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Changes of Phenolic Compound in *Pleurotus florida* (Mont.) Singer due to Effect of Plant Growth Hormones and its Effect on Crop Growth and Yield

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A B S T R A C T

The data presented in that among the two hormones, IAA and $GA_3$with different concentrations, the minimum 12 days require for spawn running stage in T_5 treatment as spraying of GA_3, @ 10ppm concentration, followed by T_6 (GA_3 15ppm) in case of control in the month of January. On the other hands, the minimum days require for pin head initiation is noted in T_5 treatment (GA_3-10ppm) which is only 18 days against 27 days in case of T_7 treatment, used as control. The harvested in 4 flushes and the data presented in the table 3 showed that the maximum yield was obtained in the first flush, than the second, third and fourth flushes. It is evident from the (Table-3) that maximum amount of total fresh weight of *P. florida* was obtained from T_5 treatment where gibberellic acid is applied at concentration of 10 ppm, representing the values 410, 371, 308 and 235 gm per bag at 1st, 2nd,3rd and 4th harvesting, respectively. The highest amount of phenolic content is found in T_6 treatment (GA_3 15ppm) with the value of 0.45 mg GAE/ml of methanolic extract which is 7.17% increased over control. The second highest amount of phenolic content is found in treatment T_2, T_3 and T_5.treatment representing the value o.44 mg GAE/ml in all the cases showing 4.76 per cent higher than control. Similarly, the data presented in the table-7 showed that the highest amount of flavenoid content was found in T_6 treatment (GA_3 15ppm) representing the value 0.128 mg QE/ml of methanolic extract. Flavenoid content in T_5 treatment (GA_3 15ppm) is 0.123 mg QE/ml of methanolic extract which is second highest among the treatments. Increase in flavenoid content over control in treatment T_6, and T_5, is 15.3% and 10.8%, over control. From the table, it is cleared that the GA_3 is more effective than the IAA.

Introduction

Mushroom is classified as vegetable in the food world, but technically they are neither vegetable nor plant. They belong to the fungi kingdom. Peoples harvested mushroom from the wild for thousand years ago as a foods and medicines. The Greeks believed that mushroom provide strength for warriors in battle and Romans regarded them as "Food of Gods" or "Gods Flesh" (Chu *et al.*, 2002), which were served only on festive occasion. Mushrooms are also known as "Flower of God" (Anderson *et al.*, 1942).

Globally, mushroom production represent only 7-8 namely *Agaricus, Lentinus, Volvariella, Pleurotus, Auricularia, Flammulina* and *Tremella* which contribute about 89% of the total world production (Chang, 2007).

Among which *Pleurotus* commonly known as Oyster mushroom (due to its oyster like shape) or *"Dhingri"* (Northern India) is the most important one which grown in a wide range of temperature (Reyes, 2003; Bayes *et al.,* 2003).

The fungus can grow wide range of agricultural and industrial wastes which are made up by cellulose hemicelluloses and lignin. These wastes can be classified into different branches such as wood residues, waste paper, grasses, agricultural residues (including straw, stalks, and bagasse), domestic wastes (lignocellulosic garbage and sewage), and municipal solid wastes (Rodriguez *et al.,* 2008).

The fungi need carbon and nitrogen source for structural and functional purposes in addition to trace elements, growth regulators and vitamins. Growth hormones influence the growth of the mushroom is a necessary aspect to be studied.

Adenipekun and Gbolagada, (2006) reported that gibberellic acid was found to be the best for the mycelial growth followed by indole acetic acid (IAA) while the poorest was 2,4 dichlorophenory acetic acid.

Application of gibberellic acid (GA), indole-3-acetic acid (IAA) and 6-benzyl amino purine (BAP) at 1 ppm, 10 ppm, 100 ppm and 200 ppm concentration in cultivated bed of *Pleurotus* sp. increased the number and weight of the sporophores including increased biological efficiency (Eswaran and Ramanujam, 1998). Keeping the above points on view, the study was undertaken as "Changes of phenolic compound in *Pleurotus florida* (Mont.) Singer due to effect of plant growth hormones and its effect on crop growth and yield in the present investigation.

Materials and Methods

Collection of culture of *Pleurotus florida*

The pure culture of *Pleurotus florida* was obtained from Mushroom Research and Development Centre, Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, which was multiplied and maintained on freshly prepared Potato Dextrose Agar (PDA) medium in laboratory for further studies.

Collection of material

Wheat straw was obtained from the Student Research Farm Chandra Shekhar Azad University of Agriculture and Technology Kanpur (208002). The empty glucose bottle, polythene bags, grain of cereals (wheat) and other chemicals, bleaching powder were procured from the Mushroom Research and Development Centre, Department of Plant pathology, Chandra Shekhar Azad University of Agriculture and Technology Kanpur (208002).

Cultivation technique

The experiment was conducted at Mushroom Research and Development Centre, Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology Kanpur during 2017-19. The golden colour wheat straw was collected from Student Research Farm of the university to conduct the experiment.

The straw was dipping in water for 12 hrs using 2.5% formaldehyde for the purpose of sterilization. Spawning was done by freshly

prepared spawn, generally 15-20 days old spawn which is considered the best for spawning. During spawning the treatment scheduled were given as follows:

Treatment details

T_1 = spraying on straw with IAA @ 5 ppm

T_2= spraying on straw with IAA @ 10 ppm

T_3= spraying on straw with IAA @ 15 ppm

T_4=spraying on straw with GA_3 @ 5 ppm

T_5 = spraying on straw with GA_3@ 10 ppm

T_6 = spraying on straw with GA_3@ 15 ppm

T_7 = Control

After spawning, the bags were then kept on iron rakes in the crop room for 10-15 days to complete the vegetative stages of mushroom. The crop room was maintained as temperature- 24 °C, relative humidity-85-90%, ventilation-0.1 to *0.5%*, visible light in the range- 360 to 420 nm

After 15 days bags are look like a covered with white mycelia mat.

The observations were recorded on the following parameters

Days taken for spawn running.

Days taken for pin head initiation.

No. of days require for harvesting.

Yield data – Number and weight of fruiting bodies per treatment.

Total phenol and flavonoid content in mushroom per treatment

Harvesting yield

The right shape for picking can be judged by the shape and size of the fruit body. The fruit bodies should be harvested before spore release. It is advisable to pick all the mushrooms at one time from a cube and the next flush will appear at one time. Harvested mushroom was weight at every time and calculated as gm / bag

Biological efficiency of substrate was calculated by using following formula-

$$\text{Biological efficiency} = \frac{\text{Fresh weight of full body}}{\text{Dry weight of substrate}} \times 100$$

Changes of phenolic compounds in mushroom due to effect of hormones

Total phenolic content

The method developed by Mau *et al.,(*2002) was used for extraction of phenolic compound and for determination of the total phenolic contents. The Folin-Ciocalteau procedure was employed and gallic acid was used as standard. Exactly, 20 µL of various concentrations of gallic acid and samples, 400 µL of 0.5 N Folin-Ciocalteu reagent and 680 µL of distilled water were mixed and vortexed.

After 3 min incubation, 400 µL of Na2CO3 (10%) solution was added and vortexed. Then the mixture was incubated for 2 h at 20 °C with interrupted shaking. Absorbance measurement was carried out at 760 nm at the end of the incubation period. The samples were performed in triplicates. A standard curve was prepared using gallic acid as a standard with different concentrations of gallic acid, and the results were expressed as mg (GAE) per g methanolic extracts (Waterhouse, 2002). Concentration of total phenolic

compounds found in sample was determined by following formula:-

$$A=\frac{c\times v}{m}$$

Where,

A = Total phenolic content

C = Concentration of gallic acid in mg/ml

V = Volume of Extract

M = Mass of extract (gm)

Total flavonoid content

The technique for preparation of mushroom extract is same as for total phenolic content (Mau *et al.*,(2002). However, total flavonoid content was measured with the aluminium chloride colorimetric assay. 1ml of aliquots and 1ml standard quercetin solution (100, 200, 400, 600, 800, 1000 µg/ml) was positioned into test tubes and 4ml of distilled water and 0.3 ml of 5 % sodium nitrite solution was added into each. After 5 minutes, 0.3 ml of 10 % aluminum chloride was added. At 6th minute, 2 ml of 1 M sodium hydroxide was added. Finally, volume was making up to 10 ml with distilled water and mix well. Orange yellowish colour was developed. The absorbance was measured at 510 nm spectrophotometer using UV-spectrophotometer. The blank was performed using distilled water. Quercetin was used as standard. The samples were performed in triplicates. The calibration curve was plotted using standard quercetin. The data of total flavonoids of dry mushroom were expressed as mg of quercetin equivalents/ 100 g of dry mass.

Concentration of total flavenoid compounds found in sample was determined as per following formula

$$A=\frac{c\times v}{m}$$

Where,

A = Total flavenoid content

C = Concentration of quercetin in mg/ml

V = Volume of Extract

M = Mass of extract (gm)

Results and Discussion

Effect of different concentrations of IAA and GA_3 on the spawn running, pin head formation harvesting days of *Pleurotus florida.*

Spawn running

Two hormones, namely, IAA and GA_3 with different concentrations along with a control were evaluated to find out their effect on spawn running days of *P. florida.* The data presented in table 1shown that among the two hormones, IAA and $GA_3$with different concentrations, the minimum 12 days require for spawn running stage in T_5 treatment as spraying of GA_3, @ 10ppm concentration, followed by T_6 (GA_3 15ppm) and T_4 (GA_3 5ppm) treatments which takes 13 days for both the cases against 19 days in case of control in the month of January. From the table, it is cleared that among the two different hormones spawn run period was quicker in gibberellic acid treatments as compare to indole acetic acid. Khandakar (2004) and Pal *et al.,* (2014) found that two to three weeks were require for spawn running. Nirdesh *et al.*, (2019) also reported that different combinations of substrates gave variable response on spawn running, pin head formation and harvesting days of *Pleurotus sajor caju.* Alam *et al.,* (2007) reported that the application of gibberellic acid showed a

positive effect on number of effective fruiting body, stalk length, stalk width, and biological and economical yield compared with IAA and NAA. Pal *et al.,* (2014) investigated that there was significant difference in growth of *P. eous* with respect to different growth regulators with the highest in gibberelic acid treatment.

Pin head formation

On the other hands, the minimum days require for pin head initiation is noted in T_5 treatment (GA_3-10ppm) which is only 18 days against 27 days in case of T_7 treatment, used as control. The treatment T_6 (GA_3 15ppm) require 19 days for pin head initiation, which was followed by T_4 treatment (GA_3 5ppm), which takes 20 days. Dey (1996) reported that GA_3 at the rate of 5-15 mg/l is very effective to form number of promodia and also to obtain a good yield. He also found that oyster mushrooms achieved maturity within two to three days after primordial initiation. Kaur and Atri (2016) found that among the different growth regulators namely IAA, IBA and GA_3 the maximum yield was obtained with GA_3 when sprayed at pin head formation as comparison to IBA and IAA. Pathamashini *et al.,* (2009) found that pin head formation is closely related to temperature and humidity. Temperature below 17^{o}C directly delays the pin head formation. Shah *et al.,* (2004) found that the fruiting bodies appeared 3 -6 weeks after pinhead formation and took 27 -34 days later after inoculation of spawn.

Harvesting days

Similarly, the minimum with 24 days require for ready to harvest of the crop is found in T_5 treatment followed by T_6 and T_4 treatment which are 25 and 26 days, respectively (Table 1). It is cleared from the table that the application of both growth hormones (IAA and GA_3) at different concentrations is effective in Oyster mushroom cultivation but gibberellic acid is more effective than indole acetic acid. Khan *et al.,* (2001) also reported that after spawn running to pin head formation took 7-8 days and fruiting body formed after 3-5 days, sporocarps may be harvested after 10-12 days. Quimio (1976, 1978) also reported that fruiting bodies appeared 3 -4 weeks after inoculation of spawn. Nirdesh *et al.,* (2019) found that the 21 – 36 days was required for ready to harvest the crop in various combinations of substrates.

Effect of various concentrations of IAA and GA_3 on growth characteristics and fruiting bodies of *Pleurotus florida*

Number of fruiting

Growth is important parameter for higher yield of any crops and mushroom is not exception from them. The data presented in the table 2, showed that the maximum number of fruiting bodies is produced in treatment T_5 (GA_3@ 10 ppm) with the value 22 per bags against 13 fruiting bodies in case of control (T_7). Treatment T_6 (GA_3 15ppm) and T_4 (GA_3 5ppm) produced equal number of fruiting bodies (17) followed by treatment T_1 which produces 16 fruiting bodies. From the table it is also cleared that GA_3 is found more effecting than IAA in respect to fruiting body formation. Alam *et al.,* (2007) also reported that the application of gibberellic acid showed a positive effect on number of effective fruiting body, stalk length, stalk width, and biological and economical yield compared with IAA and NAA. Zerihun Tsegaye (2015) found that the number of fruiting bodies recorded is related to their mycelia colonization. Sarker and Chowdhury (2013) reported that the concentration level 10 ppm and 20 ppm produced the highest number of effective fruiting body. This similar finding has also been reported by Day *et al.,* (2007).

Table.1 Effect of different concentrations of plant growth regulators on spawn running, pin head formation and number of days require for harvesting of *Pleurotus florida*

Treatment	Average number of days for spawn running*	Average number of days for pin head initiation*	No. of days require for harvesting*
T_1 = IAA@ 5ppm	14	21	31
T_2 = IAA@10ppm	15	25	29
T_3 = IAA@15ppm	16	24	27
T_4 = GA_3 @5ppm	13	20	26
T_5 = GA_3@10ppm	12	18	24
T_6 = GA3@15ppm	13	19	25
T_7 = Control	19	27	34
C.D.	3.857	4.636	5.021
SE(m)	1.134	1.363	1.476
SE(d)	1.604	1.927	2.087
C.V.	11.005	8.760	7.474

Table.2 Effect of different concentrations of plant growth regulators on fruiting ability and harvesting of *Pleurotus florida*

Treatments	Fruiting ability of *pleurotus florida* at different days				Total weight of fruiting body production (g)	Yield increase over control (%)	
	5th (g)	10th (g)	15th (g)	20th g)			
T_1 = IAA@ 5ppm	375	320	280	245	1220	37.07	
T_2 = IAA@10ppm	350	307	275	230	1162	30.56	
T_3= IAA@15ppm	340	305	245	174	1064	19.55	
T_4 = GA_3 @5ppm	388	340	298	240	1266	42.24	
T_5 = GA_3@10ppm	410	371	308	235	1324	48.76	
T_6 = GA3@15ppm	380	348	298	245	1271	42.80	
T_7 = Control	285	239	190	176	890		
C.D.	64.003	50.082	45.440	29.404	71.829		
SE(m)	18.815	14.722	13.358	8.644	21.115		
SE(d)	26.608	20.821	18.891	12.224	29.862		
C.V.	7.368	6.534	4.903	5.253	2.550		

Table.3 Effect of different concentrations of plant growth regulators on total flavonoid content of *pleurotus florida*

Treatments	Average absorbance at 415 nm	$X = \frac{Y+0.01}{1.387}$ (µg/ml)	Total flavonoid (mg QE/ml)	Increase in total flavonoid content over control (%)
T_1 = IAA@ 5ppm	0.150	0.115	0.115	3.6
T_2 = IAA@10ppm	0.157	0.120	0.120	8.1
T_3= A@15ppm	0.154	0.118	0.118	6.3
T_4 = GA_3 @5ppm	0.151	0.116	0.116	4.5
T_5 = GA_3@10ppm	0.161	0.123	0.123	10.8
T_6 = GA3@15ppm	0.168	0.128	0.128	15.3
T_7 = Control	0.145	0.111	0.111	
C.D.	0.012			
SE(m)	0.004			
SE(d)	0.006			
C.V.	4.466			

Table.4 Effect of different concentrations of plant growth regulators on Total Phenol content of *pleurotus florida*

Treatments	Average absorbance at 750 nm	$X = \frac{Y+0.063}{0.018}$ (µg/0.1ml)	Total phenol (mg GAE/ml)	Increase in total phenolic content over control (%)
T_1 IAA 5ppm	0.711	43.00	0.43	2.38
T_2 IAA 10ppm	0.729	44.00	0.44	4.76
T_3 IAA 15ppm	0.731	44.11	0.44	4.76
T_4 GA_3 5ppm	0.717	43.33	0.43	2.38
T_5 GA_3 10ppm	0.735	44.33	0.44	4.76
T_6 GA_3 15ppm	0.749	45.11	0.45	7.17
T_7 Control	0.704	42.61	0.42	
C.D.	0.018			
SE(m)	0.006			
SE(d)	0.008			
C.V.	1.413			

Weight of fruiting body

The crop of *Pleurotus florida* was harvested in 4 flushes and the data presented in the table 3 showed that the maximum yield was obtained in the first flush, than the second, third and fourth flushes. It is evident from the (Table-3) that maximum amount of total fresh weight of *P. florida* was obtained from T_5 treatment where gibberellic acid is applied at concentration of 10 ppm, representing the values 410, 371, 308 and 235 gm per bag at 1^{st}, 2^{nd},3^{rd} and 4^{th} harvesting, respectively. The treatment T_6 (GA_3 15ppm) showing 380, 348, 298 and 245 gm fresh weight of mushroom per bag at 1^{st}, 2^{nd}, 3^{rd} and 4^{th} harvesting stages,

respectively which is second highest among the treatments. The treatment T_3, where IAA @ 15 ppm was used representing 340, 305, 245 and 174 gm fresh weight of mushroom per bag at 1st, 2nd, 3rd and 4th harvesting stages, respectively which is superior over control and inferior over all other treatments. From the tables, it is also cleared that the maximum amount of fresh mushroom was obtained from 1st harvesting stages which is gradually decrease from 2nd,3rd and 4th harvesting stages in all the treatments. It is also cleared that both plant growth regulators IAA and GA_3 is produce positive effect on Oyster mushroom cultivation. The per cent increase of yield over control was as 48.76%, 42.80%, 42.24%, 37.07%, 30.56%and 19.55% in T_5, T_6, T_4, T_1, T_2 and T_3, respectively. Nirdesh *et al.,* (2019) found that *Pleurotus sajor caju* was harvested in 5 flushes, the maximum yield was obtained in the first flush, than the second and third flushes in all the treatments. They also found that the maximum yield with 1483 gm per bag was obtained from combination of substrates as 3/4 wheat straw+1/4 mustard straw+100gm wheat bran. Kaur and Atri, (2016) found that the maximum yield was obtained with GA_3 when sprayed at pin head formation as comparison to IBA and IAA. Xavier and Kumuthakalavalli (2001) reported that application of indole acitic acid (IAA), Gibberallic acid (GA_3) and kenetin (50 ppm and 100 ppm) were increased the yield up to 46.8 and 37.8 percentage respectively over the control. Ahlawat (2003) reported that indole acetic acid, indale butyric acid, cytokinin and gibberillic effective for increasing the yield of mushrooms.

Effect of different concentrations of plant growth regulators on total phenolic and flavenoid content in mushroom

Phenolic content

The data presented in the table showed that highest amount of phenolic content is found in T_6 treatment (GA_3 15ppm) with the value of 0.45 mg GAE/ml of methanolic extract which is 7.17% increased over control. The second highest amount of phenolic content is found in treatment T_2, T_3 and T_5.treatment representing the value o.44 mg GAE/ml in all the cases showing 4.76 per cent higher than control. Yim and Chye (2010) found that the phenolic contents present in the *P. ostreatus* contributed to its antioxidant activity. Park *et al.,* (2017) reported that the IAA and GA_3 at specific concentrations enhanced the growth and accumulation of total phenolic content as well as flavonoid content, including rutin and catechin.

Flavonoid Compound

Similarly, the data presented in the table - 4 showed that the highest amount of flavenoid content was found in T_6 treatment (GA_3 15ppm) representing the value 0.128 mg QE/ml of methanolic extract. Flavenoid content in T_5 treatment (GA_3 15ppm) is 0.123 mg QE/ml of methanolic extract which is second highest among the treatments. Increase in flavenoid content over control in treatment T_6, and T_5, is 15.3% and 10.8%, over control. From the table, it is cleared that the GA_3 is more effective than the IAA. Ghasmzadeh *et al.,* (2016) reported that PGRs treatments induced the accumulation of flavonoid content over control. Kim *et al.,* (2009) found that the variable content of flavenoid in dark grey, yellow and pink strain of *Pleurotus ostreatus* and according to them, dark grey strain has the highest flavenoid (2.16 mg/g) content as compared to others.

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