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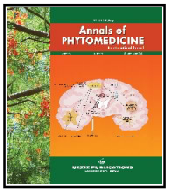

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Evaluation of different doses basis micronutrient and growth regulator against growth behaviours and yield perspective of oyster mushroom, *Pleurotus florida* (Mont.) Singer

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Abstract

Nowadays, the population is increasing hastily and arable lands are used for industrial as well as living purposes, so feeding this growing population is a dynamic challenge all over the world. Mushrooms are rich in nutrients and taste, required low-input and limited-area for their production, and have no need of fertile land for oyster mushroom, *Pleurotus florida* (Mont.) Singer cultivation. The presented data showed the effect of various concentrations of the micronutrient (Shaktiwan) and growth regulator (GA3) on the growth and yield of mushrooms. Among the treatments, T_4 [micronutrient (1%) with the spraying of GA3 (10 ppm)] recorded 16.67 days for pinhead initiation, followed by T_2 [micronutrient (0.5%) with GA3 (10 ppm)] which recorded 17.00 days, but control was taken maximum 18.33 days after spawning. The highest value of morphological growth behaviours such as the average number of fruit bodies per bag was 106.33, the length of the stalk 6.82 cm and cap diameter 9.07 cm were recorded from T_4, followed by T_2 as average number of fruit bodies per bag was 102.67, length of stalk was 5.97 cm and cap diameter was recorded 8.23 cm against control which was recorded minimum value as average fruiting bodies per bag 86.00, stalk length 4.98 cm and diameter of cap about 7.07 cm, respected to all treatments. On the other hands, the crop harvested in 3 flushes, it is evident that the maximum yield in first, second and third flush was obtained from T_4 about 405.67 g, 401.33 g and 400.67 g, respectively. Highest total yield was also obtained from T_4 about 1207.67 g, followed by T_2 and T_3 [micronutrient (1%)], which recorded 1154.00 and 1127.66 g, respectively. Control sample was harvested about 951.33 g, which recorded minimum than all the treated bags. The biological efficiency of T_4 and T_2 was highest about 120.76 per cent and 115.40 per cent, respectively.

1. Introduction

The continuous supply of healthy and nutritional foods for current huge population is a big challenge and it shall become more threat for human in upcoming time. So, it is indispensable to looks for new crops that have ability to complete demand of food and nutrition (Mehrotra, 2021). In this context, cultivation of mushroom finds favors which can be grown by landless individuals. Mushroom can be cultured on agricultural wastes. Recycling of agricultural waste, including agro-industrial waste, is one of the key areas that can help to achieve the goals of resource conservation and higher productivity. By using these wastes to cultivate mushrooms, we may increase our incomes and sustainability (Chittaragi *et al.,* 2013). On the surface of earth, approximately 200 billion tones organic matters are produces per year by the process of photosynthesis. In addition, huge amounts of agricultural wastes and industrial by-products are produced globally from farm practices and industrial food products, respectively. This leads to serious environmental pollution, though they are rich in organic compounds that are worthy of being recovered and transformed into value-added products (Sekeroglu, 2019). Thus, these days, mushroom cultivation technologies are being a promising candidate to achieve food security along with the reduction of environmental pollution apart from their nutritional and medicinal value (Sharma *et al.,* 2017).

Greeks believed that mushrooms provided warriors courage in battle, at that time Romans regarded about them as “Gods Flesh” (Chu *et al.,* 2002), which was served only on festive occasion. Mushroom is considered as “Food of the God” and is considered as a special kind of food since ancient time. It is an achlorophyllus in nature, a fungus that grows in various environments and occurs seasonally on all continents. It has a variety of specialised characteristics, including shape, size, colour, and edibility. A macro fungus called mushroom has a characteristic fruiting body that can be either epigeous or hypogeous, and enough to be seen with the naked eye, which can

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easily have harvested by hand (Kumar *et al.,* 2022). In the world of food, mushrooms are considered as vegetable, yet they are neither a plant nor a vegetable. They are members of the kingdom of fungus. Since thousands years ago, peoples used wild mushrooms as a food and medicine**.** Globally, mushroom production represents; namely, *Pleurotus, Agaricus, Volvariella, Lentinus, Flammulina, Tremella* and *Auricularia* which directly contribute around 89.0 per cent of the global production (Chang, 2007; Usha *et al.,* 2018). Among the all of them, *Pleurotus* (Oyster or Dhingri) is the most important due to its nutritious value and generally grown (Northern India) in a wide range of temperature**.**

The word meaning of 'Oyster' refers to the 'shellfish' like appearance of the sporophores (fruiting bodies). Oyster mushroom is rich in vitamin b complex, C (ascorbic acid) and protein content varies between 1.5 to 2.6 per cent. The niacin (B_3) content is ten times high than all other vegetables and few species of mushroom also exhibit health promoting activities such as; antiageing, antioxidant, antifungal, antibacterial, antiviral, anticancer and neuroprotective properties (Choudhary *et al.,* 2023). The folic acid and vitamin B_{12} was also present in oyster mushrooms which help for curing anaemia (Karuppiah *et al.,* 2021). It has most of the minerals, amino acids, proteins and enzymes required for human body (Poniedzia³ek *et al.,* 2017). *Pleurotus* species are the well-known globally for their ability to promote human health and easy cultivable methods with least input requirements.

Compared to other mushroom genera, oyster mushrooms are the easiest, quickest, and least expensive to produce, that require less preparation time and production technologies. Increase the production of oyster mushroom through the bioconversion of lingo-cellulosic agricultural and industrial wastes which provided better opportunity to exploit endless resources in the manufacture of consumable, protein-rich food that will maintain food security for people in under developed nations (Barman *et al.,* 2021; Raman *et al.,* 2021). This is grown in wide range of substrate likes paddy straw, sawdust, wheat bran, wheat straw and pulse husk *etc,*. Several researchers used diverse substrates for cultivation of 'Dhingri' mushroom (Kumar *et al.,* 2019). Single spraying of gibberellic acid reduces the time of pin head initiation and increased the overall morphological parameters of sporophores (Adenipekun and Gbolagada, 2006). Application of micronutrient and gibberellic acid which enhanced the total number and weight of the sporophores, as well as the biological efficiency, in cultivated bags of oyster mushroom**.**

2. Materials and Methods

2.1 Isolation and purification of mushroom culture (*P. florida)*

The fresh sporophores of oyster mushroom were collected from growing room (Mushroom house) of mushroom spawn laboratory (MSL), Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. These collected sporophores were used for isolation of mushroom culture, sub-cultured and kept on potato dextrose agar (PDA) media at 24 to 28^{0}C temperature in B.O.D. (Biological Oxygen Demand) incubator for further investigation.

2.2 Preparation of mushroom spawn

Healthy, uniform size and clean wheat (*Triticum aestivum*) grains were washed many times with running tap water to remove the inert materials. Cleaned and well washed grains of wheat were boiled about 30 to 40 min or until they soften. After boiling excess water was removed out and the boiled, grains were cooled on tray. To prevent cracking and clumping of the cooled grains, 0.5% calcium carbonate and 2% calcium sulphate were well mixed with the substrates on dry weight basis. Calcium carbonate and calcium sulphate mixed wheat grains were filled (350 g/pack) in well sterilized saline bottle which plugged by non-absorbent cotton plugs. These bottles (substrates filled) were sterilized in autoclave at 15 psi (pound per square inch) for 121.6^{o}C about 25 to 30 min and then allowed to cool; after that, these bottles were aseptically inoculated by mycelium bits about 8 to 12 days old pure culture of mushroom. These inoculated bottles were placed in an incubator set at 25°C to allow the mycelium running. These bottles were shaken at about 4-5 days interval for proper spreading of mycelium between the grains. About 12 to 15 days after these bottles were entirely colonized by white colored mycelium.

2.3 Substrates preparation

First of all, the substrate (fresh golden color wheat straw) was soaked in water for 10-12 h in plastic bucket cemented tank. After soaking, excess water was drained out. For sterilization, this moist substrate was filled in gunny bags and put in autoclave. The substrate was sterilized for 30-40 min in autoclave at 121.6°C at 15 psi. The substrate was cooled after sterilization and spread on well cleaned floor for removal of excess moisture.

2.4 Experimental details

The experiment was conducted in the departmental mushroom spawn laboratory's growing room. Department of Mycology and Plant Pathology, Banaras Hindu University, Varanasi, India. Micronutrient (Shaktiwan) was added in already prepared substrate, at the time of spawning and single spraying of gibberellic acid (GA_3) after pin head initiation.

Treatment details

$T_{1 =}$ Micronutrient (0.5% /bag)

$T_{2 =}$ Micronutrient (0.5%/bag) + GA_3 (10 ppm)

$T_{3 =}$ Micronutrient (1%/bag)

$T_{4 =}$ Micronutrient (1%/bag) + GA_3 (10 ppm)

$T_{5 =}$ GA_3 (10 ppm)

Control = (Untreated)

2.5 Spawning and packing

The spawning was done in a 24 h pre-fumigated room. At the time of spawning, addition of sterilized micronutrient 'Shaktiwan' which was in combination of (iron-5%, manganese-2%, copper-1%, boron-1% and small amount of zinc and sulphur) and several nutrients. Freshly prepared (milky colour and 15-20 days old) mushroom spawn was procured for spawning or seeding. Spawn of *P. florida* was mixed in well prepared substrates and filled in separate polythene bags. Each bag was contained 1.7 kg moist substrate. The filled bags were tie with rubber band and made 7-10 tiny pin size holes (1 mm) around the bags for proper aeration. Just after spawning, the bags were kept on iron racks in the growing room and maintained proper temperature and relative humidity.

2.6 Cropping

Spawned bags were kept on iron racks in the growing room for mycelium colonization of the substrate. Once the substrate fully colonized by mycelium and formed thick mycelium mate, after that, the bags were opened with least disturbance of beds and proper arranged on iron racks with a minimum distance of 15-20 cm between two beds in the growing room. Appropriate temperature (24-28^{0}C) and relative humidity (80-90%) were maintained to facilitate fruiting. The bags were sprayed with water twice (if are needed) daily during morning and afternoon to maintain moisture level of the substrate.

2.7 Fruiting and harvesting

The fruiting bodies of *P. florida* were harvested 5-10 days after pin head initiation. Removed mushroom without leaving a stub or disturbing the neighbouring fruiting bodies, it was picked by gently twisting the mushroom. Three successive times were harvested from each bag. Before spore formation, the fruiting bodies should be picked; harvested mushrooms were weighed at every time and calculated as gm/bag.

2.8 Effective performance of micronutrient and GA_3 on various observations

2.8.1 Growth behaviour

Data was recorded (days) after spawning on different parameters such as pinhead initiation, first, second, third harvesting and total cropping period.

2.8.2 Growth parameters

Growth parameters of mushroom such as total number of fruit bodies (per bag), maximum and minimum weight of fruiting body (g), average stalk length (cm), average stalk width (cm) and average cap diameter (cm) of fruiting bodies were recorded.

2.8.3 Yield parameters

The different observations on yield recorded, *viz.*, first flushing, second flushing, third flushing and total yield (g) and biological efficiency (%). The ratio of fresh weight (g) of the mushrooms (up to the third flush) to the dry weight (g) of the substrate, expressed as a percentage, was used to calculate biological efficiency (B.E.). Biological efficiency of substrate was calculated by given formula (Chang *et al.,* 1981):

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

2.9 Statistical analysis

The experiment was conducted on the basis of completely randomized design (C.R.D.) with mean of three replications. Analysis of variance (ANOVA) was used to determine the critical difference (C.D.) between the substrates with regard to size, weight, and yield of sporophores. All the examination was carried out at 5% level of significance.

3. Results

3.1 Growth behaviours and measurement

3.1.1 Initiation of pin head

The mushroom pin head first initiated from micronutrient (1%) with GA_3 (10 ppm) treated bags where it took 16.67 days, followed by micronutrient (0.5%) with GA_3 (10 ppm) 17.00, GA_3 (10 ppm) 17.33, micronutrient (0.5%) 18.00 and micronutrient (1%) 18.33 days but control sample was taken highest 18.33 days after spawning, among various series; series 1 represented pin head initiation in Figure 1. Treated bags non-significant over its control.

3.1.2 Harvesting of flushes and total cropping period (in days)

During total cropping period of oyster mushroom, three flushes were harvested. Result showed that micronutrient (1%) with GA_3 (10 ppm) was found better performance and taken minimum time for harvest 1st, 2nd and 3rd flush (20.33, 30.33 and 39.67 days, respectively. Maximum cropping period among treated was recorded from only micronutrient (1%) in 43 days but untreated bag required maximum time 44.67 days was shown in Table 1.

Table 1: Effect of micronutrient and gibberellic acid (GA_3) on growth behaviours of mushroom

Supplements	Pinhead initiation (days)	1st harvesting (days)	2nd harvesting (days)	3rd harvesting (days)	Total crop period (days)
Micronutrient (0.5%)	18.00	21.67	32.33	43.00	43.00
Micronutrient (0.5%) + GA_3(10 ppm)	17.00	20.00	31.00	41.67	41.67
Micronutrient (1%)	18.33	22.00	32.33	43.00	43.00
Micronutrient (1%) + GA_3 (10 ppm)	16.67	20.33	30.33	39.67	39.67
GA_3 (10 ppm)	17.33	21.33	32.33	42.33	42.33
Control	18.33	22.33	33.33	44.67	44.67
SE(m)	0.43	0.43	0.69	0.68	
C.D. (at 5%)	1.33	1.33	2.14	2.10	
C.V. (%)	4.23	3.50	3.76	2.78	

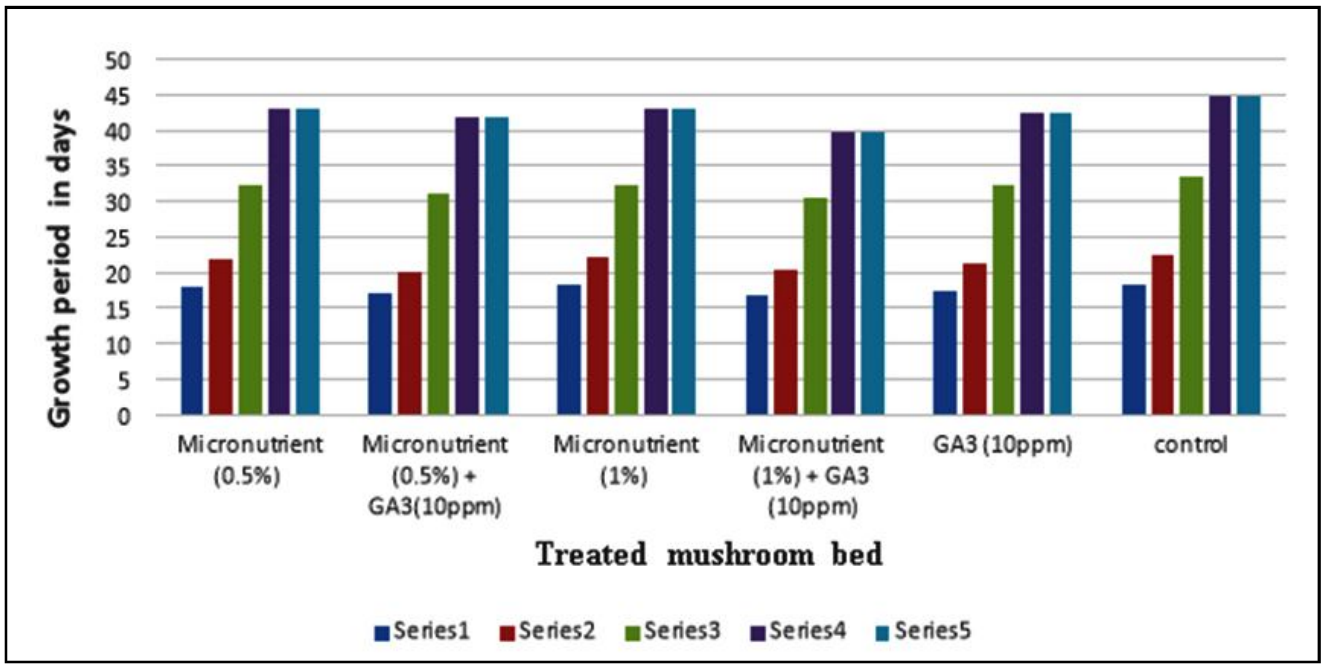

Figure 1: Effect of micronutrient and gibberellic acid (GA_3) on growth period in days.

Series 1 = Pinhead initiation, Series 2 = First harvesting, Series 3 = Second harvesting, Series 4 = Third harvesting, Series 5 = Total crop period.

3.2 Growth parameters

3.2.1 Average number of fruit bodies per bag

It is evident from the Table 2, pertaining to the number of fruit bodies harvested from the combination of micronutrient and growth regulator treated mushroom bags. Maximum number of fruit bodies was obtained from the micronutrient (1%) with GA_3 (10 ppm) treated bag where it produced 106.33 fruit bodies, followed by micronutrient (0.5%) with GA_3 (10 ppm) which produced 102.67, same in micronutrient (0.5%) and GA_3 (10 ppm) 96.33 fruit bodies while minimum number of fruit bodies about 86.00 was harvested from control (non-treated) bags.

3.2.2 Maximum and minimum weight of fruit body (sporophores)

The highest weight of fruit body recorded from T_4 treated bag that was 27.00 g, followed by T_2, (T_3) micronutrient (1%), T_5 and (T_1) micronutrient (0.5%) 26.67, 26.00, 25.67 and 22.00 g, respectively. However, minimum weighted sporophores was recorded from micronutrient (1%) with GA_3 (10 ppm) treated bags that were 5.42 g and minimum weight recorded from non-treated bags that was 4.70 g. The least maximum weight of fruit body harvested from non-treated bags about 20.67 g. Maximum and minimum weight for single sporophores significantly higher in treated bags over control.

3.2.3 Average length of stalk (cm)

The maximum average length of stalk measured from T_4 treated bag i.e. (6.82 cm), followed by micronutrient (0.5%) with GA_3 (10 ppm) (5.97 cm), micronutrient (0.5%) (5.63 cm), GA_3 (10 ppm) (5.70 cm) and minimum average stalk length was measured in micronutrients (1%) treated bags (4.87 cm), while minimum average stalk length was measured from untreated bag that was 4.98 cm.

3.2.4 Average width of stalk (cm)

The average width of stalk was different in treated and non-treated mushroom bags. The maximum stalk width was measured in micronutrient (1%) with GA_3 (10 ppm) treated bags 4.27 cm, followed by GA_3 (10 ppm) 3.92 cm, micronutrient (0.5%) with GA_3 (10 ppm) 3.88 cm, micronutrient (1%) 3.61 cm and micronutrient (0.5%) 3.53 cm, whereas in untreated bags 3.16 cm stalk width were observed.

3.2.5 Average cap diameter (cm)

The average cap diameter of mushroom fruiting body was found different size in treated and non-treated mushroom bags. The differences in mushroom cap diameter are represented in Figure 2. The highest average cap diameter of mushroom was measured in micronutrient (1%) with GA_3 (10 ppm) treated bag (9.07 cm), followed by micronutrient (0.5%) with GA_3 (10 ppm), micronutrient (0.5%), GA_3 (10 ppm) and micronutrient (1%) 8.23 cm, 7.97 cm, 7.67 cm and 7.37 cm, respectively. The least average cap diameter was measured in control (7.07 cm). Combination of micronutrient with growth regulator was found positive effect against all growth parameters. During the experiment, growth characteristics such as the weight of fruit bodies, minimum and maximum weight and their average length, width, and cap diameter of sporophores were measured. It has been concluded that average diameter of micronutrient with GA_3 treated bags are significantly different over its control.

Table 2: Effects of micronutrient and growth regulator (GA_3) on growth parameters

Supplements	Total number of fruiting body	Max. weight of fruiting body (g)	Min. weight of fruiting body (g)	Average length of fruiting body (cm)	Average width of fruiting body (cm)	Average diameter of mushroom cap(cm)
Micronutrient (0.5%)	96.33	22.00	5.26	5.63	3.53	7.97
Micronutrient (0.5%)+GA_3(10 ppm)	102.67	26.67	5.11	5.97	3.88	8.23
Micronutrient (1%)	93.00	26.00	4.36	4.87	3.61	7.37
Micronutrient (1%)+GA_3 (10 ppm)	106.33	27.00	5.42	6.82	4.27	9.07
GA_3 (10 ppm)	96.33	25.67	4.77	5.70	3.92	7.67
Control	86.00	20.67	4.70	4.98	3.16	7.07
SE(m)	5.32	1.37	0.26	0.10	0.14	0.41
C.D. (at 5%)	16.39	4.23	0.80	0.32	0.42	1.25
C.V. (%)	9.52	9.65	9.09	3.20	6.32	8.90

3.3 Yield performance and biological efficiency

3.3.1 Total yield and biological efficiency

Data pertaining to the effect of various concentrations of micronutrient and growth regulator (GA_3) on yield shown in the Table 3. The maximum yield in the first, second and third flushes was obtained from T_4 [micronutrient (1%) with GA_3 (10 ppm)] about 405.67 g, 401.33 g and 400.67 g, respectively. Maximum total yield was also obtained from treatment of micronutrient (1%) with GA_3 (10 ppm) about 1207.67 g, followed by micronutrient (0.5%) with GA_3 (10 ppm), micronutrient (1%), GA_3 (10 ppm) and micronutrient (0.5%) which recorded 1154.00, 1127.66, 1058.67 and 968.00 g, respectively. T_4 [micronutrient (1%) with GA_3 (10 ppm)] was the higher yielder with a yield of 1207.67 g/bag than that of T_2 [micronutrient (0.5%) with GA_3 (10 ppm)] which recorded 1154.00 g with the biological efficiency of 120.76 and 115.4 per cent, respectively. All the treatments performed better yield and biological efficiency as compared to un-treated mushroom bags (951.33 g), recorded in Table 3. So, it has been concluded that the biological efficiency and total yield harvested from micronutrient and GA_3 treated bags in Figure 2 was significantly higher than the control; Figure 3 representing the various steps (1-8) involved in the scientific procedure of oyster mushroom cultivation.

Table 3: Effect of micronutrient and growth regulator (gibberellic acid) on yield

Supplements	1^s flush(g)	2nd flush(g)	3rd flush(g)	Total yield(g)	Biological efficiency (%)
Micronutrient (0.5%)	330.33	330.00	307.67	968.00	96.8
Micronutrient (0.5%) + GA_3 (10 ppm)	387.33	375.67	391.00	1154.00	115.4
Micronutrient (1%)	377.33	375.00	375.33	1127.66	112.76
Micronutrient (1%) + GA_3 (10 ppm)	405.67	401.33	400.67	1207.67	120.76
GA_3 (10 ppm)	361.00	346.67	351.00	1058.67	105.867
Control	316.67	323.33	311.33	951.33	95.133
SE(m)	12.25	4.82	6.33	4.29	
C.D. (at 5%)	37.76	14.84	19.52	13.23	
C.V. (%)	5.85	2.33	3.08	2.07	

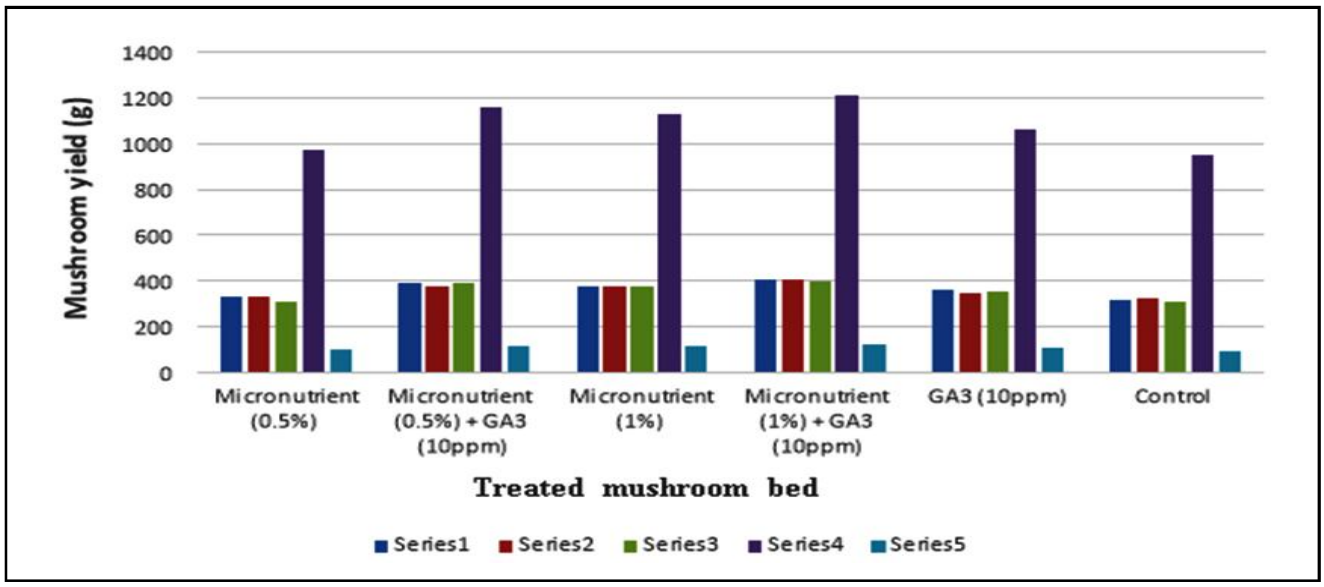

Figure 2: Effects of micronutrient and growth regulator (gibberellic acid) on yield.

Series 1= First flush, Series 2= Second flush, Series 3 = Third flush,

Series 4 = Total yield, Series 5 = Biological efficiency (%).

1 – Pure mushroom culture, 2 - Mother spawn, 3 – Substrate preparation and spawning

4 – Bags filling, 5- Spawn running, 6 – Pin head initiation

7 – Fruiting bodies of mushroom (T4) and 8- Control (untreated)

Figure 3: Different steps of (1-8) of scientific procedure of mushroom cultivation.

4. Discussion

Effect of micronutrient (Shaktiwan) and growth regulator (GA_3) with different doses (micronutrient 0.5%, 1%; GA_3 5 ppm, 10 ppm) reduced the pin head initiation period of oyster mushroom, in all treated bags over control (Table 1 and Figure 1). Among all these treatments, the minimum time recorded for pinhead initiation in bag treated from micronutrient 1% with GA_3 10 ppm, representing as 16.67 days while non-treated bags required 18.33 days for pinhead initiation day after spawning (DAS); all other, *viz.,* the time of first, second, third harvesting and total cropping period were also reduced compared to control. Growth parameters, *viz.,* total number, maximum weight, average length of stalk and average cap diameter of fruiting bodies were recorded highest in all treated bags and these all growth parameters lowest recorded in control. The highest number of fruiting body and cap diameter were harvested from bag treated by micronutrient 1% with GA_3 10 ppm, represented as 106.33 number and 9.07 cm, respectively, while control recorded lowest number of fruiting bodies and cap diameter about 86 number and 7.07 cm, respectively (Table 2).

Data of yield parameters of the present investigation showed that the total yields of all treated bags were high over the control. The highest total yield was recorded from bags treated by micronutrient (1%) along with GA_3 (10 ppm) which was 1207.67 g, while the lowest yield was recorded from control (untreated) about 951.33 g than all treated bags (Table 3 and Figure 2). The demand of oyster mushroom in the global market is rising day-by-day rapidly. Though, there are many researchers doing innovative work for enhancing the production of oyster mushroom (*P. florida)* which are most common and easily cultivable than others. In accordance with the current investigation, it can be concluded T_4 [Micronutrient (1%) with GA_3 (10 ppm)] obtained best for growth behavior and yield perspective to mushroom growers, with respect to all treatments and also over control.

Above finding is confirmed with result (Sarker *et al.,* 2013) who has concluded that gibberellic acid (GA_3) was sprayed with different doses, *viz*., 10, 30, 60, 90, and 100 ppm at the stage of pin head initiation to evaluate its effect on the growth and yield potential of *P.* sp., mushroom. Another finding was also confirmed with the findings that the effect of growth regulators on mushroom increased the number of pin heads as well as the overall size of mushroom and produced significantly higher yield (82.00% BE) against control (67.80% BE). Recently research was also confirmed that the positive effect of growth regulator (gibberellic acid and indole acidic acid with various concentrations) on reduces spawn run time and increases number of pin head and all sporophores growth parameters and yield over control.

5. Conclusion

Micronutrient and growth hormone (GA_3), both were reduced the duration of pin head initiation and mushroom bags colonized prior than untreated mushroom bags. Growth parameters of mushroom were also positively affected by application of micronutrient and growth regulator. The yield of oyster mushroom was increased due to heavy weight of fruit bodies as well as number of fruit body increased. The highest yield was obtained from mushroom bag application with micronutrient 1% with GA_3 (10 ppm), as compared to untreated mushroom bags. Present piece of work concludes that the effect of all supplemented bags by micronutrient and growth regulator performed better for growth behaviour and yield potential and it may be recommended for mushroom growers. As a result, this investigation will help to mushroom growers for selection of supplementation by micronutrient and growth hormone (GA_3) with different effective doses for enhancing the growth and yield performance of mushroom.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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