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Effect of different inorganic additives on the mycelial and spawn growth of shiitake mushroom (*Lentinula edodes*)

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Abstract
Shiitake mushroom is an edible mushroom which originated from East Asia and widely cultivated and consumed in numerous Asian countries. Present investigation was done to estimate the effect of two chemicals at different doses *viz*. magnesium sulphate and Potassium sulphate (@ 1.0, 1.5 and 2.0%) on mycelium and spawn growth. The results obtained from the present investigation show that, maximum mycelial growth of Shiitake (Le-21-01) was found in magnesium sulphate @ 2% (89.00 mm). While, minimum mycelial growth was found on potassium sulphate @ 2.0% (53.30 mm). Similarly maximum dry mycelial weight of Shiitake (Le-21-01) was observed in magnesium sulphate @ 2% (4.12 gm/100 ml) and minimum dry mycelial weight was observed on potassium sulphate @ 2.0% (1.11 mg/100 ml). In case of spawn growth, maximum vertical mycelium growth in Shiitake (Le-21-01) was found on wheat + magnesium sulphate @ 2.0%, (89.50 mm). While, minimum growth was found in wheat + potassium sulphate @ 2.0%, (66.20 mm).

Keywords: Radial growth, dry mycelial weight, spawn, mushroom, shiitake, inorganic additives, magnesium sulphate, potassium sulphate

Introduction
Mushroom has been described as “a macro fungus with a distinctive fruiting body which may be both epigenous or hypogenous and big sufficient to be visible with naked eyes and to be picked via way of means of hands" Chang and Miles (1993) [3]. World mushroom production reached up to 43 million tons in 2018-19 (Singh *et al*., 2020) [17] and may surpass 50 MT by 2025 (Singh *et al.*, 2021) [18]. The total mushroom production recorded during the year 2019-20 in India was 2.01 Lac metric tons (FAOSTAT-2020) [6]. With context to the Indian scenario of mushroom consumption, it has become an integral part of Indian food and it is being cultivated throughout the country. Haryana is the leading state (20.05 tons) followed by Odisha (19.53 tons), Maharashtra (18.38 tons) and Himachal Pradesh (14.41 tons) (Priya *et al*., 2021) [15]. Among the various mushrooms cultivated in China, total production of *Lentinula edodes* was only 11159 million kg (Singh *et al*., 2021) [18]. The word 'Shiitake' comes from two Japanese words first one is: shii, this means that oak and second one is: take, this means that mushroom. The Shiitake mushroom is likewise called the 'black forest mushroom' with inside the United States and lectin in France. 'Xiang-gu' (or Siang-gu) "The fragrant mushroom", 'Dong-gu' "The winter mushroom" and 'Hua-gu' "The blossom mushroom" or "The variegated mushroom" are a number of the Chinese names for numerous types of Shiitake mushrooms (Chen, 2001) [4]. Shiitake is known as an "elixir of life" in each Japan and China. Dried Shiitake mushrooms are wealthy in carbohydrates and protein. They contain 58-60% carbohydrates, 20-23% protein (digestible up to 80-87%), 9-10% fiber, 3-4% lipids and 4-5% ash. The mushroom is a great supply of vitamins, particularly pro-vitamin D_2 (ergosterol) 325 mg % which in the presence of ultraviolet (UV) light and high temperature yields calcitriol.

2. Material and method
2.1 Experimental site
The present investigation was conducted in Mushrooms Laboratory, Department of Plant Pathology, Sardar Vallabhbhai Pate University or Agriculture and Technology, Modipuram, Meerut 250110 (Uttar Pradesh). This university is situated on the western side of the Delhi-Dehradun highway at a distance of 10.0 km in the north of Meerut City.

The Meerut district is situated between 29^0 01'N latitude and 77^0 45'E longitude at an altitude of 237 meters above the mean sea level.

2.2 Establishment of pure culture
The culture of *Lentinula edodes* was collected from Directorate of mushroom research, Solan, Himachal Pradesh for this study. The culture of shiitake was multiply in sterilized Petri plate on Potato Dextrose Agar (PDA) for 12-15 days and transferred to PDA slants for further use with the help of single hyphal tip culture technique. These cultures tubes and Petri plates were incubated at 25±2 °C in BOD incubator.

2.3 Effect chemical additives on mycelial growth of Shiitake
For the experiments on mycelial growth, mycelial growth rate/day, dry mycelial weight and dry mycelial weight growth rate/day, the strain of shiitake (Le-21-01) has been taken. The experiments were carried out using two chemicals on different doses *viz*. magnesium sulphate and potassium sulphate (@1.0, 1.5 and 2.0%) with PDA for radial growth. PDA medium was prepared and chemicals were added in the medium before sterilization. The 20 ml medium was poured in each sterilized petri plate (90 mm) and subsequently inoculated with 9 mm disc of 7 days old culture of shiitake (Le-21-01). Inoculated plates were incubated at 25±1 °C. The observations of the mycelial growth in mm were recorded at 3 days interval till the colony covered the first full plate i.e. 90 mm. For dry mycelial weight of shiitake same above mentioned chemical additives mixed with Potato Dextrose Broth (PDB) and poured into 250 ml conical flasks @ 100 ml per flask and sterilized in autoclave at 121 ˚C, 15 psi for 15-20 minutes. Thereafter, inoculated and incubate the conical flask for 15 days in three replications of each treatment, as similar method mentioned for mycelial growth. The culture was then filtered with the help of Whatman's filter paper No.1 and the mycelium mats were dried at 60 °C in hot air oven for 48 hours before measuring the dry weight of mycelium mat, on an electronic balance. The observation of the weight in mg of dry mycelial mat was recorded for the observation.

2.4 Effect of chemical additives on spawn growth of Shiitake
For improvement in the spawn production technology, an experiment on the spawn growth was conducted. The mother spawn was prepared on wheat grains in half liter capacity wide mouthed saline glass bottles. The experiment was carried out using chemicals additives *viz*. magnesium sulphate and potassium sulphate (@ 1.0, 1.5 and 2.0% were mixed as a supplement with wheat grain. The grains were filled up to 90 mm height in the saline glass bottle in 3 replications before sterilization and followed all the steps of spawn production technology. Afterthat, 7 day old culture of shiitake (Le-2101) was inoculated by 9 mm diameter disc in individual bottle under aseptic condition. The spawn bottles were incubated without shaking at 25±1 °C in B.O.D incubator and observations were recorded on every five days interval until the first bottle completely covered by mycelial growth in anyone of the used spawn bottle for the experiment.

2.5 Statistical analysis
The Complete Randomized Design (CRD) was applied and the data thus obtained were analyzed statistically. Analysis of variance (ANOVA) technique and critical difference (CD) was calculated at five percent level of significance for comparison with other treatment (Kumar *et al*., 2022a; Kumar *et al*., 2022b) [10, 11].

3. Result and discussion
The result revealed that on 15th days, the maximum mycelial growth (Le-21-01) (89.00 mm with growth rates 5.93 mm/day) of Shiitake was observed in PDA + magnesium sulphate @ 2.0% followed by PDA + mmgnesium sulphate @ 1.5% (86.50 mm with growth rates 5.76 mm/day), while the minimum radial growth (53.30 mm with growth rates 3.55 mm/day) was found in PDA + potassium sulphate @ 2.0%. As compared to control (83.00 mm with growth rates 5.50 mm/day). Similarly in case of dry mycelium weight of shiitake (Le-21-01) at 15th days the maximum dry mycelium weight (4.12 mg/100 ml with dry mycelial weight growth rate 0.27 mg) was obtained in PDB + mgnesium sulphate @ 2.0% followed by PDB + magnesium sulphate @ 1.5% (3.18 mg/100 ml with dry mycelial weight growth rate 0.21 mg). While the minimum weight of dry mycelium was obtained in PDB + potassium sulphate @ 2.0% (1.11 mg/100 ml with dry mycelial weight growth rate 0.07 mg) than control (2.10 mg/100 ml with dry mycelial weight growth rate 0.14 mg). The result of spawn experiment indicated that on 20th days the maximum vertical mycelium growth (89.50 mm with spawn growth rate 4.47 mm/day) of shiitake (Le-21-01) was found in wheat grain + magnesium sulphate @ 2.0%, followed by wheat grain + magnesium sulphate @ 1.5%, (86.70 mm with spawn growth rate 4.33 mm/day). While, minimum growth was found in wheat grain + potassium sulphate @ 2.0%, (66.20 mm with spawn growth rate 3.31 mm/day) comparison to control (75.80 mm with spawn growth rate 3.79 mm/day). The results are almost in accordance with the findings of Huang *et al*. (2003) [8] they also found that more mycelial growth with 1 per cent $MgSO_4$, as the inorganic salts source. Singh (2012) [19] reported that maximum radial growth on 9th day of observation in magnesium sulphate (9.00 cm; 9.00 cm) and potassium sulphate (8.92 cm; 8.78 cm) supplemented medium in case of two strains of *C. indica* APK-2 and CI-6 respectively. Bhadana, (2014) [2] reported that *P. djamor*, *P. florida* showed maximum dry mycelial growth on magnesium sulphate (4.16 mg). Wang (2010) [23]; Katiyar (2018) [9] reported that 99.00 mm spawn growth at 20 days of observation in 1% magnesium sulphate. Kumar (2020) [12] found that the maximum mycelial growth (90.00 mm and 4.50 mm/day growth rate) on 20th days in strain of He-02 supplemented with magnesium sulphate @ 2% which was significantly superior to all other strains.

Table 1: Effect of different inorganic additives on mycelial growth (mm) of Shiitake (Le-21-01).

Treatment	Mycelial Growth (mm)					Growth rate (mm/day)	Dry mycelial weight (mg/100 ml)	Dry mycelial weight/day mg/day
	3rd day	6th day	9th day	12th day	15th day			
PDA+$MgSO_4$ @ 1.0%	14.20	5.54	42.80	63.30	83.20	5.54	2.37	0.15
PDA+ $MgSO_4$ @ 1.5%	14.50	5.76	45.70	64.20	86.50	5.76	3.18	0.21
PDA+ $MgSO_4$ @ 2.0%	15.70	5.93	48.80	69.20	89.00	5.93	4.12	0.27
PDA+ K_2SO_4 @ 1.0%	12.30	5.08	39.20	53.50	76.20	5.08	1.60	0.10
PDA+ K_2SO_4 @ 1.5%	13.00	4.22	37.00	49.00	63.30	4.22	1.25	0.08
PDA+ K_4SO_4 @ 2.0%	12.70	3.55	31.00	42.70	53.30	3.55	1.11	0.07
Control	13.20	5.50	44.20	62.80	83.00	5.50	2.10	0.14
C.D	0.98	1.75	2.83	2.90	1.87	-	-	-
SE (m)	0.321	0.574	0.926	0.949	0.611	-	-	-

Average of three replications

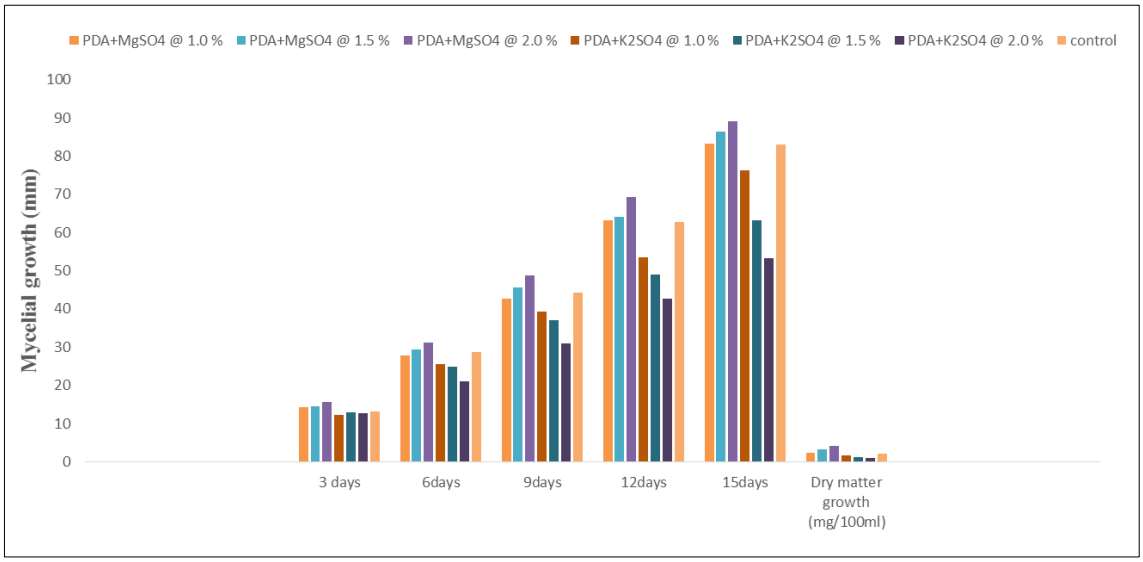

Fig 1: Effect of different inorganic additives on mycelial growth (mm) of Shiitake (Le-21-01).

Table 2: Effect of different chemical additives on spawn growth of Shiitake (Le-21-01).

Treatment	Mycelial Growth (mm)				Growth rate (mm/day)
	5th day	10th day	15th day	20th day	
Wheat grain + $MgSO_4$ @ 1.0 %	13.20	28.20	56.50	82.80	4.14
Wheat grain + $MgSO_4$ @ 1.5 %	14.70	36.00	62.60	86.70	4.33
Wheat grain + $MgSO_4$ @ 2.0 %	15.50	40.20	65.80	89.50	4.47
Wheat grain + $K2SO_4$ @ 1.0 %	12.80	22.20	47.50	76.50	3.82
Wheat grain + $K2SO_4$ @ 1.5 %	12.20	21.80	42.80	71.00	3.55
Wheat grain + $K2SO_4$ @ 2.0 %	11.00	20.50	39.20	66.20	3.31
Wheat grain (Control)	13.80	27.50	47.80	75.80	3.79
C. D	0.925	1.900	1.758	1.840	-
SE (m)	0.302	0.620	0.574	0.601	-

Average of three replications

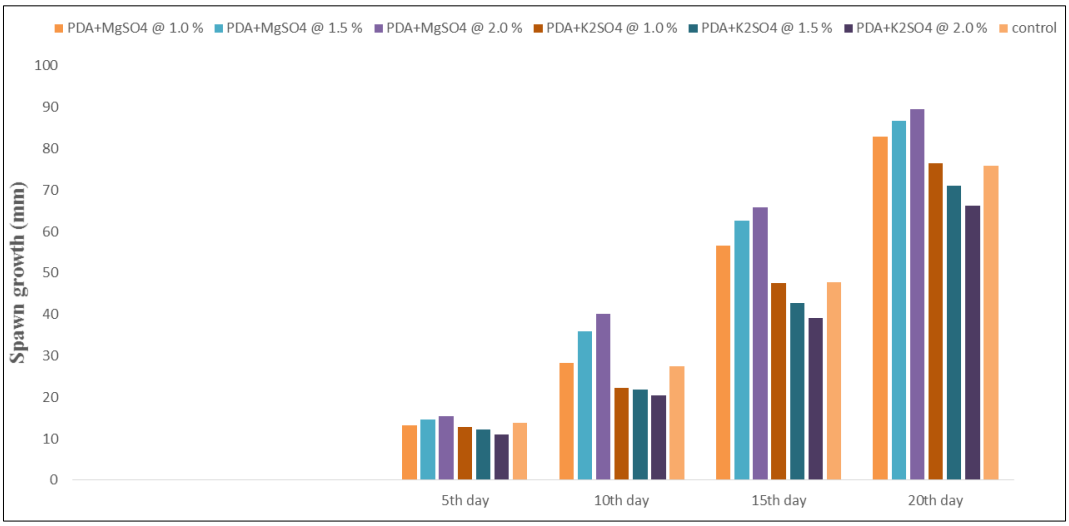

Fig 2: Effect of different inorganic additives on spawn growth (mm) of shiitake (Le-21-01).

4. Conclusion

In present investigations the maximum mycelial growth, mycelial growth rate/day, dry mycelial weight, dry mycelial weight growth rate/day, spawn growth and spawn growth rate/day of shiitake (Le-21-01) were observed with magnesium sulphate @ 2.0% in case of all these parameters. On the basis of present investigation we conclude that magnesium sulphate @ 2.0% is the best supplement for enhance the mycelial growth of shiitake (Le-21-01).

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