

Characterization of Sporophores, Spore Prints, Spines, Basidia, and Basidiospores of Seven Genotypes of *Hericium erinaceus* (Bull.: Fr.) Pers.

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ABSTRACT

In the present investigation, the morphology, dry matter, water content, fragrance of dry powder, the dimension of sporophores, basidia, and basidiospores, and length of spines of genotypes He-101, He-102, He-104, He-105, He-107, He-108, and He-109 of *Hericium erinaceus* were studied. The above genotypes were broadly categorized into clumping type (single clumping type- He-108 and multiple clumping type- He-101, He-104, He-107, He-109) and branching type (thick branching type- He-105 and diffuse branching type-He-102). The highest 29.99 % dry matter was calculated with He-102 along with 70.01% water content. The dry powder of He-101, He-104, He-105, He-108, He-109 fragrancd like vanilla essence. The cooked mushroom of even all genotypes rather gave lobster-like taste. However, He-102 and He-107 produced unpleasant raw lichen and mixed spicy-vanilla type fragrances, respectively. The dimension of each of sporophores, basidia, and basidiospores was found identical and measured 4.1-6.2 x 3.8-5.7 cm, 27-45x 5.20-7.51 μm , and 5.20-6.9 x 4.20-5.80 μm , respectively. However, the genotypes were characterized with the longest 2.08 cm (He-108), medium 1.05-1.52 cm (He-101, He-104, He-107, and He-109), and short 0.32-0.52 cm (He-102, He-105) spines. The spore prints of the genotypes were white and varied in shape, texture, and thickness.

Keywords: Morphology, Sporophores, Spine, Spore print, Basidia, Basidiospore, Fragrance, *Hericium erinaceus*.

INTRODUCTION

Hericium erinaceus (Bull.: Fr.) Pers. is a mostly saprophyte but rarely act as a weak wood degrading fungal pathogen of many deciduous trees of *Quercus* sp., *Fagus* sp., *Acer* sp., *Juglans* sp., *Ulmus* sp. etc. (Stamets, 1993; Pegler, 2003 & Fora et al., 2009) and

indeterminately grows on them. The sporophores of *Hericium* are varied in dimension from 5 to 30 cm and attached latterly on the substratum strongly with the round or subglobose, protruding, unbranched (Kuo, 2016) and rudimentary stipe.

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The sporophores consist of numerous single, 3 to 6 cm long, hanging, fleshy, tuberculate spines, which are initially white then yellowish, followed by brownish at a later stage (Thongbai et al., 2015). Kuo, 2016 mentioned the white colour of the spore print of *Hericiium erinaceus*. The water content of the sporophores is varied from 73 to 91% (Friedman, 2015). The basidiospores of 5–7 x 4–5 µm dimension are short ellipsoid, little visible ornamentation with finely roughened and amyloid (Stamets, 1993 & 2005). *Hericiium erinaceus* contains several volatile compounds. Among them, 2-methyl-3-furanthiol, 2-ethylpyrazine and 2, 6-diethylpyrazine is a principal component of the aroma of *H. erinaceus* (Miyazawa et al., 2008). Besides of pleasant fragrance, the *Hericiium erinaceus* is also a tasty mushroom.

MATERIAL AND METHODS

Genotype and growing medium

The pure culture of seven genotypes of *Hericiium erinaceus* (*He-101*, *He-102*, *He-104*, *He-105*, *He-107*, *He-108*, and *He-109*) was obtained from the Directorate Mushroom Research, Solan, and maintained on Potato Dextrose Agar (PDA) medium. The pure culture of all genotypes was used in the preparation of master spawn and subsequent commercial spawn with the standard procedure described by Gupta and Sharma, 1994. A total of 210 kg growing medium was also prepared from 70 kg of fresh dry wheat straw and 14 kg wheat bran. The dry wheat straw was soaked in water overnight followed by sun-drying for 4 hrs on a pre-disinfested cemented platform with 4% formalin. Wet wheat straw was thoroughly mixed with 14 kg wheat bran and packed in polypropylene bags @ 1.5 kg/ bag followed by autoclaving (at 20 lbs for 2 hours), inoculation (with 90 g commercial spawn /bag), and incubation (at 25°C for spawn run). Every genotype of *Hericiium eranceus* was maintained with 5 replications. Each replication contained 4 bags. The inoculated bags of growing medium were tagged with date of inoculation, rate of spawning, name of genotype, number of bags,

and number of replication. After completion of the spawn run, polyethylene bags were removed completely from the logs of the growing medium for pinhead initiation. The 8-10 days matured sporophores were selected for morphological and micrometrical study including their dry matter content, fragrance and taste. The process of spawn run was started in October around 25°C. However, the production of sporophores was led by December at 15-20°C. During production, a regular spray of water was provided every morning to maintain 90- 95% RH in the production room.

Morphology and micrometry of genotypes

Five uniform samples of 8-10 days matured freshly harvested sporophores of each *He-101*, *He-102*, *He-104*, *He-105*, *He-107*, *He-108*, and *He-109* were selected for morphological characters. Selected sporophores were split longitudinally with the help of a sharp knife and characterized the stipes (length, surface, flesh, basal end, a distal end, and branching pattern of stipe) and spines (location and length). The branches of stipe were studied similar to Sokół, et al. 2015. The arrangement of the spines formed a distinct shape/appearance of the sporophores. The texture of the sporophores was felt while holding the sporophores on the palm and recorded whether they had a wet fleshy feel, wet stemy thick feel or dry rough light feel. The dry and water content of the 100 g fresh sporophores with 3 replications of each genotype were calculated after drying them in a tray dryer at 45°C for 48 hours. The dry matter of sporophores was calculated using the formula, (%) dry matter of sporophores = (Dry weight of sporophores (g) /100 g fresh weight of sporophores) x100. However, the (%) moisture content of sporophores was measured with the formula, 100-(%) dry content of sporophores was calculated. The dried sporophores of all genotypes were powdered using Wiley grinder and confirmed their fragrance based on smell perception and memory-based comparison. The measurement of sporophores was taken using Vernier Calliper from the lower plane of the

sporophores where the main stipe is over. Since the shape of sporophores was ovoid or irregular so, an average of two or more lengths was measured at longer, shorter, and intermediate horizontal axis. The spore prints of all genotypes were also obtained. The selected sporophores of each genotype were attached to the holder in an invert position just a few cm above the piece of black paper on which spore mass was shaded. The setting was kept undisturbed for 12 hrs in an aseptic place for a successful spore print.

The micrometry of basidia and basidiospores of all genotypes of *Hericium erinaceus* was done with a compound microscope (on which Magcam DC 14 camera was attached) and computer-based Magnus Pro Image Analysis Software. Fifteen observations of each genotype for both basidia and spore micrometry were taken. In the micrometry of basidia, the selected matured spines were embedded in a piece of potato and cut into possible thinnest transverse (TS) sections with the help of a sharp blade in presence of distilled water. The possible thinnest TS of the spine was placed on the glass slides and stained with a drop of cotton blue. The prepared sections were covered with coverslips. The micrometry of basidiospores was done using 8-10 days matured sporophores. The selected sporophores of each genotype were plucked from the logs and kept for 12 hrs directly onto the clean glass slides in an aseptic place for spore shedding. The coverslips were mounted on the slide followed by the drop of water and finally covered the area of shaded spores. The prepared slides of basidia and basidiospores were observed using Magnus Pro Image Analysis Software. The respective data of the experiments were statistically analyzed using one factorial CRD of Web-Based Agricultural Statistics Software Package (WASP 2.0) at www.ccari.res.in/wasp2.0/index.php.

RESULTS AND DISCUSSION

Morphological characters of the sporophores of *Hericium erinaceus*

The sporophores of *He-101*, *He-102*, *He-104*, *He-105*, *He-107*, *He-108*, and *He-109* genotypes were matured within an average of 8-10 days from the date of their appearance on the growing media. The dimension of sporophores of all genotypes was statistically identical (table 3). Although, *He-104* had the biggest sporophore of 6.2 X 5.7cm followed by the 5.8 X 5.0 cm dimension of *He-109*. The smallest sporophore of *He-105* was measured by 4.1 X 3.8 cm. However, 5-9 cm cap diameter of *Hericium erinaceus* was recorded by Hassan, 2007. The length of spines of all genotypes was measured differently. *He-108* had the longest spines of 2.08 cm length followed by *He-101* (length 1.52 cm). Branched type sporophores had very short bristle-like spines (0.32-0.52), while clumping type had long spines. As the sporophores were grown indeterminately, the length of spines was increased by 3-4 cm. Ouali et al. (2020) described the fruit body of *Hericium erinaceus* consisting of dangling spines of 2 cm long and 2mm in diameter. Similarly, Ginns (1985) noted 1–5cm long and 2–3mm diameters of geotropic dangling spines of *Hericium erinaceus* on its lower face.

The sporophores of *He-101*, *He-102*, *He-104*, *He-105*, *He-107*, *He-108*, and *He-109* genotypes of *Hericium erinaceus* were broadly divided into two groups; 1) clumping type and 2) branch type (fig. 1). The clumping type was further divided into a single clumping type (*He-108*) and multiple clumping type (*He-101*, *He-104*, *He-107*, *He-109*). Both the clumping type sporophores were similar in many characters. Branch type sporophores were divided into thick branching type (*He-105*) and diffuse branching type (*He-102*) which differed in many characters as shown in table 1. These three groups of sporophores were differentiated on the appearance, texture, dry matter, moisture, fragrance, and taste of sporophores, length, surface, flesh, basal end, distal end, and branching pattern of stipe and location, and length spines. (table1). Ginns (1985) and Harrison (1973) stated that species in the genus *Hericium* are distinguished macroscopically by the presence of branched

or unbranched spines on which spines/tooth of various lengths are attached. Aggregation of spines formed single and multiple clumps of fruit bodies. Conde and Wolf (1972) defined *Hericium erinaceus* as large, snow-white, fleshy sporophores. Sometimes the fruit body of *Hericium erinaceus* appeared as nodular or ovoid basidioma with compact tuft-shaped, white-cream with a lateral short stipe attached on the trunk (Ouali et al., 2020). Some species of *Hericium* were described with the sporophores having thick primary branches originating from a single hard base. These primary branches were further divided into

many progressive branches and formed multitudes of ramuli of short spines (Hallenberg, 1983 & Hallenberg et al., 2013). The spore print of all genotypes having single, multiple, thick, and diffuse branching pattern of sporophores was varied in the pattern, thickness of spore mass, and shape. The basidiospores were formed externally on the tuberculate spines and at maturity, they were released in form of white spore mass. The spore print of *He-102* was insignificant in appearance due to very little in the release of spores on the black surface (Fig.1).

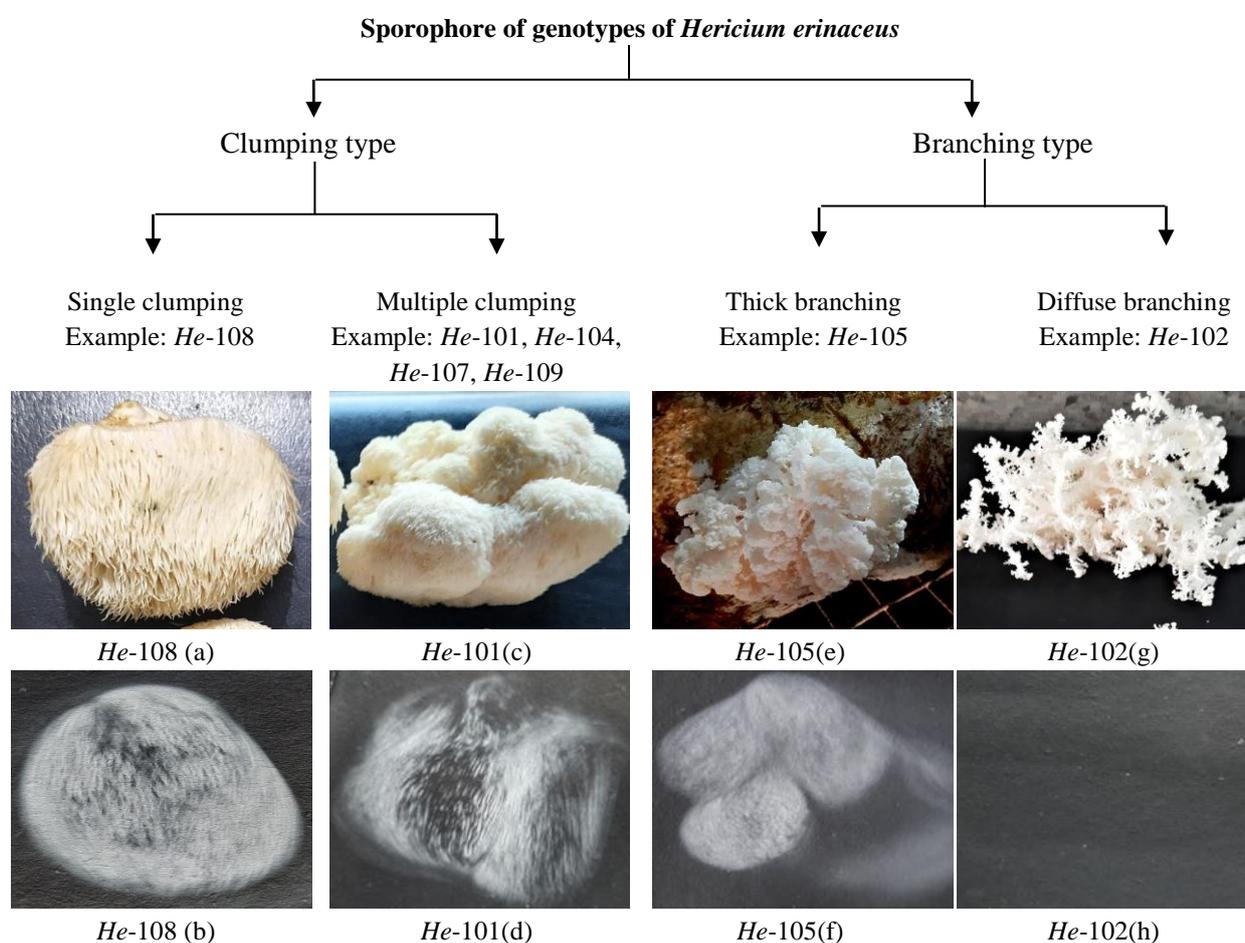


Fig. 1: Types of sporophores (a, c, e, g) and spore print (b, d, f, h)

The dry powder of *He-101, He-104, He-105, He-108, He-109* was sensed vanilla type fragrance. However, *He-102* produced unpleasant raw lichen fragrance and *He-107* was known for mixed spicy vanilla fragrance (table 2). Ahmed et al. 2008 advocated the citrus-floral and musky delicate flavor of *Hericium* species. The cooked *Hericium*

reportedly tastes as delicious to someone as lobster cooked in butter (<https://www.first-nature.com/fungi/hericium-erinaceus.php>). Thongbai, 2015 found out the seafood type taste of *Hericium* reminiscent of crab or lobster with fleshy, tough, and watery texture. Several aromatic compounds such as hericerin A, isohericenone J, isoericerin, hericerin, N-

dephenylethyl isohericerin, hericenone J, 4-(3',7'-dimethyl-2',6'-octadienyl)-2-formyl-3-hydroxy-5-methoxybenzylalcohol, erinacene D, esorcinols, erinacerins, and hericenols were reported earlier (Chaiyasut & Sivamaruthi, 2017) probably responsible for the fragrance and taste of *Hericum*.

The sporophores of all tested genotypes of *Hericum* were composed of different dry matter and moisture of their sporophores. Out of 100 g fresh weight of sporophores, a maximum of 29.99 g dry matter was obtained with *He-102*. However, a minimum of 6.25 g dry matter was recorded in the case of *He-108*. The moisture content among the genotypes was varied from 89.10 to 93.75% except for *He-102* in which 70.01% moisture content was determined. Chaiyasut and Sivamaruthi, 2017 reported 4% water content from the dry matter of *Hericum erinaceus*. The water content of the sporophores was varied from 73 to 91% (Friedman, 2015).

Micrometry of basidia and basidiospores of genotypes of *Hericum erinaceus*

During the microscopic study, club type basidia and globose to sub-globose type spores were found (Fig 3). The dimension of basidia

was found almost identical among all genotypes. Although, the *He-102* had the longest and slender type basidia with length and 45µm length X 5.20 µm width (table 3). The widest basidia of *He-107* was measured at approximately 7.51µm. Statistical analysis of the spore dimension was also done and found with no significant difference. The majority of basidiospores were dark thick walled at periphery and sub globose / mango /pear shaped. The papillae (remaining part of the sterigmata) were also appeared on the basidiospores by which they probably attached with the basidium. However, the colour of the spore print of basidiospores of all genotypes was recorded as white. The basidiospores of *He-102* were measured longest and widest with the dimension of 6.90 X 5.80µm (table 3). Harrison (1973) and Kuo (2016) found about 25–40 × 5–7µm dimension of basidia and 5.5–6.8 × 4.5–5.6µm dimension of the short ellipsoid to subglobose white coloured basidiospores. According to Ouali et al. (2020), the basidiospores (5–7µm X 4–5µm) are white in mass, short ellipsoid, little visible ornamentation with finely roughened, amyloid.

Table 1: Morphological characters sporophores of *Hericum erinaceus*

Clumping type		Branching type	
(Single and multiple clumps)	(Thick branching type)	(Diffuse branching type)	
<i>He-108, He-101, He-104, He-107, He-109</i>	<i>He-105</i>	<i>He-102</i>	
I. Stipe character			
1. Short stipe	1. Long stipe	1. Very short oblate stipe or absent	
2. Smooth stipe	2. Rough stipe	2. Rough bearing numerous bristles	
3. Fleshy stipe	3. Thick stipe	3. Thick and hard stipe	
4. The basal end was pointed and the distal end was broad and fleshy.	4. Both the ends of the stipe were almost equal in thickness and size	4. Irregular	
II. Branching pattern of the stipe			
1. No branching of the stipe. The spines were directly formed on the top end of the stipe	1. Number of branches were formed at a certain height of the stipe called primary branches. Primary branches were closely spaced and did not diffuse and grew upright, and formed thicket. Primary branches were found to be inclined at an angle at the stipe.	1. Primary branches arose from short oblate stipe or at the point of attachment to the substrate. They were widely spaced and diffused irregularly. Primary branches were formed comparatively less thicket and prostrate to the substrate.	
	2. Secondary branches were thick and aroused at a certain height of	2. Wide gap between secondary branches was observed. They were diffused, curved, and arose at different locations of primary branches in perpendicular fashion	

primary branches and formed a thicket. They were rather compact, inclined along the length of primary branches, and erected.

3. tertiary branches were occasionally noticed

or at wide-angle to primary branches.

3. Short tertiary branches were also seen that arose upright at the distal end of secondary branches and were widely spaced.

Primary and secondary branches were found to be irregular and diffused, increasing in length and width irregularly. The distal end of the secondary branches was flattened.

III. Location and length of spines.

1. Several spines were developed from the top end of the stipe and formed a broad, thick, and compact fleshy end of the stipe by joining loosely long spines together at a certain distance and then separated from each other. The spines were parallel to each other.

2. Long spines of 1.05-2.08 cm were seen.

1. The spines appeared in clusters on top of secondary branches.

2. Short spines were seen measured by 0.52 cm long.

1. Spines were seen on primary, secondary, and tertiary branches.

2. Very tiny size spines of 0.3 cm long were seen

3. Spines were compact on the top of tertiary branches and due to diffused branching gave a moss/ coral-like appearance.

IV. Shape/appearance of the sporophores

1. Many long spines together formed sporophores like Lion's mane.

1. Several spines were closed to each other and formed many clusters very similar to cauliflower curd

1. The sporophores showed moss or coral-like appearance

V. Texture

1. Wet, fleshy texture when held on the palm

1. Wet, steamy, and thick texture when held on the palm.

1. Dry, rough, light texture when held on the palm

VI. Moisture content of the fruit body

1. Moisture content was high (90.34%) in the clumping type fruit body because it had a fleshy stem with long thick spines that hold high moisture content

1. The sporophores has less moisture content (89.1%) because of stemy nature and short spines.

1. Least moisture content (70.01%) was measured because the greater part of fruit was stemy; the only distal end of the branches was fleshy and having only minute hairs.

VII. Dry matter

Dry matter was low (6.25 to 9.66%)

Moderate dry matter (10.9%)

High dry matter (29.99%)

Table 2: Dry matter, moisture content, fragrance, and taste of sporophores of *Hericium erinaceus*

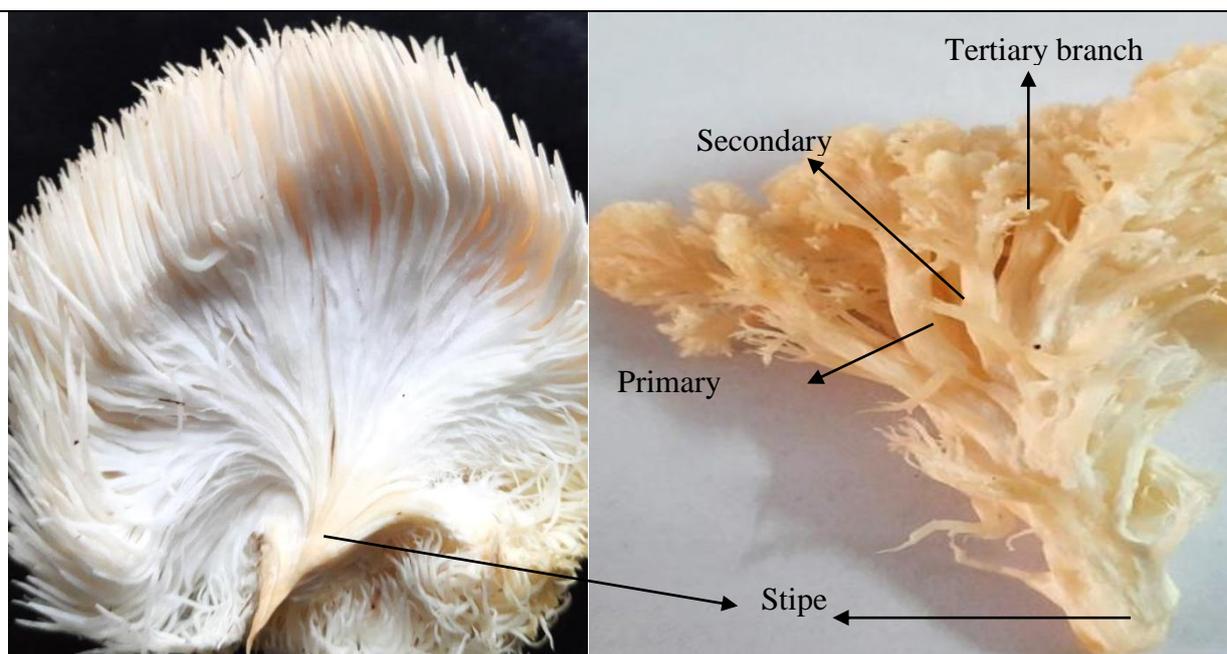
Genotype	Dry matter (%)	Moisture content (%)	Fragrance of dry powder	Taste after cooking
<i>He-101</i>	8.69 ^c	91.31 ^b	Vanilla type (Most pleasant)	The cooked sporophores of all genotypes were produced lobster-like taste except <i>He-102</i> and <i>105</i>
<i>He-102</i>	29.99 ^a	70.01 ^a	Unpleasant raw lichen type	
<i>He-104</i>	8.00 ^{cd}	92.00 ^{cd}	Vanilla type	
<i>He-105</i>	10.90 ^b	89.10 ^b	Vanilla type	
<i>He-107</i>	8.85 ^c	91.15 ^c	Mixed spicy vanilla type	
<i>He-108</i>	6.25 ^d	93.75 ^{cd}	Vanilla type	
<i>He-109</i>	9.66 ^{bc}	90.34 ^b	Vanilla type	
CD (0.05)	2.02	2.02		

Superscripts of mean values represent significant differences

Table 3: Size of sporophores, spines, basidia, and basidiospores of *Hericium erinaceus*

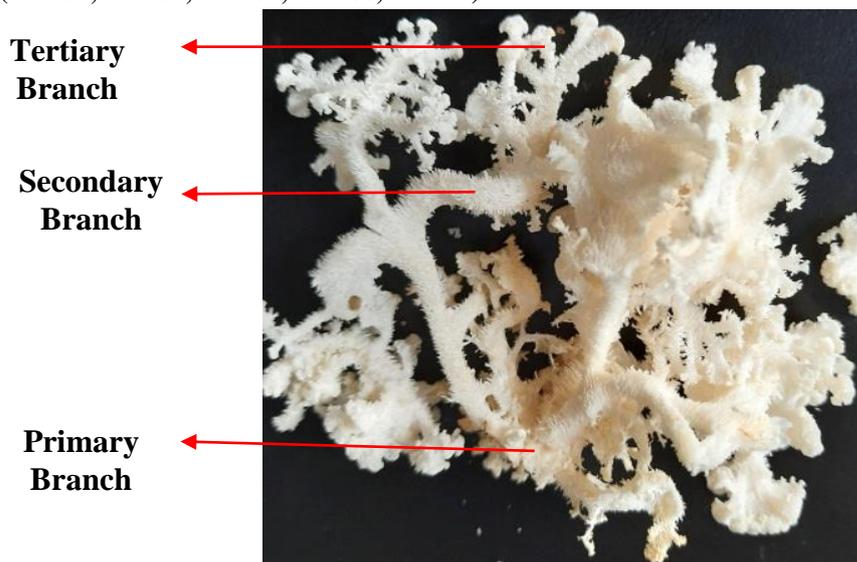
Genotype	Sporophore dimension (cm)		Spines Length (cm)	Basidia dimension (µm)		Basidiospore dimension (µm)	
	Long axis	Short axis		Length	Width	Long axis	Short axis
<i>He-101</i>	5.5	4.6	1.52 ^b	27	6.31	5.40	4.7
<i>He-102</i>	Diffuse branching		0.32 ^d	45	5.20	6.90	5.80
<i>He-104</i>	6.2	5.7	1.42 ^b	27.34	7.08	5.20	4.20
<i>He-105</i>	4.1	3.8	0.52 ^d	35.80	6.61	5.76	5.48
<i>He-107</i>	5.1	4.7	1.05 ^c	38.66	7.51	5.64	4.75
<i>He-108</i>	5.0	4.8	2.08 ^a	42.23	7.18	5.76	5.32
<i>He-109</i>	5.8	5.0	1.29 ^{bc}	31.83	6.59	5.63 ^b	4.89
CD at 5%	NS	NS	0.26	NS	NS	NS	NS

Superscripts of mean values represent significant differences



Clumping type sporophore
(*He-101, He-104, He-107, He-108, He-109*)

Thick branching type sporophore (*He-105*)



Diffuse branching type sporophores (Formed in *He-102*)

Fig. 2: Types of sporophores of *Hericium erinaceus*

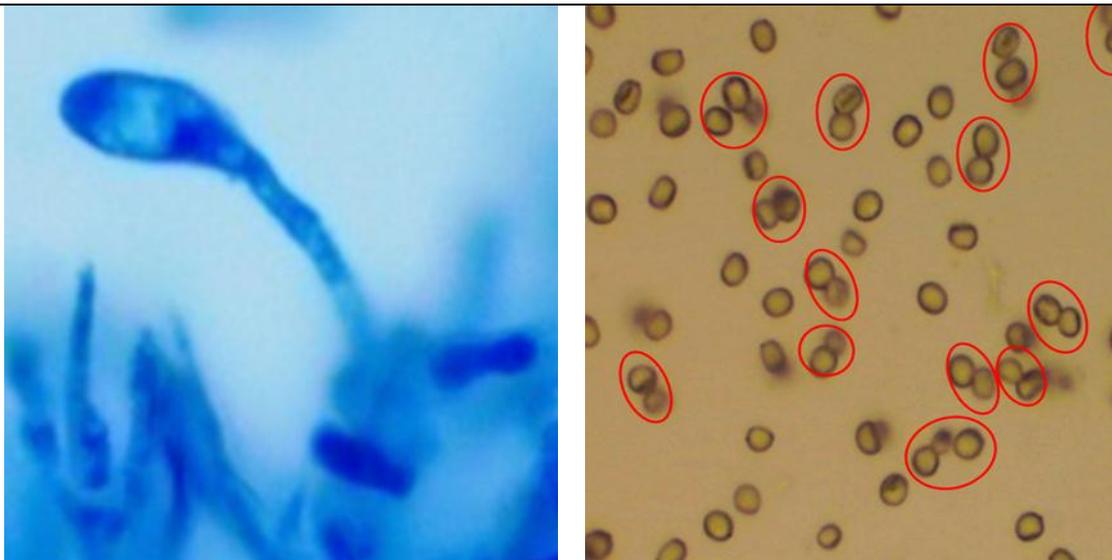


Fig. 3: Basidia of *He-102* (1) and basidiospores of *He-108* (2)

CONCLUSION

Among all genotypes of *Hericium erinaceus*, the dimension of each of sporophores, basidia, basidiospores were found identical. However, the length of spines of all genotypes was varied from 0.32 to 1.52 cm. The variation in the length of spines and branching of stipes was collectively responsible to develop different shapes and textures of the sporophores. Thus, these sporophores were grouped into clumping (single and multiple clumping type) and branching type (thick and diffuse branching). The dry matter of the sporophores was varied from 6.25 to 9.66% except for *He-102* in which a maximum of 29.99% dry matter was calculated. The fragrance of the sporophores was also studied and found that the dry powder of *He-101*, *He-104*, *He-105*, *He-108*, *He-109* were sensed vanilla type fragrance. However, *He-102* produced an unpleasant raw lichen fragrance and *He-107* was known for a mixed spicy vanilla fragrance. The taste of butter cooked sporophores of all genotypes was felt like cooked lobster except *He-102* and *He-105*. The white coloured spore prints with different pattern and thickness were noticed except the spore print of *He-102*.

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