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Original article

Standardization of Biophysical Factors Affecting the Seed Biopriming and its Influence on the Growth and Yield of Garden Cress (*Lepidium sativum* L.)

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The aim of the study was to examine the biophysical conditions necessary for seed biopriming of *Lepidium sativum* L. by investigating the different morphological parameters at various developmental stages of plant growth. In order to achieve maximum growth and yield, *Trichoderma harzianum* at a concentration 10^8 CFU/mL (colony forming unit/mL) suspended in 1% NaCl solution of pH 7 was used for the treatment of garden cress seeds and kept it for 16 hours at 20°C. Seed biopriming with the combined application of *Trichoderma asperellum* and *Trichoderma harzianum* leads to highest germination percentage (98.89 ± 0.79%), average shoot (10.10 ± 0.14 cm) and root length (8.57 ± 0.29 cm) of seedlings, seed vigour index (1,846.22 ± 47.77), seed stamina index (18.46 ± 0.48), relative water content (93.1 ± 2.77%), and seed metabolic efficiency (0.31 ± 0.02 g/g) followed by the individual application of *T. harzianum*, *T. asperellum* as compared to untreated seeds. A similar result was found at the mature stage, where combined application of fungus possesses higher biomass (12.27 ± 0.29 g/plant), number of branches (16.6 ± 0.43 branches/plant), shoot length (59.27 ± 0.42 cm), root length (12.37 ± 0.79 cm) and yield (5.55 ± 0.11 g/plant) followed by individual application of *T. harzianum*, *T. asperellum* as compared to control. Overall, biopriming of garden cress seeds with the combination of *T. harzianum* and *T. asperellum* showed a viable strategy for improving crop growth and yield.

Key words: Brassicaceae, morphological parameters, *Trichoderma asperellum*, *Trichoderma harzianum*

Vegetables represent a vital component of Indian agriculture due to their rich nutritional values, high yields, short growth cycles, and substantial contributions towards economic benefits (Schreinemachers *et al*. 2018; Noopur *et al*. 2023). Additionally, their cultivation has the potential to replace subsistence farming in various agroclimatic zones across India (Kohima *et al*. 2021). In 2021 – 2022, India achieved a total of 341.63 million tons of horticulture crops, of which vegetables contributed enormously, 200.45 million tons (Noopur *et al*. 2023). Their contribution plays a vital role in different sectors such as finance, food availability and dietary wellness (Panwar *et al*. 2019). In India, approximately 723 species of green leafy vegetables are grown and consumed in different regions (Ray & Ray 2022). Leafy vegetables are low-cost and easy to afford and can be grown outdoors and indoors with smaller space requirements. Mostly, the leafy vegetables distributed all over the world belong to the Brassicaceae family (Miller-Cebert *et al*. 2009). The Brassicaceae family includes a wide range of vegetables such as *Eruca sativa* L., *Cardamine hirsute* L., *Brassica oleracea* L. (varieties such as *gemmifera*, *capitata*, *botrytis*,

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viridis, *sabellica*, *gongylodes,* etc.), *Brassica napus* L. (varieties: *canola*, *rapseed*, *napobrassica*), *Sinapis arvensis* L., *Armoracia rusticana* G. Gaertn., B. Mey. & Scherb., *Brassica juncea* L., *Brassica rapa* L. (subspecies such as *pekinensis*, *rapa, trilocularis*, *oleifera*, *chinensis*, *glabra,* etc.), *Eutrema japonicum* (Miq.) Koidz., and *Capsella bursa-pastoris* L. (Abbaoui *et al*. 2018). Most of these species provide edible roots, leaves, stems, buds, flowers, and seeds, which can be used for different purposes such as oil production, condiments, vegetable and bio-fuel production, etc. (McVetty & Duncan 2015).

Lepidium sativum L. is one of the important plant species belonging to the Brassicaceae family and is cultivated around the world for its culinary and medicinal uses. It has a distinctive peppery flavour and is widely used in culinary ingredients such as salads, sandwiches, soups, and smoothies for its delicious taste. It is also used as a tonic against eye disease, leucorrhea, scurvy, asthma, cold and cough, as well as curative in diabetes mellitus, kidney stones, inflammations, muscular pain, etc. (Baregama & Goyal 2019). Their phytoconstituent showed anti-ameliorative effect, anti-turbid, anti-renal, antibacterial, and anticancer properties (Jain & Grover 2018). Upon germination, there is an increase in the phytochemicals that occur in the plant that support various pharmacological properties (Abdel-Aty *et al*. 2019. Garden cress is under limited cultivation because of various growth-restraining factors that are associated with soil composition, pH, temperature, and water, along with various soil-borne diseases that occur from the seed to plant level and thus decrease the germination and yield of the crop.

To overcome the constraints in the growth of the plants, at present, seeds are treated with fungicides and pesticides as well as heavily supplied with synthetic fertilisers or other chemicals that adversely affect human health. As an alternative, recently bio-priming, which is a safer and more economical approach, has gained much importance in crop enhancement for its role in activation of physiological processes without the emergence of the radicle of the seed, and it is used for uniform seed germination with better stand establishment (Moeinzadeh *et al*. 2010; Ghorbanpour & Hatami 2014). Bio-priming has been used to enhance the germination percentage, seedling length, seedling vigour index, dry matter production, seed metabolic efficiency, fresh weight, and dry weight, micronutrient uptake, grain yield, chlorophyll content, lignin deposition, antioxidant activity, and other morphological characteristics, to make the crop stands up against abiotic stress and decreases the disease severity caused by various pathogens (Mahmood *et al*. 2016; Deshmukh *et al*. 2020). The biopriming approach has been explored in diverse crops such as *Eleusine coracana* L. (Prashant *et al*. 2021), *Zea mays* L. (Karthika & Vanangamudi 2013; Sivakumar *et al*. 2017; Amruta *et al*. 2019; Singh *et al*. 2020b), *Helianthus annuus* L. (Moeinzadeh *et al*. 2010), *Phaseolus vulgaris* L. (Monalisa *et al*. 2017), *Sorghum bicolor* (L.) Moench (Manzar & Singh, 2019), *Solanum lycopersicum* L. (Bhatt *et al*. 2015), *Abelmoschus esculentus* (L.) Moench (Rai *et al*. 2019), *Lens culinaris* Medik. (Kumar *et al*. 2019), and so on. Biopriming has also demonstrated significant benefits in Brassicaceae family crops such as *Brassica oleracea* L., *Brassica napus* L., *Brassica juncea* L., *Raphanus sativus* L., etc. (Somagh *et al*. 2017; Tumpa *et al*. 2017; Umesha & Roohie 2017; Sarkar *et al*. 2022).

Currently, there is no report on the optimization of biophysical parameters that affect the biopriming in garden cress. So, keeping these things in mind, biophysical parameters like microbial type and concentration, duration of microbial exposure, temperature, priming solution composition, solution pH and adhering substrate were standardized for seed biopriming. The impact of biopriming at the seedling and plant level on morphological features such as germination percentage (GP), average shoot and root length of seedlings (ASL and ARL), seed vigour index (SVI), seed stamina index (SSI), relative water content (RWC), seed metabolic efficiency (SME) and biomass has also been undertaken in the present study.

MATERIAL AND METHODS

Seeds of garden cress (*Lepidium sativum* L.) were procured from the local market of the district Kurukshetra of Haryana state, India. Fungal strains *Trichoderma asperellum* and *Trichoderma harzianum* were procured from the ICAR-Indian Agricultural Research Institute, New Delhi. Both the cultures of *T. asperellum* and *T. harzianum* were cultured on the potato dextrose agar (PDA) media at 26°C and stored in a refrigerator at 8 – 10°C.

Seed Sterilization and Preparation of Bacterial Cell Suspension Cultures

Healthy seeds were selected and followed the earlier developed seed sterilization process (Mehta *et al*. 2022). A total of ninety sterilized seeds in three replicates (30 seeds per replicate) were taken for each fungal treatment. To prepare the fungal cell suspension cultures, both strains were grown individually in a flask containing 50 mL of potato dextrose broth (PDB) and incubated the cultures at 26 ± 2°C for 7 days. The fungal cells were harvested by centrifugation at 5,000 rpm for 10 min at room temperature, and the concentrations were adjusted to 1×10^6, 1×10^7, 1×10^8, 1×10^9, and 1×10^{10} CFU/mL (Colony forming unit/mL) in the fresh media.

Optimization of Biophysical Parameters Affecting Seed Biopriming

The sterilized seeds were treated with different concentrations (1×10^6, 1×10^7, 1×10^8, 1×10^9, and 1×10^{10} CFU/mL) of *T. harzianum* and kept in an incubator shaker at 150 rpm for a range of time periods (4, 8, 12, 16, 20, and 24 hours) and temperature ranges of 15°C, 20°C, 25°C, and 30°C. Among the other variable parameters, various priming solutions (PDB or 1% NaCl saline or Hoagland's solution) were used for fungal cell suspension preparation, and a range of pH levels (5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5) was tested. To study the effect of adhering agent on seed biopriming, various adhering agents [0.2% Carboxymethyl cellulose (CMC), 0.2% Polyvinyl pyrrolidone (PVP), and 0.2% Sucrose] were tested. After incubation of seeds with fungal suspension for each treatment, seeds were dried on a blotting paper and sown in a potting mix consisting of sand, cocopeat, and vermicompost in a 1:2:1 ratio. After the 10th day of seed sowing, the data was recorded and concluded at which parameters the highest seed germination and seedling growth occur, which was used for further analysis.

After standardization of the parameters with *T. harzianum*, the optimized protocol was also applied to *T. asperellum*, either alone or in combination with *T. harzianum*. In both cases of fungal treatment, on the 10th day of seed sowing, various growth parameters such as germination percentage (GP), average shoot and root length (ASL and ARL) of seedlings, seed vigour index (SVI), seed stamina index (SSI), seed metabolic efficiency (SME), and relative water content (RWC) were analysed. Around the 40th day of seed sowing, parameters such as biomass, number of branches, shoot and root length and seed yield were observed to evaluate the effect of the biopriming.

Evaluation Methods

Evaluation of various growth parameters for optimization of an efficient biopriming method for *L. sativum* L. seeds using *T. harzianum* has been depicted in Table 1 (Bala & Sikder 2016; Patel *et al*. 2017; Dwivedi *et al*. 2018).

Data Analysis

The experiments were conducted in three independent replicates, and post-hoc analysis was performed using Tukey Honestly Significant Difference (HSD^{a,b}) to identify homogenous subsets among the treatment groups. The analysis and graphical representation were performed using International Business Machines Corporation Statistical Package for Social Sciences (IBM SPSS) Statistics 27.0.1 at a significant level of $p \leq 0.05$ and expressed as means ± standard deviation (SD) (Sahur *et al*. 2020).

RESULTS

For sustainable agriculture, microbes are an important element that can increase the growth of the crop without using harsh chemicals and pesticides. The present study aims to increase the growth of garden cress with seed biopriming, where various conditions were analysed that affect the seed biopriming via *Trichoderma harzianum,* as depicted in Figure 1.

1. *Standardization of Biophysical Parameters Affecting Seed Biopriming*

   a) Effect of Microbial Concentration

The effect of fungal cell concentrations (10^6 to 10^{10} CFU/mL) on germination percentage (GP) and seedling growth was investigated. The highest value of GP (78.33 ± 2.36%) was obtained at a concentration of 10^8 CFU/mL with a non-significant difference at 10^9 CFU/mL (77.78 ± 1.57%), followed by significantly lower GP at concentrations 10^7, 10^{10} and 10^6 CFU/mL. The highest value of average shoot length (ASL) 5±0.14 cm and average root length (ARL) 3.57±0.25 cm was also achieved at 10^8 CFU/mL, followed by concentrations of 10^9, 10^7, 10^{10}, and 10^6 CFU/mL (Figure 1A and 1B). Thus, the highest GP and seedling length observed at concentration 10^8 CFU/mL was considered as the optimum concentration.

b) Effect of Temperature

T. harzianum at standardized concentration (10^8 CFU/mL) has showed the highest values of GP (86.67±4.16%), ASL (6.4±0.22 cm), and ARL (3.7±0.28 cm) at 20°C compared to GP (80.56±2.83%), ASL (5.03±0.13 cm) and ARL (2.5±0.14 cm) at lower temperature range (15°C). Increasing the temperature beyond 20°C to 25°C and 30°C, a noticeable drop has been observed in GP, ASL and ARL (Figure 1C and 1D). Among the temperature gradient, 20°C was considered the optimal temperature at which both the parameters GP and seedling growth were maximum.

c) Effect of Inoculation Duration

The seeds were bioprimed with *T. harzianum* (10^8 CFU/mL) at 20°C for variable time period ranging from 4 to 24 hours. A shorter period of 4 hours has resulted in the minimum GP (47.78±1.77%), ASL (3.36±0.33 cm) and ARL (3.57±0.47 cm), while an increase in the duration from 8 to 16 hours leads to an increase in the GP, ASL and ARL. The highest GP (88.33±2.72%) ASL (6.77±0.42 cm), and ARL (5.33±0.33 cm) were observed at 16 hours of duration of seed biopriming. Further increases in duration from 20 to 24 hours reduced the GP and seedling growth but maintained good health (Figure 1E and 1F).

d) Effect of Priming Solution and pH

The effects of priming solutions (Potato dextrose broth, 1% NaCl saline, and Hoagland's solution) and their pH on biopriming were studied. The results showed that when the cells of *T. harzianum* were suspended in 1% NaCl saline solution, the highest value of GP (90.00±2.72%) was obtained at pH 7.0, while at pH 7.5, a non-significant difference in the value of GP (88.89±1.03%) was observed. Similarly, the maximum value of ASL (8.27±0.05 cm) and ARL (7.73±0.34 cm) were obtained at pH 7.0, while at pH 7.5, no significant difference in the value of ASL (8.00±0.14 cm) and ARL (7.47±0.26 cm) was observed. It was observed during this study

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Evaluation of various growth parameters for optimization of efficient biopriming method for *Lepidium sativum* L. seeds using *Trichoderma harzianum*

Parameters	Evaluating method
Germination percentage (GP) [%]	Total number of seedlings/total number of seeds × 100
Average shoot length (ASL) and average root length (ARL) of the seedling [cm]	Shoot and root length of all the seedlings was measured via scale in centimetres [cm] for each treatment and the average value.
Seed vigour index (SVI)	(ASL + ARL) × GP
Seed stamina index (SSI)	SVI / 100
Relative water content (RWC) [%]	[{Fresh weight (FW) – Dry weight (DW)} / {Turgid weight (TW) – Dry weight (DW)}] ×100
Seed metabolic efficiency (SME) [g/g]	SME = Seedling dry weight [g]/Seed material respired (SMR) Seed material respired (SMR) = Seed dry weight before germination (SDW) – {Seedling dry weight + Remaining seed dry weight (RSW)}
Average biomass [g/plant]	From each treatment, ten adult plants were selected and dried in a hot air oven at 60°C until a constant biomass was obtained.
Shoot and root length of mature plant [cm]	Ten adult plants were selected from each treatment, and their average shoot and root length were measured in centimetres [cm].
Number of branches	Ten mature plants were chosen from each treatment, and their branches were counted for the evaluation of their overall growth and biomass.
Average seed yield per plant [g/plant]	Ten adult plants were chosen from each treatment, and their seeds were weighed for the yield.

that maximum response was observed at 1% NaCl with pH 7.0 and considered as the optimum solution and pH, followed by pH 7.0 of the potato dextrose broth (PDB) and Hoagland's solution (Figure 1G and 1H).

e) Effect of Adhering Substrate

The effectiveness of seed biopriming has been increased by the addition of an adhering substrate. Among the different additives used, treatment of *T. harzianum* with Polyvinyl pyrrolidone (PVP) showed the greatest value of GP (95.00 ± 1.24%), followed by Carboxymethyl cellulose (CMC) (91.67 ± 1.36%) and sucrose (90.67 ± 2.68%). Seed biopriming with adhering agent PVP showed the maximum value of ASL (9.67 ± 0.39 cm) and ARL (8.23 ± 0.29 cm), also in comparison to CMC and sucrose (Figure 1I and 1J). Thus, PVP was considered a better adhering substrate at which maximum response occurs.

f) Effect of Fungal Strain Used for Seed Biopriming

The above standardized protocol for seed biopriming using *Trichoderma harzianum* was applied to the *Trichoderma asperellum* also, either alone or co-inoculation at a concentration of 10^8 CFU/mL suspended in 1% NaCl solution having pH 7.0, PVP as adhering substrate and incubated for 16 hours at 20°C {Figure 2 (IA, IB, IC, and ID)}. Seed biopriming with fungal strain either alone or in combination, enhances GP and seedling growth parameters in comparison to untreated (Figure 1K and 1L). The combined application of *T. asperellum* + *T. harzianum* contributes to an impressive rise in the GP (98.89 ± 0.79%), followed by GP values of *T. harzianum* (95.00 ± 1.24%) and *T. asperellum* (82.22 ± 2.08%), in comparison to the control GP value (55.56 ± 3.93%). Similarly, the combined application of fungal strains boosts the ASL (10.10 ± 0.14 cm) and ARL (8.57 ± 0.29 cm), followed by individual application of *T. harzianum*, *T. asperellum* in comparison to the control ASL (5.37 ± 0.54 cm) and ARL (2.27 ± 0.53 cm). Here, the value of ASL and ARL obtained by fungal application either alone or in combination was significantly to the ASL and ARL value observed by the untreated group.

2. Effect of Seed Biopriming on the Germination-related Parameters of L. sativum

Various growth parameters like seed vigour index (SVI), seed stamina index (SSI), relative water content (RWC), and seed metabolic efficiency (SME) were observed at the seedling stage. The highest value of SVI (1,846.22 ± 47.77) and SSI (18.46 ± 0.48) was observed by the combined application of *T. asperellum* + *T. harzianum,* and non-significant to the SVI and SSI values obtained by the individual treatment of *T. harzianum* while a significant difference was noticed as compared to treatment with *T. asperellum* and control values of SVI and SSI. RWC, an indicator of water absorption, has improved significantly with seed biopriming. In this study, the combined application of *T. asperellum* + *T. harzianum* has enhanced the RWC up to the maximum value (93.1 ± 2.77%), followed by *T. harzianum* (92.2 ± 0.98%) and *T. asperellum* (88.88 ± 0.92%), and showed a significant difference compared to the control RWC (77.48 ± 1.33%). Similarly, SME has been boosted by the effect of biopriming, and the highest value of SME (0.31 ± 0.02 g/g) has been obtained by the co-inoculation, followed by *T. harzianum* and *T. asperellum* alone and control SME (0.03 ± 0.01 g/g) (Table 2).

3. Effect of Seed Biopriming on the Growth Parameters and Yield of Garden Cress

The beneficial outcomes of seed biopriming at seedling stage establish a solid framework for forecasting their beneficial effect at later plant growth stages. Seed biopriming enhanced the shoot length, root length, number of branches, biomass and yield in comparison to untreated (Table 3 and Figure 2 IIA, IIB, IIC, and IID). Seed biopriming with the co-inoculation of *T. asperellum* and *T. harzianum* resulted in a significant improvement in shoot length (59.27 ± 0.42 cm), followed by the individual application of *T. harzianum* (53.33 ± 3.81 cm), *T. asperellum* (44.63 ± 0.41 cm), and untreated shoot length (35.43 ± 0.56 cm). Similarly, the highest value of root length (12.37 ± 0.79 cm) was obtained by the combined application, closely followed by the individual application of *T. asperellum, T. harzianum,* and significantly different from the control root length (Table 3). The increase in shoot length provides a greater area for the branches; however, co-inoculation provides the maximum number of branches (16.6 ± 0.43 per plant) in comparison to other treatments. An increase in stem length and number of branches provides a greater surface area, which consequently increases the biomass. Co-inoculation has increased the biomass up to a maximum value of 12.27 ± 0.29 g/plant with a non-significant difference in value of biomass obtained by T. *harzianum* (11.6 ± 0.22 g/plant), while significant to *T. asperellum* (8.87 ± 0.29 g/plant) and control biomass (6.03 ± 0.34 g/plant). An amazing impact has been observed on the yield by seed biopriming, where the combined application resulted in the highest value of yield (5.55 ± 0.11 g/plant), which was significant compared to other treatments (Figure 2 IIIA, IIIB, IIIC, and IIID). Overall, co-inoculation of *T. asperellum* and *T. harzianum* had showed a synergistic effect and significantly boosted growth and yield measures. The findings of this study demonstrated that co-inoculation of these two fungal species may constitute a profitable approach for boosting plant productivity.

DISCUSSION

Various eco-sustainable approaches have been used for crop improvement, such as crop rotation, cross-breading, integrated pest management, use of resistant varieties and biopriming. These methodologies provide a significant effect on crop improvement in terms of nutrient enhancement, plant-soil relation, pest and disease management without the use of harmful chemicals (Maheshwari *et al.* 2020; Sharma & Bipana 2024). Among the other eco-sustainable methods, biopriming is a well-known

T a b l e 2

Effect of seed biopriming on germination-related parameters of *Lepidium sativum* L. Data based on three replicates of 30 seeds each recorded at the end of the 10th day of seed sowing*

Parameters Treatments	Seed vigour index (SVI)	Seed stamina index (SSI)	Relative water content (RWC) [%]	Seed metabolic efficiency (SME) [g/g]
Control	421.39 ± 45.66^{a}	4.21 ± 0.46^{a}	77.48 ± 1.33^{a}	0.03 ± 0.01^{a}
T. asperellum	1,049.78 ± 74.44^{b}	10.50 ± 0.74^{b}	88.88 ± 0.92^{b}	0.14 ± 0.00^{b}
T. harzianum	1,700.67 ± 50.33^{c}	17.01 ± 0.51^{c}	92.20 ± 0.98^{b}	0.25 ± 0.01^{c}
T. asperellum + T. harzianum	1,846.22 ± 47.77^{c}	18.46 ± 0.48^{c}	93.10 ± 2.77^{b}	0.31 ± 0.02^{d}

*Data are presented as mean ± standard deviation. The mean comparisons were conducted using Tukey honestly significant difference (HSD^{a,b}) and values within the same column with different alphabetical superscripts are significantly different at $p < 0.05$.

T a b l e 3

Effect of seed biopriming on different growth parameters and yield of *Lepidium sativum* L.

Parameters Treatments	Stem length [cm]	Root length [cm]	Number of branches [per plant]	Biomass [g per plant]	Yield [g per plant]
Control	35.43 ± 0.56^{a}	4.90 ± 0.22^{a}	7.33 ± 0.47^{a}	6.03 ± 0.34^{a}	2.59 ± 0.21^{a}
T. asperellum	44.63 ± 0.41^{b}	10.40 ± 0.94^{b}	11.43 ± 0.42^{b}	8.87 ± 0.29^{b}	3.56 ± 0.18^{b}
T. harzianum	53.33 ± 3.81^{c}	10.83 ± 0.45^{b}	12.40 ± 0.71^{b}	11.60 ± 0.22^{c}	4.60 ± 0.05^{c}
T. asperellum + T. harzianum	59.27 ± 0.42^{c}	12.37 ± 0.79^{b}	16.60 ± 0.43^{c}	12.27 ± 0.29^{c}	5.55 ± 0.11^{d}

*Data are presented as mean ± standard deviation. The mean comparisons were conducted using Tukey honestly significant difference (HSD^{a,b}) and values within the same column with different alphabetical superscripts are significantly different at $p < 0.05$.


A
Germination percentage [%]
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
10*6
10*7
10*8
10*9
10*10
Concentration [CFU/ml]
GP
B
ASL and ARL [cm]
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
10*6
10*7
10*8
10*9
10*10
Concentration [CFU/ml]
ASL
ARL
C
Germination percentage [%]
15
20
25
30
Temperature [°C]
GP
D
ASL and ARL [cm]
15
20
25
30
Temperature [°C]
ASL
ARL
E
Germination percentage [%]
4
8
12
16
20
24
Duration [Hours]
GP
F
ASL and ARL (cm)
4
8
12
16
20
24
Duration (Hours)
ASL
ARL
G
Germination percentage [%]
5.5
6.0
6.5
7.0
7.5
8.0
8.5
pH
PDB broth GP
Hoagland's medium GP
NaCl solution GP

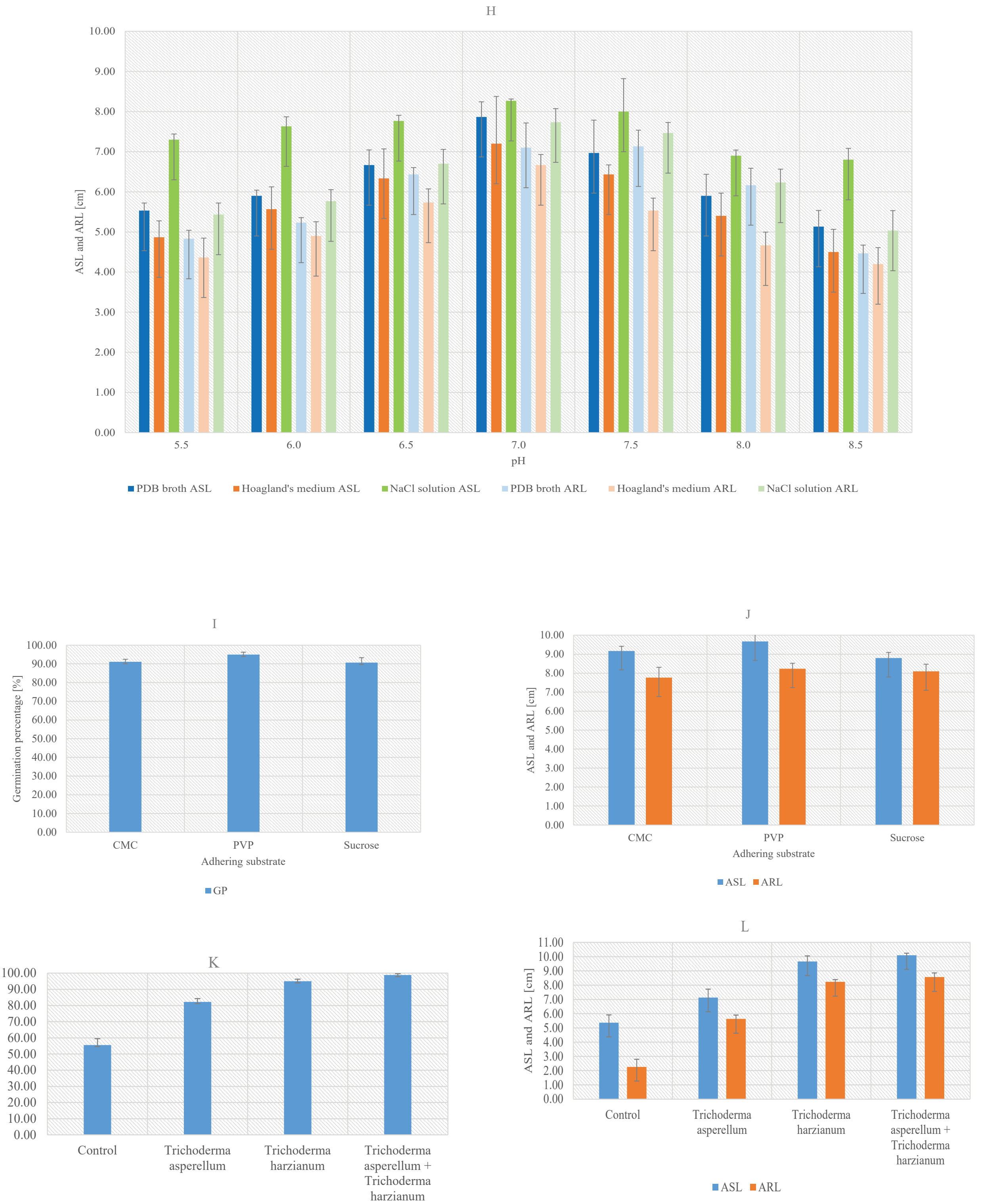

Figure 1. Standardization of various biophysical parameters affecting seed biopriming of *Lepidium sativum* L. using *Trichoderma harzianum.* Effect of concentration (A and B), temperature (C and D), duration (E and F), solution and pH (G and H), adhering substrate (I and J), and strains (K and L) on GP, ASL and ARL at the 10th day of seed sowing. Note: GP – germination percentage; ASL – average shoot length of seedling; ARL – average root length of seedling; CFU – colony forming unit; PDB – potato dextrose broth; CMC – carboxymethyl cellulose; PVP – polyvinyl pyrrolidone.

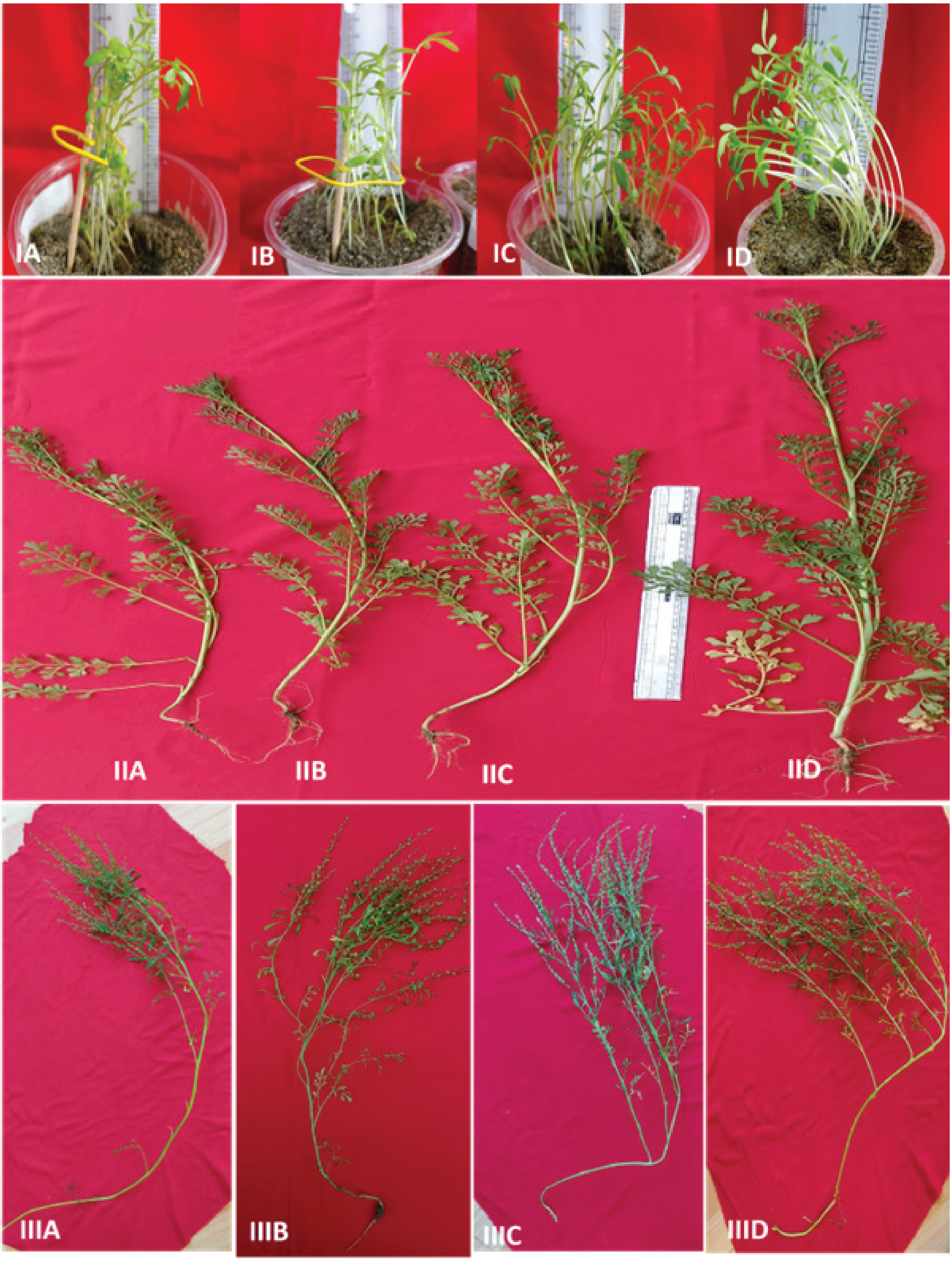

Figure 2. Effect of biopriming of *Lepidium sativum* L. at different stages (1) seedling stage at the end of 10th day (IA, IB, IC, and ID) (2) mature plant stage at the end of 45th day (IIA, IIB, IIC, and IID). (3) yield stage at approximately 90th days (IIIA, IIIB, IIIC and IIID). A – Control; B – *Trichoderma asperellum*; C – *Trichoderma harzianum* and D – *Trichoderma asperellum* + *Trichoderma harzianum*.

methodology where microbial treatment enhances the productivity of a plant by increasing its metabolic activity and alleviating the biotic and abiotic stresses (Abdel-Kader *et al*. 2024; Saha *et al*. 2025). Biopriming has demonstrable impact on morphological, physiological, and biochemical parameters and enhanced the overall plant growth and development (Prasad *et al.* 2020). It also impacts the higher grain yield (Sutariati *et al*. 2021), reduced crop losses (Khulbe 2019), salt tolerance (Rawat *et al.* 2012), seed quality (Ilyas *et al.* 2015; Forti *et al.* 2020), dormancy stress (Zulueta-Rodríguez *et al.* 2015), and reduction of pesticide use (Jurado *et al.* 2023. Generally, in various crops, biopriming has been done without much standardization of factors affecting biopriming methodology. But in our study using *L. sativum* L., we have undertaken the different biophysical parameters like concentration of microbes, temperature, duration, solution, and their pH, adhering substrate, and the combination of fungal species needed for establishing an efficient biopriming protocol. Singh *et al*. (2020a) reported that among the various concentrations, *T. asperellum* gave the maximum result in germination percentage (GP) at concentrations of 10^7 and 10^8 CFU/mL, with similarity to our findings, where 10^8 CFU/mL of *T. harzianum* showed the highest GP as compared to other treatments. Manzar & Singh (2019) reported that various strains of *Trichoderma* spp. increased the shoot and root length at 10^8 CFU/mL, our results were in correlation, where biopriming with fungal strain individually or in combination also enhanced the GP and seedling growth. At moderate concentrations (10^7 and 10^8 CFU/mL), *Trichoderma* spp. promotes the germination and overall growth of the plant, while at higher concentrations they may cause the phytotoxic effect and negatively affect the microbial activity (Iqbal *et al*. 2024; Shah *et al*. 2025). Trichoderma spp. are the most ubiquitous fungal saprophytes that can often grow as opportunistic symbionts, are eco-friendly, and are not reported to exert any environmental hazard. Soil-borne pathogens can significantly impact the yield of chilli and tomato crops. The study was conducted to explore the impact of various salts (NaCl, $MgCl_2$, $CaCl_2$, and KCl. *Trichoderma* spp. were more potent at lower temperatures and enhanced root elongation, biomass and overall plant growth, which may be due to the production of secondary metabolites (Ferrigo *et al*. 2020; Rostamikia *et al*. 2024). It coincides with our study, where lower temperature (20°C) maintains higher GP and seedling growth as compared to higher temperatures (25 – 30°C). The duration of co-cultivation with the fungal strain is also a very important factor affecting the efficacy of biopriming. Studies showed that *Trichoderma* spp. increase the GP, SVI, and seedling growth in *Glycine max* (L.) Merr. (Szemruch *et al*. 2024), *Capsicum frutescens* L. (Pavani *et al*. 2024; Rahmadina *et al*. 2024), and *Solanum lycopersicum* L. (Ariyo *et al*. 2024) at optimum duration (12 – 14 hours), and also reported that prolonged exposure of priming reduces the efficacy due to over-colonization and degrades the seed coat. In our study, a similar kind of result has been observed at a higher duration, where biopriming reduces the GP and seedling growth. Another important parameter is the solution used for the biopriming and its pH, which significantly affects the efficiency of the process. NaCl solution increases the maximum plant growth, which may be because it increases the stress-related protein that helps in better survival as compared to other treatments (Ahmad *et al*. 2015; Khomari & Davari 2017). Among the different pH ranges, it was reported that *Trichoderma* spp. activity was more prominent at neutral pH (Ferrigo *et al*. 2020) even under stress conditions (Paul & Rakshit 2023), and deviation from that optimum pH reduces the fungal enzyme production, plant growth benefits, and colonization. The effect of biopriming can be further increased by increasing the adhesion of the fungal strains with the seeds. So, the selection of a suitable adhering substance is important for achieving the maximum efficacy of the biopriming. Among the different adhering substrates, Polyvinyl pyrrolidone (PVP) acts as a better stabilizing polymer because it improves the biological integrity by reducing the precipitation of protein and aggregation of cells (Brahmaprakash *et al*. 2020).

Under the optimized conditions of biopriming, our results showed that the combined application of two *Trichoderma* species promotes GP and seedling growth as well as SVI and SSI and similar to the finding of *Brassica rapa* L. where *Trichoderma* spp. either alone or in combination increases the germination parameters, and this could be possible because of enhanced phytohormone production, siderophore production, and potassium absorption (Chen *et al*. 2021). *Trichoderma* strains have also been reported to improve nutrient uptake, root-shoot growth, and enzymatic activity, which leads to higher SVI (Mukhopadhyay & Sitansu 2012), which is in accordance with our study, where biopriming with *Trichoderma* spp. increases the SVI and SSI. The significant increase in the RWC has also been reported in our study using the *Trichoderma* spp. as biopriming agent, which is in accordance to the previous report where RWC was promoted due to the implication of *Trichoderma* spp. and increased the root growth and water content, ending up with improving water status due to some physiological and biochemical changes (Guler *et al*. 2016; Singh *et al*. 2023). Various reports also suggested that *Trichoderma* strains regulate metabolic pathways like antioxidant enzyme production, nutrient assimilation, and secondary metabolite biosynthesis (Ahmad *et al*. 2015; Khomari & Davari 2017; Paul & Rakshit 2023). It has also been reported that *Trichoderma* spp. reduces the effect of stress-related protein and reactive oxygen species (ROS) in *Brassica napus* L. (Poveda 2020; Garstecka *et al*. 2023), which promotes the plant height and yield (Hasan *et al*. 2023) accordingly in our findings *Trichoderma* spp. has increased growth and yield of garden cress. Our result showed that *Trichoderma* spp. either alone or in combination increases the number of branches, which increases the leaf number and consequently increases the yield, and resembles another study where *T. asperellum* also increased the number of leaves and consequently enhanced the yield in *Pisum sativum* L. (Singh *et al*. 2016). Garstecka *et al.* (2023) reported that *Trichoderma viride* increases the yield in *Brassica napus* L. through the mechanism of colonization around the root of non-mycorrhizal plants, similar to our findings, where *Trichoderma* spp. either alone or in combination increases the yield. Zhang *et al*. (2019) observed that an increase in root activity consequently increases the nutrient uptake and ultimately enhances the biomass of the plant. Similarly, in our study, biopriming with *Trichoderma* spp. increases the biomass of the plant. Negi *et al.* (2021) and Kumari *et al.* (2020) reported that *Trichoderma* strain increases the seed yield, and similar to our findings, biopriming with both strains individually or in combination increases the yield as compared to untreated plants.

CONCLUSIONS

The present findings established the substantial consequences of microbial treatment on promoting seed germination, seedling length, vigour index, and development of plants by thoroughly evaluating the biophysical conditions in managing seed biopriming in garden cress (*Lepidium sativum* L.). The biophysical parameters for maximum seed germination and seedling growth were optimised with *T. harzianum,* and the best response was obtained at a concentration of 10^8 CFU/mL dissolved in a 1% saline solution at pH 7.0 with PVP as adhering substrate and incubated for 16 hours at 20°C. The biopriming of garden cress with a combined application of two fungal strains, *T. asperellum* and *T. harzianum* possess a collaborative impact that contributed noteworthy improvements across various parameters at the different developmental stages from seedling to maturity of plants, which ultimately increased the growth and yield of garden cress. These findings help in future studies that seed biopriming is an eco-friendly approach for sustainable agriculture production without the use of harsh chemicals.

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