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Evaluation and standardization of Zinc and Iron biofortification techniques in Milky mushroom (*Calocybe indica*)

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ABSTRACT

Micronutrients and mineral deficiencies in the diet continue to pose serious global health challenges, especially in developing regions. The nutrients especially iron (Fe) and zinc (Zn), are often found to be insufficient in a cereal-based diet. This study evaluated the biofortification potential of the edible mushroom *Calocybe indica* through substrate supplementation with iron sulphate ($FeSO_4$) and zinc sulphate ($ZnSO_4$) under controlled cultivation conditions at Kerala Agricultural University. Substrates composed of coir pith and vermicompost were enriched with $FeSO_4$ and $ZnSO_4$ at concentrations of 2.5, 5, 10, and 20 mg/kg on a dry weight basis, with a nine-treatment Completely Randomized Design. Iron supplementation at 5 mg/kg $FeSO_4$ significantly enhanced mushroom growth and yield by approximately 18% compared to control and increased iron accumulation in fruiting bodies to 1427 ppm, demonstrating effective bioaccumulation and improved nutritional quality. However, a higher concentration of 20 mg/kg completely inhibited fruiting, indicating toxicity risks at excessive iron levels. Zinc supplementation improved yield notably, with the highest fresh weight at 2.5 mg/kg $ZnSO_4$ (367 g per bag), nearly double to the yield in control, but did not result in statistically significant increases in zinc content within fruiting bodies, likely due to stringent homeostatic regulation and limited zinc translocation under solid-state cultivation. These findings reveal distinct species- and micronutrient-specific uptake dynamics in *C. indica* and highlight the need for tailored biofortification protocols. Substrate-based micronutrient fortification emerges as a cost-effective strategy for producing nutrient-enriched mushrooms, with *C. indica* showing strong potential as a dietary iron source. Future research should focus on alternative zinc formulations, synergistic nutrient interactions, and genetic approaches to optimize zinc bioavailability and accumulation.

Keywords: *Calocybe indica*, Biofortification, Iron enrichment, Zinc enrichment, Mushroom nutrition

Micronutrient malnutrition, or "hidden hunger," remains a major global health concern, with zinc (Zn) and iron (Fe) deficiencies among the most prevalent forms (Tulchinsky, 2010). The World Health Organization (WHO, 2022) estimates that over two billion people worldwide suffer from essential micronutrient deficiencies particularly zinc and iron. These deficiencies lead to serious health issues such as anemia, impaired cognitive development, weakened immunity, stunted growth, and increased morbidity and mortality, especially among children, pregnant women, and the elderly (Bhan *et al*., 2001). Zinc is essential for enzymatic activity, DNA synthesis, immune function, and cellular signalling (Bonaventura *et al*., 2015), while iron is crucial for oxygen transport and metabolic processes. Such deficiencies are more

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severe in developing regions due to limited dietary diversity (Shankar, 2020), necessitating sustainable and cost-effective interventions.

Conventional strategies like supplementation and food fortification face limitations related to cost, accessibility, and bioavailability (Olson *et al*., 2021). Biofortification, enhancing nutrient content during crop growth, has emerged as a sustainable alternative (Dhaliwal *et al*., 2022). Mushrooms, in particular, are promising candidates due to their rapid growth and ability to efficiently accumulate minerals from substrates (Slyszyk *et al*., 2024).

Edible mushrooms such as *Pleurotus*, *Agaricus*, *Hericium*, and *Calocybe* are nutritionally rich, containing proteins, vitamins, and essential minerals (Das and Prakash, 2022). *Calocybe indica* (milky mushroom), widely cultivated in India for its thermotolerance and high yield, has significant potential for micronutrient biofortification, though it remains underexplored (Thakur, 2014). Unlike plants, mushrooms directly absorb minerals from substrates, allowing better control over biofortification (Singh and Sohrab, 2024).

Recent studies highlight successful mineral biofortification in mushrooms. Ziêba *et al*. (2020) demonstrated selenium and zinc accumulation in *Pleurotus eryngii*, enhancing antioxidant properties. Rani *et al*. (2023) and Deepali *et al*. (2023) reported increased biomass and exopolysaccharide production in zinc-fortified *Calocybe indica*. Similarly, Koreti *et al*. (2023) showed that iron supplementation improved biomass and metabolic profiles. Madaan *et al*. (2024) further reported that selenium and zinc co-fortification in *Pleurotus* species enhanced biomass, phenolics, antioxidant capacity, and protein content, though minor morphological changes such as hyphal shrinkage occurred.

Biofortification efficiency depends on mineral form, concentration, and species. Budzyñska *et al*. (2022) observed species- and salt-specific iron uptake patterns, while Budzyñska *et al*. (2021) demonstrated synergistic effects of iron and calcium. Scheid *et al*. (2020) highlighted species-dependent differences in iron bioavailability. Additionally, studies on other minerals, including lithium (Mleczko *et al*., 2017) and selenium (Bhatia *et al*., 2013; Kaur *et al*., 2018), confirm the broader potential of mushroom biofortification. Advances also include evaluating nutrient bioavailability using in vitro models (Pandey *et al*., 2020) and improving amino acid and phytochemical profiles through fortification (Fadugba *et al*., 2024). However, standardized protocols, particularly for *Calocybe indica*, remain limited.

Therefore, this study aims to optimize zinc and iron biofortification in *Calocybe indica* using zinc sulfate and iron sulfate, focusing on mineral accumulation and bioavailability. The findings are expected to support the development of nutrient-enriched functional foods as a sustainable strategy to combat global micronutrient deficiencies.

MATERIALS AND METHODS

The study was conducted over a period of six months at the Kerala Agricultural University, College of Agriculture, Vellayani. Laboratory analyses were carried at the facilities of the Department of Plant Pathology, Instrumentation labs, and the growth trials were performed in the mushroom house facility of the Instructional Farm.

Substrate Preparation

The base substrate used for the cultivation of *Calocybe indica* (Milky mushroom) was a 4:1 (w/w) ratio mixture of coir pith and vermicompost. This combination was chosen due to its favourable physical properties, including high porosity, water retention capacity, and microbial richness, which support efficient mushroom growth (Koreti *et al.,* 2023 and Rani *et al.,* 2023). Fruiting bags each of 2 kg were prepared by using the above mentioned substrate. The

coir pith was pre-wetted and then soaked for 12 hours in aqueous solutions of either zinc sulphate ($ZnSO_4$) or iron sulphate ($FeSO_4$) at concentrations of 2.5 mg/kg, 5 mg/kg, 10 mg/kg, and 20 mg/kg on a dry weight basis. The concentrations were prepared using distilled water to ensure uniform absorption of the micronutrients throughout the substrate. The substrate enrichment protocols for mineral biofortification in mushrooms reported by Budzyñska *et al.,* 2022; and Deepali *et al.,* 2023 were followed. After soaking, the substrate was thoroughly drained and sterilized using an autoclave at 121°C for 15 - 20 minutes to eliminate potential contaminants and competing microbial flora. A control substrate was prepared following the same procedure but without the addition of any micronutrients.

Experimental Design

The experiment was laid out in a Completely Randomized Design (CRD) comprising nine treatments with four replications each. The treatment structure included four different concentrations of zinc (T1 – 2.5 mg/kg $ZnSO_4$, T2 – 5 mg/kg $ZnSO_4$, T3 – 10 mg/kg $ZnSO_4$, T4 – 20 mg/kg $ZnSO_4$), four concentrations of iron (T1 – 2.5 mg/kg $FeSO_4$, T2 – 5 mg/kg $FeSO_4$, T3 – 10 mg/kg $FeSO_4$, T4 – 20 mg/kg $FeSO_4$), with T9 serving as the control (no micronutrient supplementation). Each treatment was prepared using uniformly packed polypropylene cultivation bags to maintain consistency in growing conditions. The selection of concentrations and experimental layout was adapted with necessary modifications from earlier studies on *Calocybe indica* biofortification and micronutrient supplementation (Koreti *et al.,* 2023; Deepali *et al.,* 2023).

Inoculation and Cultivation Conditions

The grain spawn of *Calocybe indica* procured from Kerala Agricultural University, Instructional farm of College of Agriculture, Vellayani was used to inoculate the fruiting blocks at a rate of 10% wet weight of the substrate. The inoculated bags were incubated under controlled environmental conditions. The temperature was maintained between 28 - 30°C, and relative humidity was maintained at 80 - 90%. Diffused natural light was provided to simulate natural growing conditions, and sufficient ventilation was ensured to prevent contamination and promote optimal mycelial growth. The bags were monitored daily for signs of colonization and contamination.

Growth Parameters and Harvesting

Mycelial growth was regularly observed, and the number of days required for complete colonization of the substrate was recorded. Fully developed sporocarps were harvested after and observations such as the number of fruiting bunches per bag and the fresh weight were recorded. Sporocarp samples from all treatments and their replicates were collected, cleaned, and air-dried for further analysis.

Sample Preparation for Mineral Analysis

Following harvest, mushroom samples were thoroughly cleaned and then dried under shade. The dried samples were ground using a stainless-steel grinder to obtain a uniform fine powder. A representative aliquot of each powdered sample was subjected to wet digestion using a mixture of concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) in a closed-vessel microwave digestion system. After complete digestion, the samples were cooled, filtered through Whatman No. 42 filter paper, and diluted with deionized water to a known volume for elemental analysis. Substrate samples were also collected from each treatment after the final mushroom harvest, dried under similar conditions, ground, and digested following the same protocol for micronutrient determination. All glassware and containers were acid-washed and rinsed with deionized water to avoid any potential contamination.

Determination of Zinc and Iron Content

The concentrations of zinc (Zn) and iron (Fe) in the digested mushroom and substrate samples were

quantified using Atomic Absorption Spectrophotometry (AAS). The instrument was calibrated using certified standard solutions of known concentrations. Appropriate blanks and quality control samples were included in the analysis to ensure accuracy and precision. The mineral content was expressed in milligrams per kilogram (mg/kg) on a dry weight basis. This methodology is consistent with established protocols for mineral analysis in mushroom biofortification studies (Budzyñska *et al.,* 2022; Koreti *et al.,* 2023).

Statistical Analysis

All collected data were statistically analysed using standard procedures. Analysis of variance (ANOVA) was performed to evaluate the significance of different treatments. Mean separation was carried out using the least significant difference (LSD) test at a 5% level of significance. Correlation analysis was also performed to examine the relationship between the zinc and iron concentrations in the substrate and their corresponding accumulation in the fruiting bodies of *Calocybe indica*. The statistical software used for analysis was SPSS and Microsoft Excel.

RESULT

Iron supplementation in growth medium

The influence of Iron enrichment on the sporocarp development in *Calocybe indica*, is illustrated in Fig. 1, while the corresponding harvested sporocarp are presented in Fig. 2. Notably, T4 (20 mg/kg $FeSO_4$)

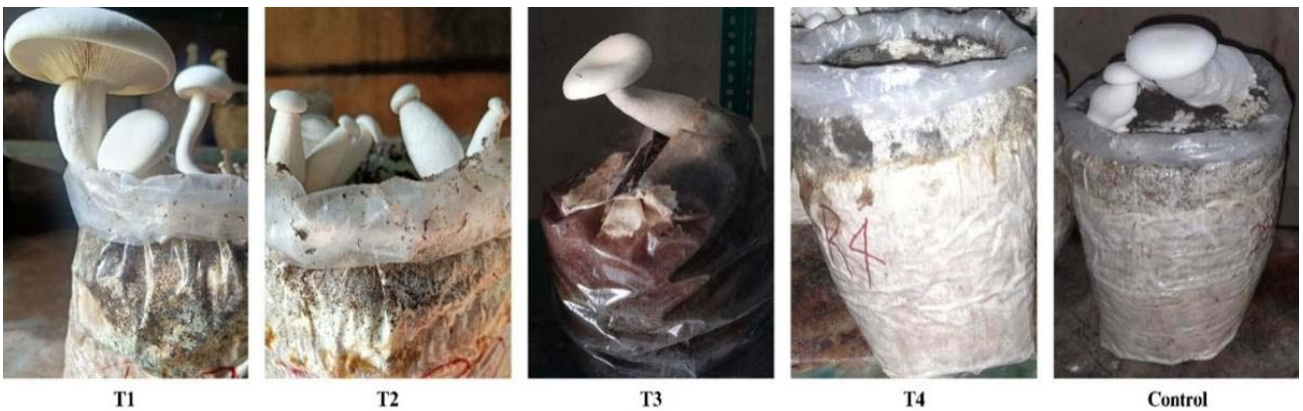

Fig. 1. Fruiting blocks of *Calocybe indica* under different iron enrichment treatments (T1, T2, T3, T4, and Control). Visible variations in fruiting body development reflect the influence of respective $FeSO_4$ concentrations. No fruiting was observed in T4

Fig. 2. Harvested sporocarps of *Calocybe indica* from different iron enrichment treatments. Treatment with 20 mg/kg $FeSO_4$, did not yield sporocarp hence the fruiting block is shown

exhibited no fruiting, indicating potential toxicity or inhibitory effects of the $FeSO_4$ salt at 20mg/kg concentration.

Iron enrichment in growth media significantly influenced the pinhead initiation period in *Calocybe indica*. As shown in Table 1, the shortest time to pinhead emergence was observed in the treatment with 5 mg/kg $FeSO_4$, at an average of 41.50 ± 0.577 days, which was significantly lower than the control (43.75 ± 0.50 days). Treatments with 2.5 mg/kg and 10 mg/kg did not exhibit any significant difference compared with that of the control. Whereas, T4 (20 mg/kg) failed to induce any pinhead, likely due to toxicity or inhibition at higher iron concentrations.

The average fresh weight of sporocarp produced was also influenced by iron concentration in the substrate (Table 1). The treatment 2.5 mg/kg $FeSO_4$ yielded the highest average sporocarp weight of 230.5 ± 1.291 g per bag, significantly outperforming the control (160.0 ± 46.188 g). T2 (5 mg/kg) also produced a moderately higher yield (190.0 ± 34.641 g) compared to control, though the difference was not statistically significant. In contrast, T3 (10 mg/kg) exhibited a marked reduction in yield (110.0 ± 29.439 g), and T4 showed no fruiting, reinforcing the observation that excessive iron levels may negatively impact mushroom productivity.

Iron accumulation in fruiting bodies increased with substrate fortification, though the efficiency varied with concentration. As presented in Table 1, the highest iron content was recorded in T2 (5 mg/kg $FeSO_4$), with 1427.0 ± 127.017 ppm, which was significantly higher than both the control (1226.5 ± 91.221 ppm) and T1 (1282.0 ± 1.291 ppm). However, T3 (10 mg/kg) recorded a lower Fe content (1195.5 ± 145.138 ppm), suggesting a possible threshold beyond which iron uptake becomes inefficient or inhibited. No data were available for T4, due to absence of fruiting bodies.

Analysis of variance (ANOVA) indicated significant differences ($p \leq 0.05$) among treatments for all measured parameters except T4, where no fruiting occurred. Mean comparisons using the Least Significant Difference (LSD) test validated the observed trends. The results demonstrate that moderate $FeSO_4$ enrichment, particularly at 5 mg/kg, can enhance both yield and nutritional value of *Calocybe indica* without negatively impacting morphological development.

Zinc supplementation in growth medium

Zinc enrichment had a significant effect on the time required for pinhead initiation (Table 2). Treatments T1 and T2 (2.5 and 5 mg/kg $ZnSO_4$) led to the early pinhead emergence with 45.0 ± 1.633 and

Table 1. Effect of iron enrichment on average pinhead initiation time, average weight of sporocarp, and accumulated iron in sporocarp of *Calocybe indica*

Treatments	Average days to Pinhead Initiation	Average Weight of sporocarp (g)	Accumulated Iron in sporocarp (ppm)
Control	43.75 ± 0.5.0^{b}	160.0 ± 46.188^{b}	1226.5 ± 91.221^{b}
T1	44.50 ± 1.00^{b}	230.5 ± 01.291^{a}	1282.0 ± 1.291^{b}
T2	41.50 ± 0.577^{a}	190.0 ± 34.641^{ab}	1427.0 ± 127.017^{a}
T3	44.00 ± 0.00^{b}	110.0 ± 29.439^{c}	1195.5 ± 145.138^{c}
T4	Not Available	Not Available	Not Available

Values represent the mean ± standard deviation (n = 4). Means followed by different letters within each column indicate significant differences at $p \leq 0.05$. "Not available" indicates no observable data due to absence of fruiting.

Table 2. Effect of zinc enrichment on average pinhead initiation time, sporocarp weight, and accumulated zinc in sporocarp of *Calocybe indica*

Treatments	Average days to Pinhead Initiation	Average Mushroom yield (g)	Accumulated Zinc in sporocarp (ppm)
Control	50.0 ± 1.414^{c}	183.25 ± 102.708^{c}	21.062 ± 7.568
T1	45.0 ± 1.633^{aa}	366.75 ± 34.865^{a}	11.900 ± 1.936
T2	45.0 ± 2.309^{aa}	286.25 ± 50.888^{ab}	32.087 ± 9.461
T3	46.0 ± 1.155^{bc}	275.00 ± 50.000^{abc}	24.525 ± 15.848
T4	47.5 ± 0.577^{b}	217.00 ± 65.818^{bc}	28.000 ± 3.580

Values represent the mean ± standard deviation (n = 4). Means followed by different letters within each column indicate significant differences at $p \leq 0.05$

45.0 ± 2.309 days, respectively, which was significantly earlier than the control (50.0 ± 1.414 days). T3 and T4 also exhibited early pinhead initiation (46.0 and 47.5 days) compared to the non-treated control blocks. Differences in fruiting bed development under various treatments are depicted in Fig. 6, and the harvested fruiting bodies are shown in Fig. 7.

Fig. 3. Fruiting bed performance of *Calocybe indica* under different Zinc enrichment treatments (T1, T2, T3, T4, and Control). Visible variations in fruiting body development reflect the influence of respective $ZnSO_4$ concentrations

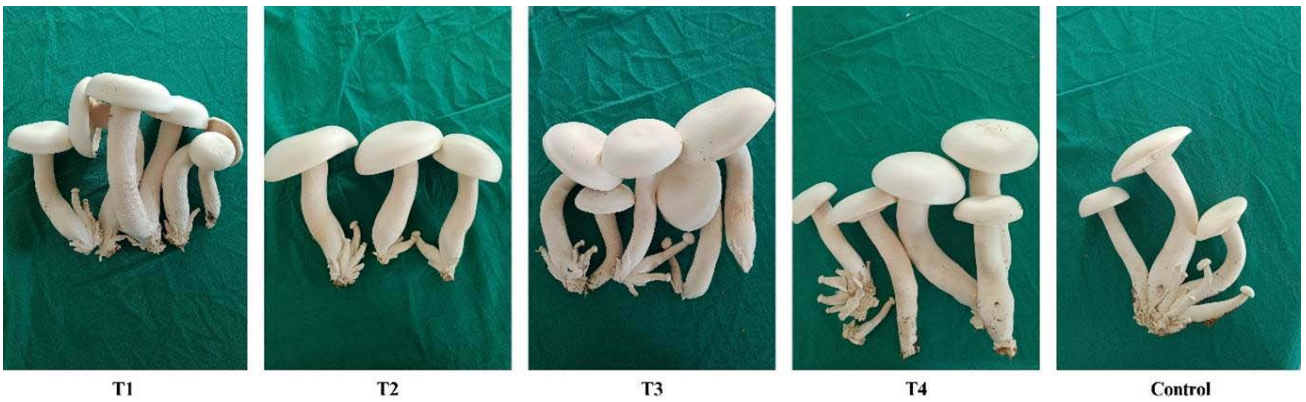

Fig. 4. Harvested fruiting bodies of *Calocybe indica* from different Zinc enrichment treatments

Mushroom yield, as measured by average fresh weight, was highest in T1 (2.5 mg/kg $ZnSO_4$), producing 366.75 ± 34.865 g per bag (Table 2, Figure 9), significantly greater than the control. T2 and T3 also showed improved yields to 286.25 and 275.00g, respectively, though differences were not always statistically significant. T4 (20 mg/kg) showed reduced yield (217.00 g), yet still higher than the control.

Zinc accumulation in the fruiting bodies varied with treatment. The highest zinc content was recorded in T2 (32.087 ± 9.461 ppm), followed by T4 (28.000 ± 3.580 ppm) and T3 (24.525 ± 15.848 ppm). In contrast, T1 showed lower Zn content (11.900 ± 1.936 ppm) than the control (21.062 ± 7.568 ppm), suggesting possible concentration-dependent uptake dynamics (Table 2, Fig. 10).

Analysis of variance (ANOVA) indicated significant differences ($p \leq 0.05$) among treatments for pinhead initiation time and mushroom yield. Mean comparisons using the Least Significant Difference (LSD) test confirmed that low-dose $ZnSO_4$ enrichment, especially at 2.5 mg/kg (T1), significantly enhanced yield performance in *Calocybe indica*. While differences in zinc accumulation were observed numerically across treatments, these were not statistically significant, suggesting that zinc uptake efficiency may not directly correspond to substrate enrichment levels within the tested range.

DISCUSSION

Micronutrient deficiencies, particularly iron (Fe) and zinc (Zn), remain a major global health issue, and biofortification offers a cost-effective dietary solution. This study evaluates the potential of *Calocybe indica* as a biofortification agent using $FeSO_4$ and $ZnSO_4$.

C. indica demonstrates a rich nutritional profile, with high protein, fiber, and iron content compared to conventional foods like spinach (Sumathy *et al*., 2015; Waseem et al., 2021). Iron supplementation showed a dose-dependent effect, where moderate concentrations (2.5–5 mg/kg) enhanced growth, yield, and iron accumulation, supporting metabolic processes such as respiration and enzyme activity (Koreti *et al.*, 2023; Levi and Rovida, 2009). However, excessive iron (20 mg/kg) inhibited fruiting due to oxidative stress, consistent with previous findings (Tunlid *et al.*, 2022; Koreti *et al.*, 2023). Similar toxicity thresholds have been reported in other mushrooms (Almeida *et al.*, 2015; Budzyñska *et al.*, 2021, 2022; Fontes *et al.*, 2013; Meniqueti *et al.*, 2021; Scheid *et al.*, 2020).

The study confirms that *C. indica* efficiently accumulates iron under optimal conditions, likely via passive or transporter-mediated uptake (Scheid *et al.*, 2020; Oyetayo *et al.*, 2021). Synergistic interactions, such as calcium co-application, may further enhance iron uptake (Budzyñska *et al.*, 2021).

In contrast, zinc biofortification was ineffective, as no significant increase in zinc accumulation was observed, however, improved yield was recorded with zinc fortification in substrate. This is attributed to strict homeostatic regulation and limited translocation mechanisms (Azevedo *et al.*, 2007; Robinson *et al.*, 2021). Zinc uptake is influenced by factors such as chemical form, growth conditions, and developmental stage (Kalucka *et al.*, 2023). While other studies reported higher zinc accumulation under liquid culture or with alternative zinc compounds (Figlas *et al.*, 2010; Ziêba *et al.*, 2020), *C. indica* under solid-state cultivation showed minimal uptake, similar to findings in *Ganoderma lucidum* (Matute *et al.*, 2011).

Species-specific differences, substrate interactions, and transporter regulation likely limit zinc accumulation (Yasmin *et al.*, 2024; Gupta *et al.*, 2016). However, *C. indica* exhibited high tolerance to zinc, suggesting potential for improved biofortification using alternative formulations such as chelated or nano-zinc (Ali *et al.*, 2024).

Overall, the study highlights that iron biofortification in *C. indica* is effective within optimal limits, whereas zinc enrichment remains challenging. With further optimization, *C. indica* can serve as a promising, scalable vehicle for dietary iron supplementation and nutritional security.

CONCLUSION

This study demonstrates the potential of *Calocybe indica* for micronutrient biofortification with iron (Fe) and zinc (Zn) through substrate enrichment. Optimal iron supplementation (5 mg/kg $FeSO_4$) significantly improved both yield and iron content in fruiting bodies, confirming its effectiveness as a sustainable nutritional enhancement strategy. However, higher iron levels inhibited growth, highlighting the importance of controlled dosing.

In contrast, zinc supplementation increased yield but did not enhance zinc accumulation, likely due to strict homeostatic regulation and limited translocation to fruiting bodies. These findings reveal the complexity of micronutrient uptake in mushrooms and the need for species- and nutrient-specific optimization.

Overall, *C. indica* shows strong potential as a functional food for iron delivery, while improving zinc bioavailability will require alternative strategies such as modified zinc formulations, synergistic interactions, and genetic approaches.

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REFERENCES

1. Ali, M.B., T. Iftikhar and H. Majeed. 2024. Green synthesis of zinc oxide nanoparticles for the industrial biofortification of (*Pleurotus pulmonarius*) mushrooms. *Heliyon* **10**. https://doi.org/10.1016/j.heliyon.2024.e37927

2. Almeida, S.M., S.H. Umeo, R.C. Marcante, M.E. Yokota, J.S. Valle, D.C. Dragunski, N.B. Colauto and G.A. Linde. 2015. Iron bioaccumulation in mycelium of *Pleurotus ostreatus*. *Braz J Microbiol* **46:** 195–200. https://doi.org/10.1590/S1517-838246120130695

3. Azevedo, M.M., A. Carvalho, C. Pascoal, F. Rodrigues and F. Cássio. 2007. Responses of antioxidant defenses to Cu and Zn stress in two aquatic fungi. *Sci Total Environ* **377:** 233–243. https://doi.org/10.1016/j.scitotenv.2007.02.027

4. Bhan, M.K., H. Sommerfelt and T. Strand. 2001. Micronutrient deficiency in children. *Br J Nutr* **85:** 199–S203. https://doi.org/10.1079/BJN2001315

5. Bonaventura, P., G. Benedetti, F. Albarède and P. Miossec. 2015. Zinc and its role in immunity and inflammation. *Autoimmun Rev* **14:** 277–285. https://doi.org/10.1016/j.autrev.2014.11.008

6. Budzyñska, S., M. Siwulski, M. G¹secka, Z. Magdziak, P. Kalaè, P. Niedzielski and M. Mleczek. 2022. Biofortification of three cultivated mushroom species with three iron salts—Potential for a new iron-rich superfood. *Molecules* **27:** 2328. https://doi.org/10.3390/molecules27072328

7. Budzyñska, S., M. Siwulski, Z. Magdziak, A. Budka, M. Glsecka, P. Kalaè, P. Rzymski, P. Niedzielski and M. Mleczek. 2021. Influence of iron addition (alone or with calcium) to elements biofortification and antioxidants in *Pholiota nameko*. *Plants* **10:** 2275. https://doi.org/10.3390/plants10112275

8. Das, S. and B. Prakash. 2022. Edible mushrooms: nutritional composition and medicinal benefits for improvement in quality life. In Research and Technological Advances in Food Science. Bhanu Prakash (ed). pp 269–300, Academic Press. https://doi.org/10.1016/B978-0-12-824369-5.00013-0

9. de Souza, D.F., M.D.C.S. da Silva, T.C. de Souza, G.C. Rocha, M.C.M. Kasuya and M.R. Eller. 2023. Effect of selenium-enriched substrate on the chemical composition, mineral bioavailability, and yield of edible mushrooms. *Biol Trace Elem Res* **201:** 3077–3087. https://doi.org/10.1007/s12011-022-03396-z

10. Deepali, P. Dipti Rani, S.K. Jadhav and N.K. Chandrawanshi. 2023. Zn fortification influential impact on the productivity of *Calocybe indica* mycelium. *Journal of Ravishankar University (Part-B: Science)* **36(2):** 158–165.

11. Dhaliwal, S.S., V. Sharma, A.K. Shukla, V. Verma, M. Kaur, Y.S. Shivay, S. Nisar, A. Gaber, M. Brestic, V. Barek and M. Skalicky. 2022. Biofortification—A frontier novel approach to enrich micronutrients in field crops to encounter the nutritional security. *Molecules* **27:** 1340. https://doi.org/10.3390/molecules27041340

12. Figlas, D., M. Oddera and N. Curvetto. 2010. Bioaccumulation and bioavailability of copper and zinc on mineral-enriched mycelium of *Grifola frondosa*. *J Med Food* **13:** 469–475. https://doi.org/10.1089/jmf.2008.0284

13. Gupta, N., H. Ram and B. Kumar. 2016. Mechanism of Zinc absorption in plants: uptake, transport, translocation and accumulation. *Rev Environ Sci Biotechnol* **15:** 89–109. https://doi.org/10.1007/s11157-016-9390-1

14. Ka³ucka, M., A. Roszczyk, M. Klimaszewska, B. Kaleta, E. Drelich, A. B³a¿ewicz, S. Górska-Jakubowska, E. Malinowska, M. Król, A.M. Prus and K. Trzeœniowska. 2023. Optimization of Se-and Zn-enriched mycelium of *Lentinula edodes* (Berk.) Pegler as a dietary supplement with immunostimulatory activity. *Nutrients* **15:** 4015. https://doi.org/10.3390/nu15184015

15. Koreti, D., S.K. Jadhav and N.K. Chandrawanshi. 2023. Mineral fortification of *Calocybe indica* (P&C) and its impact on colony morphology and metabolites production. *Ind J Aerobiol* **36:** 9–17.

16. Levi, S. and E. Rovida. 2009. The role of iron in mitochondrial function. *Biochim Biophys Acta* **1790:** 629–636. https://doi.org/10.1016/j.bbagen.2008.09.008

17. Madaan, K., S. Sharma and A. Kalia. 2024. Effect of selenium and zinc biofortification on the biochemical parameters of *Pleurotus* spp. under submerged and solid-state fermentation. *J Trace Elem Med Biol* **82:** 127365. https://doi.org/10.1016/j.jtemb.2023.127365

18. Matute, R.G., A. Serra, D. Figlas, and N. Curvetto. 2011. Copper and zinc bioaccumulation and bioavailability of *Ganoderma lucidum*. *J Med Food* **14:** 1273–1279. https://doi.org/10.1089/jmf.2010.0206

19. Meniqueti, A.B., S.P. Ruiz, M.G.I. Faria, J.S. do Valle, A.C. Gonçalves Jr, D.C. Dragunski, N.B. Colauto and G.A. Linde. 2021. Iron bioaccumulation in *Lentinus crinitus* mycelia cultivated in agroindustrial byproducts. *Waste Biomass Valorization* **12:** 4965–4974. https://doi.org/10.1007/s12649-021-01353-w

20. Mleczek, M., M. Siwulski, P. Rzymski, S. Budzyñska, M. G¹secka, P. Kalaè and P. Niedzielski. 2017. Cultivation of mushrooms for production of food biofortified with lithium. *Eur Food Res Technol* **243:** 1097–1104. https://doi.org/10.1007/s00217-016-2823-9

21. Ogidi, C.O. and K.O. Oyebode. 2023. Assessment of nutrient contents and bio-functional activities of edible fungus bio-fortified with copper, lithium and zinc. *World J Microbiol Biotechnol* **39:** 56. https://doi.org/10.1007/s11274-022-03500-4

22. Olson, R., B. Gavin-Smith, C. Ferraboschi and K. Kraemer. 2021. Food fortification: The advantages, disadvantages and lessons from sight and life programs. *Nutrients* **13:** 1118. https://doi.org/10.3390/nu13041118

23. Oyetayo, V. O., C.O. Ogidi, S.O. Bayode and F.F. Enikanselu. 2021. Evaluation of biological efficiency, nutrient contents and antioxidant activity of *Pleurotus pulmonarius* enriched with Zinc and Iron. *Indian Phytopathol* **74:** 901-910. https://doi.org/10.1007/s42360-021-00410-7

24. Robinson, J.R., O.S. Isikhuemhen and F.N. Anike. 2021. Fungal–metal interactions: a review of toxicity and homeostasis. *J Fungi* **7:** 225. https://doi.org/10.3390/jof7030225

25. Scheid, S.S., M.G.I. Faria, L.G. Velasquez, J.S. do Valle, A.C. Gonçalves Jr, D.C. Dragunski, N.B. Colauto and G.A. Linde. 2020. Iron biofortification and availability in the mycelial biomass of edible and medicinal basidiomycetes cultivated in sugarcane molasses. *Sci Rep* **10:** 12875. https://doi.org/10.1038/s41598-020-69699-0

26. Shankar, A. H. 2020. Mineral deficiencies. In Hunter's tropical medicine and emerging infectious diseases. In Hunter's Tropical Medicine and Emerging Infectious Diseases (Tenth Edition). Edward T. Ryan, David R. Hill, Tom Solomon, Naomi E. Aronson, Timothy P. Endy (eds). pp 1048–1054, Elsevier. https://doi.org/10.1016/B978-0-323-55512-8.00145-9

27. Singh, N. and S. Sohrab. 2024. Algae and fungi-based micronutrients enrichment in food. In Phytoremediation and Biofortification, pp 335–354, Apple Academic Press.

28. S³yszyk, K., M. Siwulski, A. Wiater, M. Tomczyk and A. Waœko. 2024. Biofortification of mushrooms: a promising approach. *Molecules* **29:** 4740. https://doi.org/10.3390/molecules29194740

29. Subbiah, K.A. and V. Balan. 2015. A comprehensive review of tropical milky white mushroom (*Calocybe indica* P&C). *Mycobiology* **43:** 184–194. https://doi.org/10.5941/myco.2015.43.3.184

30. Sumathy, R., R. Kumuthakalavalli and A.S. Krishnamoorthy. 2015. Proximate, vitamin, amino acid and mineral composition of milky mushroom, *Calocybe indica* (P&C). var. Apk2 commonly cultivated in Tamilnadu. *Journal of Natural Product and Plant Resources* **5(1):** 38–43. http://scholarsresearchlibrary.com/JNPPR-vol5-iss1/JNPPR-2015-5-1-38-43.pdf

31. Thakur, M. P. 2014. Present status and future prospects of tropical mushroom cultivation in India: a review. *Indian Phytopathol* **67:** 113–125.

32. Tulchinsky, T. H. 2010. Micronutrient deficiency conditions: global health issues. *Public Health Rev* **32:** 243–255. https://doi.org/10.1007/BF03391600

33. Tunlid, A., D. Floudas, M. Op De Beeck, T. Wang and P. Persson. 2022. Decomposition of soil organic matter by ectomycorrhizal fungi: Mechanisms and consequences for organic nitrogen uptake and soil carbon stabilization. *Front for Glob Change* **5:** 934409. https://doi.org/10.3389/ffgc.2022.934409

34. Umeo, S.H., M.G.I. Faria, D.C. Dragunski, J.S.D. Valle, N.B. Colauto and G.A. Linde. 2020. Iron or zinc bioaccumulated in mycelial biomass of edible basidiomycetes. *An Acad Bras Cienc* **92:** e20191350. https://doi.org/10.1590/0001-3765202020191350

35. Vieira, P.A.F., D.C. Gontijo, B.C. Vieira, E.A. Fontes, L.S. de Assunção, J.P.V. Leite, M.G.D.A. Oliveira and M.C.M. Kasuya. 2013. Antioxidant activities, total phenolics and metal contents in *Pleurotus ostreatus* mushrooms enriched with iron, zinc or lithium. *LWT Food*

Sci Technol **54:** 421–425. https://doi.org/10.1016/j.lwt.2013.06.016

36. Waseem, M., S. Akhtar, M.F. Manzoor, A.A. Mirani, Z. Ali, T. Ismail, N. Ahmad and E. Karrar. 2021. Nutritional characterization and food value addition properties of dehydrated spinach powder. *Food Sci Nutr* **9:** 1213–1221. https://doi.org/10.1002/fsn3.2110

37. Yasmin, K., M.S. Hossain and W.C. Li. 2024. Simultaneous immobilization strategy of anionic metalloids and cationic metals in agricultural systems: A review. *Chemosphere* **364:** 143106. https://doi.org/10.1016/j.chemosphere.2024.143106

38. Ziêba, P., K. Kala, A. Wlodarczyk, A. Szewczyk, E. Kunicki, A. Sêkara and B. Muszyñska. 2020. Selenium and zinc biofortification of *Pleurotus eryngii* mycelium and fruiting bodies as a tool for controlling their biological activity. *Molecules* **25:** 889. https://doi.org/10.3390/molecules25040889