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CONTENTS

Effect of ZnO Nanoparticles on Macronutrients Content of *Pleurotus sajar- caju* (Oyster Mushroom)

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ABSTRACT: In the present investigation to study the effect of ZnO nanoparticles, the culture of species of *Pleurotus sajar-caju* was multiplied by using potato dextrose agar media in MRandTC, Pantnagar. Media and substrate were supplemented with ZnO nanoparticles at different concentrations (0ppm, 20ppm, and 40ppm). It has been found that different treatments had non-significant impact on nitrogen content. However, the maximum nitrogen content (3.35%) was recorded at 20 ppm concentration of zinc oxide nanaparticles, which was 1.2% higher than control and at 40ppm 3.33% nitrogen was found. Application of ZnO nanoparticles gave 0.54% at 40 ppm more phosphorus content in the fruiting body of oyster mushrooms. It was observed that maximum potassium content (2.11%) was recorded at 20 ppm ZnO nanoparticle treatments, which was 71.54% higher than control. The sulfur content was higher at 3.23% at 20 ppm ZnO nanoparticle treatments. This study can be further used to combat macronutrients deficiency and hidden hunger.

Key words: Macronutrients, Oyster mushroom, ZnO Nanoparticles

Micronutrient deficiency is the most prevalent issue. Vitamin A and C deficiencies and deficiencies in minerals such as zinc, iodine, and iron affect roughly one-third of the world's population, causing health problems ranging from minor to life-threatening. Based on the published report, Zn insufficiency affects nearly a third of the world's population, according to estimates (Sharma *et al.,* 2009). Zn is a trace element that plays a crucial role in human health by interfering with various metabolic processes. The biological effects are based on the zinc ions (Zn^{2+}) intracellular and extracellular regulatory roles, as well as its interactions with proteins (Mar, 2019). Zn serves as a structural component of proteins as well as a cofactor in about 300 enzymes, some of which are of particular interest due to their significant nutritional and therapeutic importance (Grüngreiff *et al.,* 2019). As a result, this trace element is vital for wound healing, membrane repair, oxidative stress, coagulation, inflammation, immunological defense, tissue re-epithelialization, and other physiological and pathological processes (Lin *et al.,* 2018).

Abbreviations: PSC- *Pleurotus sajar-caju*, ZnO- Zinc Oxide,

Oyster mushrooms are a large and diversified genus of farmed mushrooms with significant commercial and therapeutic value. Oyster fungus can be found all over the world, in both tropical and temperate regions (Chang *et al.,* 2004). Oyster mushrooms are by far the easiest and least expensive to raise of all industrially cultivated edible mushrooms (Sharma *et al.,* 2017), and they grow on a variety of plant substrates, which is one of the reasons for their success. Oyster mushrooms are a good source of non-starchy carbohydrates, dietary fiber, moderate amounts of good-quality proteins, as well as the majority of important amino acids, minerals, and vitamins (Cohen *et al.,* 2002). Due to their well-known antioxidant properties, the edible mushrooms analyzed could be used directly in human diets to combat oxidative stress, whereas the inedible species may be utilized as a source of phenolic compounds that could be used as food additives or as components in pharmaceutical and cosmetic formulations (Jeena *et al.,* 2014).

A new agriculture-based approach has evolved in which staple food crops are bred and produced for higher amounts of micronutrients in the edible parts. This technique is called "biofortification (Bouis and

Welch, 2010). Biofortification, or the act of breeding nutrients into food crops, is a relatively cost-effective, long-term way of increasing micronutrient delivery. Food fortification is one of the most cost-effective, long-term solutions for mineral Fortification occurs during the manufacturing of food and raises the price of the final product. Because of these considerations, fortified foodstuffs are out of reach for the poorest individuals in rural areas (Zimmermann, 2004). Biofortification has been recommended as a long-term alternative to mineral supplementation. Biofortification is a method that increases both minerals and their bioavailability in the edible component of staple crops, with the goal of improving their mineral nutritional properties. Zn is a micronutrient that is required by humans, animals, and plants (Vanathi *et al.,* 2016). Sahu, (2018) reported that when white Oyster mushrooms are biofortified with iron sulfide nanoparticles, it increases the Fe content and also has a positive impact on economic yield. However, to date, only a few reports are available regarding the biofortification of mushrooms using nanoparticles.

MATERIALS AND METHODS

Collection and treatment of samples: The research work was carried out in the Department of Biochemistry, C.B.S.H. and Agronomy, College of Agriculture and the experiment was conducted in M.R. and T.C., G.B. Pant University of Agriculture and Technology, Pantnagar.

Application method of zinc oxide (ZnO) Nanoparticle: The Zinc oxide nanoparticle was purchased from Sisco Research Laboratories, Pvt. Ltd. India. The purity of nanoparticle was 99.99 per cent and the APS was 30 nm. To study the zinc oxide nanoparticle impact on macronutrient content of *Pleurotus spp. Pleurotus sajor-caju* was treated with different concentrations of ZnO nanoparticles, viz. 20 ppm and 40 ppm. Similar conditions were followed for untreated (control) oyster mushrooms, which were sprayed with distilled water. The minimum concentration of zinc oxide nanoparticles (20ppm, 40ppm) enhanced the mycelium radial growth on potato dextrose agar (PDA) medium and as the concentration of nanopaticles increased mycelial growth of oyster mushroom decreased.

Cultivation of Oyster Mushroom

Culture collection: The culture of the three Pleurotus spp. was procured from Mushroom Research and Training Centre (MRTC), G. B. Pant University of Agriculture and Technology, Pantnagar, US Nagar, Uttarakhand. The fungal cultures were maintained on potato dextrose agar (PDA) medium.

Preparation of Master spawn and Commercial spawn

Washing and boiling wheat grains (1:25 grain to water by volume) until they were softened was done in order to prepare master spawn. To remove excess moisture, grains were allowed to dry overnight on the sieve bed. To remove the extra moisture, the grains were thoroughly mixed with calcium sulfate and calcium carbonate at rates of 12gm and 3gm per kg of boiling wheat grains, respectively. The resulting grain combination was put into glass bottles (300g/bottle), sealed with non-absorbent cotton, and autoclaved for 20 minutes at 15 psi to sterilize them. After being removed from the autoclave, the sterilized bottles were thoroughly shaken to prevent the grains from clumping before cooling. These sterilized glass bottles were placed in a laminar flow before being inoculated with dises (5mm) of 12–15-day-old *P. sajor-caju*, culture. For 10 days, the inoculation bottles were kept at 25°C for incubation. To disrupt mycelia threads evenly across the bottle's mass of wheat grains, bottles were shaken vigorously. After 25 days after inoculation (the full spawn run), the entire mass of grains becomes covered with fine mycelia development. Additionally, master spawn was used to prepare commercial spawn.

Cultivation of oyster mushroom: In the current experiment, fresh, dry wheat straw was used as the substrate. The substrate was soaked in 1% calcium carbonate solution for 16 hours to prevent contamination from microbes. After that, the straw was removed from the solution and any extra water was drained, reducing the substrate's ultimate moisture content to about 70%. The substrate was then used for spawning. The wheat straw was then completely mixed with the commercial spawn.

Bag filling and bag opening

Substrate after mixing the spawn filled in polythene bags (60X45cm) @ 1.5 kg/bag and the polythene bag were closed tightly. Eight to ten small holes were cut at specific intervals after the polythene bag was filled and sealed to allow for the free diffusion of the gases and heat produced inside. The bags were transferred to the dark, moist incubation room, which had a temperature range of 25 to 28 °C. After the spawn run was finished, the polythene sheet was carefully removed with the help of a sharp knife, and water has been sprayed twice daily to maintain the proper humidity and promote the growth of fruiting bodies. Temperature and relative humidity were kept at 24-28 °C and 75-85 %, respectively, during cropping.

Nitrogen Estimation: The nitrogen content in mushroom samples was estimated by using the microkjeldhal method given by Jackson (1973). In this method, dried mushroom samples of 0.2g were taken and added to a digestion tube containing 1g of catalyst mixture (100g potassium sulfate, 10g cupric sulfate, and 1g metallic selenium powder in a ratio of 50:10:1, respectively) and 10 ml concentrated sulfuric acid. The digestion tubes were kept overnight for pre digestion and then the digestion was carried out in digestion assembly at 150º.The sample turned colorless or light green at the end of the digestion. The digestion tubes were allowed to cool and later fitted in the automatic ammonia distillation apparatus. At one end of the distillation unit, tubes were fitted, and 40 ml of 40% NaOH were added automatically by the digestion unit program. On the other side of the hose, 20 ml of 4% boric acid with mixed indicators (Methyl red and Bromo-cresol green) was added to a 250 ml conical flask. The digestion unit was heated by passing steam at a steady rate, and liberated ammonia was absorbed in the boric acid-containing conical flask, which turns greenish from pink. In this conical flask, nearly 125 ml of distillate were collected in 5 min. The distillate collected in a 250 ml conical flask was titrated with 0.1 N sulfuric acid, and the color changed black to pink.

Analysis of P, K, and S: Dried mushrooms were powdered in a homogenizer, and 0.5 g of dried powdered mushroom samples from different treatments were weighed in a 100 ml conical flask. Then, added 5 ml of concentrated nitric acid and allow it to stand overnight to prevent frothing and foaming. The conical flask was placed on a hot plate for 10 min. After cooling a 10 ml di-acid mixture of concentrated HNO_3 and perchloric acid ($HClO_4$) in the ratio 9:4 was added (Jackson, 1967). Then, the digestion was carried out on a hot plate until white fumes started arising and the mixture became transparent. After cooling, 5 ml of 6N HCl was added, and volume was increased to 50 ml with distilled water. Then, samples were filtered with Whatman No. 1 filter paper into a 100 ml volumetric flask, and the final volume makeup was done.

a) **Estimation of phosphorous (P):** Pipetted out 10 ml of digested sample into a 50 ml volumetric flask. Then, 10 ml of vanadate-molybdate reagent was added, and the final volume was made with distilled water and mixed well. After 30 min, absorbance was measured at 420nm under a UV-Vis spectrophotometer. (Jackson, 1967). The standards of P were prepared by taking different concentrations from a 50 ppm stock solution of P and making a volume of 50 ml after adding 10 ml of vanado-molybdate reagent. The standard curve was plotted between P concentration and absorbance at 420 mm, and the P content in samples was calculated.
b) **Estimation of potassium (K):** For estimation of total potassium in mushroom samples, the digested sample was directly measured under a flame photometer by using a red filter (Piper, 1966).
c) Estimation of sulphur (S): The total S content in oyster mushroom samples was determined by the barium chloride turbidimetric method using a spectrophotometer. In this method, 10 ml of digested sample was pipetted out into a 50 ml

volumetric flask. Then, add 1 ml of 6 N HCl and 2 ml of 0.25% gum acacia. Later, with the addition of 0.5 g of $BaCl_2$, turbidity starts and makes up the final volume. Then, the solution was allowed to react for 1 min, and the absorbance of the turbidity was measured at 490nm using a spectrophotometer (Chesnin and Yien, 1951).

Statistical Analysis: Experiments were carried out in triplicate. Data recorded from three replications were subjected to a one way analysis of variance, and critical differences were calculated at p=0.05 level.

RESULTS AND DISCUSSION

Effect of ZnO nanoparticles on Nitrogen content in the fruiting body of oyster mushroom: Data on nitrogen content in the fruiting body of *Pleurotus* mushroom as influenced by different treatments is presented in Table 1 it was indicated that different treatments had a non-significant impact on nitrogen content. However, the maximum (3.35%) nitrogen content was recorded at 20 ppm concentration, which was 1.2% higher than control. According to Yang *et al.* (2021) ZnO nanoparticles and Zn salt increased grain yield, N, P, and K uptake, and grain Zn concentration. Zinc oxide nanoparticles application enhanced nitrogen and potassium content in rice by increasing panicle number, spikelet number per panicle, and total biomass (6.8–7.6%), thereby promoting rice yield. Dimkpa *et al.* (2017) evaluated the effects of ZnO nanoparticles on macronutrient content and grain Zn enrichment. Across NPK levels and Zn exposure pathways, both Zn types increased nitrogen and potassium accumulation as compared to control plants.

Table1: Effect of ZnO nanoparticles on Nitrogen and Phosphorus content of Oyster mushroom (*Pleurotus sajar-caju)*

Treatment	Nitrogen (%)		Phosphorus (%)	
	PSC	% Increase	PSC	% Increase
20ppm	3.35±0.081	1.2	0.54±0.006	0.4
40ppm	3.3±0.015	0.60	0.47±0.007	14.89
Control	3.31±0.065		0.47±0.007	
C.D.	N/A		0.023	
SE(m)	0.061		0.007	
C.V.	3.145		2.3	

Note: Each value is expressed as mean ±standard error

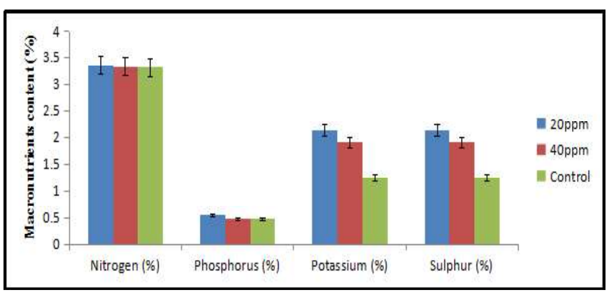

Fig. 1: Effect of ZnO nanoparticles on Macronutrients content of Oyster mushroom (*Pleurotus sajar- caju)*

Effect of ZnO nanoparticles on Phosphorus Content in the Fruiting Body of Oyster Mushroom

Application of 20 ppm ZnO nanoparticals gave (0.54%) more phosphorus content by fruiting body of oyster mushroom. Another treatment (40 ppm) gave 0.47±0.007 % more phosphorus than the control (Table1). There is an antagonistic effect of phosphorus and zinc, which was found due to the formation of zinc phosphate as an insoluble substance that decreases the availability of phosphorous in water and soil (Aboyeji *et al.,* 2020).

Effect of ZnO nanoparticles on Potassium content in the fruiting body of oyster mushroom

The potassium content in the fruiting body of an oyster mushroom sample was directly measured by a flame photometer. It was observed that maximum potassium content (2.11%) was recorded at 20 ppm ZnO nanoparticles treatments, which were 71.54%

Table 2: Effect of ZnO nanoparticles Potassium and Sulphur content of Oyster mushroom (*Pleurotus sajar- caju)*

Treatment	Potassium (%)		Sulphur (%)	
	PSC	% Increase	PSC	% Increase
20ppm	2.11±0.05	71.54	2.11±0.05	71.54
40ppm	1.91±0.021	55.28	1.91±0.021	55.28
Control	1.23±0.03		1.23±0.03	
C.D.	0.126		0.126	
SE(m)	0.036		0.036	
C.V.	3.539		3.539	

Note: Each value is expressed as mean ±standard error higher than control, followed by 40 ppm (1.91%), which was 55.28% higher than control. Phosphorus content reduced with increased concentration of ZnO nanaparticles but more potassium content was recorded as compared to control. Potassium content also decreased with increasing concentrations of the zinc oxide nanoparticles. The results indicate that a lower concentration of zinc oxide nanoparticles promotes the uptake and accumulation of potassium in *Pleurotus sajar caju* as compared to a higher concentration Sohail *et al.* (2020) observed that when the canola varieties; *Faisal canola* and Shiralee were exposed to ZnO nanoparticles, there was a significant increase in the potassium content, i.e., 1.68% and 1.72%, respectively. K plays a role in maintaining the osmotic balance between cells and interstitial fluid, which helps in lowering blood pressure, maintaining bone health, and reducing the risk of osteoporosis.

Effect of ZnO nanoparticles on sulphur content in the fruiting body of oyster mushroom: The sulphur content was higher 3.23% at 20 ppm ZnO nanaparticles treatments. However, at 40 ppm ZnO nanaparticles treatments, more than 3.22% sulphur content was observed as compared to the control of 3.20%, as depicted in Table 2**.** The data indicated that different treatments had a significant impact on sulphur content. Poudel *et al.* (2023**)** found that the Zn rate effect was observed only in the case of sulphur , where soaking the seeds in 25 ppm of Zn resulted in higher sulphur content in pea shoots than using 100 and 200 ppm Zn solutions, regardless of the Zn source used. The results indicate that a minimum concentration of zinc oxide nanoparticles promotes the uptake and accumulation of potassium.

CONCLUSION

It is well established that biofortification is a promising, cost-effective, agricultural strategy for improving the nutritional status of malnourished populations throughout the world. Oyster mushroom (*Pleurotus sajar- caju)* when fortified with zinc oxide nanoparticles at a concentration of 20 ppm, positively affected the macronutrient contents, resulted in higher nitrogen, phosphorus, potassium, and sulphur content as compared to the control. Nitrogen, potassium, phosphorus, and sulphur content improve with a minimum concentration (220 ppm) of zinc oxide nanoparticles in *Pleurotus sajar caju*. Therefore, future research would also need to focus on studies to explain the mechanisms involved in multiple nutrient interactions to mitigate the harmful effects of nutrient deficiency. This study can be further used to combat macronutrients deficiency and hidden hunger.

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