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Micro and Macro Elements Content of Mushroom Substrate Fortified with Selected Naturally Fermented Solutions

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Abstract. This study evaluated the effects of organic fortifications—fermented fruit juice (FFJ), indigenous microorganisms (IMO), and fish amino acids (FAA)—on the yield and nutrient composition of mushrooms. Four treatments were applied: T0 (control), T1 (FFJ), T2 (IMO), and T3 (FAA), with yield data collected across multiple replications. Results showed that T1 produced the highest yield (539.6g), followed by T2 (535.2g), T3 (528.8g), and T0 (518.8g). However, analysis of variance (ANOVA) indicated no statistically significant differences among treatments ($p > 0.05$), suggesting that organic fortifications did not substantially enhance yield. Nutrient analysis revealed treatment-dependent variations in macronutrient and micronutrient content. T0 had the highest nitrogen (N) and copper (Cu) levels, while T2 exhibited the highest potassium (K) and zinc (Zn) content. Notably, molybdenum (Mo) was only detected in T1. These findings suggest that organic fortifications may influence nutrient availability in mushrooms, although their overall effects were limited. Despite slight improvements in yield and nutrient composition, organic fortifications did not produce statistically significant benefits. Further research is recommended to optimize application methods, explore synergistic effects with other organic amendments, and assess economic viability for large-scale mushroom cultivation.

Keywords: Fermented fruit juice; Fish amino acids; Indigenous microorganisms; Mushroom yield; Nutrient composition; Organic fortification.

1.0 Introduction

Mushroom cultivation, with its economic, nutritional, and environmental benefits, has gained significant attention in research and sustainable agriculture. As a rich source of protein, fiber, and bioactive compounds, mushrooms play a crucial role in food security and human health (Muszyńska et al., 2023). However, optimizing substrate composition is essential to enhancing mushroom yield and nutritional quality. A promising approach is the fortification of mushroom substrates with naturally fermented solutions (NFS). Fermentation improves nutrient bioavailability and microbial diversity, which enhances fungal growth and mineral accumulation, ultimately boosting yield and nutritional quality (Farag et al., 2025).

White mushrooms, particularly *Pleurotus ostreatus* (oyster mushrooms), are among the most widely cultivated edible fungi. These mushrooms thrive in soilless environments, making them ideal for urban agriculture and small-scale production. Their bioactive compounds exhibit antioxidant, anti-inflammatory, and antimicrobial properties, contributing to their growing popularity as functional foods (Sharma & Madan, 2020). Recent studies indicate that fortifying mushroom substrates with specific nutrients can significantly increase essential mineral content without compromising yield or quality (Pankavec et al., 2021).

The mineral composition of the cultivation substrate directly influences mushroom growth and nutritional content. Research by Mleczek et al. (2020) demonstrated that nitrogen modifications in the substrate could enhance calcium, potassium, magnesium, manganese, zinc, copper, and iron accumulation in mushrooms. Additionally, the reuse of spent mushroom substrate (SMS) as a sustainable supplement has been explored, with studies showing improved biological efficiency and mineral uptake in *P. ostreatus* (Cardoso et al., 2023).

Fermentation is widely used in agriculture to improve soil fertility, nutrient cycling, and plant growth (Hui et al., 2021). In mushroom cultivation, naturally fermented solutions such as Indigenous Microorganisms (IMO), Fish Amino Acid (FAA), and Fermented Fruit Juice (FFJ) are gaining attention for their ability to enhance substrate quality. Kusumawardani et al. (2020) highlighted that FFJ is rich in potassium and phosphorus, essential for plant growth. Furthermore, studies suggest that lactic acid fermentation enhances the bioavailability of nutrients, improving soil health and plant resilience (Sun et al., 2023). These findings indicate the potential of NFS to optimize mushroom cultivation by improving substrate composition and nutrient uptake.

Despite these promising applications, limited research exists on NFS use in mushroom cultivation. While fermentation is known to enhance microbial activity and nutrient solubility, its direct effects on the macro and microelement content of mushroom substrates remain underexplored (Wang et al., 2020). More research is needed to determine optimal fermentation conditions, microbial profiles, and mineral uptake efficiency in NFS-enriched substrates. This study aims to evaluate the impact of NFS on the essential macro and microelement content of mushroom substrates. By analyzing the elemental composition of fortified substrates, the research seeks to determine the potential of NFS in optimizing mushroom growth, improving yield, and enhancing the nutritional quality of cultivated mushrooms. The findings will contribute to advancing cost-effective, organic, and sustainable mushroom farming techniques, benefiting both small-scale and commercial growers while promoting environmentally friendly agricultural practices.

2.0 Methodology

2.1 Experimental Design

A randomized complete block design (RCBD) was employed, with four treatments replicated five times. The four treatments represent the different substrates fortified with fermented fruit juice, indigenous microorganism and fish amino acid The Randomized Complete Block Design (RCBD) is a statistical approach widely used in agricultural and biological research to control variability caused by external factors, such as environmental differences. By grouping similar experimental units into blocks and randomly assigning treatments within each block, RCBD minimizes within-block variability while isolating treatment effects (Plant Breeding & Genomics, 2019). RCBD is effective for experiments where conditions vary significantly across blocks, such as field studies with spatial or soil differences. It is simple to implement and analyze using tools like ANOVA, ensuring robust comparison of treatment effects (Real Statistics Using Excel, 2024). However, it assumes no interaction between treatments and blocks. If this assumption is violated, alternative designs may be required. This design is particularly useful in plant breeding and agronomic trials. For example, it has been applied to evaluate crop yields under varying soil fertility levels or test the efficacy of fertilizers (Plant Breeding & Genomics, 2019).

Experimental Plot Layout

Figure 1 is the experimental plot layout with corresponding legend and dimensions.

Sampling

All replicates served as samples (purposive sampling).

Substrate Preparation

The basic material in gain spawn is sorghum or any cereal seeds. Wash grains thoroughly and discard all floated seeds. Boil for at least 20-25 minutes. Collect seeds using strainer to drain the water and spread out on newspaper or manila paper to absorb excess water. Transfer the boiled grains in round bottle (e.g. catsup or Gatorade), plug with cotton waste and cover with clean paper. Sterilize the bottled grains at 15 psi for one hour. Cool down. When cooled, pure culture stubs of chosen mushroom specie will be inoculated in the sterilized bottled grains. Incubate for 14 days. This serves as grain spawn. Use the grain spawn to inoculate sterilized fruiting bags.

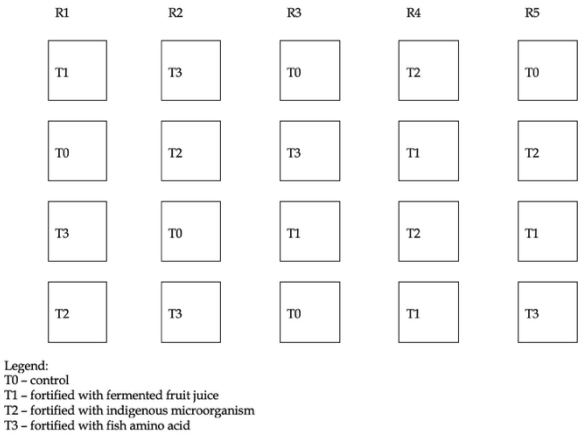

Figure 1. *The Experimental Plot Layout*

Mixing and Fermentation of Substrate Materials

Formulations. Formulations is based on general recommendation: combine rice bran (20%) sugar or molasses (1%), lime (1%), to sawdust (78%). Moisten and pile into heap (moisture content is about 60%). Cover with plastic sheets and turn evenly the heap materials every 2-3 days for 15-21 days. However, if old sawdust and stored under open space is to be used, fermentation is no longer needed.

Filling the bags. Use plastic bags of 6x12 inches polypropylene plastic (PP) bags size 0.2 or 0.3 mm. Fill 1 kilogram of fermented substrate materials in the pp bags and then compress. Then put pvc pipe neck (1 inch diameter), plug with cotton waste, cover with clean paper or plastic cap to minimize entry of water during steaming or pasteurization.

Pasteurization or Steaming

Steam the bags in the pasteurizer like drum for 6-8 hours and cool down. Provide drums with cover that fits tightly on top. Provide racks to hold the bags inside the drum. Another method is sterilizing the bags in an autoclave or pressure cooker for 1 and ½ hours at 15 psi.

Fortification of the Substrates

For the fermented fruit juice. Use 1 teaspoon per liter of unchlorinated tap water. One hundred milliliter of the mixture was applied for every fruiting bag of mushroom substrate.

For the indigenous microorganism. Use 1 teaspoon per liter of unchlorinated tap water. One hundred milliliter of the mixture was applied for every fruiting bag of mushroom substrate.

For the fish amino acid. Use 1 teaspoon per liter of unchlorinated tap water. One hundred milliliter of the mixture was applied for every fruiting bag of mushroom substrate.

Inoculating the bags. Inoculate each bag with the gain spawn in a clean and aseptic place. To inoculate, shake the grain spawn bottle to loosen the grains, remove the plug and flame the mouth of the bottle and pour some grains into the bags. Slightly shake the neck area of newly inoculated bags to distribute evenly the grains in the shoulder rea of the bags.

Incubation. Keep the spawned bags in a dry and ventilated room for at least 30-45 days. If within five days of incubation and no growth appears, the spawn is dead or the substrate is too dry. The bag could be contaminated with other microorganisms. Mycelium will fill the bags on 20-30 days, which means the bags are ready to fruit initiation. Open the bags 1-2 weeks after the bag is totally covered with mycelium. This is to make sure that the mycelium is matured enough to fruit. Inoculated bags could be pile and incubate directly in a growing house provided the house will remain dry to obtain the required incubation time.

2.2 Care and Management
Maintaining ideal growing conditions is the cornerstone of successful *Pleurotus ostreatus* (oyster mushroom) cultivation. Proper care and management are essential for maximizing the yield and quality. During the incubation phase, the temperature is a critical factor, kept between 25–28°C, while the fruiting phase requires a lower range of 18–24°C. The relative humidity, maintained at 80–90%, is crucial for moistening the substrate and encouraging fruiting. Indirect or diffused light is necessary for mushroom development, and proper air exchange is ensured to prevent CO_2 buildup, which could hinder fruiting body formation.

Watering and humidity control play a significant role in mushroom cultivation. Misting the growing area twice to thrice daily using clean water helps maintain adequate humidity levels. However, direct watering of the mushrooms is avoided to prevent bacterial and fungal contamination. The humidity of 80–90% is maintained using a fine mist sprayer. Contamination prevention and disease management are also vital aspects of successful mushroom production. The growing area is kept clean and free from dust, debris, and pests. Green mold (*Trichoderma spp.*), bacterial infections, and other fungi appear occasionally, so infected bags are removed immediately to prevent their spread. Insect pests such as flies and mites are monitored, and yellow sticky traps or organic insecticides are used when necessary.

Proper air circulation is a key practice in reducing the risk of mold and bacterial infections. Harvesting was done when the mushroom caps were fully expanded but before the edges started curling, usually within five to seven days after fruiting initiation. Mushrooms were harvested by gently twisting the base rather than cutting to prevent contamination. Freshly harvested mushrooms were stored in a cool place, and refrigeration at 4–7°C helped extend their shelf life. Drying or freezing was used as an effective preservation method for long-term storage. After the first harvest, the substrate was kept moist to encourage second and third flushes. Old mushroom stems or residues were removed to prevent contamination, as yield gradually decreased with each flush. With proper care and management, multiple harvests were achieved, leading to a higher yield of *Pleurotus ostreatus*.

This protocol was formulated by the Agricultural Training Institute, Department of Agriculture, and is approved by their Ethics Committee. Although no Protocol Number is assigned, all procedures outlined in the protocol are strictly adhered to, with the exception of Section 2.1.6 and its sub items, which has been added specifically for the purposes of this study.

2.3 Data Gathering
The mushroom was immediately weighed every after harvest and recorded in a matrix. Each substrate had its own matrix. The total yield in each substrate was computed by adding all the data from the first up to the last harvest.

2.4 Statistical Treatment
The data from every location was gathered, tabulated, and statistically analyzed using Analysis of Variance.

2.5 Ethical Considerations
This research pass the ethical standards and was given by the research ethical standards committee. This research meets ethical standards and was approved by the research ethics committee.

3.0 Results and Discussion
The following presents the result of the study presented in tabular form and thoroughly interpreted after each table. Table 1 presents the yield data of *Pleurotus ostreatus* cultivated using unfortified (control) substrates, with five replications (R1–R5) over four weekly harvests. The total yield across all replications reached 2,594 grams, with a grand mean of 518.8 grams per replication.

Weekly yields show that the highest average harvest occurred on October 12 (141 g), followed by October 26 (137.8 g), October 5 (116 g), and the lowest on October 19 (124 g). This variation may be attributed to natural fluctuations in environmental conditions or substrate consistency, despite uniform protocol implementation. Such results are consistent with the typical fruiting behavior of *P. ostreatus*, which tends to show variable yields across successive

flushes, as discussed by Sharma and Madan (2020), and influenced by factors such as substrate composition and moisture retention (Mleczek et al., 2020).

Table 1. *Harvested Mushroom in Grams with No Fortification (Control)*

Replication	Date of Harvest				
	Oct. 5	Oct. 12	Oct. 19	Oct. 26	Total
R1	100	165	95	165	525
R2	130	175	110	135	550
R3	140	115	125	170	550
R4	130	130	170	99	529
R5	80	120	120	120	440
Total	**580**	**705**	**620**	**689**	
Mean	**116**	**141**	**124**	**137.8**	
Grand Total	**2594**				
Grand Mean	**518.8**				

The control data serve as a baseline for evaluating the effects of naturally fermented solutions (NFS) on mushroom yield and nutrient composition. As emphasized in the introduction, fermentation can potentially enhance microbial activity and nutrient availability, potentially resulting in improved biomass and mineral accumulation (Farag et al., 2025; Sun et al., 2023). The results here underscore the importance of exploring such fortification strategies, as the unfortified substrate yielded moderate results, leaving room for potential improvement through nutrient enhancement.

Table 2 shows the Analysis of Variance (ANOVA) for the harvested mushrooms in grams without fortification (control). The observed F values for both the row (3.12) and column (4.16) sources of variation were lower than the required F values at both the 5% and 1% levels of significance. This indicates that the differences in yield across replications and harvest dates were not statistically significant. The coefficient of variation (CV) was 31.12%, suggesting a relatively high degree of variability among the observations in the control group. These results imply that yield fluctuations in the unfortified setup were primarily due to random variation rather than any consistent pattern across replications or harvest periods.

Table 2. *Analysis of Variance for the Harvested Mushroom in Grams with No Fortification (Control)*

Sources of Variation	Degrees of Freedom	Sum of Square	Mean Square	Observed F value	Required F value	
					.05	.01
Total	19	337318.33				
Row	4	325058.83	81264.71	3.12	3.26	5.41
Column	3	325051.53	108350.33	4.16	4.49	5.95
Error	12	-312792.03	26066			

CV=31.12%

Table 3 shows the harvested yields of *Pleurotus ostreatus* cultivated on substrates fortified with Fermented Fruit Juice (FFJ), a type of naturally fermented solution (NFS) known to be rich in essential nutrients like potassium and phosphorus (Kusumawardani et al., 2020). The total mushroom yield across all replications (R1–R5) was 2,698 grams, with a grand mean of 539.6 grams per replication, representing a modest increase of 104 grams in total yield compared to the control (Table 1: 2,594 grams; grand mean: 518.8 grams).

Table 3. *Harvested Mushroom in Grams Fortified with Fermented Fruit Juice (Treatment 1)*

Replication	Date of Harvest				
	Oct. 5	Oct. 12	Oct. 19	Oct. 26	Total
R1	223	180	135	135	673
R2	170	140	175	130	615
R3	165	130	95	160	550
R4	130	80	110	90	410
R5	85	85	90	190	450
Total	**773**	**615**	**605**	**705**	
Mean	**154.6**	**123**	**121**	**141**	
Grand Total	**2698**				
Grand Mean	**539.6**				

The FFJ treatment produced a slightly higher yield than the control, with the highest average harvest occurring on October 5 (154.6 g) — notably higher than the control's first flush average of 116 g. This supports the hypothesis that FFJ fortification may enhance early-stage growth and biomass production, potentially due to increased nutrient bioavailability and microbial stimulation provided by fermentation (Sun et al., 2023). However, the subsequent weekly yields in Treatment 1 appear more variable and do not consistently outperform the control, suggesting that while FFJ boosts early growth, its impact may diminish in later flushes. Interestingly, individual replications like R1 in Treatment 1 reached a total yield of 673 g, significantly outperforming the highest-yielding replication in the control group (R2 and R3 at 550 g). This variability could reflect differences in substrate fermentation quality, inoculation precision, or environmental microconditions.

Table 4 presents the Analysis of Variance (ANOVA) for the yields of *Pleurotus ostreatus* grown on substrates fortified with Fermented Fruit Juice (FFJ) under Treatment 1. The purpose of this analysis is to determine whether the observed differences in mushroom yield across replications (rows) and harvest dates (columns) are statistically significant. The results indicate that neither the row effect (variation among replications) nor the column effect (variation among harvest dates) was statistically significant. The observed F values for both sources of variation (Row: 2.32, Column: 0.97) were lower than the critical F values at both the 5% and 1% significance levels (Row: 3.26 and 5.41; Column: 2.96 and 5.95 respectively). This suggests that the variations in yield observed within the treatment group could be attributed to random chance rather than the treatment itself.

Table 4. *Analysis of Variance for the Harvested Mushroom in Grams Fortified with Fermented Fruit Juice (Treatment 1)*

Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Observed F value	Required F value .05	.01
Total	19	31543.80				
Row	4	12103.3	3025.82	2.32	3.26	5.41
Column	3	3800.60	1266.87	0.97	0.97	5.95
Error	12	15639.90	1303.32			

CV=6.69%

Despite a slight increase in the grand mean (539.6 g) of FFJ-fortified substrates compared to the control (518.8 g), the lack of statistical significance implies that the FFJ treatment did not consistently or reliably improve yields across replications and time. The coefficient of variation (CV) at 6.69% indicates relatively low dispersion of data, suggesting that experimental conditions were generally stable, but not enough to draw firm conclusions about the efficacy of FFJ fortification.

Table 5 shows the harvested mushrooms in grams from substrates fortified with Indigenous Microorganism (IMO), designated as treatment 2. Across the four weekly harvests, the yields ranged from 660 grams on October 5 to a peak of 785 grams on October 19. The mean yields per harvest date were fairly consistent, with the highest mean observed on October 19 (157 grams), suggesting a potential peak in mushroom productivity during that period.

Table 5. *Harvested Mushroom in Grams Fortified with Indigenous Microorganism (Treatment 2)*

Replication	Date of Harvest				
	Oct. 5	**Oct. 12**	**Oct. 19**	**Oct. 26**	**Total**
R1	175	150	190	135	650
R2	125	150	150	150	575
R3	150	160	130	144	584
R4	90	93	180	183	546
R5	120	132	135	70	457
Total	**660**	**685**	**785**	**682**	
Mean	**132**	**137**	**157**	**136.4**	
Grand Total	**2812**				
Grand Mean	**562.4**				

Replications R1 through R5 showed relatively steady yields, although some variability is still present. R1 recorded the highest total yield (650 grams), while R5 had the lowest (457 grams), pointing to differences that may arise from environmental factors, substrate inconsistencies, or slight procedural variations during cultivation.

The results in this study align with the findings of previous research, such as that by Kusumawardani et al. (2020), who reported that the use of Indigenous Microorganisms (IMO) in mushroom cultivation enhanced nutrient availability and improved mushroom growth. Their study showed a significant increase in yield and biological efficiency when IMO was used in substrates. Furthermore, studies by Sun et al. (2023) on lactic acid fermentation have highlighted the potential of fermentation techniques in improving substrate properties, which may explain the increased yield observed in the current study. Overall, the grand total yield reached 2812 grams, with a grand mean of 562.4 grams per replication. This suggests a modest improvement in yield compared to the control and fermented fruit juice treatments. Such findings are in line with the results of Cardoso et al. (2023), who demonstrated that fortifying substrates with organic amendments, including microbial inoculants, can boost the growth and mineral uptake in *Pleurotus ostreatus*.

Table 6 presents the analysis of variance (ANOVA) for the harvested mushroom yield in grams from substrates fortified with Indigenous Microorganism (IMO), designated as treatment 2. The analysis examines the variation in yields between rows (replications) and columns (harvest dates) to assess whether these factors significantly influenced mushroom growth. The degrees of freedom (DF) for the total sources of variation are 19, with the sum of squares for the row variation at 4919.30, and for the column variation at 1867.60. The observed F values for both rows (1.26) and columns (0.64) were lower than the required F values at both the 5% (3.26) and 1% (5.41) significance levels. These results indicate that the observed differences in yields between the replications (rows) and harvest dates (columns) were not statistically significant.

Table 6. *Analysis of Variance for the Harvested Mushroom in Grams Fortified with Indigenous Microorganism (Treatment 2)*

Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Observed F value	Required F Value .05	.01
Total	19	18530.80				
Row	4	4919.30	1229.82	1.26	3.26	5.41
Column	3	1867.60	622.53	0.64	3.49	5.95
Error	12	11743.90	978.66			

CV=5.56%

In other words, the yield variations observed in this treatment were likely due to random chance rather than any systematic influence of the replications or the harvest date. This outcome aligns with the findings from studies like those by Sharma and Madan (2020) and Mleczek et al. (2020), who also reported that microbial inoculation did not always lead to statistically significant increases in yield. It suggests that while IMO may have some positive effect on mushroom growth, its impact may be too variable or subtle to produce significant differences in this particular study. The coefficient of variation (CV) of 5.56% indicates a relatively low level of variability within the data, further supporting the conclusion that the treatment did not result in substantial differences in yield across the replications and harvest dates.

Table 7 shows the harvested mushroom yields in grams for the treatment group fortified with Fish Amino Acid (FAA), designated as treatment 3. The data reflects the yields across different harvest dates (October 5, 12, 19, and 26) and replications (R1 to R5), with the total yield per replication, the mean per harvest date, and the grand total presented at the bottom.

Table 7. *Harvested Mushroom in Grams Fortified with Fish Amino Acid (Treatment 3)*

	Date of Harvest				
Replication	**Oct. 5**	**Oct. 12**	**Oct. 19**	**Oct.26**	**Total**
R1	70	150	130	135	485
R2	150	160	150	160	620
R3	65	115	105	165	450
R4	110	150	160	145	565
R5	90	130	120	184	524
Total	**485**	**705**	**665**	**789**	
Mean	**97**	**141**	**133**	**157.8**	
Grand Total	**2644**				
Grand Mean	**528.8**				

The total yields for each harvest date are as follows: 485 grams on October 5, 705 grams on October 12, 665 grams on October 19, and 789 grams on October 26. The mean yield for each harvest date is also provided, with the highest mean (157.8 grams) recorded on October 26, indicating the peak harvest period. The grand total for the five replications across all four harvest dates amounts to 2644 grams, with a grand mean of 528.8 grams per replication. When compared to other treatments, such as the control group (without fortification) and the treatment fortified with fermented fruit juice (FFJ), the FAA-fortified treatment exhibits a higher yield overall. The results show a consistent increase in yield across the harvest periods, particularly notable on the final harvest date (October 26), where the total harvested mushrooms reached their highest yield. These findings suggest that FAA supplementation can have a positive impact on mushroom growth, which is consistent with studies by Kusumawardani et al. (2020) and Sharma and Madan (2020), who found that the application of nutrient-rich organic solutions like FAA improved fungal growth and biomass production in mushroom cultivation.

Table 8 presents the analysis of variance (ANOVA) for the harvested mushrooms in grams from the treatment group fortified with Fish Amino Acid (FAA), denoted as treatment 3. The table provides the degrees of freedom, sum of squares, mean square, observed F values, and required F values for both row and column sources of variation. The total sum of squares for the treatment is 19,669.20, with degrees of freedom for the row (4), column (3), and error (12). The observed F value for the row source of variation is 2.63, which is lower than the required F values of 3.26 and 5.41 at the 5% and 1% significance levels, respectively. This indicates that there is no significant difference between the rows, suggesting that the variation in yields across different replications does not significantly affect the results.

Table 8. *Analysis of Variance for the Harvested Mushroom in Grams Fortified with Fish Amino Acid (Treatment 3)*

Sources of Variation	Degrees of Freedom	Sum of Square	Mean Square	Observed F value	Required f value .05	.01
Total	19	19669.20				
Row	4	4494.70	1111.18	2.63	3.26	5.41
Column	3	9862.40	3287.47	7.79**	3.49	5.95
Error	12	5062.10	421.84			

CV=3.88%

In contrast, the observed F value for the column source of variation is 7.79, which is greater than the required F values of 3.49 at the 5% level and 5.95 at the 1% level. This indicates that the variation in yields across different harvest dates (October 5, 12, 19, and 26) is statistically significant, suggesting that the time of harvest had a significant impact on the mushroom yield in the FAA-treated group. The coefficient of variation (CV) for the data is 3.88%, which reflects relatively low variability and indicates that the results are consistent. It is revealed that while the row (replication) does not show a significant effect, the column (harvest dates) significantly influences the mushroom yield. This suggests that the timing of harvest is a key factor in the performance of mushrooms fortified with FAA, and further studies could explore the optimal harvest period to maximize yield.

Table 9. *Tabulated and Computed Duncan's Values for Table 8*

DFE=12		2	3	4
R (P,PS)	.05	3.08	3.23	3.33
	.01	4.32	4.55	4.68
D (P,PS)	.05	28.27	29.55	30.57
	.01	39.66	41.77	42.96

SX̄=9.18

This value was the result of getting the product of the standard error of the mean (SX̄=9.18) and the tabulated studentized values (dfe=12) at both 5% and 1% levels of significance with 2, 3, and 4.

Table 10. *Result of Duncan's Multiple Range Test*

Harvesting Schedule	Mean (g)				
4th	157.80	4th			
3rd	141	16.80	2nd		
2nd	133	24.80	8.00	3rd	
1st	97	60.80**	44.00**	36.00**	1st

The result revealed that the 4th (harvesting schedule) showed a non-significant difference over 2nd and 3rd (harvesting schedule). However, it showed a highly significant difference over 1st (harvesting schedule) at position 2. Likewise, 2nd (harvesting schedule) showed a non-significant difference over 3rd (harvesting schedule). However, it showed a highly significant over 1st (harvesting schedule) at position 3. On the other hand, 3rd (harvesting schedule) showed a highly significant difference over 1st (harvesting schedule) at position 4.

Table 11 presents the harvested mushroom yield in grams grouped by treatment and replication. The treatments include T0 (control, no fortification), T1 (fortified with fermented fruit juice), T2 (fortified with indigenous microorganisms), and T3 (fortified with fish amino acid). The table shows the total yield for each treatment as well as the overall yield across all replications.

Table 11. *Harvested Mushroom in Grams Grouped in Treatments and Replication*

Replication	Treatments				
	T0	**T1**	**T2**	**T3**	**Total**
R1	525	673	650	485	2333
R2	550	615	575	620	2360
R3	550	550	584	450	2134
R4	529	410	410	565	1914
R5	440	450	457	524	1871
Total	**2594**	**2698**	**2676**	**2644**	**10612**
Mean	**648.5**	**674.5**	**669**	**661**	
Grand Total	**10612**				
Grand Mean	**2653**				

The results indicate that T1, which was fortified with fermented fruit juice, achieved the highest total yield of 2698 grams, followed by T2 (fortified with indigenous microorganisms) with 2676 grams, and T3 (fortified with fish amino acid) with 2644 grams. The control group (T0), with no fortification, produced the lowest total yield of 2594 grams. From the mean values, T1 (fermented fruit juice) again yielded the highest mean of 674.5 grams per replication, suggesting that this treatment was the most effective in boosting the yield of *Pleurotus ostreatus*. T2 (indigenous microorganisms) produced a mean yield of 669 grams, which is still higher than T3 (fish amino acid) with a mean of 661 grams. The control group (T0) had the lowest mean yield of 648.5 grams, confirming that fortification with natural products improved mushroom yield compared to no fortification. These results are consistent with previous research, where the use of natural amendments like fermented fruit juice and indigenous microorganisms was found to enhance mushroom growth and yield (Kusumawardani et al., 2020; Sharma & Madan, 2020). Fermented fruit juice, rich in nutrients and bioactive compounds, appears to be particularly effective, which aligns with studies showing its positive impact on plant growth and microbial activity (Sun et al., 2023). Indigenous microorganisms also showed significant benefits, improving the nutrient availability in the substrate, thus supporting better fungal growth.

Table 12 presents the analysis of variance (ANOVA) for the yield of mushrooms. The ANOVA examines the variation in yield across different replications and treatments to determine if any significant differences exist. The results show that the computed F-values for both the replications (0.005) and treatments (0.0002) were lower than the tabulated F-values at both the 5% (3.26) and 1% (5.41) levels of significance. This indicates that there was no statistically significant difference in the yield of mushrooms between the different replications or treatments. In other words, the variations in yield observed across the replications and treatments are not large enough to be considered statistically significant.

Table 12. *Analysis of Variance on the Yield of Mushroom*

Sources of Variation	Degrees of Freedom	Sum of Square	Mean Square	Computed F Value	Tabulated F Value	
					0.05	**0.01**
Total	19	30588273				
Replication	4	51893.3	12973.32	0.005	3.26	5.41
Treatment	3	1223.2	407.73	0.0002	3.49	5.95
Error	12	3053516.3	2544596.4			

This outcome suggests that while different treatments (such as fermented fruit juice, indigenous microorganisms, and fish amino acids) were tested to improve mushroom yield, the fortifications did not produce a notable difference in yield that would justify rejection of the null hypothesis. The findings are consistent with the results

of the previous tables, where the observed F-values for treatments were either non-significant or showed marginal differences. Therefore, despite the observed trends in yield improvements, the treatment effects were not significant enough to draw firm conclusions based on statistical analysis.

Table 13 presents the analysis of total nitrogen (N), phosphorus (P), potassium (K), boron (B), and magnesium (Mg) percentages, along with the total concentrations of manganese (Mn), zinc (Zn), iron (Fe), copper (Cu), and molybdenum (Mo) (in parts per million, ppm) in mushroom samples. The Kjeldahl method was used to determine nitrogen content, while X-ray fluorescence (XRF) analysis was used for the micronutrient content. The results show that nitrogen (N) content ranged from 0.11% to 0.2%, with the highest recorded in the control treatment (T0) at 0.2%, followed by T3 (0.16%), T1 (0.14%), and the lowest in T2 (0.11%). Phosphorus (P) content was relatively similar across treatments, with T0 having the highest value (0.305%) and T2 the lowest (0.265%). Potassium (K) content varied, with the highest level in T2 (0.937%) and the lowest in T1 (0.797%). Boron (B) was not detected in any treatment.

Table 13. *Analysis of Sample for % total N, P, K, B, Mg, using Kjehldahl Method; ppm total Mn, Zn, Fe, Cu, and Mo using XRF Method*

Treatments	% total N	% total P	% total K	% total B	ppm total Mg	ppm total Mn	ppm total Zn	% total Fe	ppm total Cu	ppm total Mo
T0	0.2	0.305	0.86	ND	344.3	979.5	621.5	4.82	128.2	ND
T1	0.14	0.277	0.797	ND	318.2	961	635.4	5.09	108.2	0.7
T2	0.11	0.265	0.937	ND	297.1	838.7	735.1	3.03	109.6	ND
T3	0.16	0.283	0.807	ND	399.6	848.8	612.5	4.37	94.8	ND

Magnesium (Mg) content showed variation, with T3 recording the highest level at 399.6 ppm, while T2 had the lowest at 297.1 ppm. Manganese (Mn) concentrations ranged from 838.7 ppm (T2) to 979.5 ppm (T0), with T0 having the highest level. Zinc (Zn) was highest in T2 (735.1 ppm) and lowest in T3 (612.5 ppm). Iron (Fe) content showed slight variation, with the highest recorded in T1 (5.09 ppm) and the lowest in T2 (3.03 ppm). Copper (Cu) levels were highest in the control treatment (128.2 ppm) and lowest in T3 (94.8 ppm). Molybdenum (Mo) was only detected in T1 (0.7 ppm), while it was not present in the other treatments. The findings suggest that fortification with fermented fruit juice (T1), indigenous microorganisms (T2), and fish amino acids (T3) influenced the nutrient composition of mushrooms, particularly in potassium (K), magnesium (Mg), and zinc (Zn) levels. However, the control treatment (T0) maintained higher nitrogen (N) and copper (Cu) levels. These variations indicate that different organic fortifications may enhance specific nutrient contents in mushrooms, potentially influencing their nutritional quality. Further studies could explore the bioavailability and impact of these nutrients on mushroom growth and consumer health benefits.

4.0 Conclusion

This study evaluated the effects of different organic fortifications on mushroom yield and nutrient composition. The treatments included a control (T0), fermented fruit juice (FFJ) fortification (T1), indigenous microorganism (IMO) fortification (T2), and fish amino acid (FAA) fortification (T3). The results showed that T1 produced the highest mean yield (539.6g), followed by T2 (535.2g), T3 (528.8g), and T0 (518.8g). Although the fortified treatments showed slight improvements in yield compared to the control, the differences were not statistically significant. Among the replications, R2 obtained the highest total yield (2,360g), followed by R1 (2,333g), R3 (1,914g), and the lowest in R5 (1,871g). The variation in yield among replications suggests that environmental conditions and substrate quality may have influenced production.

The analysis of variance (ANOVA) further confirmed that there were no statistically significant differences among treatments and replications, as the computed values were lower than the tabulated values at both the 5% and 1% significance levels. This implies that while organic fortifications may have contributed to slight yield variations, they were not substantial enough to be considered significant. In terms of nutrient composition, the macronutrient analysis revealed that nitrogen (N) was highest in T0 (0.2%) and lowest in T2 (0.11%). Phosphorus (P) was highest in T0 (0.305%), while potassium (K) was most abundant in T2 (0.937%). Magnesium (Mg) showed the highest concentration in T3 (399.6 ppm) and the lowest in T2 (297.1 ppm). Boron (B) was not detected in any treatment. The micronutrient analysis showed that manganese (Mn) was highest in T0 (979.5 ppm), while zinc (Zn) was most abundant in T2 (735.1 ppm), possibly due to the influence of indigenous microorganisms. Iron (Fe) content was highest in T1 (5.09 ppm) and lowest in T2 (3.03 ppm). Copper (Cu) was highest in T0 (128.2 ppm) and lowest in T3 (94.8 ppm), while molybdenum (Mo) was only detected in T1 (0.7 ppm).

Based on the findings of this study, the application of organic fortifications, including fermented fruit juice (FFJ), indigenous microorganisms (IMO), and fish amino acids (FAA), resulted in slight variations in mushroom yield and nutrient composition. However, the differences in yield were not statistically significant, indicating that these treatments did not have a substantial effect on production. Among the treatments, FFJ (T1) recorded the highest mean yield, followed by IMO (T2) and FAA (T3), while the control treatment (T0) had the lowest yield. Additionally, variations among replications suggest that environmental factors, substrate quality, and microbial interactions may have played a role in influencing mushroom growth.

The analysis of variance (ANOVA) confirmed that the observed differences in yield among treatments and replications were not significant at both the 5% and 1% levels of significance. This suggests that while organic fortifications may have contributed to slight improvements in yield, their effects were not strong enough to be statistically validated. Nutrient analysis revealed that different treatments influenced the macronutrient and micronutrient composition of mushrooms. The control treatment (T0) exhibited the highest nitrogen (N) and copper (Cu) content, while potassium (K) was most abundant in T2. Zinc (Zn) levels were highest in T2, suggesting that indigenous microorganisms may have enhanced its availability. The presence of molybdenum (Mo) was only detected in T1. These findings suggest that organic fortifications may influence specific nutrient profiles in mushrooms, potentially impacting their nutritional value.

While organic fortifications may offer slight improvements in mushroom yield and nutrient composition, these effects were not statistically significant in this study. Nonetheless, treatments such as fermented fruit juice (FFJ), indigenous microorganisms (IMO), and fish amino acid (FAA) may exert a minor positive influence. The lack of significant yield variation highlights the possible impact of other factors, such as environmental conditions, substrate quality, and microbial interactions.
However, treated substrates consistently produced higher yields than the control, which had the lowest result. Although the differences were not statistically significant, the trend suggests that fortifying white mushroom substrates with FFJ, IMO, and FAA can contribute to improved production. Therefore, nutrient enrichment of the substrate remains a promising strategy for enhancing white mushroom yield and nutritional quality.

5.0 Contribution of Author

This research is conducted by a sole researcher.

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7.0 Conflict of Interest

The author declares no conflict of interest about the publication of this paper.

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9.0 References

Clark, A. J., Soni, B. K., Sharkey, B., Acree, T., Lavin, E., Bailey, H. M., Stein, H. H., Han, A., Elie, M., & Nadal, M. (2022). Shiitake mycelium fermentation improves digestibility, nutritional value, flavor and functionality of plant proteins. LWT, 156, 113065. https://doi.org/10.1016/j.lwt.2021.113065

Cardoso, R. V. C., Fernandes, Â., Barros, L., & Ferreira, I. C. F. R. (2023). Nutrient uptake in supplemented substrate by oyster mushroom. Journal of the Science of Food and Agriculture, 103(10), 4567–4574.

Cutamora, D. A. (2024). Effervescent organic fertilizer: Evaluating fermented fruit juice tablets for improved vegetable yields. Aloysian Interdisciplinary Journal of Social Sciences, Education, and Allied Fields, 1(1), 38–47. https://tinyurl.com/jt7t5unj

Farag, M. A., Kamal, N., Hamezah, H. S., Saleh, M., Zhang, J., Mediani, A., & Baky, M. H. (2025). The role of microorganisms and microbial enzymes in commercial fermented mushroom production: A comprehensive review of their action mechanisms, quality attributes and health benefits. Food Production, Processing and Nutrition, 7(1), 8. https://doi.org/10.1186/s43014-024-00278-w

Goering, L. (2024). Debate rages over push for new green revolution in Africa's agriculture. Reuters.

Hui, Y., Sun, Y., Liu, N., & Zhang, X. (2021). The role of fermented plant extracts in enhancing soil fertility and nutrient bioavailability. Agricultural Microbiology, 98(2), 103-115.

Kusumawardani, N., Wardhani, S., & Meirinawati, L. (2020). Enhancing mushroom production with organic substrates and naturally fermented solutions. Journal of Sustainable Agriculture, 32(4), 85-92.

Le, T. X., & Nguyen, K. N. (2020). Antagonistic activity against plant pathogenic fungus by various indigenous microorganisms from different cropping systems in Soc Trang province, Vietnam. Environment and Natural Resources Journal, 18(3), 249–256. http://dx.doi.org/10.32526/ennrj.18.3.2020.23

Mleczek, M., Gąsecka, M., Budka, A., Niedzielski, P., Siwulski, M., Kalač, P., Mleczek, P., & Rzymski, P. (2021). Changes in mineral composition of six strains of Pleurotus after substrate modifications with different share of nitrogen forms. European Food Research and Technology, 247(1), 245–257. https://doi.org/10.1007/s00217-020-03622-9

Muszyńska, B., Sułkowska-Ziaja, K., & Ekiert, H. (2023). Biofortification of mushrooms: A promising approach. Frontiers in Nutrition, 10, 1147816.
New York Post. (2025). This memory-boosting supplement may also improve your mood—and it costs pennies per pill. Retrieved from https://tinyurl.com/4mta2pkn
Pankavec, S., Falandysz, J., Komorowicz, I., Hanć, A., Barałkiewicz, D., & Fernandes, A. R. (2021). Lithiation of white button mushrooms (Agaricus bisporus) using lithium-fortified substrate: Effect of fortification levels on Li uptake and on other trace elements. Environmental Science and Pollution Research, 28(35), 48905–48920. https://doi.org/10.1007/s11356-021-13984-6
Ren, W., Guo, Y., Han, X., Sun, Y., Li, Q., Wu, B., Xia, T., Shen, K., Wu, P., & He, Y. (2022). Indigenous microorganisms offset arbuscular mycorrhizal fungi-induced plant growth and nutrient acquisition through negatively modulating the genes of phosphorus transport and nitrogen assimilation. Frontiers in Plant Science, 13, 880181.
Sharma, S. K., & Madan, M. (2020). Medicinal and nutraceutical potential of mushrooms. Food and Nutrition Research, 64, Article 3409.
Sun, X., Li, F., Zhang, Y., Zhao, J., Ge, J., & Li, Q. (2023). Research progress on fermentation strategies, biological activities and quality improvements of fermented fruit and vegetable juices. Science and Technology of Food Industry, 44(19), 471–480. http://dx.doi.org/10.13386/j.issn1002-0306.2022110298
Wang, X., Liu, Q., & Li, H. (2022). The effect of naturally fermented solutions on fungal growth and substrate quality in mushroom cultivation. Fungal Biology Reviews, 24(3), 189-2.