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Effects of Seed Soaking Treatments and Growing Media on Growth, Yield, and Disease Incidence of Sunflower Microgreens (Helianthus Annuus L.)

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ABSTRACT

Sunflower microgreens (Helianthus annuus L.) are high-value horticultural products with promising nutritional attributes; however, their productivity is often constrained by non-uniform germination and early disease incidence. This study evaluated the effects of seed soaking treatments and growing media on growth, yield, disease incidence, and vitamin C content of sunflower microgreens. The experiment was arranged in a two-factor completely randomized design with three replications. The first factor was seed soaking treatment: no soaking, soaking in distilled water at 23°C for 12 h, soaking in hot water at 50°C for 10 min, and soaking in a Trichoderma-based biological agent at 5 mL L-1 for 24 h. The second factor was growing medium: rockwool, cocopeat, and peatmoss. Observed variables included disease incidence, radicle emergence, cotyledon emergence, germination, microgreen establishment, plant height, economic fresh weight, and vitamin C content. The results showed significant treatment effects on several growth and yield variables. Seed soaking with the biological agent produced the best overall performance, particularly for germination percentage (84.89%), economic fresh weight per plant (449.78 mg), economic fresh weight per tray (21,167.78 mg), and vitamin C content (142.76 mg 100 g-1). Peatmoss supported higher vegetative growth and biomass accumulation, whereas cocopeat resulted in the highest vitamin C content (143.73 mg 100 g-1) but lower vegetative performance. These findings indicate that appropriate seed soaking and growing medium selection are essential for improving sunflower microgreen production and quality.

Keywords: biological agent, growing medium, seed treatment, sunflower microgreens, Trichoderma

INTRODUCTION

Microgreens are young edible vegetables harvested at an early developmental stage, generally 7-14 days after sowing, when cotyledons have expanded and true leaves may begin to emerge. They have gained popularity because of their short production cycle, attractive appearance, soft texture, distinctive flavor, and high nutritional potential (Thuong & Minh, 2020; Sisriana et al., 2021; Gofar et al., 2022). Microgreens are commonly consumed fresh in salads, soups, sandwiches, and as garnishes, making visual quality and uniform growth important commercial attributes.

Microgreens are frequently cultivated indoors under artificial lighting, particularly light-emitting diode (LED) systems, which can influence the accumulation of phytochemicals such as phenolic compounds, vitamins, glucosinolates, chlorophyll, and carotenoids (Zhang et al., 2020). In addition to light, the growing medium plays a critical role in microgreen production. An ideal medium should be porous, provide adequate aeration and drainage, retain sufficient moisture, and support nutrient and mineral availability (Sisriana et al., 2021). Common media used for microgreen cultivation include rockwool, cocopeat, compost, rice husk charcoal, and peatmoss.

Sunflower microgreen production often faces problems during germination, including fungal contamination and non-uniform seedling emergence. These constraints are partly related to the thick and hard seed coat of sunflower seeds, which may restrict water uptake and delay imbibition (Maulida et al., 2023). Seed soaking is a practical pre-sowing treatment that can shorten the dormancy phase, soften the seed coat, and accelerate germination. Several soaking methods may be used depending on seed characteristics and treatment objectives, including distilled water soaking, hot-water treatment to reduce physical dormancy or surface contamination, and biological agents that may stimulate early growth and suppress seed-borne pathogens.

Based on these considerations, evaluating combinations of seed soaking treatments and growing media is necessary to determine optimal cultivation practices for sunflower microgreens. This study aimed to assess the effects of different seed soaking treatments and growing media on growth, yield, disease incidence, and vitamin C content of sunflower microgreens, and to identify the most suitable treatment combinations for improving production performance.

MATERIALS AND METHODS

The study was conducted from March to April 2025 in the microgreen production room of PT Kropnation Utama Lestari, Jl. Mertasari No. 55, South Denpasar, Indonesia. Laboratory analyses were carried out at the Laboratory of Agronomy and Horticulture, Faculty of Agriculture, Udayana University. The materials used were sunflower seeds, rockwool, cocopeat, peatmoss, a Trichoderma-based biological agent, AB Mix Pramudita nutrient solution, tissue paper, filter paper, distilled water, starch, and iodine. The equipment included rectangular thinwall containers (8.4 cm x 11 cm), a one-tier microgreen rack (120 cm x 60 cm), 20 W white LED lamps, measuring cylinders, sprayers, rulers, knives, scissors, a digital balance, plastic bottles, stationery, cloth, shovels, pipettes, plastic bags, labels, a mobile phone camera, baskets, a heating pan, mortar and pestle, funnels, and beakers.

The experiment used a completely randomized design with two factors and three replications. The first factor was seed soaking treatment, consisting of four levels: no soaking (Pk), soaking in distilled water at 23°C for 12 h (Pd), soaking in hot water at 50°C for 10 min (Pp), and soaking in a Trichoderma-based biological agent for 24 h at a concentration of 5 mL L-1 (Ph). The second factor was growing medium, consisting of three levels: rockwool (Mr), cocopeat (Mc), and peatmoss (Mp).

Ten observation variables were measured: disease incidence, radicle emergence percentage, time to cotyledon emergence above the medium, cotyledon leaf emergence percentage, germination percentage, microgreen establishment percentage, microgreen height, economic fresh weight per plant, economic fresh weight per tray, and vitamin C content. Data were analyzed using analysis of variance (ANOVA) based on the F-test at the 5% level. When the interaction effect was significant, means were separated using Duncan's Multiple Range Test (DMRT) at the 5% level. When the interaction was not significant but a main factor was significant, mean separation was conducted using the least significant difference (LSD) test at the 5% level.

RESULTS AND DISCUSSION

The interaction between seed soaking treatment and growing medium significantly affected disease incidence, cotyledon leaf emergence, microgreen establishment, and microgreen height. Other variables were not significantly affected by the interaction (Table 1).

As a main factor, seed soaking treatment had a highly significant effect on disease incidence, cotyledon leaf emergence, germination percentage, microgreen establishment, plant height, economic fresh weight per plant, and economic fresh weight per tray. It also significantly affected vitamin C content. Growing medium had a highly significant effect on most observed variables, whereas its effect on vitamin C content was not significant (Table 1).

Disease incidence was significantly affected by the interaction between seed soaking treatment and growing medium. In cocopeat (Mc), disease incidence remained high and did not differ significantly among soaking treatments, ranging from 93.33% to 100.00%. This indicates that cocopeat was less responsive to soaking treatments in suppressing disease incidence. In contrast, rockwool (Mr) and peatmoss (Mp) showed clearer responses to seed soaking. The untreated control (Pk) produced high disease incidence in both media, whereas distilled water, hot-water, and biological-agent soaking reduced disease incidence, particularly in rockwool, where disease incidence decreased to approximately 10% (Table 2). The highest disease incidence occurred in the untreated seeds grown in cocopeat (PkMc), reaching 100.00%. This result suggests that without pre-sowing treatment, sunflower seeds were more vulnerable to early pathogen infection. The high moisture retention and relatively low aeration of cocopeat may have created conditions favorable for fungal and bacterial development. Therefore, the effectiveness of seed soaking in reducing disease incidence depended strongly on the growing medium.

Cotyledon leaf emergence was also significantly affected by the interaction between seed soaking treatment and growing medium (Table 3). The biological-agent soaking treatment (Ph) consistently produced the highest cotyledon leaf emergence across all media, with the highest value of 78.67% in rockwool and peatmoss.

Cocopeat generally resulted in lower cotyledon leaf emergence than rockwool and peatmoss, particularly in the untreated control, which reached only 19.33%. Distilled water and hot-water soaking produced better emergence than no soaking in several media, but their effects were lower than those of biological-agent soaking. These findings indicate that Trichoderma-based soaking was more effective in supporting early cotyledon development, especially when combined with rockwool or peatmoss.

Microgreen establishment was also affected by the interaction between seed soaking treatment and growing medium (Table 4). The biological-agent treatment produced the highest microgreen establishment across all growing media, reaching 94.67% in peatmoss. Cocopeat showed lower establishment than rockwool and peatmoss, especially under hot-water soaking, which produced only 57.33% establishment.

Table 1. Significance of the effects of seed soaking treatment and growing medium on growth and yield variables of sunflower microgreens

No.	Observed variable	Treatment		
		P	M	PM
1	Disease incidence (%)	**	**	**
2	Radicle emergence (%)	ns	**	ns
3	Time to cotyledon emergence above medium (days)	ns	**	ns
4	Cotyledon leaf emergence (%)	**	**	**
5	Germination percentage (%)	**	**	ns
6	Microgreen establishment (%)	**	**	**
7	Microgreen height (cm)	**	**	*
8	Economic fresh weight per plant (mg)	**	**	ns
9	Economic fresh weight per tray (mg)	**	**	ns
10	Vitamin C content (mg/100 g)	*	ns	ns

Notes: ns = not significant (P >= 0.05)
* = significant (P < 0.05)
** = highly significant (P < 0.01)
P = seed soaking treatment
M = growing medium

Table 2. Interaction between seed soaking treatment (P) and growing medium (M) on disease incidence (%)

Treatment	Pk	Pd	Pp	Ph	LSD 5%
Mr	99.33 a A	10.00 b C	10.67 b C	10.00 b B	7.35
Mc	100.00 a A	93.33 a A	99.33 a A	94.67 a A	7.35
Mp	98.00 a A	21.33 c B	50.00 b B	15.33 c B	7.35
LSD 5%	6.37	6.37	6.37	6.37	

Notes: Values followed by the same letters within rows or columns are not significantly different according to the LSD test at 5%.

Table 3. Interaction between seed soaking treatment (P) and growing medium (M) on cotyledon leaf emergence (%)

Treatment	Pk	Pd	Pp	Ph	LSD 5%
Mr	63.33 b A	54.00 c B	50.67 c B	78.67 a A	6.61
Mc	19.33 d C	42.67 b C	31.33 c C	73.33 a A	6.61
Mp	56.67 c B	62.00 bc A	64.00 b A	78.67 a A	6.61
LSD 5%	5.73	5.73	5.73	5.73	

Notes: Values followed by the same letters within rows or columns are not significantly different according to the LSD test at 5%.

In rockwool and peatmoss, microgreen establishment was relatively high and stable across most soaking treatments, with several treatments showing no significant differences. This result suggests that the biological-agent treatment was the most reliable for improving microgreen establishment, whereas rockwool and peatmoss provided more favorable physical conditions for sunflower microgreen growth than cocopeat.

Microgreen height was significantly affected by the interaction between seed soaking treatment and growing medium (Table 5). The biological-agent treatment produced the tallest microgreens in all media, with the highest value of 6.96 cm in peatmoss. Peatmoss showed the best overall response, especially under distilled-water and biological-agent soaking. In contrast, cocopeat consistently produced the lowest plant height across all soaking treatments, with a maximum height of only 4.23 cm under biological-agent soaking. These results confirm that the combination of seed soaking treatment and growing medium strongly influenced physical growth, with peatmoss combined with the biological agent being the most favorable treatment.

Table 4. Interaction between seed soaking treatment (P) and growing medium (M) on microgreen establishment (%)

Treatment	Pk	Pd	Pp	Ph	LSD 5%
Mr	86.67 a A	91.33 a A	88.67 a A	92.00 a A	7.69
Mc	63.33 bc B	65.33 b B	57.33 c C	94.00 a A	7.69
Mp	88.00 bc A	90.67 a AB	82.00 c B	94.67 a A	7.69
LSD 5%	6.66	6.66	6.66	6.66	

Notes: Values followed by the same letters within rows or columns are not significantly different according to the LSD test at 5%.

Table 5. Interaction between seed soaking treatment (P) and growing medium (M) on microgreen height (cm)

Treatment	Pk	Pd	Pp	Ph	LSD 5%
Mr	5.76 b A	6.09 ab B	6.10 ab A	6.43 a B	0.36
Mc	3.51 b B	3.96 ab C	3.99 ab C	4.23 a C	0.36
Mp	5.53 c AB	6.59 b A	5.25 c B	6.96 a A	0.36
LSD 5%	0.31	0.31	0.31	0.31	

Notes: Values followed by the same letters within rows or columns are not significantly different according to the LSD test at 5%.

For radicle emergence, seed soaking treatment did not produce a significant effect. Based on Table 6, radicle emergence under seed soaking treatments ranged from 95.33% to 98.00%, while the growing media ranged from 93.00% to 98.33%. The untreated control showed the highest radicle emergence among soaking treatments (98.00%), although it was not significantly different from distilled-water, hot-water, and biological-agent soaking. This indicates that different soaking treatments did not substantially alter the ability of sunflower seeds to initiate radicle growth.

The time required for cotyledons to emerge above the medium differed significantly. The untreated control produced the fastest cotyledon emergence at 3.44 days, followed by biological-agent soaking (3.56 days), distilled-water soaking (3.67 days), and hot-water soaking (3.89 days). Among growing media, peatmoss produced the fastest emergence (3.25 days), followed by rockwool (3.75 days), while cocopeat required the longest time (3.92 days). Faster cotyledon emergence may be associated with better aeration and moisture balance. Peatmoss generally has high porosity and adequate drainage, which may improve oxygen availability for the embryo and accelerate early shoot development.

Germination percentage varied clearly among treatments. The biological-agent soaking treatment produced the highest germination percentage (84.89%), which was significantly higher than distilled-water soaking (64.00%), no soaking (64.00%), and hot-water soaking (61.56%) (Table 6). For growing media, peatmoss produced the highest germination percentage (77.83%), followed by rockwool (71.33%) and cocopeat (56.67%). These results suggest that the biological-agent treatment may have reduced biotic stress, improved uniform hydration, and supported early metabolic processes during germination.

Seed soaking treatment significantly affected economic fresh weight per plant. The biological-agent treatment produced the highest value (449.78 mg), which was significantly greater than the other treatments, which ranged from 266.67 to 323.47 mg (Table 6). This increase in fresh weight may be linked to the ability of the biological agent to promote early vegetative growth. Growing medium also affected fresh weight per plant, with peatmoss producing the highest value (372.70 mg) and cocopeat the lowest (251.73 mg). These results indicate that aeration, moisture retention, and nutrient availability in the growing medium directly contributed to biomass accumulation.

The biological-agent treatment also produced the highest economic fresh weight per tray (21,167.78 mg), which was significantly different from the other treatments. The untreated control produced the lowest fresh weight per tray (11,684.44 mg), while distilled-water and hot-water treatments produced intermediate values. Growing medium also had a significant effect: peatmoss produced the highest fresh weight per tray (17,389.17 mg), followed by rockwool (16,809.17 mg), and both were significantly higher than cocopeat (11,072.50 mg). Thus, biological-agent soaking combined with peatmoss or rockwool was the most effective approach for increasing economic fresh weight of sunflower microgreens.

Vitamin C content was significantly affected by seed soaking treatment. Biological-agent soaking produced the highest vitamin C content (142.76 mg 100 g-1), followed by distilled-water soaking (140.80 mg 100 g-1) and hot-water soaking (136.89 mg 100 g-1), whereas the untreated control produced the lowest value (109.51 mg 100 g-1). Although growing medium did not significantly affect vitamin C content, cocopeat showed the numerically highest value (143.73 mg 100 g-1), followed by peatmoss (129.07 mg 100 g-1) and rockwool (124.67 mg 100 g-1). These results suggest that seed soaking and growing medium may influence physiological conditions related to vitamin C biosynthesis during early plant development.

Table 6. Main effects of seed soaking treatment (P) and growing medium (M) on radicle emergence, cotyledon emergence, germination, economic fresh weight, and vitamin C content of sunflower microgreens (Helianthus annuus L.)

Treatment	Radicle emergence (%)	Cotyledon emergence (day)	Germination (%)	Economic fresh weight per plant (mg)	Economic fresh weight per tray (mg)	Vitamin C content (mg/100 g)
Seed soaking treatment						
Pk	98.00 a	3.44 b	64.00 b	266.67 c	11684.44 c	109.51 b
Pd	95.33 a	3.67 a	64.00 b	323.47 b	14583.33 b	140.8 a
Pp	95.78 a	3.89 a	61.56 b	287.31 b	12925.56 bc	136.89 a
Ph	96.00 a	3.56 a	84.89 a	449.78 a	21167.78 a	142.76 a
LSD 5%	-	0.36	9.93	41.61	2526.70	24.12
Growing medium						
Mr	93.00 b	3.75 a	71.33 ab	370.98 a	16809.17 a	124.67 a
Mc	98.33 a	3.92 a	56.67 b	251.73 b	11072.50 b	143.73 a
Mp	97.50 a	3.25 b	77.83 a	372.70 a	17389.17 a	129.07 a
LSD 5%	2.46	0.31	8.60	36.04	2188.19	20.89

Notes: Values followed by the same letters in the same column are not significantly different according to the LSD test at 5%.

Figure 1. Experimental units of sunflower microgreens

The results presented in Tables 2-6 demonstrate that seed soaking treatment and growing medium influenced sunflower microgreen growth, yield, disease incidence, and nutritional quality. In general, the variables showed a sequential relationship: early disease suppression affected cotyledon development, cotyledon development influenced microgreen establishment, and successful establishment contributed to plant height and fresh biomass. This pattern indicates that the early germination phase is a critical foundation for subsequent microgreen performance. This finding is consistent with Gofar et al. (2022), who emphasized that early germination strongly determines the success of horticultural seedling growth, including microgreens. However, vitamin C content did not fully follow the same pattern, suggesting that nutritional quality may be influenced by physiological stress and medium-specific conditions rather than vegetative growth alone.

Seed infection can disrupt early physiological processes, particularly enzymatic activity involved in reserve mobilization within the endosperm. Seeds affected by pathogens may experience inhibited radicle and plumule growth, reducing the metabolic energy available for subsequent development. Consequently, many seeds may fail to reach later growth stages. Saputra and Mardiana (2023) reported that germination success is strongly determined by seed viability, vigor, and early environmental conditions. Similarly, Gilbert et al. (2023) reported that appropriate seed disinfestation or pre-sowing treatment can reduce the risk of seed-borne fungi and bacteria. In the present study, high disease incidence in the untreated cocopeat treatment likely contributed to poor cotyledon emergence, whereas biological-agent soaking combined with rockwool or peatmoss reduced early disease pressure and supported better cotyledon development.

Figure 2. Fungal hyphae observed during early sunflower microgreen growth on rockwool, cocopeat, and peatmoss media

Figure 3. Sunflower seed sowing and wrapping process

The main-effect results in Table 6 further clarify the relationship among variables. The biological-agent soaking treatment consistently produced superior results for most parameters, including the highest germination percentage (84.89%), economic fresh weight per plant (449.78 mg), economic fresh weight per tray (21,167.78 mg), and vitamin C content (142.76 mg 100 g-1). Peatmoss also supported strong performance, with a germination percentage of 77.83%, fresh weight per plant of 372.70 mg, and fresh weight per tray of 17,389.17 mg. Although cocopeat was less favorable for morphological growth, it produced the highest numerical vitamin C content (143.73 mg 100 g-1). This suggests that vegetative growth and biochemical quality may not always increase simultaneously. Mild stress under less favorable media conditions may trigger secondary metabolite pathways, including vitamin C biosynthesis, which functions as an antioxidant. This interpretation is consistent with Žlabur et al. (2021), who reported that mechanically induced or environmental stress can increase specialized metabolites in leafy vegetables.

The biological-agent treatment showed the most consistent positive effect on sunflower microgreen performance. Trichoderma is widely recognized as a biological control agent that may act through mycoparasitism, antibiosis, competition for space and nutrients, and induced systemic resistance (Contreras-Cornejo et al., 2024; Yao et al., 2023). It can also contribute to plant growth promotion by improving nutrient availability and stimulating plant defense-related metabolism. Comparable biofertilizer effects have been reported for other plant growth-promoting microorganisms, supporting the broader role of microbial inoculants in crop production (Adeleke & Fakoya, 2025). In this study, the combined biocontrol and growth-promoting roles of Trichoderma likely contributed to improved germination, establishment, biomass accumulation, and vitamin C content. Nevertheless, the use of biological agents requires appropriate concentration, application timing, and environmental management to avoid inconsistent performance.

Figure 4. Overall microgreen installation during the experiment

Overall, two practical directions can be drawn from the treatment responses. For growth and yield, biological-agent soaking combined with peatmoss or rockwool was the most suitable approach because it produced consistently strong microgreen establishment, plant height, and fresh biomass. For nutritional quality, cocopeat may offer potential for increasing vitamin C content, but its high disease incidence and lower vegetative performance indicate that additional disease management or medium modification would be required. These findings provide a basis for further studies on biocontrol-based seed treatment and growing-medium optimization to balance biomass production and nutritional quality in sunflower microgreen cultivation.

CONCLUSION

Based on the results and discussion, the following conclusions can be drawn: Seed soaking treatment significantly affected several growth and yield variables of sunflower microgreens. Biological-agent soaking (Ph) consistently produced the best results for germination percentage (84.89%), economic fresh weight per plant (449.78 mg), economic fresh weight per tray (21,167.78 mg), and vitamin C content (142.76 mg 100 g-1). Growing medium significantly affected microgreen growth. Peatmoss supported the highest germination percentage (77.83%) and high economic fresh weight per plant (372.70 mg), whereas cocopeat produced the highest vitamin C content (143.73 mg 100 g-1). Therefore, growing medium selection should be aligned with the production objective, either biomass productivity or nutritional enhancement. The interaction between seed soaking treatment and growing medium produced variable plant responses. Distilled-water soaking, hot-water soaking, and biological-agent soaking combined with rockwool reduced disease incidence to 10.00-10.67%, whereas biological-agent soaking combined with cocopeat, peatmoss, or rockwool produced relatively high cotyledon emergence and microgreen establishment.

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