD (P=0.05)	0.010*			0.016*		NS		74.37*			113.60*		NS	

Significant at P≤0.05; NS - Non significant at P>0.05.

Table 2: Influence of seed coating with prebiotics on rhizosphere microbial population during storage.

Treatments	Rhizosphere bacterial population (10^5 cfu / g of soil)							Rhizosphere fungal population (10^3 cfu / g of soil)						
	Months after storage							Months after storage						
	0	1	2	3	4	5	6	0	1	2	3	4	5	6
T_0 : Control	31.0	24.0	24.5	24.0	15.5	26.0	15.5	18.5	16.0	19.5	13.5	16.0	9.0	10.5
T_1: 6g kg-1	68.5	62.5	28.0	36.5	25.5	26.5	15.0	27.0	22.5	18.5	13.5	19.5	11.0	9.0
T_2: 8g kg-1	62.0	58.5	31.5	36.0	23	24.0	16.0	30.5	25.5	20.5	19.5	19.0	13	10.0
Mean	53.8	48.0	28.0	32.2	21.3	25.5	15.5	25.3	21.33	19.5	15.5	18.17	11.0	10.0
	T			M		T × M		T			M		T × M	
SE (d)	1.192			1.821		3.155		0.800			1.222		2.116	
CD (P=0.05)	2.480*			3.788*		6.561*		1.663*			2.540*		4.400*	

*Significant at P ≤0.05; NS - Non significant at P>0.05.

reducing compaction, thereby improving porosity and aeration, water permeability, creating optimal conditions for root growth and for efficient uptake of water and nutrients. Indigenous microbial strains enhance germination rates, increasing plant biomass accumulation, seed yield, resilience to abiotic stress in various agricultural crops (Rocha *et al*., 2019). During seed germination, seeds emit exudates into the spermosphere, promoting microbial proliferation, which is crucial for the successful germination of various crop seeds (Nelson, 2018). Rhizosphere microorganisms are crucial in regulating and producing plant hormones, greatly influencing growth and development of plants. Bacterial IAA (Indole 3 acetic acid) specifically enhances root growth by promoting cell elongation and division. Gibberellin initiates stem elongation and further plant growth. Ethylene helps in root hair formation and seed germination (Glick, 2012).

In the parallel study on stability of formulation, at a higher dose of newly prepared formulation (8 g kg^{-1}) significantly enhanced several growth parameters compared to the control and stored formulations. Germination rate improved slightly, with newly prepared formulation (8 g kg^{-1}) achieving the highest germination (98 %) and the highest seedling vigour index (3464). Root and shoot lengths were also most pronounced in newly prepared formulation (8 g kg^{-1}) showing 15.96 cm and 19.13 cm, respectively, with corresponding improvements in dry matter production (0.403 g/10 seedlings) (Table 3).

Additionally, microbial analysis revealed that newly prepared formulation (8 g kg^{-1}) treatment had the highest bacterial and fungal populations in both the spermosphere and rhizosphere, especially at higher dilutions, along with a peak in dehydrogenase activity (25.10 µg TPF/g soil/day), suggesting enhanced microbial and biochemical activity

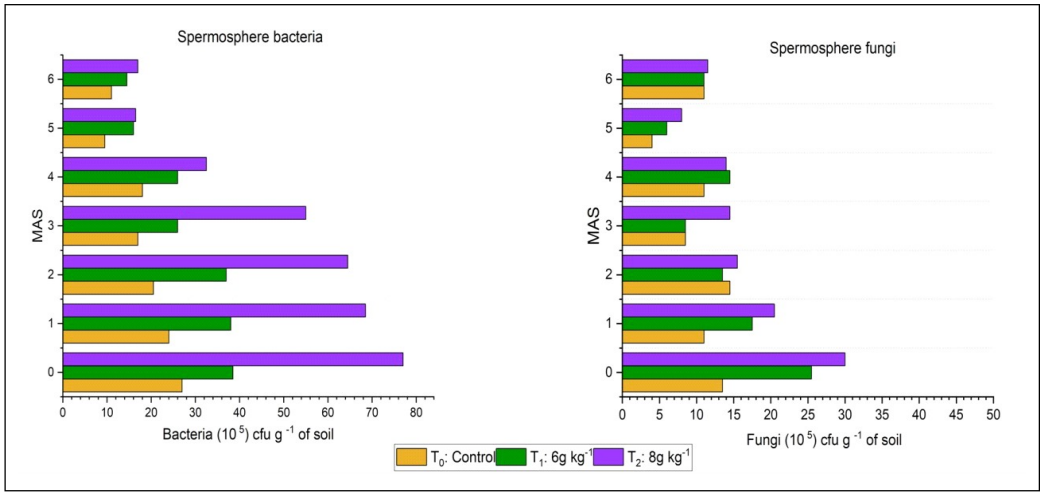


Fig 3: Influence of seed coating with prebiotics on spermosphere microbial population during storage.

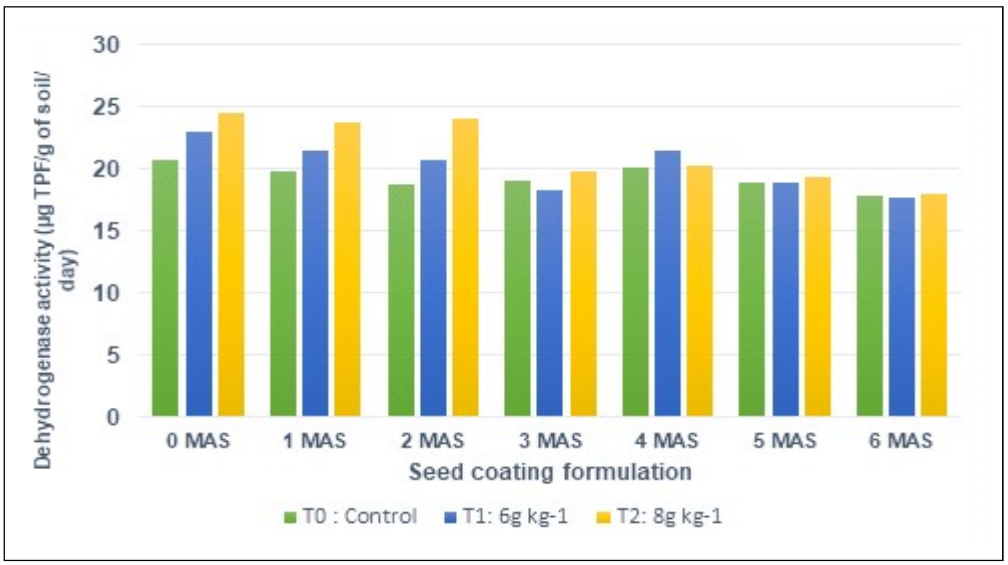


Fig 4: Influence of seed coating with prebiotics on soil dehydrogenase activity during storage.

Table 3: Comparative effect of newly prepared and 6-month stored formulation coated blackgram seeds on microbial activity and germination.

Treatments	Germination (%)	Speed of germination	Root length (cm)	Shoot length (cm)	Dry matter production (g/10 seedlings)	Seedling vigour index
T_0: Control	92	7.36	13.46	16.56	0.292	2761
T_1: (6 months stored formulation) 6 g kg^{-1}	96	7.35	13.92	18.06	0.368	3071
T_2: (6 months stored formulation) 8 g kg^{-1}	97	7.44	14.30	18.19	0.389	3085
T_3: (Freshly prepared formulation) 6 g kg^{-1}	97	7.38	14.32	18.53	0.404	3186
T_4: (Freshly prepared formulation) 8 g kg^{-1}	98	7.48	15.96	19.13	0.403	3464
SE (d)	3.676	0.176	0.977	0.558	0.030	158.35
CD	NS	NS	1.099*	1.243*	0.067*	352.84*

*Significant at P≤0.05; NS- Non significant at P>0.05.

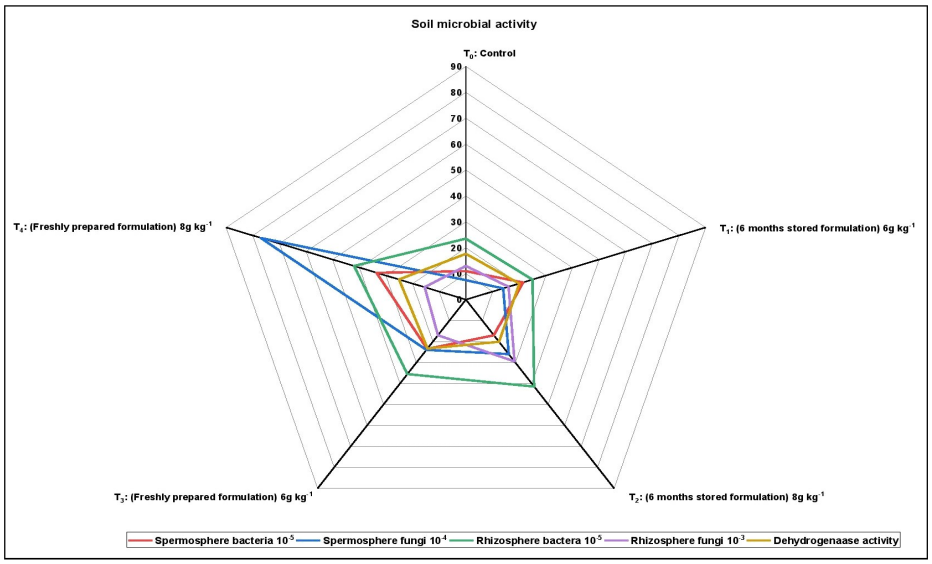


Fig 5: Comparative effect of newly prepared and 6-month stored formulation coated blackgram seeds on microbial activity and germination parameters.

in the soil (Fig 5). Prebiotics can reshape native soil microbial communities, enhancing bacterial and fungal diversity while, recruiting microorganisms with specialized ecological functions (Hellequin *et al*., 2020). Soil microbe's increases plant disease resistance by adjusting soil pH, enriching soil organic carbon and competing with pathogens for essential resources. Additionally, certain rhizosphere microbes fix atmospheric nitrogen, mobilize soil phosphorus and potassium and supply vital nutrients to plants (Wei *et al*., 2024). These findings collectively demonstrate that the newly prepared formulation, particularly at 8g kg^{-1} is more effective in promoting plant growth and microbial activity than the old formulation and untreated control.

The study showed that prebiotic seed coating, particularly at the 8 g kg^{-1} dose, in enhancing seed germination, growth parameters and microbial activity both initially and over a 6-month storage period. The sustained vigour, microbial diversity and soil enzyme activity suggest that this formulation can contribute significantly to improved plant health and productivity.

CONCLUSION

This study evaluates the impact of prebiotic seed coating on the physiological performance and soil microbial activity of blackgram (*Vigna mungo* L.), focusing on germination, vigor and microbial populations over six months. The results show that prebiotic-coated seeds, especially at

higher doses (8 g kg^{-1}), maintained superior growth parameters, microbial activity and enzyme levels compared to control seeds, supporting enhanced plant and soil health even after 6 months of storage.

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Disclaimers

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Abdul Baki, A.A. and Anderson, J.D. (1973). Vigor determination in soybean seed by multiple criteria. Crop Science. 13(6): 63.

Agricultural Statistics Division, DES, MoAF and W, (2022).

Alahmad, A., Edelman, L., Castel, L., Bernardon-Mery, A., Laval, K., Trinsoutrot-Gattin, I. and Thioye, B. (2023). Prebiotics: a solution for improving plant growth, soil health and carbon sequestration? Journal of Soil Science and Plant Nutrition. 23(4): 6647-6669.

Amirkhani, M., Netravali, A.N., Huang, W. and Taylor, A.G. (2016). Investigation of soy protein-based biostimulant seed coating for broccoli seedling and plant growth enhancement. Hort Science. 51(9): 1121-1126.

Casida, Jr., L.E., Klein, D.A. and Santoro, T. (1964). Soil dehydrogenase activity. Soil Science. 98(6): 371-376.

Chandrika, K.P., Prasad, R.D., Prasanna, S.L., Shrey, B. and Kavya, M. (2024). Impact of biopolymer-based *Trichoderma harzianum* seed coating on disease incidence and yield in oilseed crops. Heliyon. 10(19): e38816.

De La Torre-Roche, R., Cantu, J., Tamez, C., Zuverza-Mena, N., Hamdi, H., Adisa, I.O. and White, J.C. (2020). Seed biofortification by engineered nanomaterials: A pathway to alleviate malnutrition? Journal of Agricultural and Food Chemistry. 68(44): 12189-12202.

Dong, Y.J., He, M.R., Wang, Z.L., Chen, W.F., Hou, J., Qiu, X.K., Zhang, J.W. (2016). Effects of new coated release fertilizer on the growth of maize. Journal of Soil Science and Plant Nutrition. 16(3): 637-649.

Glick, B.R. (2012). Plant growth promoting bacteria: Mechanisms and applications. Scientific. 1: 963401. doi: 10.6064/2012/963401.

Hellequin, E., Monard, C., Chorin, M., Le Bris, N., Daburon, V., Klarzynski, O. and Binet, F. (2020). Responses of active soil microorganisms facing to a soil biostimulant input compared to plant legacy effects. Scientific Reports. 10(1): 13727.

Nagargade, M., Tyagi, V. and Singh, M.K. (2018). Plant growth-promoting rhizobacteria: A biological approach toward the production of sustainable agriculture. In: Role of Rhizospheric Microbes in Soil: Stress Management and Agricultural Sustainability. 1: 205-223.

Nanda, M., Kumar, V. and Sharma, D.K. (2019). Multimetal tolerance mechanisms in bacteria: The resistance strategies acquired by bacteria that can be exploited to 'clean-up' heavy metal contaminants from water. Aquatic Toxicology. 212: 1-10.

Nelson, E.B. (2018). The seed microbiome: Origins, interactions and impacts. Plant and Soil. 422: 7-34.

Parkinson, D., Gray, T.R. and Williams, S.T. (1971). Methods for Studying the Ecology of Soil Microorganisms. Oxford, Blackwell Scientific Publications for the International Biological Programme.

Pedrini, S., Balestrazzi, A., Madsen, M.D., Bhalsing, K., Hardegree, S.P., Dixon, K.W. and Kildisheva, O.A. (2020). Seed enhancement: Getting seeds restoration-ready. Restoration Ecology. 28(S3): 266-275.

Pramanik, B., Sar, P., Bharti, R., Gupta, R.K., Purkayastha, S., Sinha, S., Chattaraj, S. and Mitra, D. (2023). Multifactorial role of nanoparticles in alleviating environmental stresses for sustainable crop production and protection. Plant Physiology and Biochemistry. 201: 107831. https://doi.org/10.1016/j.plaphy.2023.107831.

Project Coordinator's Report (2023). All India Coordinated Research Project on *Kharif* Pulses; Indian Council of Agricultural Research- Indian Institute of Pulses Research: Kanpur, India. p. 208024.

Rocha, S., Antunes, J.E., Araujo, F.F., Mendes, L.W., Sousa, R.S. and Araujo, A.S. (2019). Soil microbial C: N: P ratio across physiognomies of Brazilian Cerrado Soil microbial biomass across a gradient of preserved native Cerrado. Anais da Academia Brasileira de Ciências. 91.

Shinde, P., Doddagoudar, S.R. and Vasudevan, S.N. (2017). Influence of seed polymer coating with micronutrients and foliar spray on seed yield of chickpea (*Cicer arietinum* L.). Legume Research-An International Journal. 40(4): 704-709. doi: 10.18805/lr.v0iOF.10760.

Vassileva, M., Flor-Peregrin, E., Malusá, E. and Vassilev, N. (2020). Towards better understanding of the interactions and efficient application of plant beneficial prebiotics, probiotics, postbiotics and synbiotics. Frontiers in Plant Science. 11: 1068.

Wei, X., Xie, B., Wan, C., Song, R., Zhong, W., Xin, S. and Song, K. (2024). Enhancing soil health and plant growth through microbial fertilizers: Mechanisms, benefits and sustainable agricultural practices. Agronomy. 14(3): 609.

Wu, H., Zeng, G., Liang, J., Chen, J., Xu, J., Dai, J., Li, X., Chen, M., Xu, P., Zhou, Y. and Li, F. (2016). Responses of bacterial community and functional marker genes of nitrogen cycling to biochar, compost and combined amendments in soil. Applied Microbiology and Biotechnology. 100(19): 8583-8591.