

UDC 615.322

<https://www.doi.org/10.34907/JPQAI.2020.46.81.003>

COMPARATIVE MORPHOLOGICAL AND ANATOMICAL ANALYSIS OF CHAGA AND TINDER FUNGUS AND FALSE T.F.

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A comparative morphological and anatomical study of the chaga and the tinder fungus and the false tinder fungus was conducted. Diagnostic distinctive morphological and anatomical features are established. Identified signs form the basis of the sections "External signs" and "Microscopy" of the Russian Federation State Pharmacopoeia, the XIV edition of FS.2.5.0103.18 "Chaga – Inonotus obliquus".

Keywords: chaga, tinder fungus, false tinder fungus, morphology, anatomy, macroscopic analysis, microscopic analysis

Chaga (birch fungus, *Inonotus obliquus*, timber fungus oblique) – *Inonotus obliquus* (Pers.) Pil., Hymenochaetaceae family – perennial sterile growths up to 40–50 cm in diameter that occur on living trees (usually birch), formed by a parasitic fungus [3,8]. In medical practice, birch fungus is used as a process aid in the comprehensive treatment of chronic gastritis, gastrointestinal dyskinesia of the hypokinetic type, as well as a non-specific (symptomatic) agent for oncological diseases that improves the general condition of cancer patients [5,7]. In appearance, the true tinder fungus – *Fomes fomentarius* (L.: Fr.) Fr. – Poraceae family and false tinder fungus – *Phellinus igniarius* (L.: Fr.)

Quel., Hymenochaetaceae family are similar to the chaga, impurities to chaga and can be harvested instead of chaga. [3]. In this regard, it is of interest to conduct a comparative study of chaga and tinder fungus by morphological and anatomical features in order to identify distinctive macroscopic and microscopic diagnostic signs.

Study purpose – study of the morphological and anatomical structure of chaga and impurities (true and false tinder fungi) and identification of distinctive diagnostic signs.

MATERIALS AND METHODS

The study material was samples of chaga, true tinder fungus and false tinder fungus, harvested in the Perm region in the autumn-winter period of 2016–2017. Macroscopic and microscopic analysis was performed according to the generally accepted methods of the State Pharmacopoeia, XIV edition [4].

Chaga pieces are very dense, so to prepare slices, the raw material was pre-soaked in a mixture of "96% ethyl alcohol – 1 part: glycerine – 1 part" for 3–7 days, after which longitudinal and cross sections were made by 30 microslides from each sample.

To prepare powder specimens, pieces of raw material with a diameter of 1–2 mm were boiled in a 5% sodium hydroxide solution, then washed with water and placed on a slide in a drop of chloral hydrate solution.

The study of anatomical features was performed using a Biomed-6 microscope, magnification 640x, 400x, 160x. Microphotographs were performed using a DCM 510 digital camera in the Scope Photo program. Cell sizes were measured using a Motic microscope in the Motic Educator program. The results were processed in Microsoft Excel.

RESULTS AND DISCUSSION

Chaga and tinder fungus are parasitic fungi that infect various types of trees, and are capable of long-term existence.

Chaga is a stenotrophic plant, shows a high degree of specialization in relation to birch wood [1,2,9], is less common on alder, rowan-tree, beech and some other hardwoods, develops on living trees [2,10].

Tinder fungus is a poorly specialized eurythroff that inhabits coniferous (fir) or deciduous (oak, maple, linden, birch, alder, poplar, aspen, willow) trees [6,9], and develops on dead trunks, stumps, and trees [3,8].

According to the literature data, the false tinder fungus does not have a strict substrate specialization [9]. In Europe, the preferred substrate for *Phellinus igniarius* is birch wood, the species was observed somewhat less frequently on alder and willow [6, 9]. However, sometimes along with birch, hornbeam, willow, and maple are considered as preferred substrates in the Urals, while aspen, willow, and rowan-trees are considered in Western Siberia [2]. False tinder fungus develops on live trees, fallen trunks, stumps [2,8].

Thus, along with the chaga both the true tinder fungus and false tinder fungus can be seen on the birch.

Inonotus obliquus (chaga) has an annual fruit body, widely spread, developing under the bark, reaching 3–4 meters long along the trunk and 10–50 cm wide (Fig. 1a), depending on the thickness of the affected trunk. In the fresh state, the fruit body is soft-skinned, later fibrous and cracking, in the dry state, it is hard and brittle, easily separated by parts from the substrate. The fruit body of the chaga under the bark is pale brown at first, as it matures, it destroys the bark of the tree and becomes dark brown, gradually drying out.

The development of the fruit body on the trunk of a living tree is usually preceded by the formation of sterile growths (forma sterilis (Van.) Nikol), called "chaga" (Fig. 1b). The size of the growths, depending on their age, can range from the size of a walnut to 40–50 cm in diameter. Chaga has a nodulous shape, very hard, woody body. The outer surface is uneven, cracking, black, and hard. The inner tissue is rusty-brown with white spots, somewhat softer than the outer surface. The weight of the nodule can range from a few grams to ten kilograms [2,6].

Fruit bodies of tinder fungus are perennial, sessile, of characteristic hoof-shaped. The fruit body is attached to the tree trunk only by its upper central part. Stipe is missing. The fruit body is large, in old fungi – up to 40 cm wide and up to 20 cm high, covered with a hard crust. The crust is matt, uneven, wavy, with concentric rollers, darker in the depressions, at first gently velvety-nappy, then naked, almost smooth. Small cracks may occur on the surface. The color of the pileus varies from light gray to dark gray in old fungi, sometimes light beige tones. The flesh is dense, soft, resembles a cork, occasionally woody. The cut is velvety-suede. Color is brown, rich reddish-brown, less often a nutty shade. The hymenophore is tubular with fairly large rounded pores, light in color, in which basidiospores are formed. When pressed, it darkens. The pores are rounded with entire pubescent edges 3–4 per 1 mm [3,6,8].

Fruit bodies of false tinder fungi are perennial (they can grow for decades), sessile, rounded



FIG. 1. *Inonotus obliquus*: a – fruit body beneath the bark of birch, b – asexual form

when young, then they get a characteristic hoof-like appearance, sometimes they are pillow-shaped or flat, prostrate. The fruit body is very firmly attached to the trunk of the affected tree, by this way false tinder fungus differs from the true tinder fungus. Stipe is missing. In fruit bodies, cracking is quite common, and the presence of radial cracks is characteristic. The tissue of the fruit body is very hard, woody, reddish-brown, rust-colored or chestnut-brown. The fruit body is medium-sized, reaches 20–26 cm wide in old fungus, covered with a hard crust. The crust is matt, uneven, with concentric rollers. The color of the pileus varies from dark gray to almost black or brownish-black in old fungi. The outer (growing) roller sometimes has a more noticeable brownish tint. The hymenophore is tubular, inside the same color as the tissue. Every year a new layer of hymenophore grows, and the old layers eventually overgrown with white hypha. Outside, the surface of the hymenophore is rusty brown to rich chestnut color. The pores are all-edged,

rounded, often with grayish pubescence along the edge, 2–6 per 1 mm [2,6,8].

Based on the literature data and morphological characteristics of the raw materials we have harvested, we have compiled a comparative table of the distinctive characteristics of chaga and tinder fungi species (see table).

According to morphological characteristics, the chaga differs from the true and false tinder fungi by the absence of tubular hymenophore and the shape of the fungus. The nature of the outer surface and color of the chaga and true and false tinder fungi are close.

According to the generally accepted structure of the pharmacopoeial monograph, microscopic analysis is mandatory when determining the identity of raw materials, as well as performing microphotographs.

When studying the anatomical structure of the cross and longitudinal section of the chaga, we considered the outer, middle and inner layers, which differ in consistency and

DISTINCTIVE MORPHOLOGICAL FEATURES OF CHAGA AND TRUE AND FALSE TINDER FUNGI

Feature	Chaga	True tinder fungus	False tinder fungus
Form	nodulous	hoof-shaped	hoof-shaped
Outer surface color	Black	from light gray to dark gray, sometimes light beige tones	from dark grey to almost black or brownish black
Outer surface character	uneven, dehiscing	uneven, wavy, with concentric rollers, small cracks may take place	uneven, with concentric rollers, cracking in the form of radial cracks often takes place
Hymenophore	not available	hymenophore tubular, light color	hymenophore tubular, from rusty brown to rich chestnut color

color. In the outer layer, a dense interweaving of hyphae is found, in the middle and in the inner layer, the hyphae are located more loosely (Fig. 2). There is no tubular hymenophore. The inner layer may contain holes of different diameters in the range from 73 to 240 microns, located randomly (Fig. 3).

In contrast to the chaga, the true and false tinder fungi have a tubular hymenophore, which on the longitudinal section has the form of longitudinal strands consisting of a dense intertwining of hyphae (Fig. 4). On the cross section of the hymenophore, there are ordered rounded pores of size from 128 to 148 microns (Fig.5). In the raw material harvested in the autumn period, basidia with basidiospores can be found in the hymenophore (Fig. 6).

When studying the chaga powder specimens, a branched cell mycelium is visible along the edges and on the surface of the pieces, spores are absent (Fig. 7). In the specimens of true tinder fungus and false tinder fungus, there are hyphae often with spores (Fig. 8).

Since the powder specimens have a particle size of 1–2 mm, it is not possible to detect the presence of hymenophore, so you should pay

attention to the mycelium and the presence or absence of spores.

Thus, it is established that the common anatomical feature of chaga and true and false tinder fungi is the presence of branched cell mycelium. A distinctive feature of chaga is the absence of tubular hymenophore and spores, which are found in tinder fungus specimens.

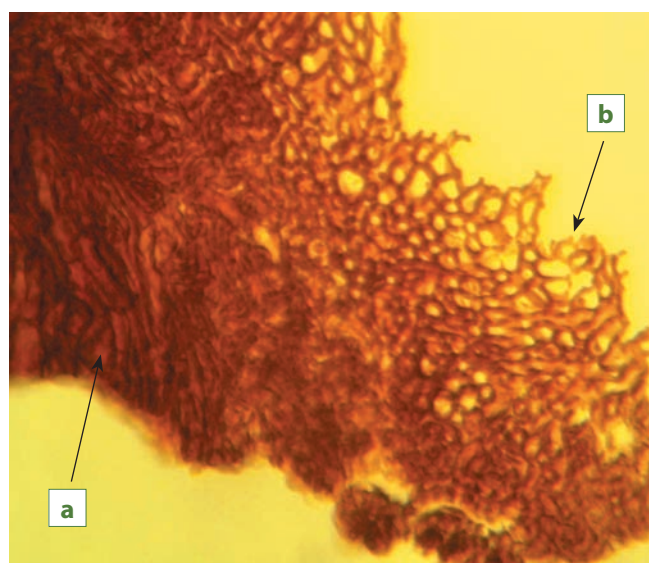


FIG. 2. A fragment of a cross-section of the fungus (microphotography): a – outer layer (dense interweaving of hyphae); b – inner layer (hyphae are located loosely) (640x)

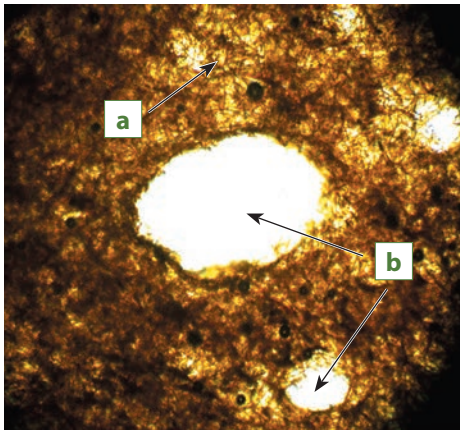


FIG. 3. A fragment of a cross-section of the inner layer of chaga (microphotography): a - mycelium; b - holes (640x)

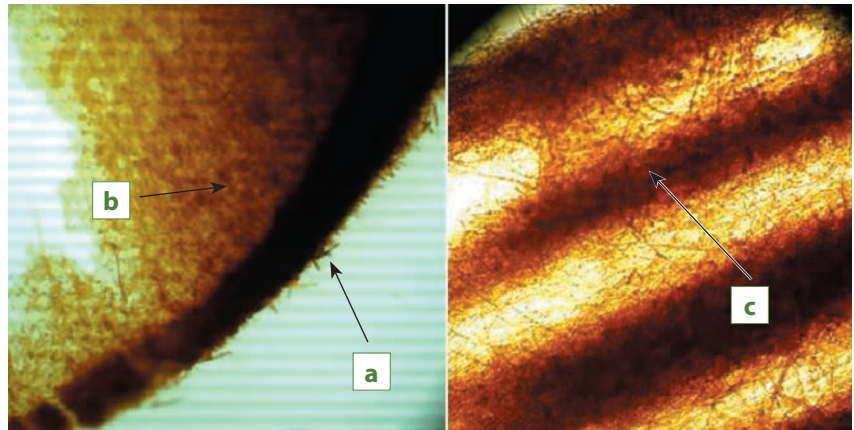


FIG. 4. A fragment of a longitudinal section of tinder fungus (microphotography): a – an outer layer (dense interweaving of hyphae), b – a middle layer (hyphae are located loosely), c – a tubular hymenophore (160x)

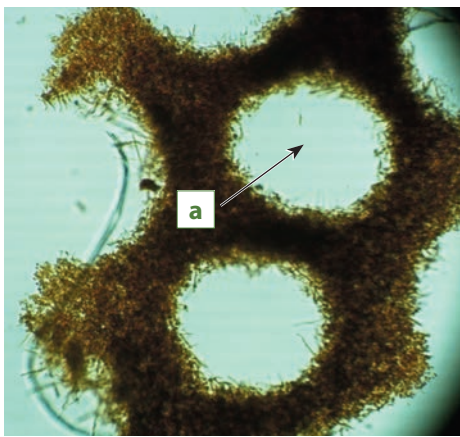


FIG. 5. A fragment of a tinder fungus cross-section (microphotography): a – pores of the tubular hymenophore (160x)

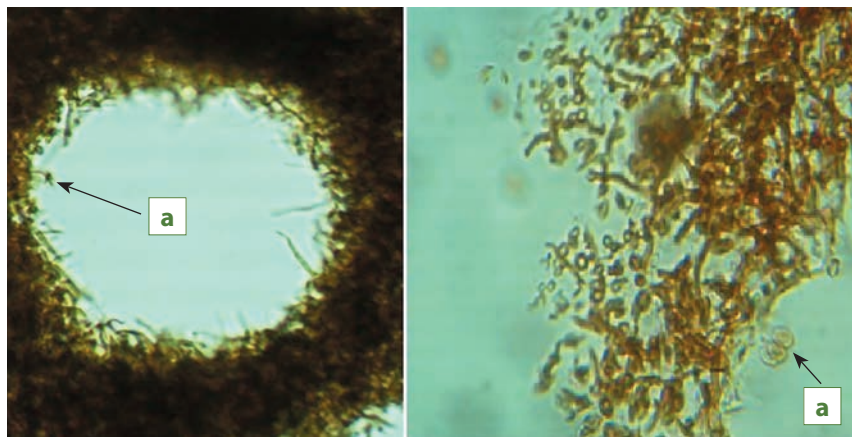


FIG. 6. A fragment of a tinder fungus cross-section (microphotography): a – basidia with basidiospores (400x, 800x)



FIG. 7. A fragment of the fungus powder (microphotography): a – branched cell mycelium (800x)

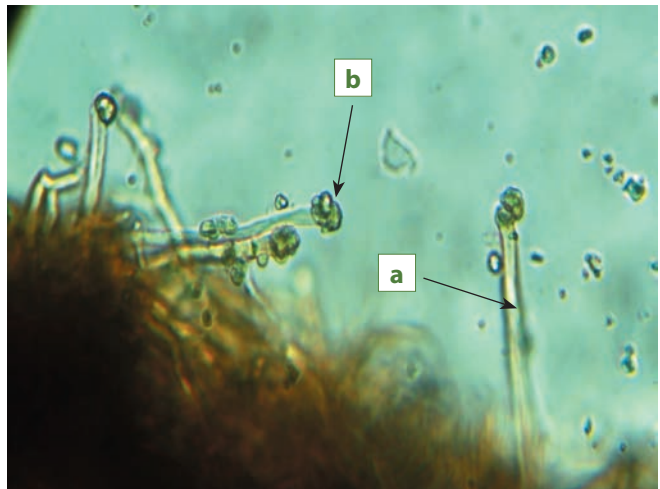


FIG. 8. Fragment of tinder fungus powder (microphotography): a – hyphae, b – spores (640x; 160x)

CONCLUSION

1. Comparative morphological and anatomical study of chaga and true and false tinder fungi was carried out.

2. Morphological and anatomical diagnostic signs have been established to distinguish chaga from true and false tinder fungi

3. The study results are included in the State Pharmacopoeia, XIV edition of FS. 2. 5. 0103. 18 "Chaga – *Inonotus obliquus*", section "External features" and "Microscopy".

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