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Shivani
Department of Plant Pathology, College of Agriculture, G.B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

KPS Kushwaha
Department of Plant Pathology, College of Agriculture, G.B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Arun Kushwaha
Department of Plant Pathology, College of Agriculture, G.B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Omkar Singh
Department of Plant Pathology, College of Agriculture, G.B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Anjali Patil
Department of Biotechnology, Division of life sciences, HNB Garhwal University, Srinagar, Uttarakhand, India

Corresponding Author:
Shivani
Department of Plant Pathology, College of Agriculture, G.B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Effect of zinc sulphate nanoparticle on productivity of oyster mushroom (*Pleurotus florida*)

Shivani, KPS Kushwaha, Arun Kushwaha, Omkar Singh and Anjali Patil

Abstract
Mushrooms have been considered as functional foods since the time immemorial owing to its nutritional and medicinal properties. Similar to the agricultural crops, the biofortification of mushrooms using nanoparticles is an emerging technology in the field of mushroom cultivation. It has been widely adopted to delineate micronutrient deficiencies and increasing biological yield of mushrooms. Among the cultivated mushrooms, oyster mushroom is highly popular as they are rich in nutrients, antioxidants and trace minerals. The present investigation was carried out to at the Mushroom Research and Training Centre, GBPUAT, Pantnagar, to determine the impact of zinc sulphate nanoparticles at four different concentrations *viz.*, 10 to 40 ppm on the biological yield of oyster mushroom. The results revealed that all the tested concentration had significantly higher yield as compared to the control. Among the tested concentrations, 10 ppm concentration significantly increased the total yield and biological efficiency of oyster mushroom. It was followed by 20 ppm, 30 ppm and 40 ppm, respectively. The total yield was 1088.33g, 964.98g, 889.98g and 874.99g in 10, 20, 30 and 40 ppm respectively as compared to 724.98g in control. Similar trend was observed in the biological efficiency as well with the 10ppm concentration having the highest biological efficiency of 108.83 percent which was followed by 20 ppm (96.50%), 30 ppm (89.00%) and 40 ppm (87.50%). The results obtained in the study can be used for increasing the production of the oyster mushroom in the country as well to decrease the zinc malnutrition.

Keywords: Mushroom, *Pleurotus florida*, zinc sulphate nanoparticle, biological yield

Introduction
For years, mushrooms have been part of the fungal diversity. Mushrooms are cultivated as an important agriculture product worldwide. China, USA, Netherlands, Poland, Spain, France, Italy, Ireland, Canada and UK are the leading producers. In India, the mushroom production is about 1.45 lakh metric tons (about 0.4% of the world production). The food experts also appreciated the food value of mushroom because of its less calorific value (27–30 kcal/100 g), low amount of fat (1.3–8% of dry weight mushrooms) and digestible carbohydrate and very high content of protein (20-40% on dry weight basis) with the balanced composition of vitamins and minerals like phosphorous, potassium, sodium, calcium, magnesium, zinc, iron, copper, manganese etc. They also contain 5–15% dry matter and are rich in fiber (Mattila *et al*., 2002) [17]. Among all cultivated edible mushrooms, Oyster mushrooms are one of the most popular edible mushrooms and belong to the genus *Pleurotus* and the family *Pleurotaceae* (Kong, 2004) [14]. Oyster mushroom is in the second position (after button mushrrom) in the world and its cultivation has increased during the last decade (Royse, 2002; Shelly, *et al*., 2009) [22, 24]. It is widely cultivated due to its medicinal and organoleptic properties, the cultivation is simple with lowcost production and higher biological (Chirinang and Intarapichet, K. 2009) [4]. Size differs among species and within the same species if cultivated under different climatic and nutritional conditions. At present, India produces about 10,000 tons of oyster mushroom annually. It is mostly cultivated in Orissa, Karnataka, Maharashtra, Andhra Pradesh, Madhya Pradesh, West Bengal and in the North-Eastern States of Meghalaya, Tripura, Manipur, Mizoram and Assam. (Jha, 2011) [10]. *Pleurotus florida* (also known as white rot basidiomycete, abalone or tree mushroom) which cultivated on variety of agricultural lignocellulosic wastes is a delicious edible mushroom having immunostimulating and antitumour compounds (Khanna and Garcha, 1981) [13]. It forms innumerable primordia scattered over the entire substrate, which mature into medium sized (average 5 cm diam.) funnel shaped fruitbodies with elongated stems. It produces myriad of extracellular enzymes and can be used as a source of lignin-degrading enzymes for the biotreatment of wastes and effluents. Certain novel techniques are used to achieve high production rates and yields of crops.

In the field of biology, nanoparticles have a variety of applications as vaccine or drug delivery systems, minerals, antibacterials, etc. Now, the nanotechnology is also being used in agriculture. It serves as the modern technology for precision agriculture, whereby strategies are formulated and streamed towards meeting with food demands of the increasing human population. Applying nanotechnology in agriculture will help in bridging the gap in nutrient loss and fortification of crops. Reports suggested that the use of nanotechnology in agriculture have been grown exponentially in past (Lowry *et al*., 2019; Kah *et al*., 2018, 2019) [16, 11, 12]. To alleviate the micronutrient deficiency in human diet, this technology can be a boon. Studies are going on to biofortify crops to reduce the micronutrient deficiency. Among all the essential micronutrients, Zn plays a vital role in many physiological activities and nowadays its deficiency is a worldwide problem, causing a severe threat on crop production and human health (Farooq *et al*., 2018) [9]. According to Kopittke *et al*. (2019) [15] and Phattarakul *et al*. (2012) [19] 50% of the global agricultural soil is deficient in available Zn and approximately 30% of the global population is subjected to Zn deficiency, especially in developing countries, respectively. The primary sources of zinc micronutrients for fertilizer fortification are zinc oxides (ZnO) and zinc sulfates ($ZnSO_4H_2O$ or $ZnSO_4.7H_2O$). Many previous studies reported that the application of Zn increased the grain yield (Dimkpa *et al*., 2017; Du *et al*., 2019 and Subbaiah *et al*., 2016) [5, 6, 7, 26]. Several studies have suggested that nanoparticles have positive effects on overall germination, growth and performance on different crops. For example, seed germination, seedling growth, photosynthetic efficiency, biomass, total protein, sugar, nitrogen and micronutrients have been efficiently increased in several crop plants; *viz*., *Spinacia oleracea* (Srivastava *et al*., 2014) [25], *Cucumis sativus* (Moghaddasi *et al*., 2017) [18], *Solanum lycopersicum* (Faizan *et al*., 2018) [8], and *Triticum aestivum* (Zhang *et al*., 2018) [28]. Moghaddasi *et al*. (2017) [18] reported that *C. sativus* grown in gel chamber showed increased shoot and root biomass with ZnONPs (1 mg/L), and increased shoot length with ZnONPs compared to bulk ZnO. Therefore, keeping in view the above facts in mind the present investigations on biofortification of *Pleurotus* mushroom using zinc sulphate nanoparticle were carried out.

Materials and Methods

Culture collection

The culture of *P. florida* was procured from Mushroom Research and Training Centre (MRTC), G. B. Pant University of Agriculture and Technology, Pantnagar, US Nagar, Uttarakhand on 06, September 2019. The fungal cultures maintained on Potato dextrose agar (PDA) medium and was stored at 4ºC till use.

Preparation of Master Spawn

Wheat grains obtained from the CRC (Crop Research and Training Centre) were washed and boiled (grain: water; 1:25, w/v) till they get softened. Grains were dried on the sieve bed overnight to remove extra moisture. The grains were thoroughly mixed with calcium sulphate and calcium carbonate @ 12 g and 3 g per kg boiled wheat grains, respectively, to absorb the excess moisture. The resultant mixture of grains was filled in the glass bottle (300 g/bottle) and plugged with non-absorbent cotton and sterilized in an autoclave at 15 psi for 20 minutes. Sterilized bottles were taken out of autoclave and shaken properly to avoid the clumping of the grains before cooling them. These sterilized glass bottles were kept in a laminar flow and then inoculated with the 12-15 days old culture of *Pleurotus florida* in the form of discs (5mm). The inoculated bottles were incubated at 25°C for 10 days. Bottles were shaken vigorously to be disrupted mycelia threads evenly in entire mass of wheat grains in bottle. Entire mass of grains gets covered with fine mycelia growth after 25 days of inoculation (complete spawn run). Master spawn was further used in preparation of commercial spawn.

Preparation of commercial spawn

Same method was used as per the preparation of master spawn, but propylene bags were used instead of glass bottles.

Substrate Preparation

Fresh and dry wheat straw was used as substrate in the present experiment. To avoid microbial contamination, the substrate was immersed in 1% calcium carbonate solution for 16 hours. Then straw was taken out from the solution and excess water was drained out, so that the final moisture content of the substrate is approximately 70 per cent. This substrate was then used for spawning.

Spawning of *P. florida*

The commercial spawn was then thoroughly mixed with the treated wheat straw (approx. 2.0%).

Bag filling

Spawned substrate (substrate after mixing with the spawn) was filled in polythene bags (60 X45cm) and closed tightly. After filling and closing polythene bag, 8-10 small holes were made at the certain distance for free diffusion of gases and heat generated inside. The bags were transferred to the dark incubation room with temperature 25-28°C and humidity 70-80%.

Bag opening

After complete spawn run, polythene bags were removed carefully and regularly watered for maintaining proper humidity and to promote development of fruiting bodies. At this stage, temperature and relative humidity was ranged between 24-28^{0}C and 75-85%, repectively.

Application of Zinc sulphate ($ZnSo_4$) Nanoparticle

After complete spawn run, bags of *P. florida* were treated with different concentrations of $ZnSo_4$ nanoparticles *viz.*, 10 ppm, 20 ppm, 30 ppm and 40 ppm. Different concentrations of zinc sulphate nanoparticles solution were prepared by diluting stock solution in double distilled water followed by proper mixing. Different concentrations of the working solutions were sprayed on the spawn run substrate @ 12.5 ml per Kg substrate and three replicates of each treatment were taken for the experiment.

Harvesting

Harvesting was done when the caps of mature mushroom became flat and curled inward. The matured fruiting bodies of *P. florida* were harvested by hand pick up in clockwise or anti clockwise rotation so that the fruit body is pulled out without leaving any stub. The harvested fruit body was weighed and recorded individually. The observation was observed up to 5th harvesting.

Mushroom yield and biological efficiency

Total yield was calculated as the fresh weight of mushrooms harvested up to fifth flush per Kg of dry substrate. Yield data of different treatments were recorded on basis of their number of fruiting bodies and the fresh weight. Biological efficiency (BE) was determined by the ratio of fresh weight (g) of mushrooms to dry weight (g) of substrate and expressed as percentage (Chang *et al*., 1981) [3].

$$\text{Biological efficiency \%} = \frac{\text{Fresh weight (g) of mushrooms harvested}}{\text{Dry weight (g) of substrate}} \text{X100}$$

Statistical Analysis

In this experiment, a minimum of three replicates were used and the data obtained was subjected to one factorial CRD, using SPSS 16.0 for windows.

Results and Discussion

Biological yield of *Pleurotus florida*

The mushrooms with all the treatments sprouted in clusters and had the white color fruiting body that is characteristic for the species. The first flush or harvest occurred after 15 days of incubation on the substrate (Table 1). These were averaged to the nearest day from the replicates. The data regarding number of flushes, flush wise yield, total yield and their means are presented in Table 1. The highest yield in the first flush was obtained in 10 ppm (260.00 g), followed by 20 ppm (231.66 g). In 30 and 40 ppm the yield was 215.00 g each and in control treatment 203.33 g fresh mushroom was obtained. Similar trend was observed in second, third, fourth and fifth harvest with 10 ppm concentration yielding 255.00 g, 221.66 g, 185.00 and 171.66 g, respectively. In second to fourth flush the second highest yield was obtained in 20 ppm followed by 30 and 40 ppm. In case of fifth flush the 40 ppm (128.33 g) concentration had higher yield than 30 ppm (121.66 g). The control had the lowest yield with 203.33 g, 188.3 g, 141.66 g, 113.33 g and 78.33 g in first to fifth flush, respectively. The highest total yield was obtained in 10 ppm (1088.33 g), which was followed by 20 ppm (964.98 g), 30 ppm (889.98 g) and 40 ppm (874.99 g) which was significantly higher than the control (724.98 g) furthermore the 10 ppm also recorded the highest biological efficiency (108.83) with 50 percent increase in yield over control. The 20ppm concentration recorded 96.50 percent biological efficiency and 33 percent increase in yield over control. The biological efficiency of the control was 72.50 percent only.

The results of this study demonstrated that the minimal i.e., 10 ppm concentration of zinc sulphate nanoparticle significantly contributes in increasing the biological yield of *P. florida* by 50% followed by 20 ppm, 30 ppm and 40 ppm i.e., 33%, 23% and 21% respectively. The results of the study were in corroboration with the findings of Sahu, (2018) [23] where maximum yield of *Pleurotus florida* was recorded at 7 ppm concentration of iron sulfide nanoparticle, with 73% higher economic yield as compared to control, whereas 43% and 5% yield were increased in 14 ppm and 21 ppm concentrations, respectively. Similarly, the enhancement of crop yield with the application of zinc sulphate nanoparticles have been reported by Du *et al.* (2019) [6, 7]. According to them the treatment of ZnO nanoparticles increase the yield and biomass of wheat by 56 & 63 percent and 55 and 72 percent respectively as compared to control. According to Bakhtiari *et al.* (2015) [2] wheat plants treated with 0.04% iron oxide nanoparticles showed a significant increase in grain yield. Arora *et al*. (2012) [1] reported that plants treated with 10 ppm of gold nanoparticles caused 19% increase in seed yield of *Brassica juncea* under field conditions. This confirms previous observations that *Brassica juncea* seedlings treated with 4 ppm iron sulfide nanoparticles at 30 DAS recorded increase in seed yield (Rawat *et al*., 2017) [20]. According to Rossi *et al*. (2019) [21] After the zinc oxide nanoparticle treatment in coffee plants the fresh weight of leaves and roots were increased by 37 percent and 95 percent, respectively as compared to control. ZnO NPs application enhanced NPK content in rice, with subsequence increasing panicle number (3.8–10.3%), spikelet number per panicle (2.2–4.7%), and total biomass (6.8–7.6%), thereby promoting the rice yield. Compared with conventional fertilization (Yang *et al*., 2021) [27].

Table 1: Biological yield of Pleurotus Florida per 2 kg of wet substrate treated with different concentrations of zinc sulphate nanoparticles

	1st flush	2nd flush	3rd flush	4th flush	5th flush	Total Yield	Biological efficiency	Percent increase
T1	260.00±5.77	255.00±2.88	221.66±4.40	185.00±2.88	171.66±6.00	1088.33±3.32	108.83	50%
T2	231.66±6.00	218.33±7.26	198.33±4.41	168.33±4.41	148.33±6.00	964.98±18.02	96.50	33%
T3	215.00±8.66	208.33±9.28	193.33±8.81	151.66±6.00	121.66±4.41	889.98±27.53	89.00	23%
T4	215.00±5.77	205.00±2.88	183.33±1.66	143.33±6.00	128.33±4.41	874.99±16.07	87.50	21%
T5	203.33±6.00	188.33±4.41	141.66±6.00	113.33±4.41	78.33±4.41	724.98±10.00	72.50	
CD (5%)	20.87	18.88	17.8	15.6	16.31	54.4		
CV	5.03	4.78	5.14	5.55	6.82	3.24		

Values are mean of triplicates (±S.E.M) are statistically significant different ($p < 0.05$) from each other. T1=10 ppm, T2=20 ppm, T3=30 ppm, T4=40 ppm and T5 = Untreated (0 ppm)

Conclusion

The application of ZnSo4 nanoparticles at10 ppm helps in enhancing the yield and biological efficiency of *Pleurotus florida.* Moreover, this will help in biofortification of zinc in these mushrooms and in turn help in alleviating zinc deficiency and ensuring food and nutrition security in the country.

Further Research

Further research needs to be carried out on other economically important oyster mushrooms like *Pleurotus sajor- caju, P. eous, P. pulmonaris* etc. Moreover, detail investigation is also required to understand the effect of zinc nanoparticle on the nutritional and biochemical properties. The enhancement of the zinc and other micronutrient content also needs to be validated to scientific investigations.

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