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Influence of *Trichoderma viride* Application on Seed Quality Attributes in Black Soybean

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

The present study investigates the influence of *Trichoderma viride* application on seed quality attributes of Black soybean (*Glycine max* L.), a crop recognized for its nutritional and functional benefits. The experiment was conducted using local germplasm collected from the Tehri district, Uttarakhand, under controlled laboratory conditions at the Plantica – Indian Academy of Rural Development, Dehradun. Five treatments comprising different durations of seed soaking in *Trichoderma viride* suspension (1 to 4 hours) and an untreated control were evaluated using a Completely Randomized Design (CRD) with four replications. Key seed quality parameters, including germination percentage, seedling length, shoot and root growth, fresh and dry biomass, and seedling vigor indices, were assessed in accordance with ISTA guidelines.
Seeds treated with *Trichoderma viride* showed significant improvements compared to the control in terms of germination and seedling vigor. Notably, the 1-hour soaking treatment (T2) recorded the

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highest germination percentage (97%), while the 2-hour (T3) and 3-hour (T4) treatments significantly enhanced seedling length and biomass accumulation, respectively. These enhancements are attributed to the bio stimulatory and bio protective effects of *Trichoderma viride*, including phytohormone production, nutrient solubilization, and suppression of soil-borne pathogens.

The findings show the potential of *Trichoderma viride* as an effective and eco-friendly bio-priming agent for improving seed quality attributes and promoting sustainable black soybean production under local conditions.

Keywords: Trichoderma viride; Glycine max L.; phytohormone; seedling vigor index; germplasm.

1. INTRODUCTION

Black soybean (*Glycine max* L.), a variant of the common soybean, is botanically classified as an annual, self-pollinated leguminous crop belonging to the family Fabaceae. It is characterized by its distinct black seed coat, which is rich in anthocyanins and other bioactive compounds that distinguish it nutritionally and functionally from conventional yellow soybeans (Messina et al*.,* 2009). Morphologically, black soybean exhibits typical soybean plant architecture, with trifoliate leaves, hairy stems, and pods containing two to four seeds (Lee et al., 2019). It thrives best in temperate to subtropical regions, requiring well-drained loamy soils with adequate organic matter and a pH range of 6.0–7.5 for optimal growth (Singh et al., 2010). In India, Black soybean is primarily grown in the hilly regions of Uttarakhand, Himachal Pradesh, and parts of the northeastern states, where it serves as an important component of traditional farming systems. Though the total cultivated area of black soybean is relatively limited compared to commercial yellow soybean, it holds significant cultural, nutritional, and economic value for subsistence farmers in these regions (Rawal et al., 2015). Productivity levels vary depending on agro-climatic conditions and management practices, with yields generally ranging between 1.0 and 1.5 tonnes per hectare under traditional low-input systems, though improved agronomic practices and high-yielding local varieties have demonstrated higher yield potentials (Joshi et al., 2020). Nutritionally, Back soybean is renowned for its high protein content, typically ranging from 35% to 42%, and it is an excellent source of essential amino acids, particularly lysine and leucine, which complement cereal-based diets (Kumar et al., 2017). Additionally, it contains 18–20% oil rich in unsaturated fatty acids, particularly linoleic and oleic acids, contributing to its functional food value (FAO, 2022). Beyond macronutrients, black soybean seeds are notable for their elevated levels of phenolic compounds, isoflavones, and antioxidants, which have been associated with various health benefits; including anti-inflammatory and anti-carcinogenic properties (Xu and Chang, 2008). These attributes have led to growing interest in Black soybean as a nutritionally dense, climate-resilient crop with potential to contribute to food and nutritional security in marginal hill and mountain farming systems (Kim et al., 2017).

In recent years, the integration of biological inputs has emerged as a promising alternative to conventional chemical treatments in sustainable agriculture. Among these, *Trichoderma viride*, a well-known soil-borne filamentous fungus, has demonstrated multiple beneficial roles, including the suppression of phytopathogens, enhancement of nutrient availability, and promotion of plant growth (Kumar et al., 2020). Seed biopriming with *Trichoderma viride* has been shown to improve germination rates, seedling vigor, and stress tolerance in various crops through mechanisms such as phytohormone production, enzymatic activity, and induction of systemic resistance.

Despite substantial research on soybean improvement, studies focusing specifically on the influence of *Trichoderma viride* on the seed quality attributes of black soybean are relatively limited. Optimizing seed treatment protocols with *Trichoderma viride* could offer an effective, low-cost strategy to improve seed germination and seedling vigor, thereby contributing to enhanced crop productivity and sustainability.

Therefore, the present study aims to assess the effect of *Trichoderma viride* seed treatment on key seed quality parameters in Black soybean, including germination percentage, seedling growth, biomass accumulation, and vigor indices. The results are expected to provide valuable insights into the practical application of *Trichoderma viride* in sustainable Black soybean cultivation systems.

2. MATERIALS AND METHODS

The present investigation was carried out in the Seed Science and Technology Research Laboratory of Plantica – Indian Academy of Rural Development (IARD), located in Dehradun, Uttarakhand, India. To ensure the genetic relevance and adaptability of the experimental material, locally cultivated Black soybean (*Glycine max* L.) germplasm was collected directly from farmer fields in the Tehri district of Uttarakhand. This approach aimed to maintain regional suitability and practical applicability of the findings for local farming communities.

The experiment was designed following a Completely Randomized Design (CRD) design to minimize experimental error and enhance the precision and reproducibility of the results. The study comprised five treatments, each replicated four times. The treatments included four durations of seed soaking in a freshly prepared *Trichoderma viride* suspension and one untreated control for comparative assessment.

For the seed treatment, healthy and uniformly sized Black soybean seeds were selected and surface-sterilized using a 1% sodium hypochlorite solution for two minutes, followed by thorough rinsing with sterile distilled water to remove any residual disinfectant. The sterilized seeds were then soaked in an aqueous suspension of *Trichoderma viride* at a standardized concentration, with soaking durations set at 1 hour (T2), 2 hours (T3), 3 hours (T4), and 4 hours (T5). The control treatment (T1) consisted of untreated seeds soaked in sterile distilled water for the equivalent duration.

Following treatment, the seeds were air-dried under shade to bring the surface moisture to a manageable level suitable for testing. Standard germination tests were performed according to the guidelines prescribed by the International Seed Testing Association (ISTA). For each treatment, 100 seeds were placed in sterilized germination trays lined with moistened paper towels and maintained under controlled laboratory conditions at optimal temperature (20^0C) and humidity levels (75 %) appropriate for soybean germination.

Observations including germination percentage, seedling length, shoot length, root length, fresh and dry biomass, and seedling vigor indices were recorded. Germination counts were recorded at regular intervals, and measurements of seedling growth were taken at the final count. Seedling vigor indices were calculated using standard formulas combining germination percentage and seedling length data.

Germination percentage = (Number of Germinated seeds)/ (Total Number of Seeds) X 100

The data obtained were subjected to analysis of variance (ANOVA) to determine the significance of treatment effects, and mean comparisons were made using the appropriate statistical tests to identify the most effective soaking duration. This methodological approach aimed to generate robust and reliable evidence on the effectiveness of *Trichoderma viride* as a bio-priming agent for enhancing seed quality attributes in black soybean under local conditions.

3. RESULTS

The present investigation clearly showed that the application of *Trichoderma viride* exerted a significant and consistently positive influence on key seed quality attributes of Black soybean (*Glycine max* L.). The findings provide experimental evidence for the effectiveness of this biological agent in improving germination percentage, seedling growth parameters, and overall seedling vigor, all of which are critical factors for successful crop establishment, especially under resource-limited and sustainable cultivation practices.

The results indicated that seeds treated with *Trichoderma viride* exhibited enhanced physiological responses and superior early growth performance when compared to the untreated control. This improvement underscores the potential of *Trichoderma viride* as a reliable biopriming agent capable of promoting uniform seedling emergence and establishing a strong foundation for subsequent crop growth and productivity.

Among the various treatment durations, T_2 recorded the highest germination percentage (97.00%), which represents a substantial improvement over the T_1 that showed the lowest germination percentage at 88.75%. This significant increase in germination is consistent with previous reports that highlight the ability of *Trichoderma* species to stimulate seed metabolic processes through the production of phytohormones such as auxins, gibberellins, and cytokinins (Harman et al., 2004; Hermosa et al., 2012). These hormones are known to activate hydrolytic enzymes that break down stored food reserves within the seed endosperm, thereby providing the necessary energy for faster and more uniform radicle protrusion.

Further, the data clearly showed that both shoot and root lengths increased progressively with longer soaking durations, highlighting the additional benefits of extended exposure to *Trichoderma viride*. The maximum shoot length was observed in T_4 (7.195 cm), closely followed by T_3 (6.995 cm), indicating that these treatments not only promoted quicker germination but also supported robust post-germination vegetative growth. Root length displayed a comparable trend, with the highest mean values recorded in T_3 (3.1075 cm) and T_4 (2.79 cm), both of which were significantly superior to the control treatment.

The increase in root and shoot lengths can be attributed to the multifaceted mechanisms of *Trichoderma viride*, which include the solubilization of essential macro- and micronutrients, production of siderophores, and suppression of soil-borne pathogens through mycoparasitism and antibiosis (El-Komy, 2001; Singh et al., 2019). These synergistic effects create a healthier rhizosphere environment, enabling the developing seedlings to establish a more extensive root system, which in turn enhances water and nutrient uptake. An efficient root system supports better shoot development by ensuring adequate translocation of nutrients and photosynthates, ultimately leading to increased biomass accumulation.

Seedling length, a composite indicator encompassing both root and shoot development, reflected the overall growth vigor imparted by the treatments. The maximum seedling length was recorded for the T_3, which produced an average length of 10.0875 cm, followed by T_4 (9.5925 cm) and T_2 (8.37 cm). In contrast, the shortest seedlings were recorded in the untreated control group (6.575 cm). These results are in line with the findings of Rajput et al. (2022), who reported that *Trichoderma* seed treatments in leguminous crops consistently improved seedling length, thus enhancing the seedlings capacity to compete with weeds and withstand initial abiotic stress factors during crop establishment.

The study further demonstrated significant positive effects of *Trichoderma viride* treatments on fresh and dry biomass accumulation. The highest fresh biomass weight was recorded in T_4 (9.1425 g), which was nearly double that of the untreated control (4.6925 g). A similar trend was observed in dry biomass, where T_4 also recorded the maximum dry weight (0.64 g), emphasizing the enhanced efficiency of treated seedlings in converting absorbed water and nutrients into cellular material. This increase in biomass aligns with earlier reports by Benítez et al. (2004) and Shoresh et al. (2010), who highlighted that *Trichoderma* spp. promotes higher photosynthetic efficiency, chlorophyll retention, and delay in senescence, thereby improving the net assimilation rate and overall plant vigor.

Moreover, the seedling vigor indices, which integrate germination percentage and seedling growth into a single measure of seed performance, were markedly enhanced by *Trichoderma viride* treatments. The highest seedling vigor index based on length (SVI-L) was observed in T_3 (932.2625), while the highest seedling vigor index based on mass (SVI-M) was recorded in T_4 (58.7725). These elevated SVI values indicate that the treated seeds possessed greater physiological and metabolic strength, which is a crucial determinant of field emergence, uniform crop stand, and resilience against environmental stressors. These observations corroborate the findings of Gajera and Vakharia (2010), who reported that *Trichoderma viride* treatments significantly improved seed vigor indices in several legume species by enhancing enzymatic activities and metabolic pathways that sustain seedling growth.

The cumulative results of this study demonstrate that the positive effects of *Trichoderma viride* can be scientifically attributed to its complex modes of action. These include the secretion of bioactive metabolites that function as plant growth regulators, enhanced nutrient bioavailability through solubilization of phosphates and micronutrients, and the suppression of deleterious soil microflora through competitive exclusion and mycoparasitism (Harman et al., 2004; Shoresh et al., 2010). Such interactions contribute to an improved rhizosphere environment, ensuring that emerging seedlings have optimal access to nutrients and are better protected from early-season diseases.

Overall, the findings of the present investigation strongly support the integration of *Trichoderma viride* seed treatment into sustainable seed management practices for black soybean cultivation. The significant improvements in germination, seedling growth, biomass accumulation, and vigor indices demonstrate that *Trichoderma viride* holds substantial promise as an eco-friendly and cost-effective biopriming agent for enhancing early crop establishment, particularly under organic and low-input farming systems.

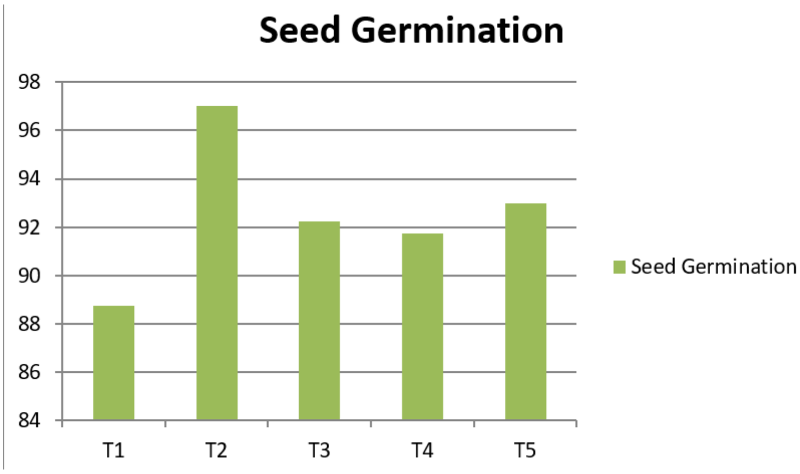

Fig. 1 (a). Performance of various *Trichoderma viride* treatments on seed germination in black soybean

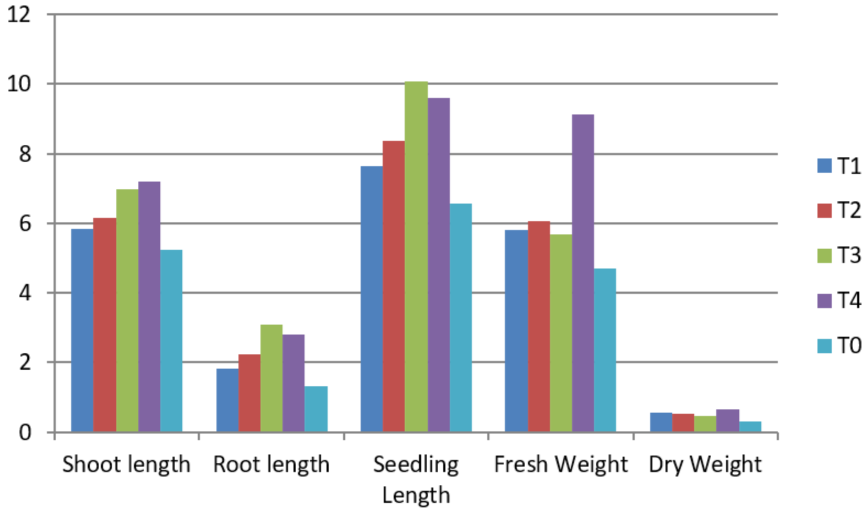

Fig. 1 (b). Performance of various *Trichoderma viride* treatments on seed quality parameters in black soybean

Table 1. Performance of various *Trichoderma viride* treatments on seed quality parameters in black soybean

Treatment	Seed Germination	Shoot length	Root length	Seedling Length	Fresh Weight	Dry Weight	SVI-Mass	SVI - Length
T1	88.75	5.83	1.82	7.65	5.82	0.55	49.16	677.81
T2	97	6.14	2.23	8.37	6.05	0.52	51.17	804.66
T3	92.25	6.99	3.10	10.08	5.67	0.47	43.71	932.26
T4	91.75	7.19	2.79	9.59	9.14	0.64	58.77	880.41
T5	93	5.24	1.33	6.57	4.69	0.31	30.51	611.11
Mean	92.55	6.28	2.25	8.45	6.27	0.5	46.66	781.25
C.V.	4.05	3.74	8.39	9.15	5.94	10.83	17.38	26.02
C.D. (5%)	4.96	0.21	0.47	0.68	0.3	0.056	6.69	253.82
S. E.	1.7	0.11	0.224	0.366	0.16	0.026	3.19	94.17

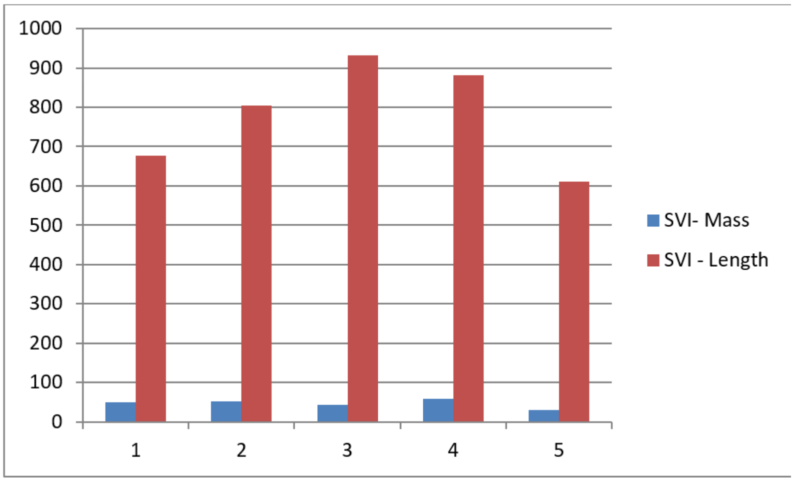

Fig. 2. Bar graph showing performance of various *Trichoderma viride* treatments on vigour index in black soybean

4. DISCUSSION

The results of the present study provide compelling evidence that seed treatment with *Trichoderma viride* can substantially enhance seed quality attributes and early seedling performance in black soybean (*Glycine max* L.). The observed improvements in germination percentage, seedling length, root and shoot growth, biomass accumulation, and seedling vigor indices clearly demonstrate the multifaceted benefits of *Trichoderma viride* as a bio-priming agent.

The significant increase in germination percentage among treated seeds, particularly in the 1-hour soaking treatment (T_2), highlights the role of *Trichoderma viride* in stimulating early metabolic activity during germination. This finding aligns with previous reports indicating that *Trichoderma* species produce plant growth-promoting phytohormones such as auxins, gibberellins, and cytokinins, which accelerate the activation of hydrolytic enzymes necessary for mobilizing stored seed reserves (Harman et al., 2004; Hermosa et al., 2012). The enhanced enzymatic activity ensures faster radicle emergence and higher final germination percentages, which are critical for establishing a uniform crop stand.

The positive response of shoot and root lengths to increasing durations of soaking further underscores the effectiveness of *Trichoderma viride* in promoting post-germination growth. Treatments T_3 (2-hour soak) and T_4 (3-hour soak) significantly improved root and shoot development compared to the untreated control. These results are consistent with studies by El-Komy (2001) and Singh et al. (2019), which reported that *Trichoderma* spp. improve nutrient solubilization, promote efficient nutrient uptake, and suppress soil-borne pathogens through mycoparasitism and antibiosis. Such mechanisms create a healthier rhizosphere, enabling seedlings to develop stronger root systems that can access water and nutrients more effectively, directly supporting shoot elongation and above-ground biomass production.

The enhanced seedling length observed in the treated seeds confirms the cumulative benefit of improved root and shoot growth. Longer seedlings are generally more vigorous and resilient to early-stage biotic and abiotic stresses, increasing their competitiveness against weeds and contributing to robust stand establishment in the field. This observation is in line with findings by Rajput et al. (2022), who noted similar improvements in seedling growth in legumes treated with *Trichoderma*.

Fresh and dry biomass weights also demonstrated significant gains in response to *Trichoderma viride* application. The highest fresh and dry weights recorded in the T_4 treatment reflect the efficient conversion of absorbed nutrients into cellular biomass. Such an increase suggests not only better root and shoot growth but also a likely improvement in photosynthetic efficiency and chlorophyll retention, as supported by earlier work (Benítez et al., 2004; Shoresh et al., 2010). Greater biomass accumulation during the seedling stage is directly linked to enhanced early vigor, which is essential for maintaining crop performance under field conditions.

The markedly higher seedling vigor indices observed across *Trichoderma viride* treatments further substantiate the fungus's capacity to boost both metabolic activity and structural robustness of seedlings. High vigor indices imply that seeds are physiologically active and capable of rapid establishment, which can translate to better crop emergence, uniformity, and yield stability. Similar trends were reported by Gajera and Vakharia (2010), who found that *Trichoderma viride* seed priming significantly improved vigor indices in various leguminous crops.

The underlying mechanisms driving these positive effects are well documented. *Trichoderma viride* functions through multiple synergistic pathways, including phytohormone production, nutrient solubilization, siderophore production, mycoparasitism of pathogens, and induction of systemic resistance (Harman et al., 2004; Shoresh et al., 2010). Collectively, these attributes improve seed metabolic activity, strengthen the root system, and protect seedlings from early-season soil-borne diseases, creating favorable conditions for successful crop establishment.

Taken together, the findings of this study reinforce the practical potential of integrating *Trichoderma viride* seed treatment into sustainable seed quality management strategies for black soybean cultivation. By reducing reliance on synthetic chemical treatments and enhancing seedling vigor naturally, *Trichoderma viride* biopriming aligns well with the principles of organic and low-input agriculture.

However, while the laboratory results are promising, field validation under different agro-climatic conditions is essential to confirm the consistency and scalability of these benefits. Future research should explore the interactive effects of *Trichoderma viride* with other biocontrol agents or organic amendments, as well as its performance under varying soil health and management practices.

Overall, this study contributes valuable evidence supporting the use of *Trichoderma viride* as an eco-friendly and cost-effective tool for enhancing seed quality, promoting sustainable soybean production, and supporting resilient agricultural systems.

5. CONCLUSION

The present study demonstrates that treating Black soybean (*Glycine max* L.) seeds with *Trichoderma viride* significantly improves seed quality by enhancing germination percentage, seedling growth, biomass accumulation, and seedling vigor. Soaking seeds for one hour resulted in the highest germination (97%), showing that even a short duration is effective for stimulating early germination through the production of growth-promoting substances like auxins and gibberellins. A two-hour soaking period produced the longest seedlings and roots, supporting better nutrient uptake and early plant establishment, while a three-hour soak yielded the greatest fresh and dry biomass and the highest vigor index based on seedling mass, indicating robust physiological activity and stronger seedlings. These benefits are attributed to *Trichoderma viride*'s ability to suppress harmful soil pathogens, colonize root surfaces, enhance nutrient availability, and promote hormone production, which together strengthen seedling growth and establishment. The results support existing evidence that *Trichoderma viride* is an effective bioagent for improving seed performance in various crops and highlight its practical potential as an eco-friendly alternative to chemical seed treatments. Based on these findings, it is recommended that farmers and seed producers adopt *Trichoderma viride* seed soaking for 1–3 hours before sowing to enhance germination and seedling vigor, and integrate it with other good agricultural practices for sustainable soybean production. Further field trials under diverse conditions are encouraged to validate these laboratory results and refine recommendations for wider use, supporting sustainable agriculture and higher productivity for farming communities.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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