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Effect of substrate additives on oyster mushroom growth (*Pleurotus sp.*): cultivation timeline and reuse of the spent substrate for radish germination.

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Abstract

Oyster mushrooms (*Pleurotus* spp.) convert agricultural residues into food, but the value of low-cost additives to paddy straw and the fate of the spent substrate are rarely examined together. This study compared three locally available additives — seaweed extract (20 mL of a 2 g L^{-1} solution injected after mycelium set), egg shell powder (2 g per bag) and coir pith (20 g dry weight per bag) — with a paddy straw control in a completely randomised design with two bags per treatment. The spent substrate of each treatment was then tested as a component of a vermicompost–soil medium for radish (*Raphanus sativus*) germination. Egg shell powder gave the earliest pinning (17.5 ± 0.7 days vs. 23.5 ± 3.5 days in the control; −25.5%, Dunnett P = 0.068). Coir pith significantly delayed pinning (32.0 days, +36.2%, P = 0.022); only one of its two bags fruited, and it gave the fewest harvest cycles (0.5 ± 0.7 vs. 3.0 ± 0.0 per bag; P = 0.098). Seaweed extract did not differ from the control in any timeline measure. In the reuse trial (200 seeds per tray), germination ranged from 69.5% (coir pith) to 81.0% (seaweed extract) against 73.0% in the untreated medium. No pairwise difference reached significance (seaweed extract vs. baseline, P = 0.057; overall $\chi^2(4) = 7.99$, P = 0.092), and 74.6% of all 1,000 seeds germinated. Egg shell powder is a promising low-cost additive for earlier fruiting, coir pith is unsuitable as a bulk amendment at this rate, and spent *Pleurotus* substrate is a usable component of a diluted growing medium.

1. Introduction

Mushroom-forming fungi convert low-value lignocellulosic residues such as straw, sawdust and wood chips into high-quality food (Grimm & Wösten, 2018). Oyster mushrooms of the genus *Pleurotus* account for about one quarter of all cultivated mushrooms (Raman *et al.*, 2021), and *P. ostreatus* is the second most cultivated species after *Agaricus bisporus* (De Mastro *et al.*, 2023). Their lignin-modifying enzymes, including laccase and versatile peroxidases, allow rapid colonisation of diverse agro-residues, and the fruiting bodies are rich in protein, dietary fibre, essential amino acids, vitamins and minerals (Raman *et al.*, 2021). Because they need simple substrates and are less sensitive to pathogens than other cultivated mushrooms (De Mastro *et al.*, 2023), they are the usual choice for small farms and teaching laboratories.

The substrate supplies the carbon, nitrogen and minerals for fruiting, and colonisation time and yield change with its composition and condition (Bellettini *et al.*, 2019). Straw is the most common basal material, but its nitrogen content is low; in *P. ostreatus* grown on wheat straw, biological efficiency fell from 26.28% to 15.49% as the straw aged, and nitrogen was limiting in all media tested (De Mastro *et al.*, 2023). Supplementing a basal substrate with small amounts of another material is a common correction, and inexpensive, locally sourced options suit small growers.

Three such additives are of interest. Egg shell is a calcium-rich food waste: rice-straw substrates supplemented with calcium from lime, egg shell or oyster shell raised the yield, biological efficiency and calcium content of *P. ostreatus* (Julian *et al.*, 2018). The dose matters, since up to 2% calcinated oyster shell powder did not delay spawn run or primordium formation in *P. eryngii*, whereas larger amounts suppressed growth (Choi *et al.*, 2011). Coir pith, a by-product of coconut fibre extraction, is lignin-rich, low in nitrogen and highly water-retentive; in a Tamil Nadu comparison of five

substrates, sawdust and sugarcane trash outperformed it on biological efficiency (Sathiyaseelan *et al.*, 2024). Seaweed extracts are established crop biostimulants (Ali *et al.*, 2021; Khan *et al.*, 2009), but in mushroom cultivation seaweed has mainly been mixed into the substrate: 5% seaweed in rice straw raised biological efficiency by 22%, while 10% and 20% reduced it (Hausiku & Mupambwa, 2018). Applying a dilute extract after the mycelium has set has not been reported.

Every crop also leaves a spent substrate, which can serve as compost, feed, a substrate for other fungi or a raw material for enzymes and building products (Economou *et al.*, 2017; Grimm & Wösten, 2018). For small farms the most direct use is as part of a plant growing medium, alongside vermicompost, whose positive effect on plant growth is well documented (Blouin *et al.*, 2019). Whether spent material from different additive treatments performs equally in such a medium is rarely tested.

Two gaps therefore justify this work. Substrate studies emphasise final yield over the timing of pinning, first harvest and later harvest cycles, and they usually ignore the fate of the spent substrate. This study followed the same bags from packing to reuse with two objectives: (1) to compare the cultivation timeline of *Pleurotus* sp. on paddy straw amended with seaweed extract, egg shell powder or coir pith against a paddy straw control; and (2) to assess the spent substrate of each treatment as a growing medium for radish (*Raphanus sativus*), against an untreated vermicompost–soil medium and the paddy straw control. The work was also run as a hands-on, student-led model of low-cost cultivation.

2. Materials and Methods

2.1 Site, design and treatments

The experiment was carried out at the Department of Zoology and Research Centre, Sarah Tucker College, Tirunelveli, from 31 July to 13 September 2026, in a room in the college animal house. A single-factor completely randomised design was used, with four treatments and two replicate bags per treatment (eight bags). All bags contained paddy straw; the treatments are listed in Table 1.

Table 1. Treatments and bag allocation.

Treatment	Bags	Substrate and additive (per bag)	Packed / additive applied
Control	A2, A3	Paddy straw only	1 Aug
Seaweed extract	A0, A10	Paddy straw; 20 mL seaweed extract (2 g L^{-1}) injected after mycelium set	7 Aug; extract on 18 and 25 Aug
Egg shell powder	B1, B2	Paddy straw + 2 g egg shell powder	1 Aug
Coir pith	C1, C2	Paddy straw + 20 g coir pith (dry weight)	31 Jul

2.2 Room preparation, substrate and spawning

The room was fumigated with potassium permanganate crystals and 3% formaldehyde solution, and work began 24 h later. Paddy straw from nearby villages was soaked overnight, boiled until steam was released from its surface, and drained. Spawn of *Pleurotus* sp. was obtained from Tamil Nadu Agricultural University. Bags (2 ft × 1 ft polythene, >50 µm) were packed by the layering method with alternate layers of straw and spawn, using about 200–210 g spawn per bag (filled weight 700–900 g). Egg shell powder and coir pith were mixed in at packing. The packing date was day 0 for each bag.

2.3 Crop management and seaweed extract

Bags hung in the same room at about 26 °C and 80–90% relative humidity, with diffuse light for 8–12 h a day and ventilation through mesh-covered openings. Humidity was maintained by hanging water-soaked gunny bags around the mushroom bags daily rather than by frequent watering. Nano seaweed extract was diluted to 2 g in 1 L of water, and 20 mL was injected into each seaweed bag with a syringe on day 11 and day 18 after packing.

2.4 Control of cobweb infection

Cobweb infection appeared during early colonisation in bags B1, B2, C1 and C2. Each was treated once on 10 August with 10 mL of 3% hydrogen peroxide (diluted from a 6% topical solution) by syringe, and no further infection was seen. This was a sanitation step, not an experimental factor, and was not analysed.

2.5 Cultivation timeline

Dates of pinning (first bud formation), fruiting and harvest were recorded for every bag. Days to first pinning and days to first harvest were counted from each bag's packing date. Harvest cycles per bag were

the number of separate pinning–fruiting–harvest records up to 13 September 2026.

2.6 Reuse of spent substrate for radish germination

After the last harvest, the spent substrate of one representative bag per treatment was used fresh, without drying, in a growing-medium tray. Five trays (1.5 ft × 0.75 ft) were filled with a base medium of vermicompost and garden soil (1:1 by volume): Tray 1, base medium only (Normal baseline); Trays 2–5, base medium plus the spent substrate of the control, seaweed extract, egg shell powder and coir pith bags respectively. Two hundred radish seeds were sown per tray (1,000 in total) at a uniform shallow depth and spacing. All trays were kept together under the same shade and humidity and watered lightly on alternate days. A seed was scored as germinated when its radicle or cotyledon emerged above the surface, and final counts were taken 10 days after sowing, when germination had plateaued. Germination (%) = seeds germinated ÷ seeds sown × 100.

2.7 Statistical analysis

Timeline data were analysed by one-way ANOVA, followed by Dunnett's test comparing each treatment with the control; results are mean ± SD, with significance at $P < 0.05$. A treatment with fewer than two observations was described but not tested. Because each germination tray was a single unreplicated planting, germination counts were analysed as proportions: each spent-substrate tray was compared with the Normal baseline and with the Control tray by a two-proportion z-test (pooled proportion), and a chi-square test of independence was run across all five trays. A non-significant result was read as a difference not confirmed, not as evidence of equality. Analyses used Python (SciPy).

3. Results

3.1 Cultivation timeline

All eight bags formed pins and seven produced fruiting bodies; bag C1 (coir pith) pinned on 1 September but never fruited (Table 2). In the other bags, harvest followed pinning within a median of 2 days.

Table 2. First pinning, first harvest and harvest cycles for each bag (days after packing in brackets).

Bag	Treatment	Packed	First pinning (day)	First harvest (day)	Harvest cycles	Last harvest
A2	Control	1 Aug	27 Aug (26)	29 Aug (28)	3	12 Sep
A3	Control	1 Aug	22 Aug (21)	24 Aug (23)	3	4 Sep
A0	Seaweed extract	7 Aug	28 Aug (21)	30 Aug (23)	2	7 Sep
A10	Seaweed extract	7 Aug	29 Aug (22)	31 Aug (24)	3	13 Sep
B1	Egg shell powder	1 Aug	19 Aug (18)	25 Aug (24)	2	4 Sep
B2	Egg shell powder	1 Aug	18 Aug (17)	20 Aug (19)	4	13 Sep
C1	Coir pith	31 Jul	1 Sep (32)	No fruiting	0	—
C2	Coir pith	31 Jul	1 Sep (32)	4 Sep (35)	1	4 Sep

Treatments differed significantly in days to first pinning (ANOVA, $F(3,4) = 22.16$, $P = 0.006$; Table 3). Egg shell powder pinned earliest, 6.0 days (25.5%) ahead of the control, a difference just short of significance ($P = 0.068$). Seaweed extract pinned 2.0 days earlier than the control ($P = 0.610$). Coir pith pinned 8.5 days (36.2%) later than the control, the only significant difference ($P = 0.022$).

Table 3. Effect of treatment on the cultivation timeline (mean ± SD, n = 2 bags). Brackets give the difference from the control and the Dunnett P value.

Treatment	Days to first pinning	Days to first harvest	Harvest cycles per bag
Control	23.5 ± 3.5 (reference)	25.5 ± 3.5 (reference)	3.0 ± 0.0 (reference)
Seaweed extract	21.5 ± 0.7 (−8.5%; P = 0.610)	23.5 ± 0.7 (−7.8%; P = 0.741)	2.5 ± 0.7 (−16.7%; P = 0.888)
Egg shell powder	17.5 ± 0.7 (−25.5%; P = 0.068)	21.5 ± 3.5 (−15.7%; P = 0.400)	3.0 ± 1.4 (0.0%; P = 1.000)
Coir pith	32.0 ± 0.0* (+36.2%; P = 0.022)	35 (C2 only; n = 1, not tested)	0.5 ± 0.7 (−83.3%; P = 0.098)
One-way ANOVA	$F(3,4) = 22.16$, $P = 0.006$	$F(2,3) = 0.94$, $P = 0.482$†	$F(3,4) = 3.78$, $P = 0.116$

* Significantly different from the control (Dunnett's test, P < 0.05). † Control, seaweed extract and egg shell powder only.

First harvest came 25.5 ± 3.5 days after packing in the control, 23.5 ± 0.7 days with seaweed extract and 21.5 ± 3.5 days with egg shell powder, with no overall treatment effect (F(2,3) = 0.94, P = 0.482). The single coir pith harvest (C2, day 35) was later than any other bag (days 19–28). Egg shell powder bags gave the first harvest of the trial (day 19), followed by the control and seaweed extract bags from day 23 (Figure 2). Harvest cycles per bag were 3.0 in the control and egg shell powder treatments, 2.5 with seaweed extract and 0.5 with coir pith; the coir pith shortfall (−83.3%) was not significant (P = 0.098), and the overall ANOVA was not significant (F(3,4) = 3.78, P = 0.116; Figure 1). All bags fruited in the control, seaweed extract and egg shell powder treatments, against one of two in coir pith (Figure 3); Figure 4 sets each timeline measure against the control on a common percentage scale. Means include the bags treated with hydrogen peroxide (B1, B2, C1, C2).

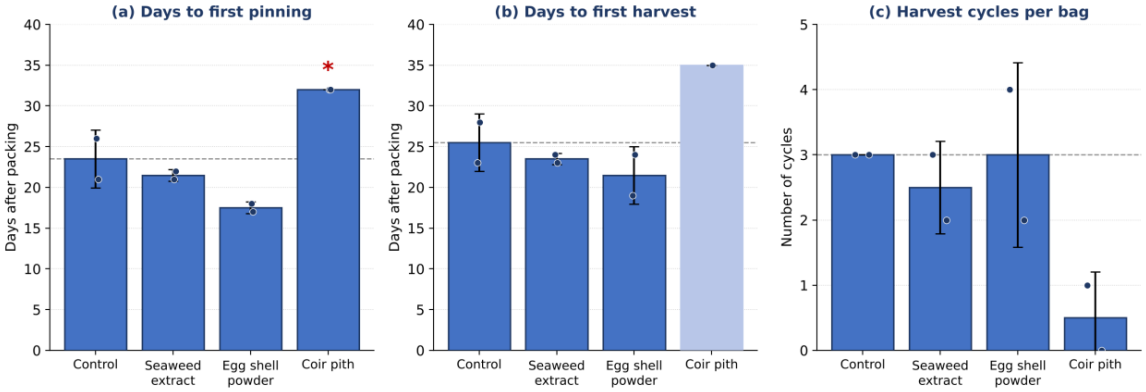


Figure 1. (a) Days to first pinning, (b) days to first harvest and (c) harvest cycles per bag. Bars show mean ± SD of two bags; circles show individual bags; the dashed line marks the control mean. * Significantly different from the control (Dunnett's test, P < 0.05). The pale bar in (b) is a single bag (C2) and was not tested.

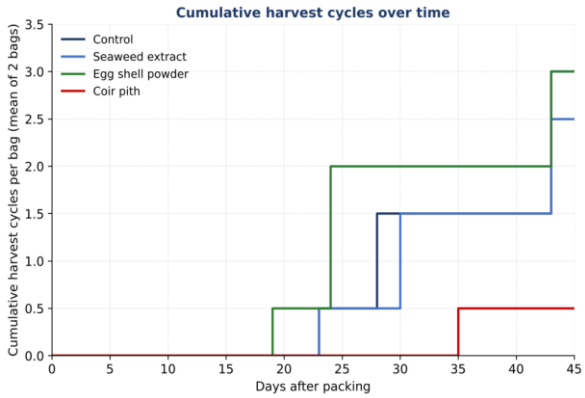


Figure 2. Cumulative number of harvest cycles per bag (mean of two bags) against days after packing for each treatment.

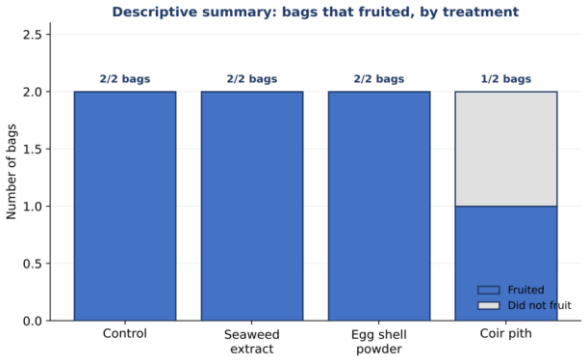


Figure 3. Number of bags that fruited in each treatment, out of two bags per treatment.

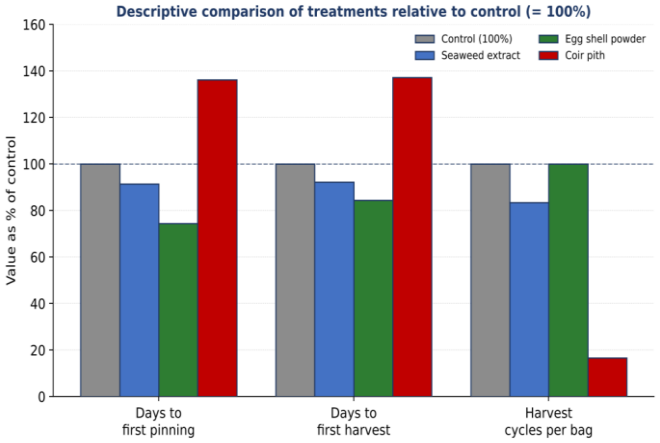


Figure 4. The three timeline measures expressed as a percentage of the control value (control = 100%) for each treatment.

3.2 Germination in spent-substrate growing media

Germination 10 days after sowing ranged from 69.5% to 81.0% across the five trays (Table 4). The seaweed extract spent substrate gave the highest germination (81.0%), 8.0 percentage points above the Normal baseline (73.0%) and 4.5 points above the Control tray (76.5%). The egg shell powder tray equalled the baseline (73.0%). The coir pith tray gave the lowest value (69.5%), 3.5 points below the baseline and 7.0 points below the Control tray.

Table 4. Radish germination per tray (200 seeds sown) and two-proportion z-tests against the Normal baseline and the Control tray.

Tray	Growing medium	Germinated (of 200)	Germination (%)	vs Normal: z (P)	vs Control: z (P)
1	Normal (vermicompost + soil only)	146	73.0	—	—
2	Control (paddy straw) spent substrate	153	76.5	0.81 (0.421)	—
3	Seaweed extract spent substrate	162	81.0	1.90 (0.057)	1.10 (0.271)
4	Egg shell powder spent substrate	146	73.0	0.00 (1.000)	−0.81 (0.421)
5	Coir pith spent substrate	139	69.5	−0.77 (0.439)	−1.58 (0.115)

Overall chi-square test across all five trays: $\chi^2(4) = 7.99$, $P = 0.092$.

No pairwise comparison reached $P < 0.05$; the seaweed extract tray against the baseline came closest ($z = 1.90$, $P = 0.057$). The overall chi-square test was also not significant. Pooled across trays, 746 of 1,000 seeds germinated (74.6%; Figure 7). The ranking was seaweed extract > control > Normal = egg shell powder > coir pith (Figures 5, 6 and 8), consistent with a mild beneficial trend for seaweed extract and a mild adverse trend for coir pith that the data do not confirm.

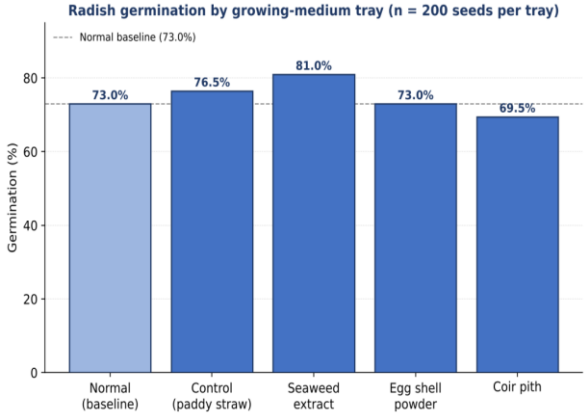


Figure 5. Radish germination percentage by growing-medium tray (200 seeds sown per tray). The dashed line marks the Normal baseline.

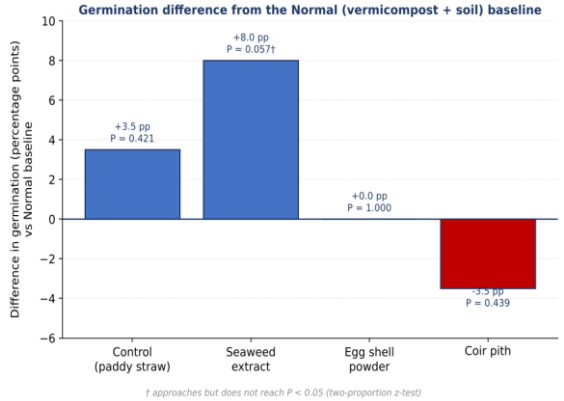


Figure 6. Germination difference (percentage points) of each spent-substrate tray from the Normal baseline, with the two-proportion z-test P value for each comparison.

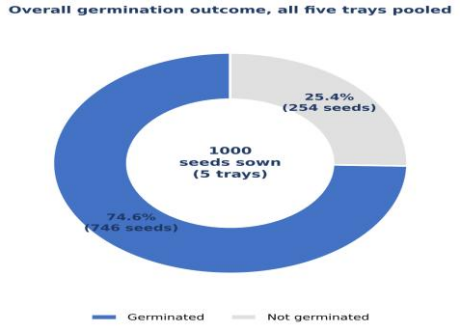


Figure 7. Overall germination outcome with all five trays pooled (1,000 seeds sown in total).

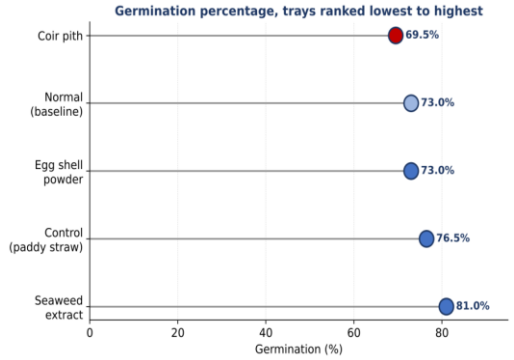


Figure 8. Germination percentage of the five growing-medium trays, ranked from lowest to highest.

4. Discussion

4.1 Eggshell powder and earlier pinning

Egg shell powder brought pinning forward by 6.0 days (25.5%), the largest acceleration in the trial, although it stopped just short of significance (P = 0.068). This agrees with calcium supplementation studies in *Pleurotus*: calcium from egg shell, oyster shell or lime raised yield and biological efficiency on rice straw (Julian *et al.*, 2018), and 2% calcinated oyster shell did not delay spawn run or primordium formation in *P. eryngii*, whereas higher doses suppressed growth (Choi *et al.*, 2011). The 2 g per bag used here is a modest dose relative to substrate weight, consistent with the absence of any growth suppression. A plausible mechanism is a shift in ligninolytic enzyme activity: laccase and manganese peroxidase in *P. florida* peak at fruit-body initiation and change with oyster shell concentration (Naraian *et al.*, 2014). Calcium may also act directly on hyphal growth and signalling. The present data cannot separate a direct calcium effect from one acting through enzymes or substrate pH.

Both egg shell powder bags, and both coir pith bags, received hydrogen peroxide for cobweb infection, whereas no control or seaweed extract bag did. The egg shell powder result therefore cannot be fully separated from the sanitation step. However, the coir pith bags received the same treatment and pinned later rather than earlier, which argues against hydrogen peroxide alone explaining the earlier pinning.

4.2 Seaweed extract: a neutral result tied to the method of delivery

Seaweed extract did not differ from the control in any timeline measure. Its biostimulant effects in crops depend strongly on concentration, timing and method of delivery (Ali *et al.*, 2021; Khan *et al.*, 2009), and the one comparable mushroom study incorporated seaweed into the substrate before spawning, finding a benefit at 5% and losses at 10–20% (Hausiku & Mupambwa, 2018). Here a dilute extract was injected only after colonisation was

largely complete, so the fungus met it late and in small amounts. The neutral result is best read as a first data point on post-colonisation delivery, not as evidence that seaweed has no value for this crop. The seaweed bags were also packed six days later than the others; timeline values were counted from each bag's own packing date, but room conditions over the two periods were not identical.

4.3 Coir pith and the limits of a bulking agent

Coir pith was the only clearly unfavourable treatment: pinning was delayed by 8.5 days (P = 0.022), one of two bags never fruited, and harvest cycles were the lowest. This matches the five-substrate comparison in which coir pith ranked below sawdust and sugarcane trash (Sathiyaseelan *et al.*, 2024). Its high lignin and low nitrogen would be expected to slow colonisation when added in bulk (20 g per bag). Its high water-holding capacity offers a second explanation: excess substrate moisture above the recommended 55–65% is linked to lower biological efficiency (De Mastro *et al.*, 2023). A wetter, slower-colonising substrate may also have been more open to cobweb infection, in which case the poor timeline and the infection reflect the same underlying condition.

4.4 Reuse of spent substrate

No spent substrate made the growing medium unsuitable for radish: germination stayed within 69.5–81.0%, and no tray differed significantly from the untreated baseline. This supports the view that spent mushroom substrate is a resource rather than a waste (Grimm & Wösten, 2018). The direction of the differences is still informative. The seaweed extract tray gave the highest germination (P = 0.057), plausibly because residual biostimulant compounds (Ali *et al.*, 2021; Khan *et al.*, 2009) persisted in the spent material and acted alongside the vermicompost base, which itself promotes plant growth (Blouin *et al.*, 2019). This would mean the extract had a measurable effect on the second crop even though it did not shift the mushroom timeline.

The coir pith tray gave the lowest germination, in line with its weak cultivation performance. Poudel *et al.* (2023) found that spent mushroom compost used at a high share of a microgreen medium reduced radish germination through high salinity and electrical conductivity, and recommended keeping it below 50% of the medium. Zhang *et al.* (2012) likewise found that spent substrate outperformed a peat control for tomato and cucumber seedlings only at a favourable blending ratio. The present design, which diluted spent substrate in a 1:1 vermicompost–soil base, is consistent with that guidance, and the narrow spread of outcomes suggests dilution protected germination. The control and egg shell powder substrates neither improved nor reduced germination at this ratio.

4.5 Limitations and practical value

The study used two bags per treatment and one unreplicated tray per medium. Non-significant differences, such as the egg shell powder effect on pinning (P = 0.068), are therefore unconfirmed rather than absent, and a larger trial could well confirm them. The proportion tests on the trays describe chance variation in seed counts but cannot estimate variation between replicate trays; two or three trays per medium would allow this. The amount of spent substrate added per tray, yield weights and the sanitation imbalance across treatments should be standardised in a follow-up. Even so, a 6-day gain in pinning or an 8-point gain in germination is large enough to matter to a grower. The whole cycle — room preparation, substrate treatment, bagging, monitoring, harvesting and reuse of the spent material — was run by undergraduates with local inputs within one term, which supports its use as a low-cost, low-waste student enterprise model.

5. Conclusion

On paddy straw, 2 g of egg shell powder per bag brought first pinning of *Pleurotus* sp. forward by 25.5% without reducing harvest cycles, making it a promising low-cost additive, although the effect needs confirmation in a larger trial. Seaweed extract injected after mycelium set had no measurable effect on the cultivation timeline. Coir pith at 20 g per bag significantly delayed pinning and reduced fruiting, so it is not recommended as a bulk amendment. Spent substrate from every treatment, diluted in a vermicompost–soil medium, supported radish germination of 69.5–81.0%, with a non-significant advantage for the seaweed extract substrate. Spent *Pleurotus* substrate is therefore a usable growing-medium component, which supports a low-waste, student-run cultivation model.

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