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Effects of inorganic additives on the mycelial and spawn growth of lion's mane mushroom (*Hericium* species)

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Abstract
Lion's mane mushroom (*Hericium* species) is common saprophytic fungi found on decaying trees throughout the Northern United States and Canada. The present investigation was done to find out the effect of two different inorganic additives *viz*. magnesium sulphate and calcium sulphate at different doses @ 1.5, 2 and 2.5% on mycelial growth, mycelial growth rate/day, dry mycelial growth, dry mycelial growth rate/day, spawn growth and spawn growth rate/day of Lion's mane mushroom. The results obtained from present investigation show that, the maximum mycelial growth (90.00 mm. and growth rate 5.00 mm/day), dry mycelial weight (3.00 mg/50 ml. and dry mycelial growth rate 0.166 mg/day) and spawn growth (90.00 mm. and growth rate 3.750 mm/day) of *Hericium* sp. (He-02.) were recorded among the both strain in case of all these parameter in treatment number one which received magnesium sulphate @ 2.5% while, minimum mycelial growth (80.33 mm. with 4.46 mm growth rate/day), dry mycelial weight (2.30 mg/50 ml. with dry mycelial growth rate 0.127 mg/day) and spawn growth (62.00 mm. with growth rate 2.583 mm/day) of *Hericium* sp. (He-04.) were recorded among the both strain in case of all these parameter in treatment number seven which serve as control (only PDA without any additive). Keeping in view the above nutritional and medicinal importance of Lion's mane mushroom (*Hericium* sp.), and possibilities of cultivating these mushrooms in the rural as well as urban areas of the country, the present investigation was undertaken.

Keywords: Radial growth, dry mycelial weight, spawn, inorganic additives, magnesium sulphate, calcium sulphate

Introduction
Mushrooms are delicious, nutritionally rich, medicinally important and non-conventional sources of human food. Mushroom production is regarded as the second most important commercial microbial technology next to yeast for large scale production (Chang, 1999) [1]. There are over 70,000 fungi species in the world, out of which only 2000 (31 genera) species are edible (Wakota and Temesgen, 2018) [19]. Mushrooms can be defined as a macro fungus which has a distinctive fruiting body. These fruiting bodies can be either epigenous i.e. above the ground or hypogenous i.e. below the ground. They are edible fleshy fungi which are devoid of chlorophyll and thus cannot make their own food (Chang and Miles, 1991) [2]. The total mushroom production recorded during the year 2019-20 in India was 2.01 lakh metric tonnes (FAOSTAT-2020) [3]. World mushroom production increased to 43 million tons in 2018-19 and may surpass 50 MT by 2025 (Singh *et al*., 2021) [15].

Hericium sp. (Bull.: Fr.) Pers., commonly known as the monkey head mushroom, is considered as one of the best edible and medical mushrooms belonging to the family Hericiaceae, order Russulales, and class Agaricomycetes (Kirk *et al*., 2008) [7]. Lion's mane mushroom is known by different names all over the world. In Japan, *Hericium erinaceus* is called as "Yamabushitake", Yamabushi literally means "mountain priest". In China, the mushroom is called by the name Houtou, which means "monkey head". This mushroom is also known as "Lion's Mane", "Monkey's Mushroom" (Thongbai, 2015) [18]. Lion's mane mushroom consists of certain unique bioactive compounds like Hericenones A and Hericenones B which have helped in the growth and development of the neurons and other accessory structure, it helps in the coordination of the neurons which are associated with the complex neuro degenerative diseases like Alzheimer's disease, depression disorder, anxiety (Jiang, 2014) [8].

Experimental site

The experiments were carried out in the Mushroom laboratory of Department of Plant Pathology, Sardar Vallabhbhai Patel University of agriculture and technology, Meerut, Uttar Pradesh during the year 2021-22, which is located on the western side of the Delhi-ehradun highway (NH-58) located at the distance of 10 km away from the Meerut city. Meerut is located between 29 degree 01'N latitude and 77 degree 45'E longitude at an altitude of 237 meters above the mean sea level.

Establishment of the pure culture

The pure cultures of Lion's mane mushroom (*Hercium* sp.) were were purified and maintained by single hyphal tip method. For this purpose, the cultures were grown in sterilized Petri plate on Potato Dextrose Agar Medium (PDA) for 8-10 days. Single branched hyphae from the periphery of the growing colony were marked under low power (10x) in the compound microscope and transferred to PDA slants. These tubes were incubated at 21-24 ℃ for about a week, again sub cultured on PDA and then stored in a refrigerator at 5-10 ℃ for further use.

Materials and Methods

The present investigation was carried on two strains of *Hericium* sp. i.e., He-02 and He-04. The experiments were conducted to find out the effect of different inorganic additives on the mycelial growth of *Hericium* sp. The basal media was selected Potato Dextrose Agar (PDA) media. Different inorganic additives i.e., $MgSO_4$ and $CaSO_4$ were selected. Different doses of these inorganic additives i.e., $MgSO_4$ at 1.5%, 2% and 2.5% was added in the basal media. The media was then sterilized and poured into the petri plates of 90 mm of diameter @ 20 ml of media per plate. These plates were then inoculated centrally with 9 mm diameter disc of one week old culture of *Hericium* (He-02 and He-04) and incubated at 25±2 ^{0}C. All the treatments were replicated in 3 times to take the observations of mycelial growth after each 72 hours until the plate is full. For the purpose of dry mycelial growth Potato Dextrose Broth (PDB) was selected as the basal media and same above mentioned different inorganic additives and their doses were added and the seventh treatment was taken as control (PDB). The media was then poured into 250 ml conical flasks @ 50 ml per flask. These treatments were replicated with 3 replications. These flasks were then sterilized at 121 ^{0}C at 15 psi for 20 minutes. These flasks were then inoculated with different strains of *Hericium* (He-02 and He-03) with 9 mm of disc. The flasks were then incubated at 25±2 ^{0}C. The culture was then filtered with Whatman's filter paper No.1 and the mycelium collected was dried at 60 ^{0}C in hot air oven for 48 hours followed by measuring the dry mycelial growth, on the electronic weighing balance. The weight of the dry mycelial mat was recorded for the observation (Kumar *et al.* 2022a; Kumar *et al.* 2022b and Kumar *et al.* 2019) [13, 10, 19].

For the study of spawn growth of Lion's mane mushroom (*Hericium* sp.) the spawn was prepared in the glass bottles (Kumar *et al.* 2022a and Katiyar, *et al.* 2018) [13, 6]. The experiments were conducted to find out the effect of two different chemicals at different percentage as additives on spawn growth of Lion's mane mushroom (*Hericium* sp.). For the present experiment, MgSO4 at 1.5%, 2% and 2.5% and CaSO4 at 1.5%, 2% and 2.5% were added to wheat and wheat alone was taken as control. The spawn were prepared as described above. The grains were filled up to 90 mm in bottle in 3 replications. The cultures of two strains of *Hericium* sp. (He-02 and He-04) were inoculated by 9 mm disc in individual bottle. After inoculation spawn bottles were placed in the BOD incubator at 25±2 °C. Observation for spawn growth (mm) and spawn growth rate (mm/day) were recorded at every 6 days interval till the any bottle completely covered with mycelial growth.

Statistical analysis

The suitable statistical design completely 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; Kumar *et al*., 2019; Katiyar, *et al.* 2018) [13, 10, 19].

Table 1: Effect of different inorganic additives on the mycelial growth of *Hericium* sp., (He-02 and He-04)

S. No.	Treatment	3rd day		6th day		9th day		12th day		15th day		18th day			Growth rate (mm/day)
		He -02	He -04	He -02	He -04	He -02	He -04	He -02	He -04	He -02	He -04	He 02	He -04	He -02	He -04
1.	PDA+$MgSO_4$ @ 2.5%	15.66	16.66	24.33	24.00	37.33	37.00	52.33	61.00	70.33	67.33	90.00	88.00	5.00	4.88
2.	PDA+$CaSO_4$ @ 2.5%	15.33	15.33	23.66	21.66	35.33	33.00	50.66	48.00	68.67	67.00	88.00	86.66	4.88	4.81
3.	PDA+$MgSO_4$ @ 2%	15.00	14.33	22.66	21.33	34.66	32.00	49.66	47.33	68.33	65.00	87.33	84.00	4.85	4.66
4.	PDA+$CaSO_4$ @ 2%	14.33	13.33	22.33	20.33	34.66	30.66	49.33	45.33	66.00	63.33	84.00	83.00	4.66	4.61
5.	PDA+$MgSO_4$ @ 1.5%	12.66	12.00	19.00	18.33	31.00	29.33	44.33	42.33	62.33	61.66	83.33	82.00	4.62	4.55
6.	PDA+$CaSO_4$ @ 1.5%	12.00	12.00	19.66	18.33	29.33	28.33	41.00	40.66	62.00	61.00	82.00	80.66	4.55	4.48
7.	Potato dextrose agar (control)	13.66	12.66	21.66	19.33	32.00	31.33	45.66	45.33	63.00	61.00	81.33	80.33	4.51	4.46
8.	CD @ 5%	1.929	2.441	1.637	1.591	2.379	1.768	2.183	2.251	2.701	2.113	1.494	1.850	-	-
9.	SE(m)	0.630	0.797	0.535	0.591	0.777	0.577	0.713	0.735	0.882	0.690	0.488	0.604	-	-

Table 2: Effect of different inorganic additives on the dry mycelial growth of *Hericium* sp., (He-02 and He-04)

S. N.	Treatment	Dry matter growth (mg/50ml)		Dry matter growth rate (mg/day)	
		He-02	He-04	He-02	He-04
1.	PDA+$MgSO_4$ @ 2.5%	3.00	2.90	0.166	0.161
2.	PDA+$CaSO_4$ @ 2.5%	2.90	2.81	0.161	0.156
3.	PDA+$MgSO_4$ @ 2%	2.70	2.68	0.150	0.148
4.	PDA+$CaSO_4$ @ 2%	2.65	2.60	0.147	0.144
5.	PDA+$MgSO_4$ @ 1.5%	2.59	2.50	0.143	0.138
6.	PDA+$CaSO_4$ @ 1.5%	2.55	2.45	0.141	0.136
7.	Potato dextrose agar (control)	2.40	2.30	0.133	0.127
8.	CD @5%	0.25	0.20	-	-
9.	SE(m)	0.08	0.05	-	-

Table 3: Effect of different inorganic additives on the spawn growth of *Hericium* sp., (He-02 and He-04)

S. No.	Treatment	6th day		12th day		18th day		24th day		Growth rate (mm/day)	
		He-02	He-04	He-02	He-04	He-02	He-04	He-02	He-04	He-04	He-04
1.	PDA+$MgSO_4$ @ 2.5%	20.00	19.66	37.66	38.66	64.00	61.00	90.00	90.00	3.750	3.750
2.	PDA+$CaSO_4$ @ 2.5%	18.00	17.33	35.66	33.00	61.00	59.00	87.66	86.33	3.652	3.597
3.	PDA+$MgSO_4$ @ 2%	16.66	16.00	36.00	33.00	59.00	58.33	85.00	82.33	3.541	3.235
4.	PDA+$CaSO_4$ @ 2%	16.00	15.33	35.00	30.00	55.00	52.33	83.00	77.66	3.458	3.430
5.	PDA+$MgSO_4$ @ 1.5%	14.66	12.33	24.66	23.00	40.33	41.00	69.33	67.66	2.888	2.819
6.	PDA+$CaSO_4$ @ 1.5%	14.00	12.00	23.00	20.33	33.00	31.66	68.33	66.66	2.847	2.777
7.	Potato dextrose agar (control)	15.00	14.66	26.33	25.33	43.33	41.66	63.66	62.00	2.652	2.583
8.	CD @5%	2.148	1.280	1.682	2.078	2.440	2.217	1.014	2.201	-	-
9.	SE(m)	0.701	0.148	0.549	0.678	0.797	0.724	0.984	0.816	-	-

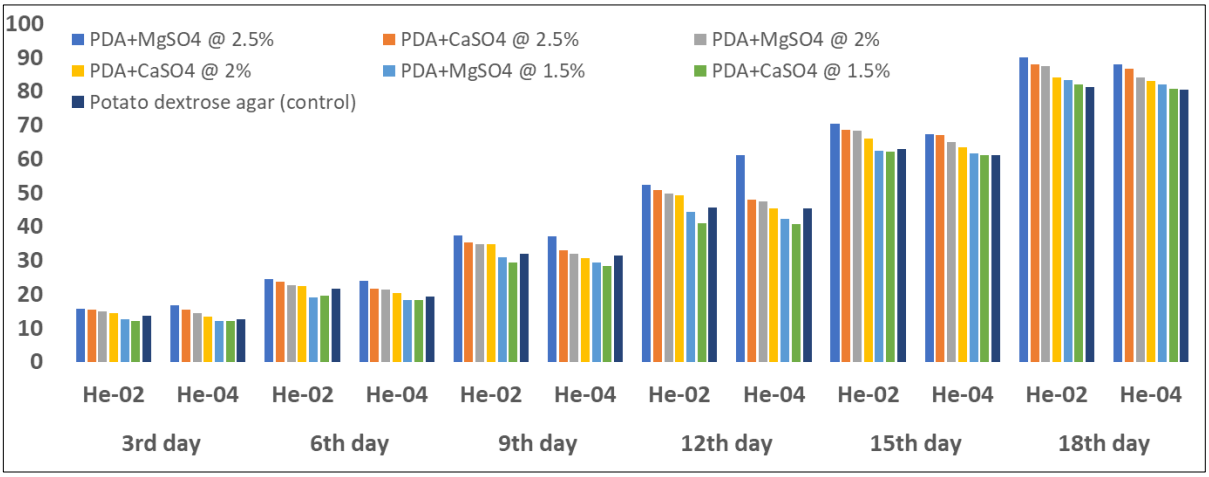

Fig 1: Effect of different inorganic additives on the mycelial growth of *Hericium* sp., (He-02 and He-04).

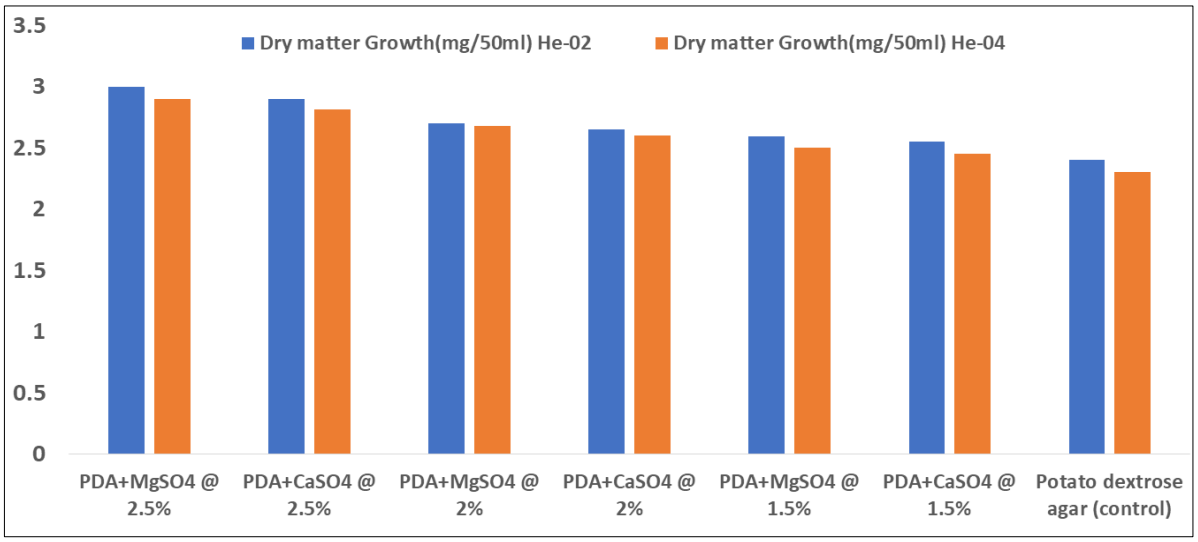

Fig 2: Effect of different inorganic additives on the dry mycelial growth of *Hericium* sp., (He-02 and He-04).

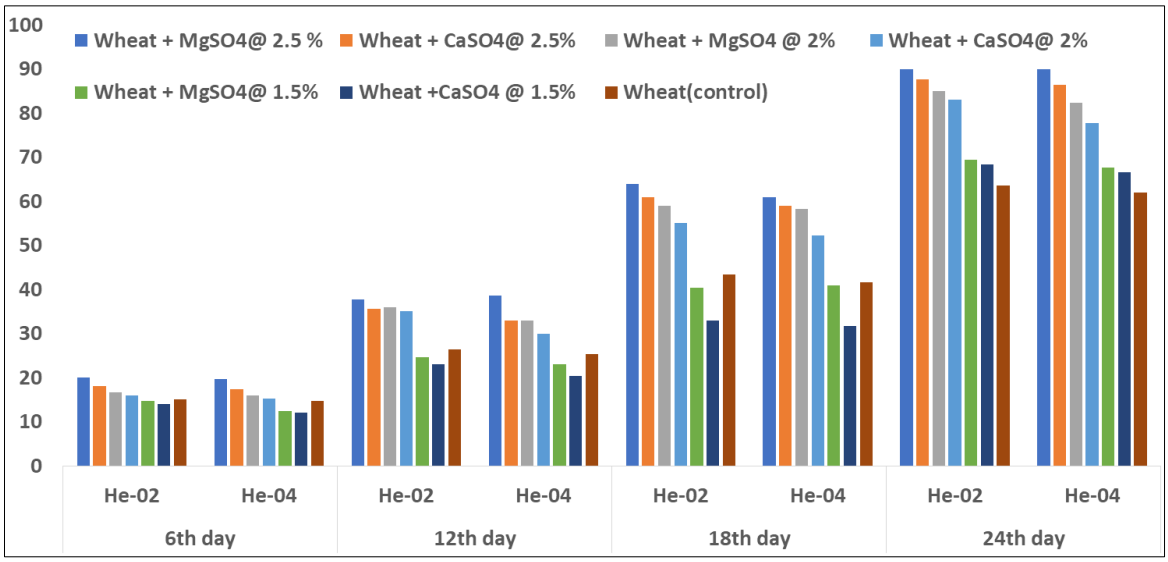

Fig 3: Effect of different inorganic additives on the spawn growth of *Hericium* sp., (He-02 and He-04).

Results and Discussion

The results revealed that on the 18th day of inoculation, the maximum mycelial growth (90.00 mm with growth rate 5.00 mm/day and 88.00 mm with growth rate 4.88 mm/day) were observed in case of PDA + MgSO4 @ 2.5% in case both strain of *Hericium* sp. (He-02 and He-04) followed by PDA + CaSO4 @ 2.5% (88.00 mm with growth rate 4.88 mm/day and 86.66 mm with growth rate 4.81 mm/day) respectively. While, minimum mycelial growth was observed in PDA media i.e., control (81.33 mm with growth rate 4.51 mm/day and 80.33 mm with growth rate of 4.46 mm/day) in case both strain of *Hericium* sp. (He-02 and He-04).

Regarding the dry weight of dry mycelium, both strains of *Hericium* sp. (He-02 and He-04) were observed at 18th day. Results revealed that in case both strain of *Hericium* sp. (He-02 and He-04) the maximum dry weight (3.00 mg/50 ml with growth rate 0.166 mg/day and 2.90 mg/50 ml with growth rate 0.161 mg/day) were observed in case of PDB + MgSO4 @ 2.5% followed by PDB + CaSO4 @ 2.5% (2.90 mg/50 ml with growth rate of 0.161 mg/day and 2.81 mg/50 ml with growth rate of 0.156 mg/day) Whereas, minimum dry weight were observed in case of Potato dextrose broth (2.40 mg/50 ml with growth rate of 0.133 mg/day and 2.30 mg/50 ml with growth rate of 0.127 mg/day). The results were in accordance with Kumar *et al.* (2020) [11] they observed that (90.00 mm) mycelia growth of *Hericium* sp. in He-02 strain of *Hericium* sp. with PDA + MgSO4 @ 2% after the 20th day of inoculation. Kumar, (2013) [8] also reported that maximum (87.33 mm) and (87.66 mm) mycelial growth of *Calocybe indica* (APK-2) in magnesium sulphate and calcium carbonate @ 1%, at 9th day respectively. The observations of spawn growth were recorded on 24th day after inoculation in the bottles. In the present investigation the results exhibited that in case both strain of *Hericium* sp. (He-02 and He-04) the maximum spawn growth were observed in Wheat + MgSO4 @ 2.5% (90.00 mm with growth rate of 3.75 mm/day and 90.00 mm with growth rate of 3.75 mm/day) followed by Wheat + CaSO4 @ 2.5% (87.66 mm with growth rate of 3.652 mm/day and 86.33 mm with growth rate of 3.597) order by. The minimum spawn growth was observed in case of control (63.66 mm with growth rate of 2.652 mm/day and 62.00 mm with growth rate of 2.583 mm) in case of both strain of *Hericium* sp. (He-02 and He-04).

The result was in accordance with the findings of Kumar, (2020) [11] who observed maximum mycelial growth (90 mm) strain of He-02 with MgSO4 @ 2% on 20th days. Kachroo *et al.*, (1997) [13] obtained maximum yield in magnesium sulphate. Kumar *et al.*, (2018) [14] observed maximum mycelial growth of *Pleurotus* species in magnesium sulphate (69 mm) followed by potassium dihydrogen orthophosphate (66 mm).

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