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Effect of different growth regulators on spawn growth on production of *Pleurotus* spp. (*P. djamor* and *P. sajor-caju*)

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Abstract
Present study was use of different growth regulators for spawn improvement and production of *Pleurotus* spp. (*P. djamor* and *P. sajor-caju*). Growth regulators such as GA, IAA and NAA used @ 10 and 20 ppm in seven treatments with three replication. In case of *P. djamor*, maximum spawn growth (90.00 mm) was recorded in GA 20 ppm and minimum spawn growth (74.00 mm) was recorded control. While in case of *P. sajor-caju*, maximum spawn growth (90.00 mm) was recorded in GA 20 ppm. However minimum spawn growth (67.00 mm) was recorded in control. In case of *P. djamor* maximum growth rate (6.00 mm/day) was observed in GA 20 ppm and minimum growth rate (4.93 mm/day) was recorded in control. While in *P. sajor-caju* maximum growth rate (6.00 mm/day) was recorded in GA 20 ppm. However minimum growth rate (4.46 mm/day) was recorded in control. In case of *P. djamor* maximum yield was observed in GA @ 20 ppm (514.33g/kg of dry substrates with 51.33% biological efficiency.). In case of *P. sajor-caju* maximum yield was observed in GA @ 20 ppm (700g/kg of dry substrates with 70.00% biological efficiency.).

Keywords: Growth regulators, Spawn, *Pleurotus* spp. and biological efficiency

Introduction
Mushroom are those fungi that form large visible fructifications which have been of long interest to scientists as well as to public due to their important role in human life mushrooms have been considered as an ingredient of gourmet cuisine across the globe; especially for their unique flavor and have been valued by human kind as a culinary wonder. Mushrooms are considered as a delicacy with high nutritional value, and they are also accepted as nutraceutical foods; they are of considerable interest because of their organoleptic merit, medicinal properties, and economic significance (Chang & Miles, 2008; Ergonul, *et al*. 2013) [4, 5]. Fresh mushrooms are highly perishable because they contain about 87 to 95% water. Due to their high moisture content they cannot be stored for more than 24 hr at ambient conditions. Hence, they need to be preserved by some method. Efficient preservation methods may extend shelf life and diversify the product for consumers. Preservation may also be useful if mushrooms are to be used as an ingredient for the production of other foods like dehydrated instant meals.
The common name "oyster mushroom" comes from the white shell-like appearance of the fruiting body. The fruiting bodies of this mushroom are with different shades of white, cream, grey, yellow, pink or light brown depending upon the species. Oyster mushroom is the third grown mushroom in the world and ranks second in India. For the successful cultivation of oyster mushroom on a small scale or commercial scale, one of the most important requirements is the mushroom seed (spawn). Which is a pure culture of the mycelium grown on a special medium. The production of spawn is done by professionals in the laboratory under controlled conditions or temperature, light and humidity. The mushroom cultivation and its yield depend on large extent on the purity and quality of the spawn used. Grain spawn is sterilized grain that has been inoculated with spores or a sterile culture of mycelium. Many types of grain (wheat, millets, rye etc.) can be used for spawn preparation. Considering the importance of growth regulators on the yield of mushroom, the experiment was carried out to find out optimum concentration of GA, IAA and NAA for maximizing growth and yield of mushroom.

Material and Methods
The present study was carried out in department of plant pathology, S. V. P. U. A. & T. Meerut-250110 (UP) during 2018-019.

The isolate of *Pleurotus* spp. (*P. djamor* and *P. sajor-caju*) was collected DMR solan. The district Meerut is situated between 29o 01'N latitude and 77o 45'E longitude at an altitude of 237 meters above the mean sea level. The district Meerut falls under north western plains sub-region of Upper Gangatic plains. This district is spread over a geographical area of 2564 km^2. The general climate of Meerut is semi-arid and sub-tropical, characterized by very hot summer and cold winters. The maximum temperature shoots up to 440C during summer whereas, minimum temperature remaining up to 70C and below during winter season.

Establishment and maintenance of pure culture

The culture of different *Pleurotus* species *viz. P. djamor*, and *P. sajor-caju* used in the present investigations were collected from mushroom research laboratory SVPUA&T Meerut. The cultures of *Pleurotus* species were further purified by single hyphal tip method. For this purpose, the cultures were grown in sterilized petri plates on potato dextrose agar (PDA) medium for 8 days. Single branched hyphae from the periphery of the growing colony were marked under low power (10x) of compound microscope and transferred to PDA slants for maintenance. These culture tubes were incubated at 24±10C for about a week and again subculture on PDA medium and then stored in a refrigerator at 050±1C for further use.

Spawn Production Technology

For study on spawn growth, grain spawn of *P. djamor* and *P. sajor-caju* were prepared by using the standard spawn production technology. The spawn was prepared on wheat grains in the wide mouthed glass bottles of 500 ml capacity. The wheat grains were cleaned to remove any broken, shriveled grains either by sieving or winnowing or by hand picking of undesired grains. The grains were then soaked overnight in clean water and then washed. Now, they were boiled in water for 15 minutes taking care that grains should not split but remain slightly hard after boiling. The boiled grains were spread in thin layer over a wire net to remove excessive water and enable them to cool about 25 ± 2oC. The cooled grains were then mixed with 1.2 per cent commercial grade gypsum ($CaSO^4$) and 0.3 per cent calcium carbonate ($CaCO^3$). Gypsum prevents the sticking of wheat grains together and calcium carbonate maintains the pH in the range of about 6.5.

The grains were then filled in clean glass bottle up to 2/3rd of its capacity. The bottles were plugged with non-absorbent cotton and covered with butter paper. These bottles were then sterilized at 1210C (1.1 Kg/cm^2 pressure) for 2 hours on two consecutive days. Sterilized bottles were taken out from the autoclave, while still hot, the bottles were shaken to avoid clumping of grains. Sterilized bottles were inoculated with few 9 mm discs of a 10 days old culture of *P. sajor-caju* and *P. djamor* Inoculated bottles were incubated at 25±10C and shaking was done after 7 days. Entire grains were covered with fine mycelial growth after 18±2 days. This spawn is known as mother spawn or master spawn. The spawn prepared for commercial use was prepared on sterilized grains in polypropylene bags inoculated with the use of a spoon full of mother spawn.

Use of different growth regulators

The experiment was conduct to find out the effect of different growth regulator on improvement of spawn of oyster mushroom. In this experiment different types of growth regulator were used of spawn improvement and yield, such as GA @ 10 ppm, GA @ 20 ppm, IAA @ 10 ppm, IAA @ 20 ppm NAA @ 10 ppm NAA @ 20 ppm and control were taken.

Results and discussion

This experiment was conducted to study the effect of different growth regulator on spawn growth of *P. djamor* and *P. sajor-caju*. The observations were recorded on 5th, 10th, and 15thdays, receptively as shown in (Table 1).

On 5th day in case of *P. djamor*, maximum spawn growth (36.66 mm) was recorded in GA 20 ppm, NAA 20 ppm and IAA 20 ppm followed by GA 10 ppm (35.66 mm) and minimum spawn growth (32.00 mm) was recorded in control. While in case of *P. sajor- caju*, maximum spawn growth (37.33 mm) was recorded in GA 20 ppm and followed by GA 10 ppm (30.66 mm). However minimum spawn growth (24.00 mm) was recorded in NAA 10 ppm.

On 10th day in case of *P. djamor*, maximum spawn growth (55.66 mm) was recorded in GA 20 ppm followed by IAA 20 ppm (55.00 mm) and minimum spawn growth (47.66 mm) was recorded control. While in case of *P. sajor-caju*, maximum spawn growth (54.66 mm) was recorded in GA 20 ppm and followed by GA 10 ppm (49.00 mm). However minimum spawn growth (32.00 mm) was recorded in control.

On 15th day in case of *P. djamor*, maximum spawn growth (90.00 mm) was recorded in GA 20 ppm and NAA 20 ppm followed by GA 10 ppm and NAA 10 ppm at (87.66 mm) and minimum spawn growth (74.00 mm) was recorded control. While in case of *P. sajor- caju*, maximum spawn growth (90.00 mm) was recorded in GA 20 ppm and followed by NAA 20 ppm at (79.66 mm). However minimum spawn growth (67.00 mm) was recorded in control.

In case of *P. djamor* maximum growth rate (6.00 mm/day) was observed in GA 20 ppm and NAA 20 ppm followed by GA 10 ppm and NAA 10 ppm (5.84 mm/day) and minimum growth rate (4.93 mm/day) was recorded in control. While in *P. sajor-caju* maximum growth rate (6.00 mm/day) was recorded in GA 20 ppm followed by NAA 20 ppm (5.30 mm/day). However minimum growth rate (4.46 mm/day) was recorded in control.

The result revealed that among all different growth regulator on improvement of spawn growth of *Pleurotus* species In case of *P. djamor* maximum spawn growth (90.00 mm) was recorded in GA 20 ppm and NAA 20 ppm followed by similar GA 10 ppm and NAA 10 ppm at (87.66 mm) and minimum spawn growth (74.00 mm) was recorded in control. In case of *P. sajor-caju* maximum spawn growth (90.00 mm) was recorded in GA 20 ppm and followed by NAA 20 ppm (79.66 mm) however minimum spawn growth (67.00 mm) was recorded in control.

The results were in accordance with the findings of Pal *et al.* (2014) [11] The effect of different growth regulators on spawn quality and yield of *P. eous* five different growth regulators *i.e.* naphthalene acetic acid (NAA), gibberelic acid (GA), cytokinine, 2,4- dichlorophenoxy acetic acid (2, 4-D), and indolebutaric acid (IBA) were evaluated. Mukhopadhyay *et al.*, (2005) [10] evaluate the di-ammonium hydrogen phosphate and plant growth hormone *viz.* indole 3 acetic acid (IAA), gibberelic acid (GA) and kinetin for biomass production of *P. sajor-caju*. The hormone, at different concentrations, increased the biomass of *P. sajor-caju* by 15-26%. Maximum enhancement was observed with IAA.

Table 1: Effect of different growth regulators on spawn growth of *Pleurotus* spp. (*P. djamor* and *P. sajor-caju*)

S. No.	Treatment	5thDay Dose	*P.djamor*	*P.sajor- caju*	Spawn growth (mm) 10thDay *P.djamor*	*P.sajor- caju*	15thD ay *P.djamor*	*P.sajor- caju*	15th day Growth rate (mm/day) *P.djamor*	*P.sajor- caju*
1	Gibberellic acid	10	35.66	30.66	54.00	49.00	87.66	72.33	5.84	4.82
2	Gibberellic acid	20	36.66	37.33	55.66	54.66	90.00	90.00	6.00	6.00
3	Indol acetic acid	10	33.00	29.00	53.00	46.00	83.00	70.00	5.53	4.66
4	Indol acetic acid	20	36.66	30.00	55.00	48.66	87.00	75.66	5.8	5.04
5	Nepthelic acetic acid	10	34.66	24.00	53.33	33.33	87.66	77.33	5.84	5.15
6	Nepthelic acetic acid	20	36.66	26.33	54.00	37.33	90.00	79.66	6.00	5.31
7	Control		32.00	26.33	47.66	32.00	74.00	67.00	4.93	4.46
	CD at 5%		1.44	2.93	2.31	3.80	3.25	4.02	-	-
	SE (M)		0.47	0.95	0.75	1.24	1.06	1.31	-	-

Yield

The experiment was conducted to find out effect of different growth regulators on oyster mushroom (*P. djamor* and *P. sajor-caju*) observations on mycelial ramification in wheat substrates, days for spawn run, days for pin head formation, days for first harvesting, number of fruiting bodies and total yield were recorded as shown in Table: 2.

In case of *P. djamor* maximum yield was observed in GA @ 20 ppm (514.33g/kg of dry substrates with 51.33% biological efficiency.) Which was followed by GA @ 10 ppm (510.00g/kg of dry substrates with 51.00% biological efficiency). While minimum yield was observed in control (413.33g/kg dry substrates with 41.33% biological efficiency.) Which was followed by NAA @10 ppm (455.00 g/kg of dry substrates with 45.50% biological efficiency) which was statistically lower than all other treatment the minimum days for spawn run (14.00 days) were observed in GA @ 20 ppm which was statistically at par with GA @ 10 ppm (14.20). While maximum days for spawn run (19.58 days) were observed in control at par with IAA @ 10 ppm (15.50) the minimum days for pin head formation (18.00 days) were observed in GA @ 20 ppm which was statistically at par, with GA @ 10 ppm (18.20 days). Maximum days for pin head formation (23.76 days) were observed in control which was statistically higher than all other treatment. Minimum days for first harvesting (22.00 days) were observed in GA @ 20 ppm which was statistically lower than all other treatment. While maximum days for first harvesting (27.75 days) were observed in control which was statistically higher than all other treatment. The maximum numbers of fruiting bodies (122.00) were observed in GA @ 10 ppm which was significantly higher than all other treatment. While minimum number of fruiting bodies (91.00 days) were observed in control. Which was significantly lower than all other treatment the maximum average weight of fruiting bodies (25.00 days) was observed in control. And it was significantly higher than all other treatment. While minimum average weight of fruiting bodies (14.00 days) was observed in GA 10 ppm.

In case of *P. sajor-caju* maximum yield was observed in GA @ 20 ppm (700g/kg of dry substrates with 70.00% biological efficiency.). And was followed by GA @ 10 ppm (663.33g/kg of dry substrates with 66.33% biological efficiency). While minimum yield was observed in control (473g/kg dry substrates with 47.33% biological efficiency.) and it followed by IAA @ 20 ppm (533.33 g/kg of dry substrates with 55.33% biological efficiency) which was statistically lower than all other treatment. Minimum days for spawn run (16.50 days) were observed in GA @ 20 ppm which was statistically at par with GA @ 10 ppm (17.00 days). While maximum days for spawn run (20.28 days) were observed in control at par with IAA @ 20 ppm (18.25 days). The minimum days for pin head formation (20.00 days) were observed in GA 20 ppm which was statistically at par, with GA 10 ppm (21.20 days). Maximum days for pin head formation (24.16 days) were observed in control which was statistically higher than all other treatment. The minimum days for first harvesting (24.20days) were observed in GA @ 20 ppm which was statistically lower than all other treatment. While maximum days for first harvesting (28.25 days) were observed in control which was statistically higher than all other treatment. The Maximum numbers of fruiting bodies (152.00) were observed in NAA 10 ppm and it significantly higher than all other treatment. While minimum numbers of fruiting bodies (125.00) were observed in control and it significantly lower than all other treatment. Maximum average weight of fruiting bodies (25.15) was observed in GA @ 20 ppm which was significantly higher than all treatment. While minimum average weight of fruiting bodies (19.65) was observed NAA @ 10 ppm. Which was significantly lower than all other treatment as shows.

The results were in accordance with the findings of Hong (1978) [9] recommended dose of (0.1 ppm) Indole Acetic Acid (IAA) to support maximum growth of *P. ostreatus* followed by 10 ppm Gibberellic Acid (GA) and 0.1 and 0.01 ppm of Kinetin. Fasidi and Jonathan (1994) [7] reported highest mycelia growth of *Pleurotus* spp. when the basal medium was supplemented with Gibberellic Acid. Reddy *et al*. (2002) [13] observed most favorable growth of the *P. ostreatus* with the addition of IAA at 5 ppm.

Eswaran and Ramabadran (2002) [6] recorded the yield performance of *P. eous* using plant hormone, Gibberellic Acid (GA), Indole-3-Acetic Acid (IAA) and 6- Benzyl Amino Purine (BA) at 1, 10, 100 and 100 ppm. Among all spraying 100 ppm GA has recorded maximum productivity of *Pleurotus spp*.

Alam *et al.* (2007) [1] found that application of GA^3 increased the biological and economic yield by 18% and 16%, respectively. Hans (1997) [8] stated that application of GA^3 in high concentration increased biological and economical yield by increasing cell division and enlargement as it was evident from the study since application of GA^3 resulted in enlargement of pileus diameter and pileus girth and elongation of stalk length. Barclay (1985) [3] suggested that hormone application timing and concentration might be critical and important factor for production of mushroom.

Sarker and Chowdhury (2013). Gibberellic acid (GA^3) was sprayed with eleven doses viz. 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppm at the primordia initiation stage to evaluate its effect on the growth and yield performance of Oyster Mushroom. At 10 ppm level GA^3 gave the highest economic yield and dry weight. Application of GA^3 increased the fresh economic yields to about 30% and 34%, while 80% and 115% dry weights increase occurred compared to the control at first and second harvests, respectively. GA^3 showed a positive effect on number of effective fruiting body, stalk

length, pileus diameter, biological yield, economic yield and dry economic yield. The result suggested that GA^3 at 10 ppm/packet would be the best possible concentration for production of Oyster Mushroom.

Table 2: Effect of different growth regulators on yield of *Pleurotus* spp. (*P. djamor* and *P. sajor-caju)*

S. No	Growth regulator (ppm)	DFSR		DFPF		DFFH		NOFB		Yield (g/kg dry substrate)		Average weight (gm./FB)		Biological efficiency (%)	
		P. djamor	*P. sajor-caju*	*P. djamor*	*P. sajor-caju*	*P. djamor*	*P. sajor-caju*	*P. djamor*	*P. sajor-caju*	*P. djamor*	*P. sajor- caju*	*P. djamor*	*P. sajor-caju*	*P. djamor*	*P. sajor-caju*
1	Gibberellic acid @ 10	14.20	17.00	18.20	21.20	22.40	25.00	122.00	133.00	510.00	663.33	14.00	22.00	51.00	66.33
2	Gibberellic acid @ 20	14.00	16.50	18.00	20.00	22.00	24.20	123.50	143.00	514.33	700.00	18.00	25.15	51.43	70.00
3	Indol acetic acid @ 10	15.50	18.25	19.52	22.85	24.10	27.00	103.00	134.86	455.00	566.66	17.00	21.55	45.50	56.66
4	Indol acetic acid @ 20	15.20	17.82	19.00	22.26	24.00	27.00	110.10	132.33	489.00	553.33	18.66	20.56	48.90	55.33
5	Nepthelic acetic acid @ 10	14.52	17.50	18.60	22.10	23.50	25.84	117.66	152.33	496.00	577.00	17.55	19.65	49.60	57.70
6	Nepthelic acetic acid @ 20	14.25	17.22	18.28	21.85	23.45	25.28	121.66	144.00	503.33	657.00	19.25	20.35	50.33	65.70
7	Control	19.58	20.28	23.76	24.16	27.25	28.25	91.66	125.00	413.33	473.33	19.00	20.45	41.33	47.33
CD at 5%		2.67	1.77	2.67	1.67	2.67	1.77	3.53	5.25	12.19	9.51				
SE		0.87	0.58	0.87	0.54	0.87	0.58	1.15	1.71	3.98	3.10				

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