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EVALUATION OF BASIL SEED (*OCIMUM BASILICUM* L.)

HODNOTENIE SEMIEN BAZALKY PRAVEJ (*OCIMUM BASILICUM* L.)

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Ten basil (*Ocimum basilicum* L., family *Lamiaceae*) seed populations from Serbia were evaluated in this paper. The following parameters were analyzed: germination energy, total germination, mass of 1 000 seeds (absolute seed weight), and content of fatty oil in the seed and plumpness of seed (length, width and thickness). Furthermore, mycobiota was identified on the seed. All examined populations were characterized by high average values for seed germination. The highest average seed germination was determined in the population T-7 and amounts to 96.6%. The greatest mass of 1 000 seeds was determined in population T-7 and it was 1.71 g. The seed of examined populations is characterized by high content of fatty oil. The greatest content was recorded in population T-2 and it was 25.29%. The analysis of seed dimensions established significant differences between average values for length, width and thickness, i.e. plumpness of seeds. Population T-7 was characterized by largest seeds. Five fungi genders were isolated and identified on the seed. Species from Alternaria gender were identified in the seed samples of all examined basis populations. Species from *Penicillium* gender were isolated from the sample of seeds of eight populations. *Aspergillus* spp. was identified in the seed sample of two populations. Fungi *Trichoderma* spp., *F. oxysporum* and *F. verticillioides* were identified in the seed sample of one basil population.

Key words: seed, basil, germination, fatty oil, mycobiota

Basil (*Ocimum basilicum* L., family *Lamiaceae*) has been grown traditionally in Serbia as a decorative, medicinal, seasoning and ritual herb. With Orthodox people it has a religious and ritual meaning, and in India and Pakistan basil is a herb dedicated to Goddess Tulsa (Nazim et al., 2009). Gender *Ocimum* comprises a large number of species (60 – 150), which differ mutually according to their general morphological characteristics, contents and chemical composition of essential oil. Difficulties in the classification of *Ocimum* species are the polymorphic character of the plant and cross-breading within the species, which resulted in a large number of sub-species, varieties and types. Basil was brought to Serbia in the 12th century and since then it has been adjusted to the existing agroecological conditions and differentiated into a large number of types and populations (Jelačić, et al., 2011).

Top herbaceous end of the blossoming plant *Basilici herba* and essential oil *Basilici aetheroleum* are used for medicinal purposes, as seasoning and food preservative, as a raw material in food, pharmaceutical and perfume industry.

Secondary metabolites from *Ocimum species* possess an exceptional biological activity and have: bactericide, fungicide, repellent, antiinflammatory, antioxidative, antidiarrheic, chemopreventive and radioprotective effect (Opalchenova and Obreshova, 2003; Lukmanul Hakkim et. al., 2008; Gajula et al., 2009; Runyoro et. al., 2010).

Basil seed has been used in traditional medicine for the treatment of collics, ulcus, dyspepsia, diarrhea and other illnesses. In Iran and other parts of Asia basil seed is used to prepare beverages (Sharbat) and ice desserts (Faloodeh). Seed has also been used as a raw material for the preparation of various products used in cosmetics and represents a major source of dietary fibres. It is used in cosmetics, perfume and food industry. Basil seed is black in colour and oval in shape (Hosseini-Parvar at al., 2010).

Angers at al. (1996) have studied the content and quality of fatty oil of different *Ocimum* species seeds. The use of basil seed fatty oil as a raw material in cosmetic and perfume industry has been studied by Domokos and Perédi (1993). Razavi at al. (2010) studied some physical and mechanical characteristics of basil seed. Soltani et al. (2006) and Padurariu at al. (2010) studied the effect of magnetic field and various concentrations of heavy metals on the basil seed germination.

Basil seed mycobiota was studied by Reis at al. (2007) and Kruppa and Russomanno (2008).

Bearing in mind the research conducted so far, there is a great interest to perform evaluation of basil seed of the populations grown in Serbia. Basil seed evaluation includes determination of individual parameters of seed quality, content of fatty oil in the seed and examination of pathogens (mycobiota) on the seed.

Material and methods

Seeds from ten basil populations collected on the territory of the Republic of Serbia and representing a part of multidisciplinary studies conducted in the period from 1996 to 2010 have been used as a material for this research. Taxonomic qualification of the collected populations was determined according to Ivanovaís key. The selected populations have been marked under codes from T-1 to T-10 and deposited in the Serbian Plant Genes Bank and at the Institute for Crop Farming and Vegetable Growing of the Faculty of Agriculture in Belgrade.

Basil seed produced in 2008 was used for this research. Basil crop was established by direct seed sowing during the month of May. After germination, the plants were thinned out to final distance, which was 50 cm between the lines and 25 cm within the line. During vegetation, the following measures of crop tending were applied: digging up, elimination of atypical plants, covering with earth and watering. Seed harvest was performed in September.

Examining basil seed germination was performed by standard laboratory method on a filter paper moistened with 0.2% solution of KNO_3. The seed was incubated for 14 days at the temperature of 20 – 30 °C and relative humidity of 95%. On the seventh day of incubation germination energy (GE) was evaluated, and on the fourteenth day of total germination (TG), i.e. the number of typical young plants (ISTA, 2009). Examining germination was performed on 100 seeds from each population in five repetitions (5 × 100).

Mass of 1 000 seeds was determined by measuring on a technical scale in 10 repetitions.

Plumpness of seeds (length, width and thickness) was determined by measuring on a digital nonius (Digital caliper, model: DC-5159) in 30 repetitions. Determining the content of fatty oil in the seed was performed by standard laboratory method on a device according to Soxhlet in three repetitions (Ph.Yug.IV).The content of oil in the seed was calculated based on the formula:

$$\% \textit{ fatty oil} = (a - b) \times 100/Mq$$

where:

a – weight of dish with fatty oil (g)
b – weight of empty dish (g)
Mq – measured quantity of sample (g)

Isolation and identification of fungi on basil seed were performed in the following way:

– 100 seeds from each basil sample were rinsed for one hour under the jet of tap water and then sterilized in 1% solution of sodium hypochlorite, three times rinsed with distilled water and dried up between two layers of soft paper. Eight seeds were distributed into Petri boxes with potato dextrose agar (PDA), kept for seven days under laboratory conditions. After the incubation period, seed samples were examined under minor magnification of stereomicroscope.

For the purposes of reliable identification of certain species of fungi, fragments of mycelia developed around the sample of seeds were transferred in sterile conditions to PDA and agar with sterile fragments of carnation leaf (CLA). The resown cultures on PDA were incubated in dark at 25°C, while they were incubated on CLA while changing light (combined fluorescent and NUV light) and dark at 12 hour intervals. PDA and CLA agars were prepared according to methods described by Burgess et al. (1994).

The intensity of occurrence (I) of certain species of fungi on the basil seed was calculated using the following equation:

$$I = \textit{number of seeds on which fungus was established} \times 100 / \textit{total number of seeds}$$

Analysis of experimental results was conducted by means of descriptive and analytical statistics with statistical package STATISTICA. Of the central tendency indices arithmetical mean. Data variability was quantified through standard deviation. Testing the differences between the treatments was conducted by method analysis of variance (ANOVA), LSD-test,

Results and discussion

The main index of the seed vitality, from which its useful value largely depends, is its germination.

The energy of seed germination of the examined basil populations was determined by counting seedlings on the seventh day (Table 1). Average values of germination energy ranged from 78.6% (T-1) to 93.6% (T-6). On the fourteenth day total germination was calculated and it ranged from 83.6% (T-1) to 96.6% (T-7). The differences in the value of total germination between the examined basil populations were around the interval of 13%. Padurariu et al. (2010) examined the seed germination of different basil varieties, and the obtained values ranged from 66.7% to 96.7%.

Table 1 Germination energy and total germination of seeds basil populations

Basil populations (1)	Germination energy in % (2)	Total germination in % (3)
T-1	78.6 ±2.80	83.6 ±1.67
T-2	85.6 ±2.29	91.2 ±0.84
T-3	80.2 ±2.17	89.8 ±1.48
T-4	86.8 ±2.09	91.8 ±1.59
T-5	81.8 ±2.49	87.0 ±1.58
T-6	93.6 ±1.52	94.8 ±1.10
T-7	91.6 ±1.52	96.6 ±1.82
T-8	83.0 ±2.08	85.0 ±1.87
T-9	86.6 ±1.14	90.2 ±1.10
T-10	90.0 ±2.74	94.6 ±1.51
LSD 0.05 LSD 0.01	3.55 4.75	2.23 2.98

Tabuľka 1 Energia klíčivosti a celková klíčivosť semien populácií bazalky
(1) populácia bazalky, (2) energia klíčivosti, (3) celková klíčivosť

According to Habán et al. (2007) and Kišgeci at al. (2010) the mass of 1 000 basil seeds was 1.2 to 1.8 g. In our studies the lowest average value of the mass of 1 000 seeds was obtained in the population T-8 and it was 1.12 g, whereas the highest average value of 1.71 g was obtained in the population T-7 (Table 2). In the studies of Razavi, (2010) the average value of mass of 1 000 seeds was 2.13 g.

Table 2 Mass of 1 000 seeds and content of fatty oil

Basil populations (1)	Mass of 1000 seeds in g (2)	Content of fatty oil in % (3)
T-1	1.22 ±0.03	20.77 ±0.10
T-2	1.32 ±0.07	25.29 ±0.06
T-3	1.51 ±0.06	19.71 ±0.07
T-4	1.52 ±0.04	21.22 ±0.10
T-5	1.44 ±0.04	20.49 ±0.07
T-6	1.43 ±0.07	24.24 ±0.19
T-7	1.71 ±0.04	15.82 ±0.10
T-8	1.12 ±0.03	12.91 ±0.07
T-9	1.25 ±0.02	12.48 ±0.12
T-10	1.29 ±0.03	16.85 ±0.11
LSD 0.05 LSD 0.01	0.07 0.10	0.18 0.25

Tabuľka 2 Hmotnosť tisíc semien a obsah oleja
(1) populácie bazalky, (2) hmotnosť tisíc semien, (3) obsah oleja

The content of fatty oil in the seed of examined populations ranged (Table 2) from 12.48% (T-9) to 25.29% (T-2). Angers et al. (1996) examined the content of fatty oil in different species of *Ocimum* gender and they established that the content of oil was the lowest in the species *Ocimum canum* and it was 18%, and the highest percentage, 26%, was recorded in the specie *Ocimum basilicum.*

By examining plumpness, i.e. dimensions of seeds the following results have been obtained (Table 3). The length of seed ranged from 2.31 mm (T-8) to 2.64 mm (T-7). The values of the width of seed ranged from 1.30 mm (T-8) to 1.54 mm (T-7). The thickness of seed ranged from 0.99 mm (T-8) to 1.14 mm (T-7).

Table 3 Dimensions of seeds basil populations

Basil populations (1)	Length (2)	Width (3)	Thickness (4)
T-1	2.39 ±0.07	1.39 ±0.03	1.02 ± 0.04
T-2	2.40 ±0.05	1.37 ±0.09	1.02 ±0.03
T-3	2.39 ±0.08	1.42 ±0.07	1.08 ±0.06
T-4	2.41 ±0.06	1.40 ±0.08	1.03 ±0.06
T-5	2.42 ±0.07	1.44 ±0.05	1.04 ±0.03
T-6	2.35 ±0.05	1.31 ±0.07	1.03 ±0.05
T-7	2.64 ±0.08	1.54 ±0.04	1.14 ±0.04
T-8	2.13 ±0.07	1.29 ±0.04	0.99 ±0.04
T-9	2.35 ±0.07	1.37 ±0.07	1.02 ±0.04
T-10	2.34 ±0.05	1.30 ±0.03	1.00 ±0.04
LSD 0.05 LSD 0.01	0.048 0.064	0.043 0.057	0.032 0.042

Tabuľka 3 Rozmery semien populácií bazalky
(1) populácie bazalky, (2) dĺžka, (3) šírka, (4) hrúbka

In the research of Hosseini-Parvar, (2010) somewhat higher average values of seed dimensions have been obtained (length 3.11mm, width 1.82mm and thickness 1.34 mm).

Five fungi genders were isolated and identified on the examined seed of ten domestic basil populations (Table 4). In all seed samples species from *Alternaria* gender were identified, in the percentage of 12.5 to 68.8%. Species from gender *Penicillim* were isolated from eight seed samples in the intensity of 2.1 to 6.3%. *Aspergillus* spp. was identified in two samples, and *Trichoderma* spp. (4.2%), *F. oxysporum* and *F. verticilliodes* (2.1%) were identified each in one sample respectively.

In comparison to data from scientific literature, a significantly lower number of fungi were isolated from domestic population of basil than from the commercial basil seed grown in Brazil (Reis et al., 2007; Kruppa and Russomanno, 2008). Reis et al. (2007) isolated 11 species of fungi from basil seed. The frequency of certain fungi varied significantly, depending on the examined variety of basil. The most frequent species was *A. alternata*, as it was isolated from all examined varieties of basil, and it had the highest intensity of occurrence (up to 57.5%). Apart from *Cladosporium* spp. (1.5 – 21.0%), *F. oxysporum* (0.8 – 9.3%) and *Fusarium* spp. (1.5 – 5.0%), all other species had the intensity much below 5% (2.8 – 4.3% *Myrothesium roridum*, 2.5 – 2.8% *Phoma* spp., 1.8% *Stemphilium* spp., 0.5 – 3.0% *Rhizopus stolonifer*, 1.8 – 2.8% *Helminthosporium* spp., 0.5 – 1.8% *Colletotrichum gloeosporioides* and 0.5 – 2.8% *Aspergillus* spp.).

Kruppa and Russomanno (2008) isolated and identified 22 species from basil seed. The most frequently isolated species were *Rhizopus* spp. (46.4%) and *Cladosporium* spp. (42.9%) and *Alternaria alternata* (32.1%). *Curvularia lunata* and *Epicoccum purpurascens* (25.0%) were isolated in the same intensity, while other isolated species were 17.9% (*Aspergillus* spp.), 14.3% (*Phoma* spp.), 10.7% (*Fusarium solani, Trichoderma* spp.), 7.1% (*Colletotrichum* spp., *Curvularia* spp., *Exserohilum* spp., *Fusarium* spp., *Penicillium* spp., *Pithomyces chatarum*) and (*Acremoniella* spp., *Bipolaris sorokiniana, Colletotrichum gloeosporioides, Curvularia inaqualis, Fusarium equiseti, F. oxysporum*).

Species from gender *Fusarium* were, in some Mediterranean countries (Italy, France, Israel, etc.) economically significant pathogens of basil, since they caused withering and rotting of root of this medicinal herb (Minuto et al., 2002). The seed is the cause of fast spread of pathogens to many countries, as a way of maintenance of its vitality (Gamliel and Yarden, 1998). The most significant worldwide basil pathogen is *F. oxysporum* f. sp. *basilici* which causes poor germination, low growth and basil leaf chlorosis, especially when transplanted more than once or twice (Minuto et al., 2002). In Israel, basil fusariosis is the result of combination of effects of climatic conditions and modes of picking (Gamliel and Yarden, 1998).

Conclusions

All examined basil populations are characterized by high average values for seed germination. The highest average seed germination was established in population T-7 and it was 96.6%. In population T-7 the greatest mass of 1000 seeds was determined, being 1.71g. The seeds of examined populations are characterized by high content of fatty oil. The greatest content was recorded in population T-2, being 25.29%. The analysis of seed dimensions established significant differences of average values

Table 4 The intensity of the fungi of seeds basil populations

Types of fungi (1)	Basil populations in % (2)									
	T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10
Alternaria spp.	39.6	60.1	29.2	68.8	56.3	56.3	29.2	12.5	18.8	14.6
Aspergillus spp.	–	–	–	–	–	–	–	–	2.1	2.1
Fusarium oxysporum	–	–	–	–	–	–	2.1	–	–	–
Fusarium verticillioides	–	–	–	–	–	2.1	–	–	–	–
Penicillium spp.	–	–	2.1	4.2	2.1	6.3	2.1	2.1	6.3	6.3
Stemphylium botryosum	–	–	–	–	–	–	–	2.1	–	–
Trichoderma spp.	–	–	–	–	–	4.2	–	–	–	–

Tabuľka 4 Napadnutie semien bazalky hubami
(1) typ húb, (2) populácie bazalky

in terms of their length, width and thickness of seeds. Population T-7 is characterized by largest seeds. Five fungi genders were isolated on the examined seed of ten basil populations. Species from gender *Alternaria* were identified in all samples of seeds from the examined basil populations. Species from gender *Penicillim* were isolated from the samples of seeds of eight populations. Fungi *Trichoderma* spp., *F. oxysporum* and *F. verticillioides* were identified on the samples of seeds of one population of basil each.

Súhrn

V práci bolo hodnotených 10 populácií semien bazalky pravej (*Ocimum basilicum* L., čeľaď *Lamiaceae*) zo Srbska. Analyzovali sa nasledovné parametre: energia klíčenia, celková klíčivosť, hmotnosť tisíc semien (absolútna váha semien), obsah oleja v semene a rozmer semien (dĺžka, šírka a hrúbka). Ďalej bola identifikovaná mikrobiota semien. Všetky hodnotené populácie boli charakteristické vysokou priemernou hodnotou klíčivosti semien. Najvyššia priemerná klíčivosť na úrovni 96,6% ako aj najvyššia HTZ 1,71 g bola zistená v populácii T-7. Semená hodnotených populácii mali vysoký obsah oleja. Najvyšší obsah oleja bol zaznamenaný v populácii T-2 na úrovni 25,29%. Analýzou rozmeru semien boli zistené preukazné rozdiely priemerných hodnôt dĺžky, šírky a hrúbky tzn. bacuľatosti semien. Populácia T7 bola charakteristická najväčšími rozmermi semien. Na semenách bolo identifikovaných päť rodov húb. Druhy rodu *Alternaria* boli identifikované vo všetkých základných populáciách. Druhy rodu *Penicillium* boli identifikované vo vzorkách deviatich populácií. *Aspergillus* spp. boli identifikované v dvoch populáciách semien bazalky. Huby *Trichoderma* spp., *F. oxysporum* a *F. verticillioides* boli zistené iba na vzorke jednej populácie bazalky.

Kľúčové slová: semeno, bazalka, klíčenie, olej, mikrobiota

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