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Effect of supplements and growth regulators on the productivity of Jew's ear mushroom (*Auricularia auricula-judae*)

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Abstract
Among the factors attributed to the productivity of mushrooms, type of substrate and supplements used for its cultivation is substantially important. The present study highlights the effects of different supplements viz. rice bran (10%), bengal gram powder (10%), poultry manure (10%), spent mushroom compost (10%), urea (2%) and ammonium sulphate (2%) and different growth regulators viz. Indole acetic acid (IAA), indole-3-butyric acid (IBA), gibberellic acid (GA), naphthalene acetic acid (NAA) and kinetin (10 ppm each), on the growth and yield parameters of Jew's ear mushroom (*Auricularia auricula-judae*). Experiment was done by polybag method of cultivation during the monsoon seasons of 2021-22. The results from this study revealed that rice bran was the best supplement (total yield, 308.42 ± 16.36 g/kg) and GA was the best growth regulator (total fresh yield, 413.37±28.08 g/kg) for the growth and yield enhancement of Jew's ear mushroom. Among the supplements used, spent mushroom compost was found to be the poor yielder with biological efficiency of 16.59% and in case of growth regulators, kinetin was found to show minimum yield (BE-28.22%). Faster spawn run was observed in beds supplemented with urea whereas among growth regulators, GA showed faster spawn run compared to control.

Keywords: Jew's ear mushroom, supplements, growth regulators, yield, biological efficiency

Introduction
Mushrooms are of considerable interest because of their organoleptic properties, medicinal values, and economic merits. *Auricularia* is a well-known genus among the edible mushroom world, sourced as both cultivated and wild forms. The genus *Auricularia* belongs to the family *Auriculariaceae*, fruiting bodies of which are typically ear-shaped and gelatinous, with a smooth, wrinkled, or veined under surface and an upper surface that ranges from faintly downy to noticeably hirsute. All species flourish on woods. These mushrooms are utilized as both nutrient rich foods as well as medicinal sources for which they occupy a prominent place in the Asian traditional medicine. Cultivated species of *Auricularia* can be grown under a wide range of conditions which allows a worldwide production of the same (Bandara *et al*., 2019) [2]. Different species of *Auricularia* like *A. auricula-judae*, *A. polytricha*, *A. cornea* and *A. mesenterica* are being cultivated worldwide. Among them *A. auricula-judae* also referred to as wood ear or Juda's ear is one of the top five most widely grown edible mushrooms worldwide (Chen *et al*., 2021) [4]. *Auricularia* is currently the second most extensively cultivated fungus in China which comprises two major species (*A. auricula* and *A. polytricha*) and had a roughly 92% increase in production since 2010 (Royse *et al*., 2017) [10]. Supplementation of the substrate with various materials is recommended prior to spawning for enhancement of yield of mushrooms (Hadwan *et al*., 1997) [7]. Ahlawat (2011) [1] came to the conclusion from his experiment on the use of growth regulators for mushroom yield enhancement that commercial preparations of IBA stimulate mushroom mycelial growth under *in vitro* conditions, and the spraying of such formulations on mushroom beds stimulates early pinning as well as higher yield of mushrooms. Vidyareshmi and Lulu (2008) [11] reported that the biological efficiency of A*uricularia* cultivated in raw substrate was very less compared to others. Hence the present study is envisaged to observe the influence of different supplements and growth regulators on the growth and yield of Jew's ear mushroom.

Materials and Methods
The study was conducted at department of Plant Pathology, College of Agriculture Vellayani, Thiruvananthapuram, Kerala, during the North East and South West monsoon seasons of 2021 and 2022.

Mushroom collection and spawn preparation

Native isolates of *A. auricula-judae* collected from different Agro Ecological Units (AEU 8, AEU9 and AEU 12) of Southern Kerala were subjected to *in vitro* culture in PDA media by standard tissue isolation method (Gogoi *et al*., 2019) [5]. The best isolates from this were used for spawn production and further study. Paddy grains were used as the substrate for spawn production. Paddy grains were soaked in water for 12-18h and boiled till the grains partially split open. The grains were then dried and mixed with $CaCO_3$ at the rate of 40-50 g/kg. The grains were then filled (250 g each) in polypropylene cover (30 cm x 15 cm), sealed properly and autoclaved at a temperature of 121 ℃ and 1.055 kg/cm² pressure for 2 hours. Sterilized grains were then inoculated with bits of fully grown mushroom mycelium under LAF (Laminar Air Flow chamber), sealed tightly, labelled properly with the date of inoculation and kept for incubation at a temperature of 25-27 ℃.

Study on the effect of supplements on the growth and yield of *A. auricular*

Rubber saw dust and rubber wood chips in 1:1 proportion added with 2% calcium carbonate was used as the basic substrate to which 6 supplements namely rice bran (10%), bengal gram powder (10%), urea (2%), ammonium sulphate (2%), poultry manure (10%), spent mushroom compost (10%) were added to each treatment. Polythene bags of 150-gauge thickness and 60 cm x 30 cm dimension was used for cultivation. Rubber sawdust and wood chips were softened by soaking them in water for 12 hours, and they were then sterilized by boiling. Sterilized substrate was then sun dried until it achieves a moisture content of around 60%. Disinfection of supplements to be added was also done by autoclaving them at a temperature of 121 ℃ and 1.055 kg/cm² pressure for 2 hours. Mixing of substrates and supplements along with $CaCO_3$ were carried out in a clean surface and spawning (125 g spawn/kg) done as 4 layers in a circular manner at the periphery. The inoculated bags were fastened tightly at the top and pierced all over with 25 to 30 pin holes, labelled and maintained for incubation in a dark room at 28 ℃ and ~60% relative humidity. After the complete mycelial colonization, the slits were made on the polybag using a sharp razor at sides and were shifted to cropping room after primordial initiation providing a higher relative humidity above 80%. Watering was also done on these beds on a regular basis. Days for complete mycelial colonization, pinhead formation, first harvest and total yield were recorded and percentage biological efficiency (BE) for each treatment was calculated.

$$BE = \frac{\text{Fresh weight of fruit bodies per bag (g)}}{\text{Dry weight of spent substrate (g)}} \times 100$$

Table 1: Treatment details; substrate-supplement combination

Treatments	Substrate + Supplement combination
T_1	Rubber saw dust (500g) + Rubber wood chips (500g) + 20g $CaCO_3$ + Rice bran (100g)
T_2	Rubber saw dust (500g) + Rubber wood chips (500g) + 20g $CaCO_3$ + Bengal gram powder (100g)
T_3	Rubber saw dust (500g) + Rubber wood chips (500g) + 20g $CaCO_3$ + Urea (20g)
T_4	Rubber saw dust (500g) + Rubber wood chips (500g) + 20g $CaCO_3$ + Ammonium sulphate (20g)
T_5	Rubber saw dust (500g) + Rubber wood chips (500g) + 20g $CaCO_3$ + Poultry manure (100g)
T_6	Rubber saw dust (500g) + Rubber wood chips (500g) + 20g $CaCO_3$ + Spent mushroom compost (100g)
T_7 (control)	Rubber saw dust (500g) + Rubber wood chips (500g) + 20g $CaCO_3$

Study on effect of growth regulators on the growth and yield of *A. Auricula*

Best performing treatment in the above-mentioned study was taken as the basic substrate-supplement combination for testing the effectiveness of growth regulator on the production of *A. Auricula.* At the time of spawning, the substrate was slathered separately with a solution of Indole acetic acid (IAA), naphthalene acetic acid (NAA), gibberellic acid (GA), kinetin, and indole-3-butyric acid (IBA) at a concentration of 10 ppm for each growth regulator and the growth regulators (IAA, NAA, GA Kinetin and IBA @ 10 ppm) were sprayed at the time of pin head formation to observe the effect of growth regulators on the growth and yield of *A. Auricula*.

Statistical analysis

The data collected from the completely randomized design (CRD) of experiments with three replications for each treatment were subjected to analysis of variance using GRAPES version 1.1.0. Mean separation done using LSD and effects were declared at a level of significance 5% (Gopinath *et al*., 2020) [6].

Results and Discussion

Effect of supplements on the growth and yield of *A. Auricula*

The various supplements tested under this study were significantly different (p >0.05) in the growth and yield attributes of *A. auricula.* Substrates supplemented with urea took comparatively less time (36.00±1.73 days) for completion of mycelial growth in beds followed by rice bran (39.33±2.52) where as those substrates supplemented with ammonium sulphate took more days (44.33±1.15) for complete spawn run which is on par with the control. Pinhead formation occurred earlier in rice bran amended beds (43.00±2.00) which is on par with that of beds amended with urea (43.33±1.15) than other treatments whereas beds amended with ammonium sulphate took maximum days (49.67±1.53) to produce pinheads.

Minimum number of days for first flush was in case of beds amended with rice bran which is on par with that of urea supplemented beds. Total crop period was also highest in case of rice bran amended beds (93.67±2.52) and lowest in beds supplemented with spent mushroom compost (Table 2). Number of sporocarps produced per kilo gram dry matted substrate, average sporocarp fresh weight, total yield and thereby biological efficiency were all found to be significantly higher in case of beds supplemented with rice bran followed by that of urea. A similar study conducted by Priya and Geetha (2017) [9] in another species of *Auricularia* (*A. polytricha)* also showed rice bran as a best supplement for yield enhancement. Comparatively lesser number of sporocarps (22.33±4.06) was produced in beds amended with spent mushroom compost. Average sporocarp weight, total yield as well as biological efficiency were also found to be lesser in spent mushroom

compost supplemented beds which were found to be on par with the non-amended beds (Table 3).

Effect of growth regulators on the growth and yield of *A. Auricula*

Study on the effectiveness of growth regulator on production of *Auricularia* with rice bran supplemented beds (best performing supplement in first study) as check showed significantly different ($p > 0.05$) results in the growth and yield attributes of *A. Auricula* (Table 4). Earlier spawn run was observed in case of beds incorporated with gibberellic acid (33.67±1.16) and significant delay in spawn run was observed in case of control (39.33±2.52) which is on par with that of NAA (38.67±1.16). GA incorporated substrate also showed earlier pinhead formation as well as minimum days for first flush. IAA and IBA smeared beds took comparatively more intervals between complete spawn run and pinhead formation. It might be because of the induction of more vegetative growth by auxins that delays production of pinheads. Maximum crop period was observed in case of beds incorporated with IAA (96.33 ± 2.08) whereas kinetin showed a minimum crop period of 88.67±2.08 days. Fresh yield of the mushrooms from one-kilogram dry matter of substrate was found to be significantly higher in case of GA treated beds (413.37± 28.08) compared to other growth regulators and untreated control bed. Minimum yield was observed in case of beds treated with kinetin (282.22±14.37) and was lower than the untreated control (Table 5). To our knowledge, this is the first study regarding the effect of growth regulators on yield parameters of *A. auricula.* Similar results were obtained in cultivation of *Pleurotus eous* with growth regulators by Pal *et al*., (2014) [8]. Faster spawn run and yield enhancement in GA treated beds might be due to the rapid stimulatory effect of GA in cell division and cell elongation (Camara *et al*., 2015) [3].

Table 2: Effect of different supplements on growth of Jew's ear mushroom

S. No.	Supplements	Days for complete spawn run	Days for pinhead formation	Days for first harvest	Total crop period (days)
1.	Rice bran	39.33 ± 2.52^{b}	43.00 ± 2.00^{c}	50.00 ± 2.00^{c}	93.67 ± 2.52^{a}
2.	Bengal gram powder	41.33 ± 2.52^{ab}	46.67 ± 2.08^{b}	54.00 ± 2.65^{b}	92.00 ± 2.00^{abc}
3.	Urea	36.00 ± 1.73^{c}	43.33 ± 1.15^{c}	50.67 ± 1.53^{c}	92.67 ± 2.31^{ab}
4.	Ammonium sulphate	44.33 ± 1.15^{a}	49.67 ± 1.53^{a}	58.33 ± 1.15^{a}	89.33 ± 4.04^{bcd}
5.	Poultry manure	42.67 ± 1.15^{a}	48.00 ± 1.00^{ab}	55.67 ± 0.58^{ab}	87.33 ± 2.08^{d}
6.	Spent mushroom compost	42.33 ± 1.53^{ab}	47.67 ± 1.53^{ab}	55.67 ± 1.53^{ab}	86.67 ± 2.52^{abc}
7.	Control	43.00 ± 2.00^{a}	48.33 ± 2.08^{ab}	56.33 ± 1.53^{ab}	88.00 ± 2.00^{cd}
CD at 5%		3.287	2.935	2.935	4.522
SE (m)		1.084	0.968	0.968	1.491

Table 3: Effect of different supplements on yield of Jew's ear mushroom

S. No.	Supplements	No. of sporocarps	Sporocarp fresh weight(g)	Total yield (fresh weight-g)	Biological efficiency (%)
1	Rice bran	40.33 ± 7.03^{a}	8.86 ± 0.91^{a}	308.42 ± 16.36^{a}	31.17
2	Bengal gram powder	33.00 ± 4.61^{abc}	8.08 ± 0.15^{ab}	235.61 ± 19.01^{c}	23.56
3	Urea	37.00 ± 3.61^{ab}	8.45 ± 0.40^{a}	271.76 ± 13.03^{b}	27.17
4	Ammonium sulphate	29.67 ± 6.03^{bcd}	7.58 ± 0.40^{bc}	215.48 ± 20.31^{cd}	21.55
5	Poultry manure	27.00 ± 4.61^{cd}	7.28 ± 0.29^{cd}	198.47 ± 22.92^{de}	19.85
6	Spent mushroom compost	22.33 ± 4.06^{d}	6.62 ± 0.36^{d}	165.87 ± 15.29^{e}	16.59
7	Control	24.00 ± 5.36^{d}	7.02 ± 0.09^{cd}	173.46 ± 27.43^{c}	17.34
CD at 5%		7.518	0.784	34.698	
SE (m)		2.478	0.258	11.439	

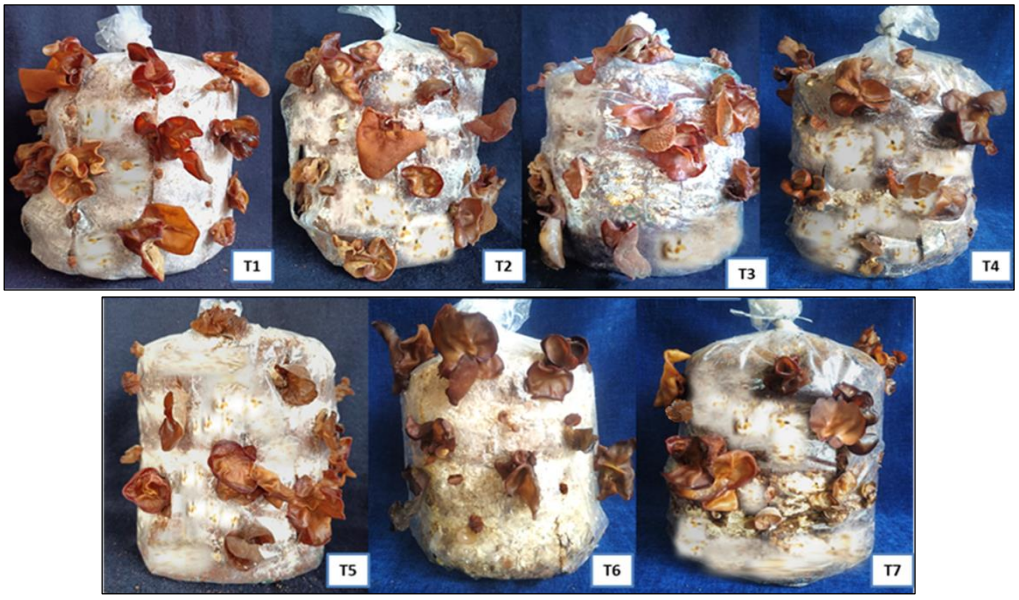

Fig 1: Effect of supplements in production of Jew's ear mushroom; Supplements used; T1-Rice bran, T2-Bengal gram powder, T3-Urea, T4-Ammonium sulphate, T5-Poultry manure, T6- Spent mushroom compost, T7-Control (Non amended substrate)

Table 4: Effect of growth regulators on growth of Jew's ear mushroom

S. No.	Growth regulators	Days for complete spawn run	Days for pinhead formation	Days for first harvest	Total crop period (days)
1	IAA	35.67 ± 2.08^{bc}	44.33 ± 0.58^{a}	51.33 ± 0.58^{a}	96.33 ± 2.08^{a}
2	NAA	38.67 ± 1.16^{a}	42.33 ± 0.58^{bc}	48.67 ± 0.58^{bc}	91.67 ± 2.08^{bc}
3	GA	33.67 ± 1.16^{c}	37.33 ± 0.58 ^{d}	44.33 ± 0.58^{d}	93.00 ± 2.65^{ab}
4	Kinetin	37.33 ± 1.16^{ab}	41.33 ± 1.16^{c}	48.00 ± 1.00^{c}	88.67 ± 2.08^{c}
5	IBA	35.67 ± 1.16^{bc}	43.33 ± 0.58^{ab}	51.00 ± 1.00^{a}	92.00 ± 3.00^{bc}
6	Control	39.33 ± 2.52^{a}	43.00 ± 2.00 ^{abc}	50.00 ± 2.00^{ab}	93.67 ± 2.52^{ab}
CD at 5%		2.905	2.011	1.922	4.317
SE (m)		0.943	0.653	0.624	1.401

Table 5: Effect of growth regulators on yield of Jew's ear mushroom

S. No.	Growth regulators	No. of sporocarps	Sporocarp fresh weight(g)	Total yield (fresh weight-g)	Biological efficiency (%)
1	IAA	42.67 ± 2.08^{b}	10.42 ± 1.25^{ab}	352.40 ± 20.10^{b}	35.24
2	NAA	42.33 ± 3.06^{b}	7.79 ± 0.52^{c}	313.07 ± 13.23^{cd}	31.31
3	GA	49.33 ± 4.16^{a}	11.72 ± 1.37^{a}	413.37 ± 28.08^{a}	41.34
4	Kinetin	35.33 ± 1.53^{c}	8.69 ± 1.16^{bc}	282.22 ± 14.37^{d}	28.22
5	IBA	39.00 ± 3.61^{bc}	8.92 ± 0.41^{bc}	330.01 ± 18.08^{bc}	33.00
6	Control	40.33 ± 6.03^{bc}	8.86 ± 0.91^{bc}	308.42 ± 16.36^{cd}	30.84
CD at 5%		6.603	1.783	33.82	
SE (m)		2.143	0.579	10.98	

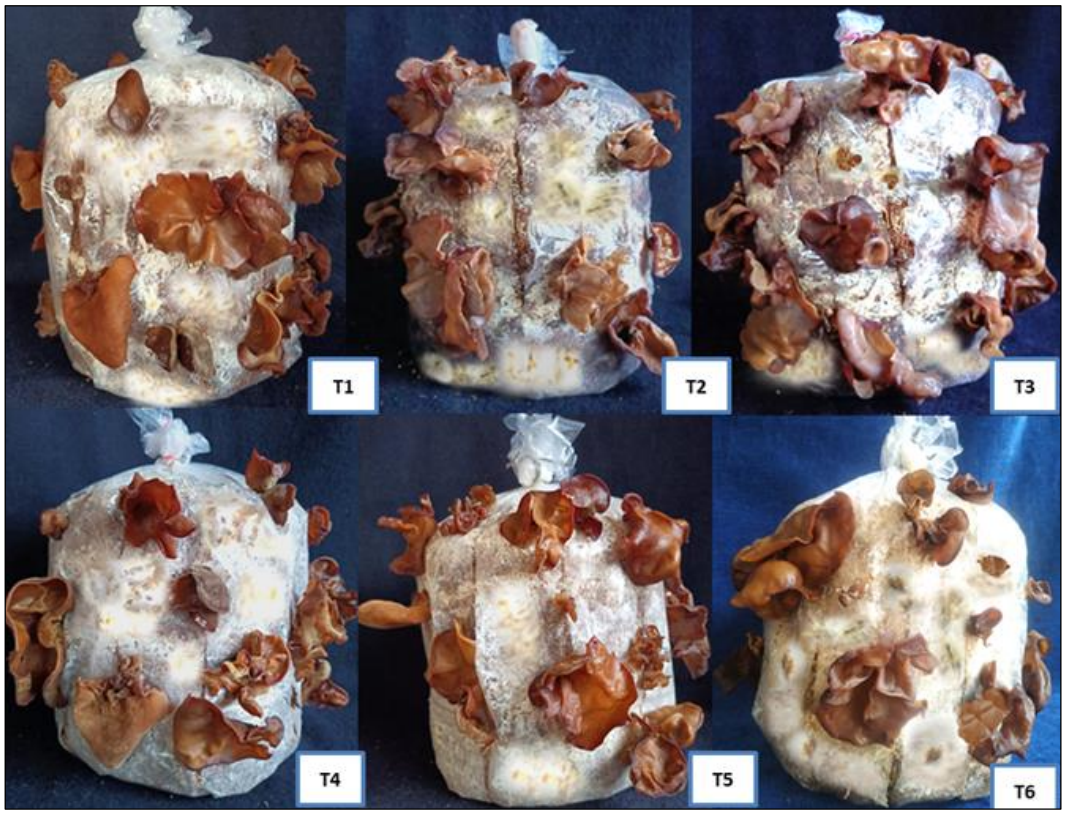

Fig 2: Effect of growth regulators in production of Jew's ear mushroom; Treatments: -T_1-IAA, T_2-NAA, T_3-GA, T_4-Kinetin, T_5-IBA, T_6-Control

Conclusion

From the present study, it is concluded that the growth, yield and thereby biological efficiency of Jew's ear mushroom in rubber saw dust-based substrate is higher when supplemented with rice bran followed by urea. Gibberellic acid is found to be the best growth regulator for the yield enhancement of *A. auricula.* Kinetin is found to reduce the fruiting body production of this mushroom whereas naphthalene acetic acid has no significant effect. Hence the study reveals that both supplementation and application of certain growth regulators influences the growth and yield of *A. auricula.* Effect of supplementation on the proximate composition of this mushroom can be done as a future line of work.

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