

Morphological and anatomical study of seeds of Chernushka sowing (*Nigella sativa* L.) and *Nigella damascena* L.

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Morphoanatomic study seeds *Nigella sativa* L. and *Nigella damascena* L.

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SUMMARY

*Nigella damascena* L. (*Damascus nigella*) and *N. sativa* L. (including sowing) belong to the Ranunculaceae (Buttercup) family and are used in world practice as food and medicinal plants.

Morphological and microscopic studies of whole and crushed seeds of two types of nigella were carried out, allowing to establish the authenticity of raw materials in order to further include the data obtained in the section "Microscopy" of the draft pharmacopoeial monograph.

Key words: sowing nigella, damask nigella, seeds, microscopy, authenticity, diagnostic signs.

RESUME

*Nigella damascena* L. and *N. sativa* L. belong to the family Ranunculaceae is found in the world to use as food and medicinal plants.

Morphological and microscopic study of whole and crushed seeds of two types of *Nigella* allows authenticate materials to further integration of the data in the section "Identification" project pharmacopoeia article.

Keywords: *Nigella damascena*, *Nigella sativa*, seeds, microscopy, authenticity, diagnostic features.

INTRODUCTION

*Nigella damascena* L. (*Damascus nigella*) and *N. sativa* L. (sowing) belong to the Ranunculaceae (Buttercup) family. The biological characteristic feature of the black nigella, which distinguishes it from other types of nigella, are the fruits - leaflets, which, when ripe, do not crack on their own, but only under mechanical action. When grown commercially, it is a very valuable quality that significantly reduces seed yield losses.

In Russia, the official raw material is only the seeds of chernushka damascus (VFS 42-1691-87), for the production of the enzyme preparation "Nigedaza". However, the pharmacological activity of the seeds of chernushka damaska and sowing is due not only to the presence of lipolytic enzymes, but also to other groups of biologically active compounds, in particular, fatty oil,

a component of essential oil - thymoquinone, carbohydrates, which makes it possible to use the considered plants for the treatment of nosological forms of diseases, including socially significant ones, such as malignant neoplasms, diabetes mellitus, infectious diseases, HIV [1].

Therefore, it is urgent to develop a draft FS "Chernushki seeds" for the State Fund of the XII edition in accordance with modern requirements. For this, it is necessary to develop characteristics of the authenticity of raw materials. The data available in the scientific literature on this issue, in particular the structure of the seeds of the damask damask, are fragmentary, sometimes mutually exclusive [2, 3, 4], in this regard, they require clarification, and there is no characteristic of the authenticity of the seeds of the nigella sowing.

The aim of the work was to conduct morphological and anatomical studies samples of seeds of two types of nigella, collected from cultivated plants in various regions of the country and abroad, to establish diagnostic signs.

**MATERIALS AND RESEARCH METHOD** The material of the study was the following samples: seeds of black cottages - *Semina Nigellae sativae* (country of growth: Russia (Stavropol Territory), Ukraine, Uzbekistan, India, Turkey, Morocco in the period from 2004 to 2010); damask black seeds - *Semina Nigellae damascenae* (Ukraine, Russia (Stavropol Territory), Morocco in the period from 2006 to 2010).

To determine the external signs, the raw materials were examined with the naked eye and using a magnifying glass (10x) or a stereomicroscope (8x, 16x) in accordance with the section "Methods of analysis of medicinal plant materials, article" Seeds "[5].

Microscopic analysis was performed on fresh, fixed (a mixture of alcohol and glycerol) and dried plant material [6].

Preparations for microscopic examination were prepared in accordance with the articles of the State Fund of the XI ed. [5] and studied using a Biolam microscope. Micrographs were obtained using a Motic DM-111 microscope with a built-in digital camera at magnifications of 40-, 100-, 400-, 1000- with permission 640x480 pixels. The photographs were processed on a computer using the Adobe Photoshop CS and CorelDRAW X3 software.

For a complete specification investigated objects used histochemical reactions with Sudan III reagent. Drops of fatty and essential oil were colored orange-yellow. To distinguish essential oils from fatty ones, temporary preparations were stained with an aqueous solution of methylene blue. After a few minutes, they were examined in water or glycerin. The essential oil turned blue.

During micro-sublimation of the powder, the formation of a red plaque on the walls of the test tube was observed, which acquired a blue coloration (quinones) upon the addition of 5% sodium hydroxide solution.

## RESULTS AND DISCUSSION

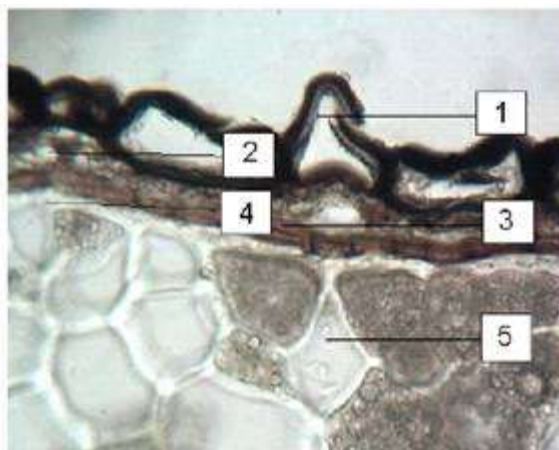
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As a result of the research, it was found that whole seeds of nigella are ovoid, flattened, three or less often tetrahedral, pointed on one side. The surface of the seeds is bare, slightly transversely wrinkled, granular, matte between the edges. The seed scar is poorly visible. The seed coat is hard, tightly fitting to the embryo, the endosperm is poorly developed, the embryo consists of two cotyledons and occupies almost the entire seed. Length - 2–3 mm, width up to 1 mm, thickness in the middle part 1.5–2 mm. The color is black, the smell is peculiar, the taste of the seed peeled from the woody part of the peel is slimy, the taste of the water extract is spicy and pungent. The weight of 100 seeds is  $0.26 \pm 0.02$  g.

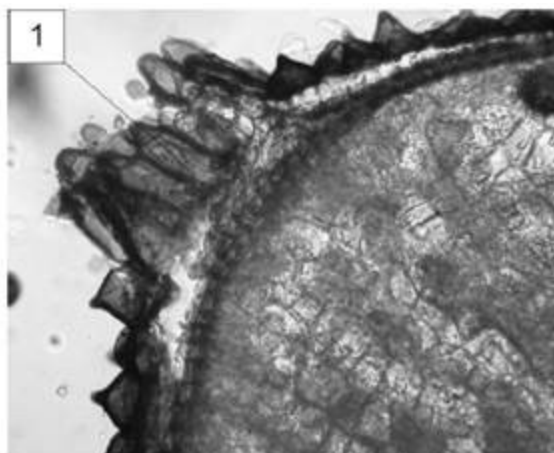
The study of the powder revealed that it is a black mixture passing through a sieve with holes of 0.5 mm in diameter, with a strong, aromatic odor and a bitter-spicy taste.

It should be noted that the aforementioned morphological characters are characteristic of both types of nigella seeds.

Microdiagnostic studies of whole raw materials showed that a three-layer seed coat in the form of a dark brown strip, endosperm and an embryo are visible on a cross section of seeds. At high magnification, the layers of the seed coat are distinguished (Fig. 1). The first layer is a single layer of epidermis. The epidermis consists of large, tightly closed, radially elongated cells with uniformly thickened walls. Epidermal cells are weakly sinuous, rectangular cells alternate with triangular cells. In the places where the wrinkles are located, the epidermal cells have a conical shape to the apex drawn into the papilla (Fig. 2).



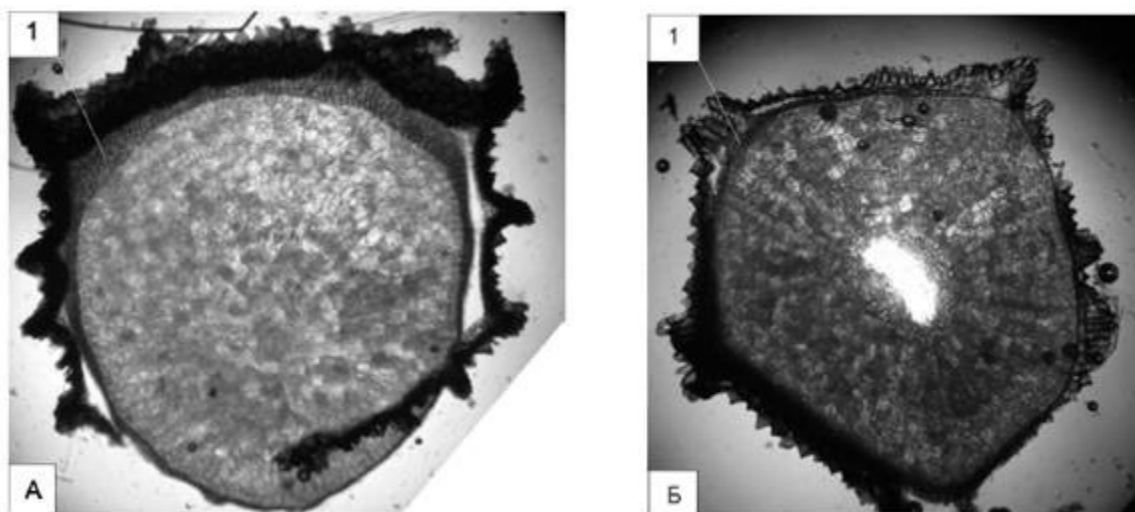
Rice. 1. Micrograph of a fragment of a cross section of a nigella seed (enlarged. x250): 1 - epidermis, 2 - structureless layer, 3 - pigment layer, 4 - inner epidermis of the seed coat, 5 - parenchyma cells.



Rice. 2. Micrograph of a fragment of a cross-section of the seed coat in places location of wrinkles (magnification x100): 1 - cells of the epidermis of a conical shape.

The second layer - structureless, located under the epidermis, consists of thin-walled parenchymal colorless palisade-like collapsed cells.

The third layer - pigment, consists of tangently elongated cells with irregular thickenings and containing brown pigment. The layer thickness is 2-4 rows along the perimeter of the seeds, in the area of wrinkles it expands to 6 rows (Fig. 3).



Rice. 3. Micrograph of a cross section of a seed (enlarged x40): A - blackies damask, B - seed nigella, 1 - pigment layer

In young seeds, there is a well-defined endosperm between the seed coat and the embryo. In the process of seed development, the endosperm decreases and is already presented in the form of a single layer of small tangently elongated cells.

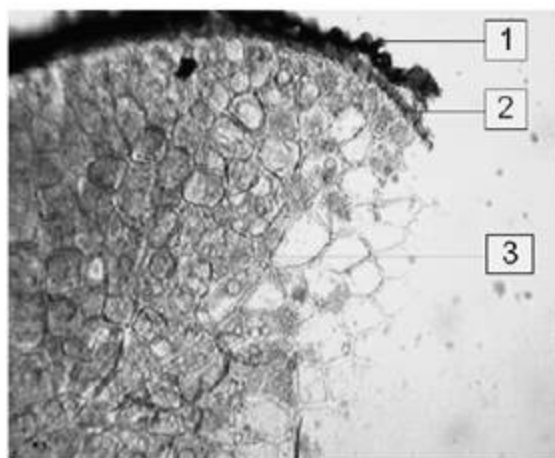
Endosperm cells are polygonal, thin-walled, contain aleurone grains and drops of fatty and essential oils (Fig. 4). Reaction with Sudan III solution

positive, negative with Lugol's solution.

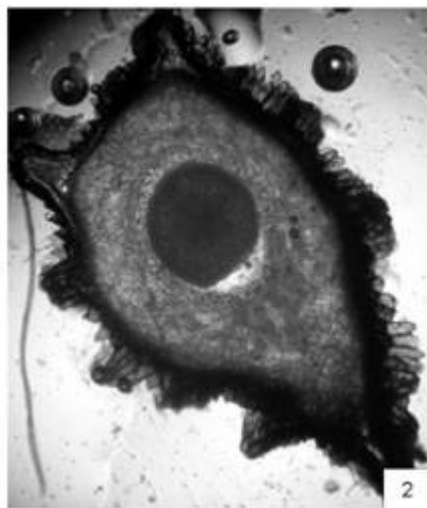
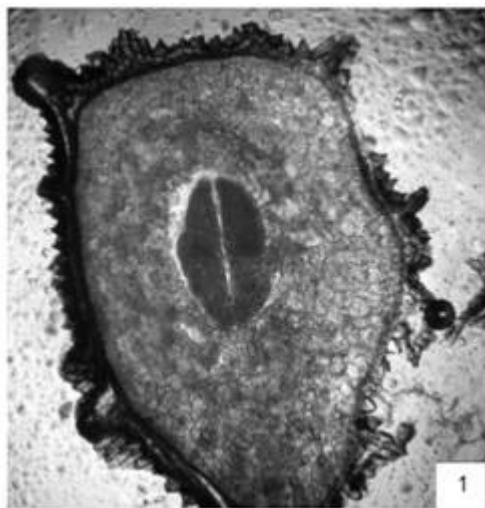
The embryo consists of two cotyledons with a root (Fig. 5).

In a comparative aspect, no microscopic differences were found between the seeds of chernushka damaska and sowing. The data obtained are of practical interest, since they make it possible to judge the authenticity of the raw materials.

In the course of research work, it was revealed that the diagnosis of raw materials does not cause difficulties in fractions of 0.5-1.0 and 0.31-0.5 mm. With an increase in the size of the raw material in the powder, there are more diagnostically insignificant particles (fragments of the seed coat in the longitudinal and cross sections, small groups and individual parenchymal cells). Therefore, diagnostics in smaller fractions is difficult.



Rice. 4. Micrograph of a fragment of a transverse section of a seed (enlarged x100): 1 - epidermis, 2 - pigment layer, 3 - parenchymal cells, stained with reagent Sudan III



Rice. 5. Micrographs of a cross-section of seeds through the embryo (enlarged x40): 1 - c region of the kidney, 2 - in the region of the root.

When examining the preparations of the powder under a microscope, one can see: scraps of seed coat, the epidermis of which consists of thin-walled polygonal cells, light brown cells of the pigment layer, embryo tissue with drops of fatty oil.

#### CONCLUSIONS

1. Diagnostic signs of seeds of Chernushka sowing and Damascus are the three-layer structure of the seed coat, the structure of the epidermis, the pigment layer, as well as endosperm cells with fatty, essential oil and aleurone grains.

2. Revealed morphological and anatomical signs of raw materials "Chernushki seeds" allow to confirm its authenticity regardless of the region of procurement and are included in the sections of the pharmacopoeial article "Chernushki seeds" ("External signs", "Microscopy") for the SP of the XII edition.

3. As a result of the research, microscopic diagnostic traits of seeds of chernushka damascus and for the first time identified for seeds of chernushka sowing.

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