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POTENTIAL OF SOME MEDICINAL AND FODDER CROPS TO ALLEVIATE SOIL SICKNESS IN THE OLD *PRUNUS PERSICA* VAR. *PERSICA* (L.) BATSCH AND *MALUS DOMESTICA* BORKH. TREE MONOCULTURES

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The effect of nine species of cover crops (calendula, eastern galega, tartary buckwheat, mustard, tansy phacelia, lemon balm, golden marigold, sainfoin, blue fenugreek) on allelopathic and biochemical characteristics of the rhizosphere soil from-under 30-year-old *Malus domestica* Borkh. and *Prunus persica* var. *persica* (L.) Batsch plantations in model laboratory experiments were studied. The samples of the rhizosphere soil were collected at the beginning of the growing season (fruit trees bud development), dried, sieved and placed into the plastic pots. The seeds of the tested cover crops were sown into the pots. After the emergence of the seedlings 10 plants per pot were left and cultivated for 4 months under laboratory conditions. The contents of the nutrients, organic carbon and soil acidity were determined at the beginning of the experiments. Every two weeks after the start of the experiments, the allelopathic activity of the soil was assessed using bioassays on the cress root growth and radish seeds germination. The number of free phenolics in soil solution was determined at the beginning and the end of the experiments. Analysis of the content of soil nutrients showed that the content of manganese and iron 3–6-fold exceeded the optimum level, and the content of free phenolics in soil solution exceeded the phytotoxic threshold almost 10-fold. Growing of cover crops improved the allelopathic and biochemical regime of the studied soil. The most effective were *Tagetes tenuifolia*, *Phacelia tanacetifolia*, and *Sinapis alba*. The size of the positive effect was correlated with the duration of cultivation of the cover crops in the soil. We consider the above-mentioned plant species promising for further study of their potential to restore soil fertility in the old apple and peach orchards.

Keywords: cover crops, soil sickness, old monoculture, apple tree, peach tree

Introduction

Presently soil sickness (SS), which is also known in agronomy as “soil fatigue” or “replant disease problem”, in the orchards of fruit trees is a global problem, causing huge economic losses for fruit producers and significantly limiting the development of fruit production

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(Benizri et al., 2005; Lü et al., 2018). SS affects most of the fruit trees including both pome and stone fruit trees after long-term (commonly 20-ty years and more) cultivation of the same orchard species in the same field. As a result, a general decline in the growth and productivity, vigor and performance of the fruit trees are observed, without causes clearly defined (Benizri et al., 2005; Thakur et al., 2018).

Plant ecologists refer to SS as a negative plant-soil feedback emphasizing the mutual negative interactions between plant and soil (Cesarano et al., 2017). The problem is specific to a plant genus or species and persists for up to 20 to 30 years. The problem of SS in the fruit orchards was reported in the literature for more than two centuries ago. However, until the present, the causes of this phenomenon have not been clearly defined yet. The decline in the fruit yield has been attributed to phytotoxic allelochemicals, depletion of mineral nutrients, phytopathogenic fungi, bacteria, insects, and nematodes (Benizri et al., 2005). However, none of the mentioned above factors fully explain the species-specificity, as well as the long durability of SS. A better understanding of the factors causing SS is a necessary step to develop eco-friendly solutions to overcome this problem.

Analysis of the available literature data on SS in apple and peach orchards revealed that one of the underlying causative mechanisms of this problem is the accumulation of phytotoxic phenolics in the rhizosphere soil. Particularly, phlorizin, phenolic acids, vanillic aldehyde, etc. were found to be responsible for SS in old apple monoculture. While in the rhizosphere soil from under old peach tree phytotoxic concentrations of catechin, amygdalin, and their derivatives were reported (Moroz, 1990; Liu et al., 2008; Yin et al., 2016). The mentioned above allelopathic inhibitors showed phytotoxic effects on a wide range of higher plants (Reigosa and Pazos-Malvido, 2007; Yin et al., 2016). It has also been shown that the decay products of root and leaf residues of apple and peach trees stimulate the development of phytopathogenic and producing phytotoxic substances microorganisms and inhibit agronomically beneficial microflora (Čatská et al., 1982; Benizri et al., 2005). Particularly, in the rhizosphere soil of older apple trees, an increase in the counts of micromycetes and actinomycetes and a decrease in bacterial counts were found in agreement with the decreasing pH of the rhizosphere soil. The number of phytotoxic micromycetes was higher in 'sick' soil as compared with control soil in which apple trees had not been grown for at least 15 years. The latter, via their metabolites, affected negatively the microbial equilibrium and biological activity of the soil and thus also the growth, development, and health of the plants (Čatská et al., 1982). Benizri et al. (2005) noted a shift in the structure of bacterial communities in "sick" peach tree rhizosphere soil with an increase in phytotoxic microorganisms capable of producing toxic cyanide compounds and inhibition of beneficial microbiota. The inhibition of the growth of agronomically useful microflora leads to enhanced development of soil-borne pathogenic microbiota (Manici et al., 2003). The mentioned changes in soil biochemical and biological characteristics impair soil structure and cause depletion or immobilization of nutrients which, in turn, worsen further growth condition for cultivated plants (Moroz, 1990; Politycka and Adamska, 2003).

In this context, chemical or/and biological removal of phytotoxins is of utmost importance because it affects not only plant vigor and growth, but also the structure and performance of soil microbiota. Present agricultural practices aimed to combat or reduce SS in old apple

and peach tree orchards include soil fumigation with EDTA (ethylenediaminetetraacetic acid), fertilization, soil chemical sterilization, biocontrol using plants with high phytosanitary potential, arbuscular mycorrhiza and other microbial agents (Moroz, 1990; Singh et al., 2017; Lü et al., 2018; Thakur et al., 2018). Sufficient research has been done on chemical control with fumigation, however, research is needed to develop environment-friendly biological methods and to restore soil fertility (Singh et al., 2017; Thakur et al., 2018). There is also a need to develop an integrated approach combining chemical and biological control of factors causing SS (Singh et al., 2017; Thakur et al., 2018).

In this aspect, the use of cover crops with phytosanitary potential could be a promising solution to the problem of SS in fruit orchards. Cover cropping is environmentally friendly, cost-effective and is less vulnerable to weather fluctuation than microbial preparations. Today cover cropping is widely used to manage soil fertility, water retention capacity, chemical, and physical characteristics, for control of weeds, pests, diseases, as well as an increase in biodiversity and stability of agroecosystems. It is a major challenge in the sustainable management of fruit orchards, because of its complex effect on soil biological, biochemical, agrochemical and physical characteristics. When properly fit cover crops not only alter allelopathic and biochemical regime of the "sick" soil but also enrich the soil with additional organic matter, which stimulates microorganisms involved in mineralization processes and suppresses phytopathogenic and phytotoxic strains (Manici et al., 2015; Zhou et al., 2019). The changes in soil microbial community affect further detoxification and mineralization processes and contribute to the restoration of the balance of nutrients. Besides, their phytomass after incorporation into the soil creates additional moisture reserves (Rodrigues et al., 2018).

In the long-term field studies performed by Moroz (1990) the positive results of cultivation of legumes and cruciferous cover crops in old peach and apple tree orchards for the alleviation of SS were obtained. The best effect was achieved when in the spring under cover of cereals clover or alfalfa were sown and cultivated for 2 years. The described intercropping practice promoted the accumulation of organic matter in the soil, activated microbiological processes and improved allelopathic regime (Moroz, 1990).

The positive effect of the permanent cover of alfalfa plus fescue mixture (*Medicago sativa* + *Festuca arundinacea* Schribn.); strawberry clover (*Trifolium fragiferum* L.), common vetch (*Vicia sativa* L.) on the mineral nutrients balance in the soil, apple tree growth and yield as compared to control (apple trees are grown with natural vegetation of grasses and legumes) was shown by Sánchez et al. (2007).

Conducted by Alicia Morugán-Coronado et al. (2020) meta-analysis to assess the effect of soil management techniques on soil properties and crop yields in fruit crops (including peach, almond, avocado, citrus, grapevine, etc.) in Mediterranean region highlighted the overall positive effects of permanent intercropping (namely, maintenance of a permanent cover crop in the alleys, such as aromatics *Thymus* sp, *Lavandula* sp, *Salvia* sp, *Rosmarinus* sp, *Brachypodium* sp, *Asparagus* sp or natural grass), and annual intercropping (cover crops in the alleys that are annually harvested or incorporated into the soil) on soil organic

matter and nitrogen content. The mentioned agricultural practices make the agroecosystem more resilient to drought and erosion events, with no negative effects on fruit yield. The authors concluded that the incorporation of such cropping systems and practices in policy measures could provide a meaningful contribution to securing the long-term soil quality of Mediterranean orchards. The analysis used the results of 187 experimental treatments from 46 peer-reviewed articles, including the countries of Spain, Italy, France, Portugal, Greece, Turkey, Slovenia, Tunisia, Chile and the United States of America.

The impact of three cover crops with phytosanitary potential (namely, barley (*Hordeum vulgare* L. cv. Tidone), alfalfa (*Medicago sativa* L. cv. Europe), marigold (*Tagetes patula* L. cv. Disco Marinetta) on the soil microbial community colonizing apple trees in orchards suffering SS was studied by Manici et al. (2015). All studied cover crops showed the potential to increase soil microbial diversity in long-term permanent cropping systems and to manipulate root colonizing fungi involved in crop health. The most effective in this respect was marigold, which increased the abundance of nonpathogenic root inhabiting fungi and stimulated the growth of young apple seedlings more than other cover crops. The author concludes that the appropriate use of a cover crop pre-plant can reduce the inoculum of soil-borne pathogens and give an effective advantage in the early post-plant stage, during which young fruit trees are most susceptible to root rot fungal pathogens (Manici et al., 2015). Tongy Zhou et al. (2019) demonstrated that the incorporation of cover crops (mixed herbs or red clover) into the soil in apple orchards improved the chemical composition and had a positive influence on microbial communities.

The objective of our study was to evaluate the potential of nine medicinal and fodder crops to alleviate soil sickness in the 30-year-old peach and apple tree monocultures.

Material and methodology

Soil sampling

Soil samples (gray podzol) for this study were taken at a depth of between 20 and 50 cm from under 30-year-old apple trees (*Malus domestica* Borkh., cv. Slava Pobediteliam) and peach trees (*Prunus persica* var. *persica* (L.) Batsch, cv. Druzhba) at the beginning of the growing season (bud development stage) cultivated in a fruit orchard at the M.M. Gryshko National Botanical Garden (Kyiv, Ukraine).

Experimental set-up

Pot experiments were conducted in the laboratory conditions at the Department of Allelopathy of the M.M. Gryshko National Botanical Garden. Air-dried and 2-mm sieved soil was placed into the 1 l plastic pots. The latter were arranged in a randomized block design and maintained at 22–28 °C with a 12/12 light cycle, soil moisture of 60% of full physical soil water capacity for 4 mo.

Seeds of calendula (*Calendula officinalis* L.), eastern galega (*Galega orientalis* L.), tartary buckwheat (*Fagopyrum tataricum* L.), mustard (*Sinapis alba* L.), tansy phacelia (*Phacelia tanacetifolia* Benth.,), lemon balm (*Melissa officinalis* L.), golden marigold (*Tagetes tenuifolia*

Cav.), sainfoin (*Onobrychis arenaria* (Kit.) DC.), blue fenugreek (*Trigonella caerulea* (L.) Ser.) were surface-sterilized with 1% sodium hypochlorite and sown into the pots. After the emergence of seedlings, they were thinned to 10 plants per pot.

Assessment of soil characteristics

The amounts of nutrients, organic carbon, and soil acidity were determined at the beginning of the experiments. The content of biogenic elements in soil samples was analyzed using the method described by G. Ya. Rinkis and V.F. Nollendorf (1982) with the help of spectrophotometer ICAP 6300 DUO. The acidity of soil solution was determined using ion meter HI 2211 (HANNA Instruments).

Every two weeks after the start of the experiment, the allelopathic activity of the soil was determined using bioassays on cress (*Lepidium sativum* L.) radicle elongation and radish (*Raphanus sativus* L., cv. Krasny s belym konchikom) seeds germination (Grodzinski, 1991). As a control, we used the same fruit tree rhizosphere soil exposed to the same temperature, light and watering conditions but without cover crops. Seeds of the test plants were surface-sterilized with 1% sodium hypochlorite. For germination tests and root elongation tests, seeds were sown on Whatman no.1 paper disks placed over the tested soil samples in Petri dishes with 10-cm-diam. All control and test treatments were replicated three times. Germination was assessed after 48 hours; radicle length was measured 3 days after cress seeds were sown.

The content of free phenolics in the soil solution was determined at the beginning and the end of the experiments. Phenolic allelochemicals were extracted from soil samples with methanol and their amount was determined by the method described in (Grodzinski et al., 1988) with the help of spectrophotometer Specord 200, Analytic Yena 2003.

Statistic analysis

Statistical analysis on quantitative data and visualization of the results was performed using descriptive statistics and ANOVA with the help of STATISTICA 10.0 software (StatSoft Inc., Tulsa, Oklahoma, USA) and Microsoft Excel 7.0. Mean germination and mean root elongation indices were compared to their respective controls. The results of the assessment of the allelopathic activity of soil samples presented on figures are % to their respective values in control. The least significant differences test (LSD at $p < 0.01$) was used to compare the means of different treatments.

Results and discussion

Analysis of the nutrients contained in the soil collected from under 30-year-old apple and peach trees showed that the content of manganese under the apple trees was within 179.4 mg/l of soil. This is 20% above the phytotoxicity threshold of this metal in soil solution (150 mg/l of soil) according to (Bityutskiy, 2014). Iron content in soil under peach 375 mg/l by 25% exceeded the phytotoxicity threshold of this element in soil solution (300 mg/l soil, according to Bityutskiy (2014) (Table 1).

Table 1 The content of mineral nutrients (mg/l of soil), hummus (C%), free phenolics (mg/l of soil), pH in soil from under 30-year-old apple and peach trees plantations, p <0.01

Fruit tree	NH_4^+	NO_3^-	P	K	Ca	Mg	Fe	S	Mn	pH	C%	Total phenolics
Apple tree	194.7	7.5	163.5	382.5	5,831.0	518.2	258.5	77.4	179.4	7.21	2.05	120
Peach tree	31.7	3.8	172.4	447.0	2,165.0	263.8	375.0	49.2	115.6	6.9	2.96	140

Note: C% – hummus %

The content of phenolic allelochemicals in the tested soil samples was 120 and 140 mg per liter, which is almost an order of magnitude higher than the phytotoxicity threshold of these substances – 15–20 mg/l (Reigosa and Pazos-Malvido, 2007; Perveen et al., 2019). Phenolic compounds are a major group of allelochemicals ranging from phenols, hydroxy acids, aldehydes, benzoic acids cinnamic acids, coumarins, tannins, and flavonoids. They are produced by various plant species and their inhibitory effects on higher plants have been well reported (Makoi and Ndakidemi, 2007; Reigosa and Pazos-Malvido, 2007; Liu et al., 2008; Yin et al., 2016). Apparently, the phytotoxicity of the studied soil samples is mainly due to the accumulation of free phenolic compounds. Taking into account the ability of phenolics to form chelate complexes with metals (Makoi and Ndakidemi, 2007), and thus retaining them in the soil medium, it is possible to predict that the toxic effect of the excess concentrations of manganese and iron was also significant.

The results of the allelopathic analysis showed that *Ph. tanacetifolia* and *T. tenuifolia* most effectively improved soil allelopathic regime in apple orchards as compared to the other studied cover crops (Figure 1). A significant stimulating effect was observed after a month of cultivation of these cover crops and lasted until the end of the experiments. Somewhat less effective was *Trigonella caerulea*. Cress radicle elongation was the most responsive to the tested cover crops allelochemicals as compared to radish seed germination. The former bioassay is considered to be one of the most sensitive to phenolic allelochemicals (Perveen et al., 2019).

The results of the allelopathic analysis were in good agreement with the data of the assessment of free phenolics content in the soil solution (Figure 2). The lowest amount of phenolics was revealed after the cultivation of *T. tenuifolia*. A slightly higher content was detected after the cultivation of *S. alba*, *C. officinalis*, *T. caerulea* and *Ph. tanacetifolia*. The highest amount of phenolics was found after the cultivation of *O. arenaria*.

Similar results were obtained for soil samples collected from under 30-year-old peach trees. All studied cover crops significantly improved the allelopathic and biochemical regimes of this soil (Figure 3 and 4). The greatest positive effect was observed at 3–4 months of cultivation *Ph. tanacetifolia* and *S. alba*.

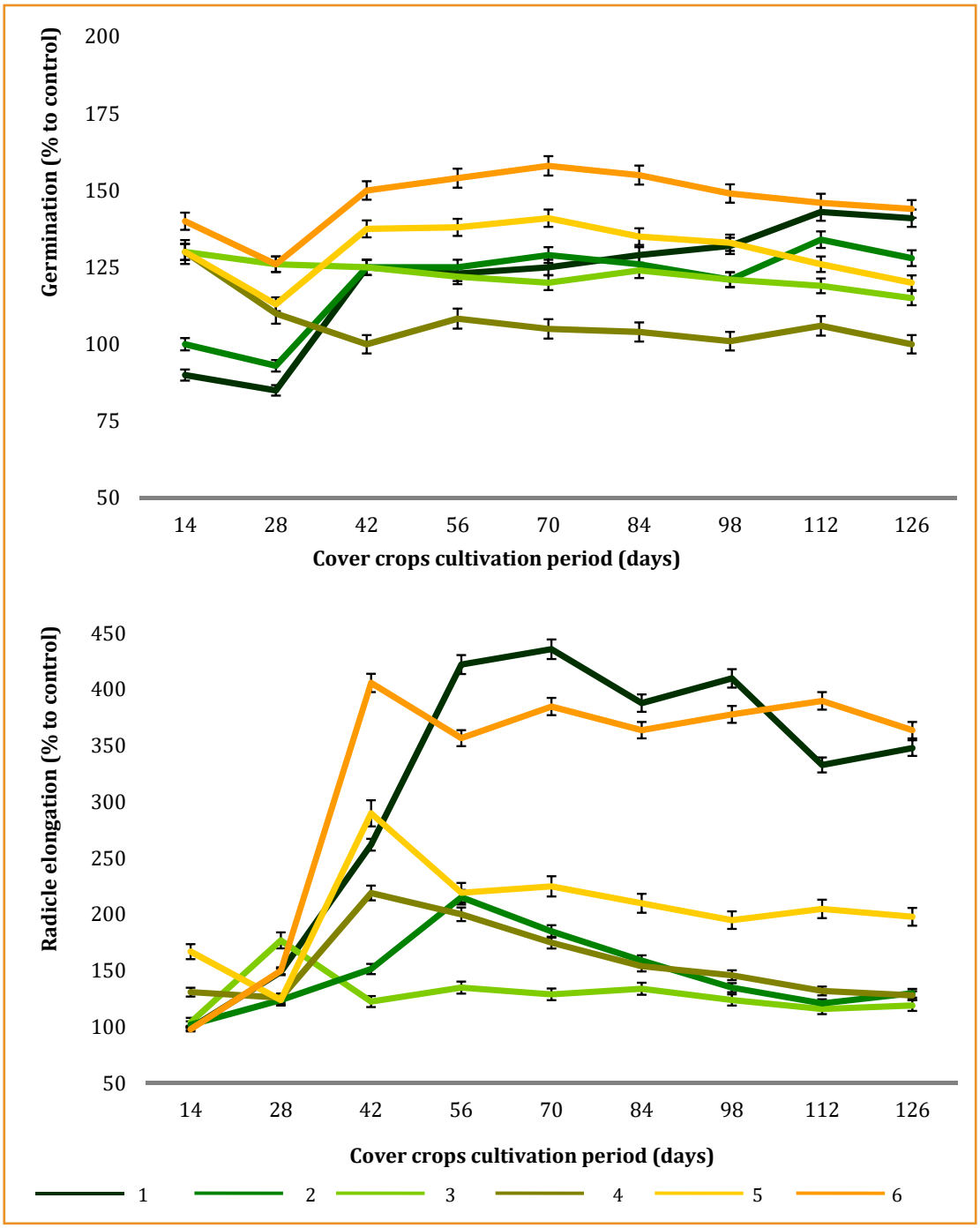

Figure 1 Effect of cover crops cultivation on the allelopathic activity of the soil from-under 30-year-old apple trees. Bioassays: radish seed germination and cress radicle elongation
1 – *Tagetes tenuifolia*; 2 – *Onobrychis arenaria*; 3 – *Sinapis alba*; 4 – *Calendula officinalis*; 5 – *Trigonella caerulea*; 6 – *Phacelia tanacetifolia*. Vertical bars – the least significant difference at p <0.01%

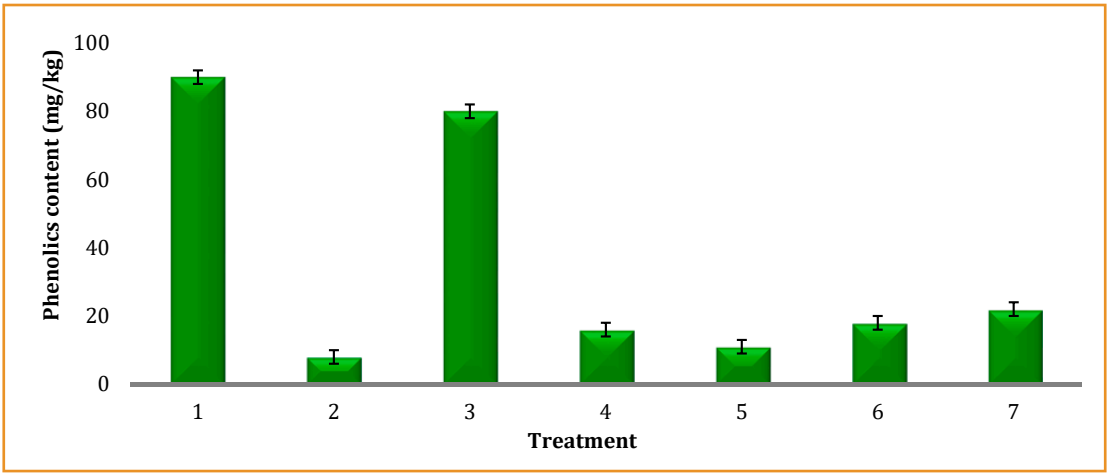

Figure 2 Effect of cover crops cultivation on the content of free phenolics in the soil from-under 30-year-old apple trees after 126 days of cultivation of cover crops
1 – control (without crops); 2 – *Tagetes tenuifolia*; 3 – *Onobrychis arenaria*; 4 – *Sinapis alba*; 5 – *Calendula officinalis*; 6 – *Trigonella caerulea*; 7 – *Phacelia tanacetifolia*. Vertical bars – the least significant difference at p <0.01

It should also be noted that all the cover crops studied except *Galega orientalis* and *Onobrychis arenaria* significantly reduced the content of free phenolics in soil solution. This not only helped to reduce the phytotoxic properties of the soil but also contributed to the diversity of soil microbiota, including agronomically useful microorganisms. In addition, the observed decrease in the amount of total phenolics increases the mobility of toxic metals such as manganese and iron, facilitating their washing out of the soil solution.

A positive effect of marigold species on the allelopathic and biochemical characteristics of soil was shown in our previous studies (Didyk and Mashkovska, 2006). According to the results obtained by Manici et al. (2015) marigold used as a pre-plant in apple orchards demonstrated the highest stimulative effect on apple rootstock plantlets growth as well as the increase in the abundance of nonpathogenic root inhabiting fungi more compared to the other tested cover crops. Besides, marigolds (*Tagetes* spp.) have been shown to suppress certain nematode species (Kimpinski et al., 2000) and some noxious weeds (Didyk and Mashkovska, 2006). Tansy phacelia is commonly used as a cover crop in arable rotations and is considered to be particularly effective in conditioning soil structure, in sandy loam and clay soil (Bacq-Labreuil et al., 2019). Root exudations of tansy phacelia were also shown to control root-lesion nematodes (Kimpinski et al., 2000), and the infection by such soil-borne fungi as *Alternaria alternata*, *Fusarium oxysporum*, *F. culmorum* etc. (Patkowska and Konopiński, 2011). Mustard exometabolites contain glucosinolates which could be further hydrolyzed by the enzyme myrosinase to form isothiocyanates (ITCs), compounds toxic to a variety of soil-borne plant pests, including nematodes, bacteria, fungi, and weeds (Matthiessen and Kirkegaard, 2006; Hossain et al., 2015; Brennan and Smith, 2018). Another reason for this suppressive effect could be a change in the structure of the soil microflora (Hossain et al., 2015). Allelochemicals of *S. alba* were shown to selectively inhibit soil-borne pathogens without major side effects

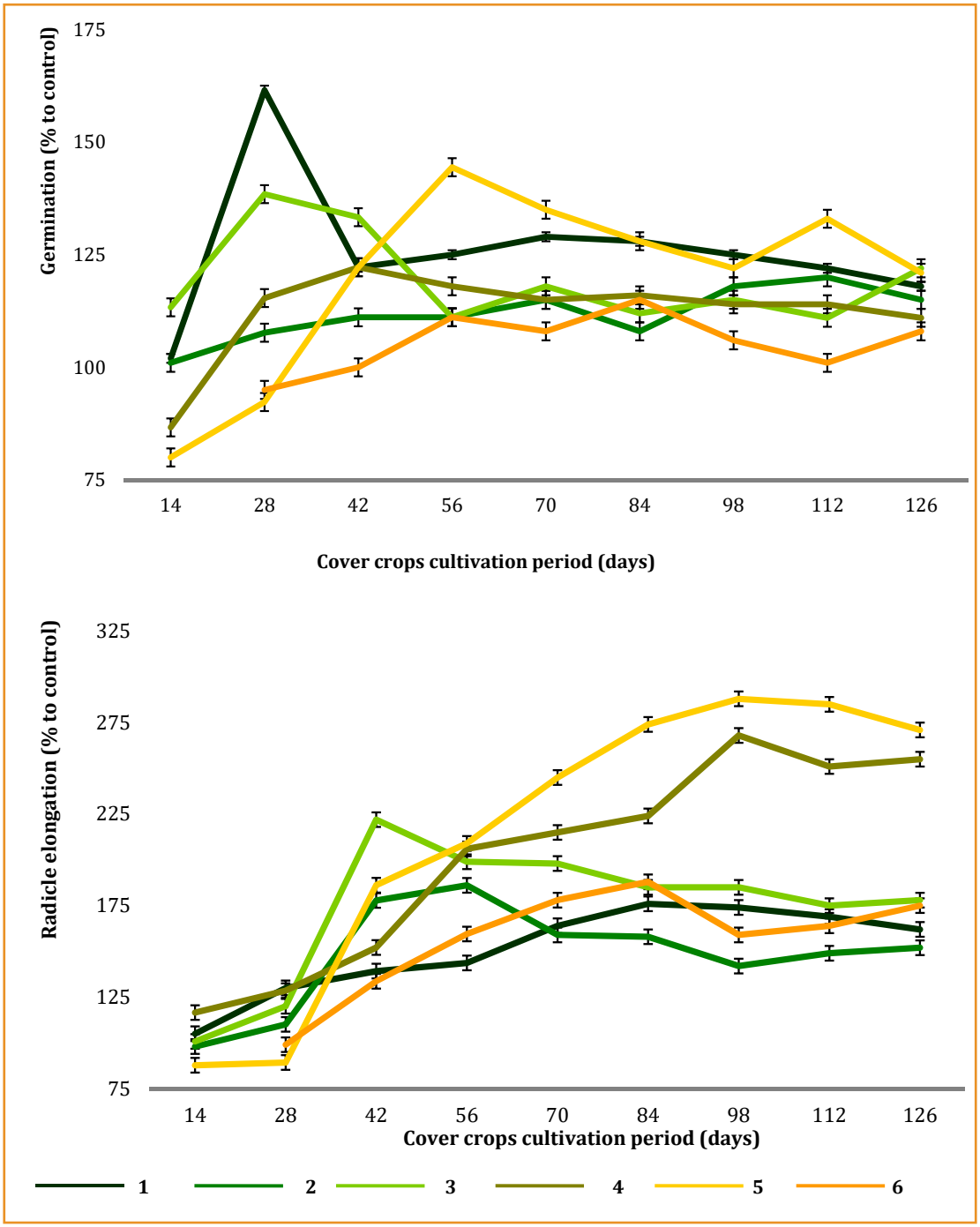

Figure 3 Effect of cover crops cultivation on the allelopathic activity of the soil from-under 30-year-old peach trees. Bioassays: radish seed germination and cress radicle elongation
1 – *Calendula officinalis*; 2 – *Galega orientalis*; 3 – *Fagopyrum tataricum*; 4 – *Sinapis alba*; 5 – *Phacelia tanacetifolia*; 6 – *Melissa officinalis*. Vertical bars – the least significant difference at p <0.01

on the genetic potential of beneficial soil microorganisms involved in N cycling (Hossain et al., 2015). Besides they stimulate the development of antagonistic organisms towards pathogenic fungi in soil (Matthiessen and Kirkegaard, 2006). The present study demonstrated the good potential of the mentioned above cover crop species to reduce soil phytotoxicity caused by an accumulation of phenolic allelochemicals in old apple and peach tree monocultures. Due to the mentioned above features marigolds, tansy phacelia, and mustard present the most promise for further studies of their effectiveness in alleviating soil sickness in the old fruit orchards.

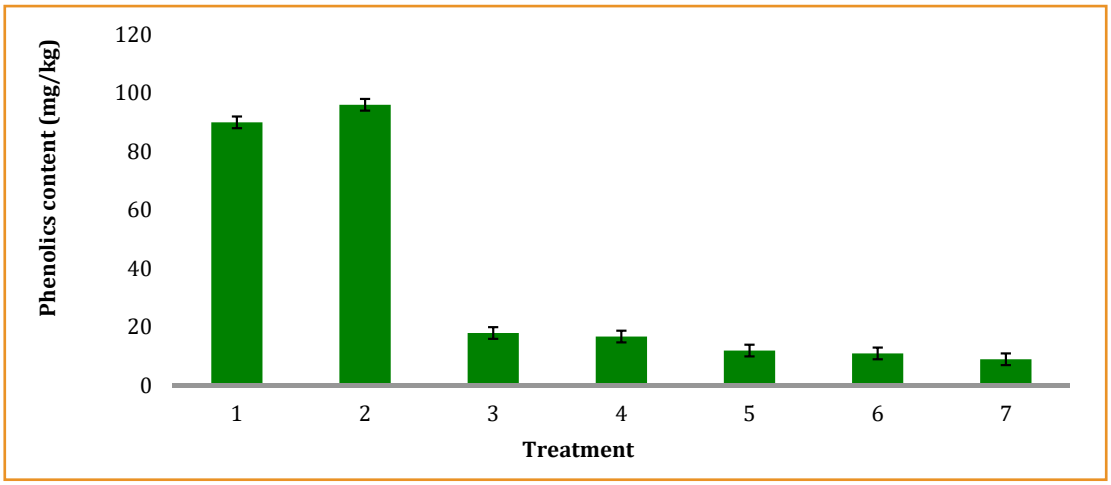

Figure 4 Effect of cover crops cultivation on the content of free phenolics in the soil from-under 30-year-old peach trees after 126 days of cultivation of cover crops
1 – control; 2 – *Galega orientalis*; 3 – *Calendula officinalis*; 4 – *Fagopyrum tataricum*; 5 – *Sinapis alba*; 6 – *Phacelia tanacetifolia*; 7 – *Melissa officinalis*. Vertical bars – the least significant difference at p <0.01

Soil quality is one of the central factors that control yield and crop health in agro-ecosystems. Amelioration of soil allelopathic and biochemical regimes control its microbocenoses, organic matter dynamics, nutrient mineralization and suppression of pathogens. Today cover crops are widely used for increasing crop health in sustainable and organic agriculture (Moroz, 1990; Manici et al., 2015; Morugán-Coronado et al., 2020). Primary benefits from cover crops conventionally include improving soil quality (soil macroporosity, water retention, nutrient supply, etc.), erosion (Bacq-Labreuil et al., 2019) and weed control (Didyk and Mashkovska, 2006; Manici et al., 2015). The results of our study demonstrated the effectiveness of cover cropping in the alleviation of soil sickness caused by the accumulation of phytotoxic allelochemicals.

Conclusions

The data obtained indicate that SS in the investigated 30-year-old plantations of apple and peach trees are associated with the accumulation of toxic concentrations of phenolic allelochemicals, as well as toxic metals of Mn and Fe. Cultivation of cover crops improved the allelopathic and biochemical regime of the SS. In particular, it significantly reduced the concentrations of phytotoxic phenolics and improved the biological activity of soil. The

highest ameliorative effect was observed after the cultivation of *Tagetes tenuifolia*, *Phacelia tanacetifolia*, and *Sinapis alba*. We consider these cover crops to be promising as eco-friendly solutions to alleviate SS in the apple tree and peach tree plantations.

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EVALUATION OF SOME BIOCHEMICAL PARAMETERS OF RAW OF *ARTEMISIA* SPP. (ASTERACEAE BERCHT. & J. PRESL.)

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This study was aimed to investigate the biochemical composition of plant raw material of *Artemisia* spp. in conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine (Kyiv) at both budding and flowering stages. It was investigated the following species of *Artemisia* L. genus: *A. abrotanum* L., *A. annua* L., *A. argyi* H. Lev. & Vaniot, *A. austriaca* Jacq., *A. japonica* Thunb., *A. ludoviciana* Nutt., and *A. maritima* L. The content of dry matter determined by measuring till constant weight, the total content of reducing sugars by Bertrand method, tannins with indigo carmine, titratable acidity by titration with sodium hydroxide, and ascorbic acid with 2.6-dichlorophenol-indophenol, and the content of carotene on spectrophotometer with Kalosh petrol. At the stage of budding content of dry matter was from 26.72 (*A. annua*) to 48.63 (*A. maritima*) %, the content of reducing sugars from 5.11 (*A. austriaca*) to 8.93 (*A. maritima*) %, the titratable acidity from 2.06 (*A. abrotanum*) to 3.52 (*A. japonica*), the tannin content from 2.77 (*A. abrotanum*) to 5.1 (*A. ludoviciana*) %, ascorbic acid content from 11.65 (*A. argyi*) to 37.68 (*A. japonica*) mg%, and the content of carotene from 0.09 (*A. ludoviciana*) to 0.56 (*A. abrotanum*) mg%. At the stage of flowering, dry matter in raw was from 31.64 (*A. annua*) to 42.74 (*A. austriaca*) %, the content of sugars from 6.8 (*A. austriaca*) to 8.23 (*A. annua*) %, titratable acidity from 2.8 (*A. abrotanum*) to 4.66 (*A. annua*) %, tannin content from 4.22 (*A. austriaca*) to 6.36 (*A. annua*) %, the ascorbic acid content from 12.93 (*A. abrotanum*) to 65.18 (*A. annua*) mg%, and carotene content from 0.14 (*A. austriaca*) to 0.22 (*A. annua*) mg%. Also, at the period of budding very strong correlation was between titratable acidity and tannin content ($r = 0.824$), moderate correlation between dry matter and sugars content ($r = 0.581$). At the stage of flowering determined a very strong correlation between sugars and tannin content ($r = 0.890$), titratable acidity and tannins ($r = 0.957$), titratable acidity and ascorbic acid content ($r = 0.999$), tannins and ascorbic acid content ($r = 0.966$). In the M.M. Gryshko National Botanical Garden of the NAS of Ukraine just plants of *A. abrotanum*, *A. annua*, and *A. austriaca* passed in the period of flowering. Obtained data can be used for the deep further biochemical and pharmacological study.

Keywords: *Artemisia*, biochemical composition, correlation

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Introduction

Artemisia L. genus belongs to Asteraceae Bercht. & J. Presl. family and concludes more than 200 species (Isani et al., 2019). Species of *Artemisia* spp. exhibited antioxidant, antimicrobial, and anti-inflammatory activities (Pavithra et al., 2018; Moacă et al., 2019). The secondary metabolite artemisinin from *A. annua* L. a unique sesquiterpene lactone demonstrated antimalarial properties (Knudsmark et al., 2014; Czehowski et al., 2019). *A. annua* is an important medicinal plant widely used in Africa for the treatment of malaria and other diseases (Chukwurah Nkachukwu et al., 2014). Also, this species has been used for centuries in Traditional Chinese Medicine. Among 600 phytochemicals identified in *A. annua*, the most dominated are sesquiterpenes, flavonoids, and coumarins (Isani et al., 2019). Some reviews demonstrated the antioxidant activity of *A. annua* but *A. ludoviciana* had a higher value of this parameter (Lutgen, 2018). Also, the antimicrobial activity of *A. abrotanum* described against gram-positive and gram-negative bacteria and *Candida albicans* (Ivashchenko et al., 2014).

Among secondary metabolites of *A. nilagarica* organs determined alkaloids (the most in shoot buds), saponins (the most in stems), steroids (the most in roots), phenols (the most in leaves), etc. (Nganthoi et al., 2016). Powder of *A. annua* content gross energy 3,876.7 kcal/kg, total fat 3.04%, cellulose 27.61%, ash 8.90%, amino acids (aspartic acid 1.77%, glutamic acid 1.74%, leucine 1.32%, threonine 1.26%, arginine 1.02%, rest of amino acids was less 1% of content) (Panaite et al., 2018).

This study aimed to determine the peculiarities of biochemical compound accumulation in raw of different species of *Artemisia* L. genus in conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine.

Material and methodology

Biological material

In this study investigated following species of *Artemisia* L. genus: *A. abrotanum* L., *A. annua* L., *A. argyi* H. Lev. & Vaniot, *A. austriaca* Jacq., *A. japonica* Thunb., *A. ludoviciana* Nutt., *A. maritima* L. Plants collected from the experimental collection of Department of Cultural Flora in M.M. Gryshko National Botanical Garden of the NAS of Ukraine (NBG) at the stage of budding and flowering during 2019–2020. Biochemical analyses were conducted in the laboratory of Department Cultural Flora of M.M. Gryshko National Botanical Garden. All investigated plants are perennial.

Biochemical analyses

Dry matter determination

Plant samples were dried in drying oven at the 105 °C till constant weight in aluminum boxes. Results are given in percentages (Hrytsajenko et al., 2003).

The total content of sugars determination

The total content of sugars was investigated by Bertrand's method in water extracts. 4 g of fresh mass mixed and homogenized with distilled water (approximately 50 ml) in the 100 ml test-tubes and heated in the water bath at 70 °C during 15–20 min. After cooling in the obtained mixtures added 1 ml of the phosphate-oxalate mixture. After this was added 1.5 ml of lead acetate. The obtained mixture brings to the mark (100 ml) with water. After filtration from obtained solution took 50 ml and mixed with 8 ml of 20% HCl (at the 70 °C in a water bath for 5 min) and after cooling was neutralized by 12% NaOH and brought to the mark by distilled water (100 ml). 3 ml of obtained solution mixed with 6 ml of Fehling's solution reagent (6 min boiling in the water bath). Obtained mixture analyzed for the total content of sugars. Results are given by percentages (Hrytsajenko et al., 2003).

The total content of ascorbic acid

Determination of ascorbic acid content conducted by method offered by K. Murri. 2 g of fresh mass mixed with 50 ml of 2% oxalic acid. Obtained mixture put into the dark for 20 min. Content of ascorbic acid of obtained extracts determined by a 2.6-dichlorophenol-indophenol method that based on the reduction properties of ascorbic acid. Obtained results expressed in the mg% DW (Hrytsajenko et al., 2003).

The total content of carotene

The concentration of total carotene determined according to Pleshkov (1985) using extraction with rubber solvent (petrol). 1 g of absolutely dried raw mixed with 20 ml of Kalosha petrol for 2 hours. After this obtained filtrate measured spectrophotometrically at the wavelength 440 nm at the Unico spectrophotometer. Obtained results expressed in mg% DW.

The total content of tannins

The content of tannins was determined with indigo carmine as an indicator (Yermakov et al., 1972). 5 g of fresh mass mixed with distilled water (approximately 50 ml) in 100 ml taste-tubes. Obtained mixture heated in the water bath at 70 °C for 2 hours. After cooling, adding water to the 100 ml and following filtration 10 ml of filtrate used for determination of the total content of tannins. This procedure used 700 ml distilled water and 25 ml of 1% solvent of indigo carmine. Obtained results expressed in %.

The total content of organic acids

The total content of organic acids determined with phenolphthalein and results calculated with a malic acid coefficient (Krishchenko, 1983). 10 ml of filtrate (the same procedure described for the determination of total content of tannins) titrated with 1 N solvent of NaOH in presence of phenolphthalein. Obtained results expressed in percentages.

Statistical analysis

The mean values of three replicates and the standard deviation are given. Data submitted with ANOVA and differences between means compared using Tukey-Kramer test ($\alpha = 0.05$). Correlation analysis performed using Pearson's criterion.

Results and discussion

Our investigation of biochemical composition and antioxidant activity of plant extracts of representatives of Asteraceae, among which *Artemisia dracunculus* L., *Rhaponticum carthamoides* Willd., *Serratula coronata* L., *Scorzonera hispanica* L., *Silphium* spp. have studied (Korablova and Rys, 2012; Andrushchenko et al., 2018; Vergun et al., 2018; Ivashchenko et al., 2019; Rakhmetov et al., 2019; Vergun et al., 2019).

We found the content of dry matter for plants of *Artemisia* spp. from 26.72 (*A. annua*) to 48.63 (*A. maritima*) % at the stage of budding (Figure 1). Sugars are the most important regulators of many physiological processes such as photosynthesis, seed germination, flowering and processes under abiotic stresses (salt, drought, and cold stresses) (Sami et al., 2016). The study of *Triticum aestivum* showed that tolerant genotypes elevated reducing sugars, while susceptible plants had decline sugar content (Khan and Naqvi, 2012). The content of reducing sugars was from 5.11 (*A. austriaca*) to 8.93 (*A. maritima*) %. The titrable acidity values of investigated plants determined from 2.06 (*A. abrotanum*) to 3.52 (*A. japonica*) %.

One of the most important secondary metabolites tannins plays an important role in the growth of plants and act as protective compounds. They characterized by antimicrobial, antihelmintic and protein bypassed effects (Hassanpour et al., 2011). The above-ground part of plants identified tannin content from 2.77 (*A. abrotanum*) to 5.1 (*A. ludoviciana*) % at the stage of budding (Figure 1).

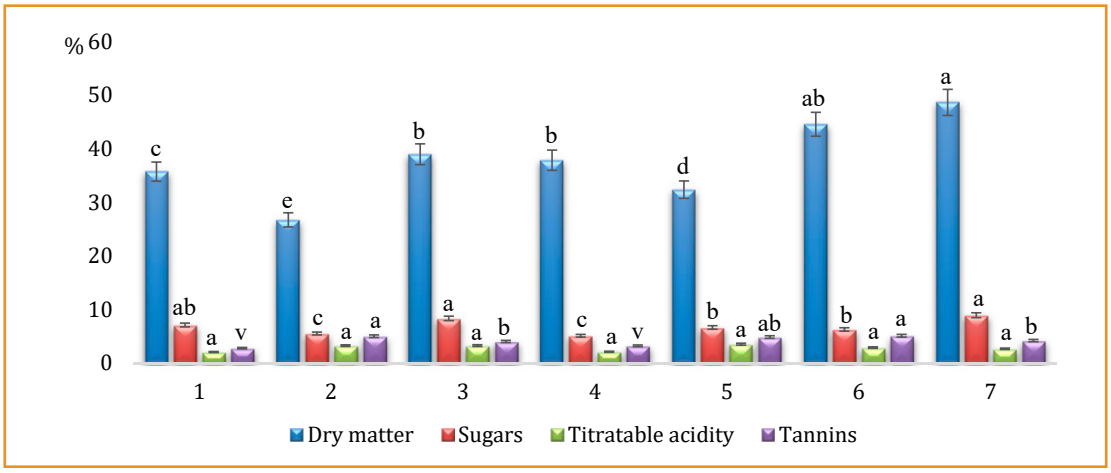

Figure 1 The content of dry matter, reducing sugars, tannins, and titrable acidity in the plant raw material of *Artemisia* L. species at the period of budding
1 – *A. abrotanum*; 2 – *A. annua*; 3 – *A. argyi*; 4 – *A. austriaca*; 5 – *A. japonica*; 6 – *A. ludoviciana*; 7 – *A. maritima* (means in columns followed by different letters are different at $p = 0.05$. Each value represents the mean of three independent experiments (±SD))

According to Iqbal et al. (2012), the content of carbohydrates was 8.3%, fat 6.09%, fiber 14.2%, total tannins 0.61%. Tannins are naturally occurring polyphenol compounds that form complexes with proteins (Singh et al., 2012). As reported Lutgen (2018), the content of

tannins in different organs of various species of *Artemisia* from Turkey, Iran, Algeria varied but leaves of *A. annua* had a higher content of tannins in approximately 10 times. Singh et al. (2012) determined the content of tannins expressed on gallic acid equivalent to 30.44 mg/g in the water extract. The dry matter of *A. annua* powder in an investigation of Panaite et al. (2018) was 88.30%. Also, we determined the accumulation of ascorbic acid and carotene concentration in the above-ground part of the investigated plants (Figure 2).

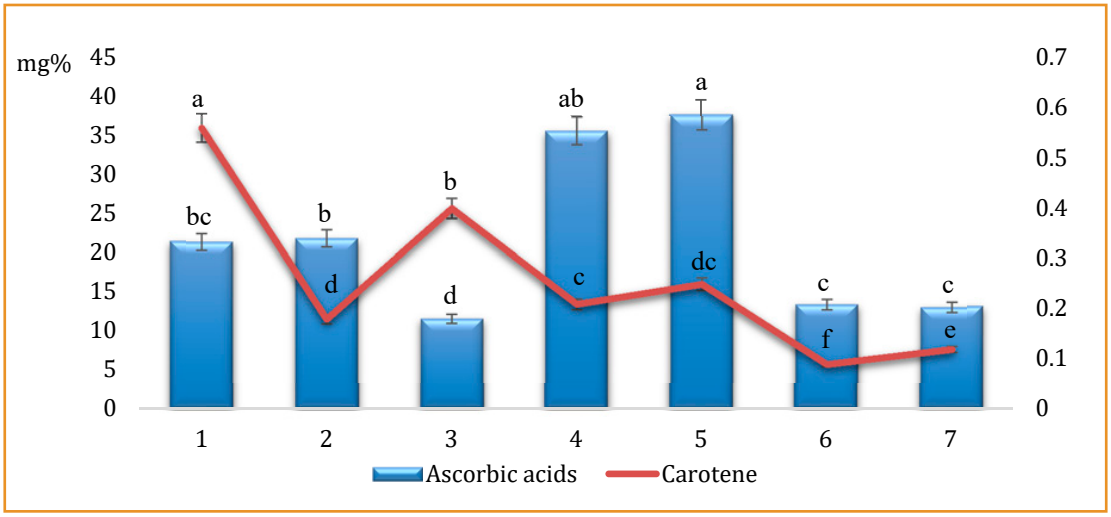

Figure 2 The content of ascorbic acid and carotene in the plant raw material of *Artemisia* L. species at the period of budding
1 – *A. abrotanum*; 2 – *A. annua*; 3 – *A. argyi*; 4 – *A. austriaca*; 5 – *A. japonica*; 6 – *A. ludoviciana*; 7 – *A. maritima* (means in columns followed by different letters are different at $p = 0.05$. Each value represents the mean of three independent experiments (±SD))

Ascorbic acid content at the stage of budding was from 11.65 (*A. argyi*) to 37.68 (*A. japonica*) mg%. We identified the content of carotene from 0.09 (*A. ludoviciana*) to 0.56 (*A. abrotanum*) mg%.

Smoylovska et al. (2010) found the content of ascorbic acid in *A. absinthium* during vegetation 0.035–0.113% (its matches 35–113 mg%). The most content of ascorbic acid determined in the full spring vegetation and budding stage.

Observations on plant growth showed that three species only passed to the next stage of growth (flowering): *A. abrotanum*, *A. annua*, and *A. austriaca*. We found that the content of dry matter of three investigated plant species at the stage of flowering was from 31.64 (*A. annua*) to 42.74 (*A. austriaca*) % (Figure 3).

Content of sugars in our study was from 6.8 (*A. austriaca*) to 8.23 (*A. annua*) %, titrable acidity from 2.8 (*A. abrotanum*) to 4.66 (*A. annua*) %, and tannin content from 4.22 (*A. austriaca*) to 6.36 (*A. annua*) %. It should be noted that dry matter content, sugars content and titratable acidity for *A. abrotanum* at the stage of flowering was approximately the same as at the stage of budding, while the content of tannins at the stage of flowering increased 1.7 times. The

values of these investigated parameters for *A. annua*, *A. austriaca* increased in the stage of flowering comparing with a budding stage.

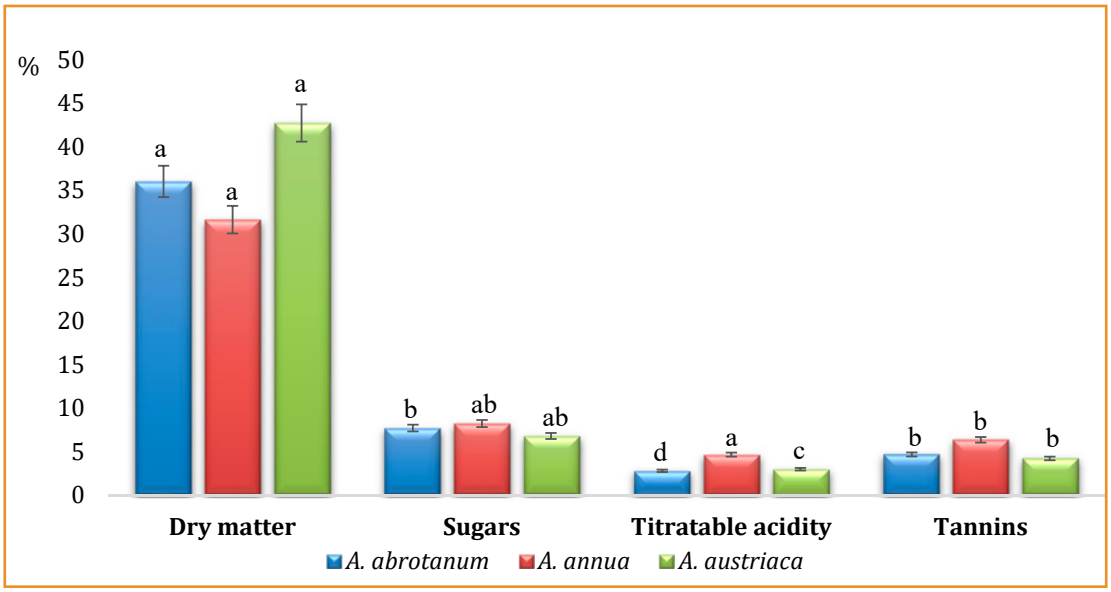

Figure 3 The content of dry matter, reducing sugars, tannins, and titrable acidity in the plant raw material of *Artemisia* L. species at the period of flowering (means in columns followed by different letters are different at $p = 0.05$. Each value represents the mean of three independent experiments (±SD))

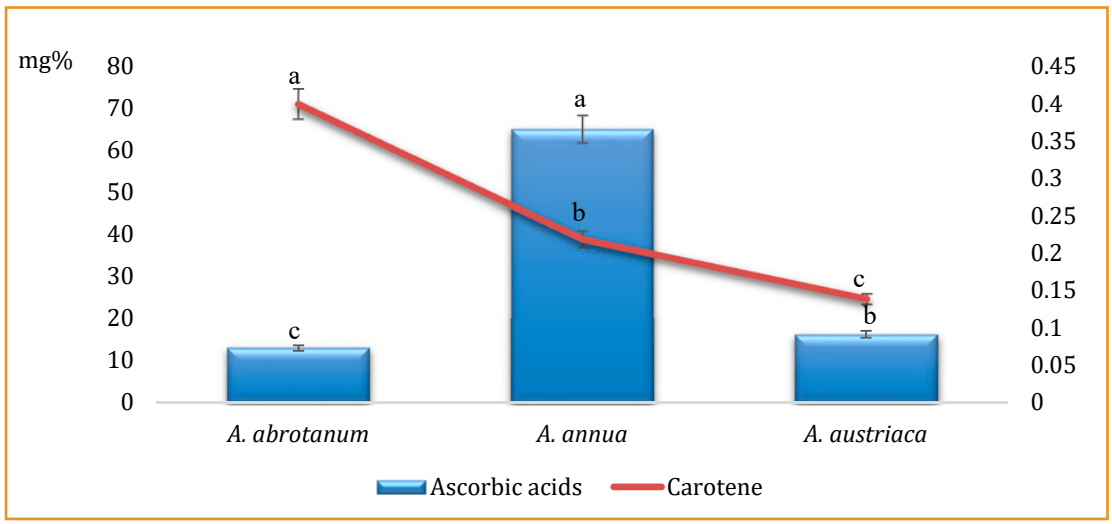

Figure 4 The content of ascorbic acid and ß-carotene in the plant raw material of *Artemisia* L. species at the period of flowering (means in columns followed by different letters are different at $p = 0.05$. Each value represents the mean of three independent experiments (±SD))

The ascorbic acid content in raw of investigated species at the stage of flowering was from 12.93 (*A. abrotanum*) to 65.18 (*A. annua*) mg% (Figure 4). At the stage of flowering carotene accumulated in the range from 0.14 (*A. austriaca*) to 0.22 (*A. annua*) mg%. Content of ascorbic acid and carotene decreased from the budding stage to flowering for *A. abrotanum* and *A. austriaca*, while for *A. annua* increased. Taking into account previous studies with *A. dracunculus*, the highest content of ascorbic acid found in the leaves at the stage of spring vegetation up to 730 mg%, the least content found in stems of this species at the stage of flowering 24.6 mg% (Korablova, 2003).

We also conducted a correlation analysis between the accumulation of investigated compounds in two stages of growth (Table 1). We found that at the period of budding very strong correlation was between titratable acidity and tannin content (r = 0.824), moderate correlation between dry matter and sugars content (r = 0.581), a weak correlation between sugars and titratable acidity (r = 0.127), and very weak correlation was between carotene and ascorbic acid content (r = 0.023).

Table 1 Correlation analysis between investigated parameters of *Artemisia* spp.

Parameter	Dry matter	Sugar content	Titratable acidity	Tannin content	Ascorbic acid content
Budding					
Sugar content	0.581*	1			
Titratable acidity	-0.311*	0.127*	1		
Tannin content	-0.094	-0.081	0.824*	1	
Ascorbic acid content	-0.527*	-0.622*	-0.053*	-0.142	1
ß-carotene content	-0.299	0.202*	-0.278	-0.690	0.023*
Flowering					
Sugar content	-0.999	1			
Titrable acidity	-0.743*	0.720*	1		
Tannin content	-0.905*	0.890	0.957*	1	
Ascorbic acid content	-0.764	0.741*	0.999*	0.966*	1
ß-carotene content	-0.413	0.445*	-0.302	-0.013*	-0.272

Note: significant according to the t-test (p <0.05)

At the stage of flowering determined a very strong correlation between sugars and tannin content (r = 0.890), titratable acidity and tannins (r = 0.957), titratable acidity and ascorbic acid content (r = 0.999), tannins and ascorbic acid content (r = 0.966). Strong correlation found sugars and titratable acidity (r = 0.720), sugars and ascorbic acid content (r = 0.741). The moderate correlation found between sugars and carotene (r = 0.445). Rest relations determined as negative correlated.

Conclusions

Taking into account obtained data of the biochemical composition of *Artemisia* L. spp., it should be noted that in the M.M. Gryshko National Botanical Garden of the NAS of Ukraine just plants of *A. abrotanum*, *A. annua*, and *A. austriaca* passed in the period of flowering. Investigation of the biochemical composition of *Artemisia* species showed a very strong correlation between titratable acidity and tannin content in both budding and flowering stages, sugars and tannin content and ascorbic acid with tannin content and ascorbic acid with titratable acidity. The most content of dry matter and sugars at the budding stage was maximal in raw of *A. maritima*, titratable acidity and ascorbic acids in raw of *A. japonica*, tannin content in raw *A. ludoviciana*, carotene content in raw of *A. abrotanum* (as well as in flowering stage). At the flowering stage, the high content of dry matter found in raw *A. austriaca*, the total content of sugars, tannins, ascorbic acid, and titratable acidity in raw *A. annua*. Obtained data can be used for the deep further biochemical and pharmacological study.

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SPECTRAL QUALITY OF SUPPLEMENTAL LED GROW LIGHT ALTERS CHILLING TOLERANCE IN ORNAMENTAL PLANTS

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The spectral properties of light changes the direction and intensity of metabolic processes in the plant, which allows it to adapt to changing environmental conditions. The influence of the spectral properties of light on plant physiology is still not fully understood, and a significant part of the research is devoted to the short-term effects of processing with monochromatic light in growth chambers. The purpose of this study was to determine whether the spectral modifications of additional LED lighting in greenhouse production can alter the characteristics of ornamental plants after growing, which is susceptible to chilling injuries. We grew snapdragons (*Antirrhinum majus nanum* L.) cv. Flora Shower White, tagetes (*Tagetes patula* L.) cv. Karmen, and multi-flowered petunia (*Petunia hybrida* L.) cv. Mambo blue in greenhouse conditions, using two different additional methods of irradiation with LEDs: 100% red (λ = 600 nm) (RL variant) and 100% blue (λ = 400 nm) (BL variant), followed by modeling the effect on the seedling of short-term cooling. The plants studied in the experiment are often used in urban landscaping and are exposed to low positive and zero temperatures, especially in the first days after transplanting into open ground. Morphological indicators and levels of hormonal regulators (salicylic acid (SA) and abscisic acid (ABA)) and water-soluble sugars, as well as changes in the selective permeability of cell membranes, were determined at the end of the exposure period. Light treatment showed a noticeable effect on the resistance of seedlings to cooling. Short-term exposure to low temperatures caused a violation of the semipermeability of cell membranes in all experimental variants, but it was minimal in plants under exposure to spectral light: the electrolyte yield decreased by 50% in comparison with the control in petunias, up to 37% in snapdragon and up to 37% in tagetes eighteen %. In the test variants, after cooling, the yield of potassium ions and the content of salicylic acid decreased – by 9 and 14% (RL) and 21, respectively, by 37% (BL), and the content of water-soluble monosugars increased. After cooling, tagetes quickly restored turgor in the RL variant, but in the control and when using BL this process was slowed down, there was damage on the leaf edges. Plants of snapdragons and petunias under illumination (RL and BL) quickly recovered, they maintained a habitus close to the original, and budding began. In control plants, restoration proceeded more slowly; their decorative qualities were low. When transplanted into the open ground (at night temperatures of 2–5 °C), the survival rate of ornamental plants after exposure was 80–100% versus 60–70% in the

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control. Additional illumination with narrow-spectrum light when growing seedlings of ornamental plants can reduce the death of plants from spring frosts.

Keywords: snapdragon, tagetes, petunia LED light, chilling injury, cell membranes, water-soluble carbohydrates, salicylic acid

Введение

Проблема повышения устойчивости декоративных растений к неблагоприятным условиям среды имеет важное научное и практическое значение. При этом одним из актуальных направлений остаются исследования, направленные на выявление механизмов адаптации растительных организмов к низким температурам. Так как низкотемпературный стресс и вызванные им повреждения растительных тканей являются одним из основных факторов ограничивающих рост растений, их продуктивность, а также распространение по климатическим зонам (Turchaninova, 2006; Trunova, 2007). Этот фактор не теряет своего значения и при озеленении городов, когда рассаду декоративных теплолюбивых растений пересаживают из оранжерейных условий в грунт. Формирование холодоустойчивости декоративных растений обусловлено различными изменениями клеточного метаболизма, в том числе увеличением содержания сахаров, синтезом стрессовых гормонов, изменением свойств мембран, процессов дыхания, фотосинтеза и др. (Tarchevsky, 2001; Avercheva et al., 2009).

В настоящее время к числу приоритетных направлений современной агробиотехнологии относится изучение механизмов регуляции роста и устойчивости растений к разным по природе неблагоприятным факторам среды под влиянием различных факторов, например, таких как узкополостный спектральный свет. Известно, что спектральный состав света меняет интенсивность и направленность метаболических процессов в растении (Olle and Viršilė, 2013; Avercheva et al., 2009). Интенсивность освещения, его продолжительность и длина волны могут вызвать экспрессию определенных генов и синтез ряда новых веществ, влияющих на генеративные органы растений (Causin et al., 2006; Vanninen et al., 2010; Colquhoun et al., 2013). Однако физиолого-биохимические основы адаптации остаются недостаточно изученными. Существует предположение, что узкоспектральный свет связан с активизацией COR генов, инициирующих синтез белков холодового стресса. В этот каскад взаимосвязанных преобразований вовлекается несколько светозависимых реакций, что существенно корректирует метаболизм растений; меняется гормональный и углеводный статус его клеток, проницаемость их мембран, активизируется или ингибируется работа отдельных ферментов (Chinnusamy et al., 2006; Crosatti et al., 2013). В этот каскад взаимосвязанных преобразований вовлекаются несколько светозависимых реакций, что существенно корректирует метаболизм растения: меняется гормональный и углеводный статус клеток, проницаемость их мембран, активируется или ингибируется работа некоторых ферментов (Kreslavsky et al., 2007; Trotta et al., 2014; Van Gelderen et al., 2018). Повышение устойчивости растений

к гипотермии связано с подавлением окислительного стресса за счет связывания активных форм кислорода (АФК) и свободных радикалов, благодаря действию антиоксидантных ферментов и накоплению низкомолекулярных органических антиоксидантов (Pennycooke et al., 2005; Patton et al., 2007). При адаптации к холодовому стрессу растения аккумулируют стресс-протекторные вещества – аминокислоты, растворимые сахара, сахароспирты и другие метаболиты (Patton et al., 2007; Van Gelderen et al., 2018).

Высказывается мнение, что COR-гены активируются при облучении красным (λ = 660 нм) и синим (λ = 400 нм) светом, то есть в регуляцию экспрессии этих генов вовлекаются световоспринимающие рецепторы фитохромы и криптохромы (Crossatti et al., 1999; Zhao et al., 2014). Информация о стабилизирующем влиянии красного и синего света при регуляции экспрессии генов в стрессовых условиях позволяет предположить наличие первичных элиситорных сигналов различной природы. Адаптацию к понижению температуры моделирует ряд взаимосвязанных процессов, позволяющих растению изменить клеточный и метаболический гомеостаз (Rejeb et al., 2014). Существенную роль в работе этих каскадных механизмов играют салициловая (СК) и абсцизовая (АБК) кислоты. СК – один из метаболитов, инициирующих экспрессию генов синтеза ферментов антиоксидантной защиты, что позволяет контролировать АФК, сохранить целостность клеточных мембран и окислительно-восстановительный статус клеток растения (Janda et al., 2007; Mahdavian et al., 2008; Yuan and Lin, 2014). Накопление АБК в тканях включает АБК-сигнальный каскад, который завершается экспрессией COR-генов, определяющую холодовую толерантность вида (Shi and Yang, 2014).

В представленной работе мы изучали влияние узкополосного красно-синего спектрального освещения на рост и развитие рассады декоративных растений и в дальнейшем определяли влияет ли данная подсветка на формирование устойчивости рассады к непродолжительному воздействию низких положительных температур при весенних заморозках.

Материалы и методика

Объекты исследования

В качестве объектов исследования были взяты декоративные растения, часто используемые в открытом грунте при озеленении городов – львиный зев (*Antirrhinum majus nanum* L.), низкорослый сорт Flora Shower White, тагетис (*Tagetes patula* L.), низкорослый сорт Karmen, и многоцветковая петуния (*Petunia hybrida* L.) сорта Mambo blue.

Растения в стадии 5 – 7-го листа высаживали в сосуды с песком (по 5 растений, в каждом варианте по 15 сосудов). Растения выращивали в следующих условиях: Контроль (1-й вариант – К) – растения, выращенные при естественном освещении. Вариант 2 – к естественному свету добавляли красный (max λ = 600 нм) (КС) свет; вариант 3 – к естественному свету добавляли синий (max λ = 400 нм) (СС) свет. В качестве

дополнительных источников света использовали светодиодные лампы модели ПС-2 (УСС-12) («Фокус», Россия). Интенсивность (плотность фотонов) для КС составляла $2{,}58\times10^{18}$ и для синего – $6{,}04\times10^{18}$ фотонов/(м^{2}Łс). Подсветку растений проводили ежедневно по 12 ч. Полив дистиллированной водой осуществляли ежедневно, подкормку проводили 1 раз в неделю 150 мл питательной смесью Кнопа (0,25 г фосфата калия, 0,25 г сульфата магния, 1 г кальциевой селитры и 0,125 г хлористого калия на 1 л дистиллированной воды). После окончания подсветки половину растений из каждого варианта (КС, СС, К) помещали на 24 ч в камеру с температурой 2 °С, оставшиеся растения оставляли без охлаждения. Пробы для биохимических анализов брали на 35-й (после окончания подсветки) и 37-й день (на 2-й день после воздействия холодового стресса).

Определение функционального состояния мембран

Для определения функционального состояния мембран клеток навеску листьев 0,3 г помещали в бидистиллят, выдерживали 24 ч в термостате при температуре 26 °С и измеряли электропроводность элюата, определяли в нем содержание ионов K^+ потенциометрически с использованием ионоселективных электродов (ИТАН, ООО «НПП «Томьаналит»», Россия); ионоселективный электрод Элит-031, ООО «НИКО Аналит», Россия) по ранее опубликованной методике (Kondratieva et al., 2011).

Определение содержания сахаров

Содержание моносахаров определяли спектрофотометрически (Specol 1300, «Analytik Jena AG», Германия) с помощью пикриновой кислоты (Kondratieva et al., 2008). Количество салициловой (СК) и абсцизовой (АБК) кислот анализировали из одной навески: 2 г сырых листьев экстрагировали этанолом (80 %), экстракт упаривали до водной фазы, которую делили на две равные по объему части. Для выделения СК и АБК экстракт очищали по модифицированной в лаборатории методике (Shelepova et al., 2012). На заключительном этапе использовали изократическую высокоэффективную жидкостную хроматографию (изократический хроматограф Стайер, ЗАО «Аквилон», Россия) с колонкой RP-18 (250/4,6 мм) («Phenomenex, Inc.», США).

Статистические методы

Статистическую обработку данных проводили с применением программы PAST v3.0 (Hammer et al., 2001). Определяли средние значения изучаемых показателей (M), стандартные ошибки средних (±SEM) и доверительный интервал при 95 % доверительном уровне ($t_{0,05}$ ±SEM). Достоверность различий между вариантами оценивали в программе PAST v3.0 методом непараметрической (критерий парных сравнений Шапиро-Уилкоксона) статистики. Различия между вариантами считали достоверными при $p \leq 0{,}05$.

Результаты и дискуссия

Перепады суточных температур в весенний и осенний периоды часто вызывают повреждения и даже гибель растений. Температура, использованная нами для

моделирования холодового стресса (2 °С), не летальна для изучаемых растений, но может вызвать существенные повреждения листьев (Petrovskaya-Baranova, 1983).

После окончания подсветки КС и СС светом особых различий по габитусу и морфологии у тагетиса по вариантам не было. И в опыте, и в контроле отмечали хороший тургор листьев, почти у 30 % растений началась бутонизация. У тагетиса, львиного зева и петунии после подсветки КС увеличилась биомасса надземной части растений (соответственно в 1,2, 1,3 и 1,6 раза) и корней (в 1,5; 1,2 и 1,8 раза) по сравнению с контролем (Рисунок 1). А после подсветки СС у всех трех видов декоративных растений увеличилась биомасса корней – в 1,8 раза у тагетиса и в 2,2 раза у львиного зева и петунии. Сроки начала цветения в опытных вариантах не отличались от таковых в контроле, но продуктивность цветения возросла на 19–23 % по сравнению с контролем. В мае при пересадке в открытый грунт (при дневной температуре 10–12 °С и ночной 2–5 °С) приживаемость растений в вариантах КС и СС была от 100 % (тагетис, петуния) до 80 % (львиный зев), тогда как у контрольных растений она составила 60–70 %.

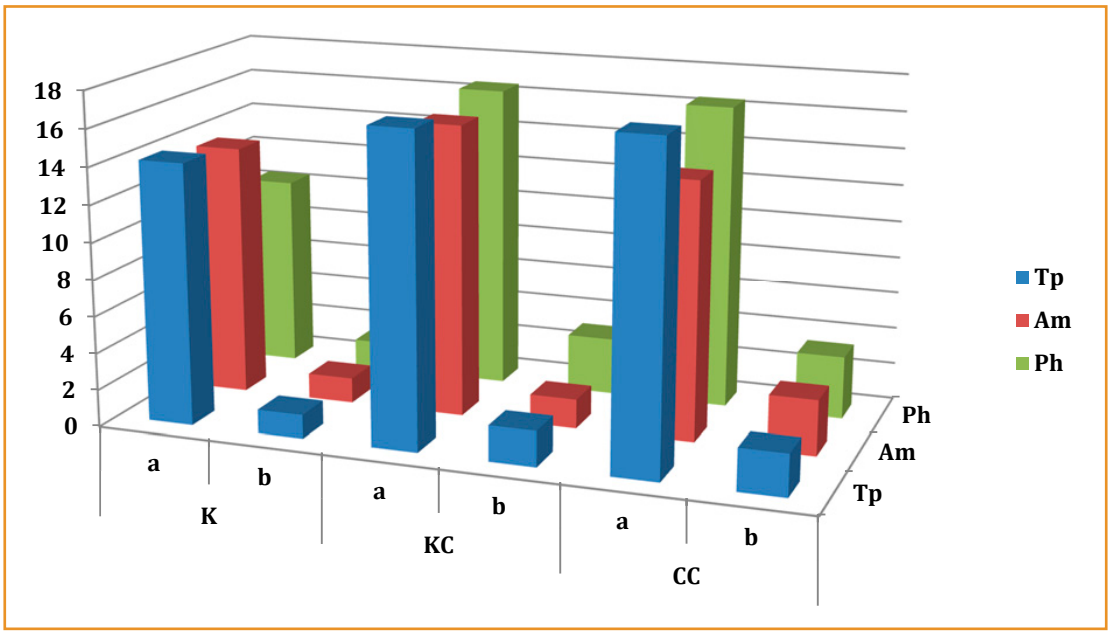

Рисунок 1 Влияние разных видов подсветка узкоспектральным светом на биомассу растений (г) варианты опыта – К (контроль); КС (красный свет); СС (синий свет). a – надземная часть растений; b – корни растений. Тр – тагетис (*Tagetes patula* L.), сорт Karmen; Am – львиный зев (*Antirrhinum majus nanum* L.), сорт Flora Shower White; Ph – петуния (*Petunia hybrida* L.), сорт Mambo blue

Figure 1 The influence of different types of illumination by LED light on the biomass of plants (g) experimental options – K (control); KC (red light); CC (blue light). a – aerial part of plants; b – plant roots. Tp – tagetes (*Tagetes patula* L.), cultivar Karmen; Am – snapdragon (*Antirrhinum majus nanum* L.), cultivar Flora Shower White; Ph – petunia (*Petunia hybrida* L.), cultivar Mambo blue

Важным показателем стрессоустойчивости растений служит состояние мембранной системы клеток листьев. Сохранение избирательной проницаемости плазмалеммы

для некоторых ионов и для молекул воды позволяет поддерживать гомеостаз клеток (Tarchevsky, 2001). Для его сохранения в клетке важно накопление ионов натрия в вакуоле, поддержание физиологической концентрации ионов калия и высокого соотношения K+/Na+ в цитоплазме (Munns and Tester, 2008), то есть увеличение выхода ионов калия указывает на негативные изменения во внутренней среде клетки. В тканях листьев у тагетиса после окончания досветки избирательная проницаемость мембран снизилась в варианте КС (27,4 µСм/мл) и осталась в пределах контроля (41,0 µСм/мл) в случае СС (40,6 µСм/мл), тогда как у растений львиного зева и петунии выход электролитов под воздействием КС возрос по сравнению с контролем незначительно (на 4,4 и 2,2 µСм/мл, соответственно), а в случае СС – значительно (на 24,5 и 10,3 µСм/мл, соответственно). При этом выход ионов калия существенно снизился в обоих вариантах опыта относительно контроля, что позволило сохранить высокое соотношение K^+/Na^+ в цитоплазме клеток растений, подвергавшихся подсветке (Рисунок 2).

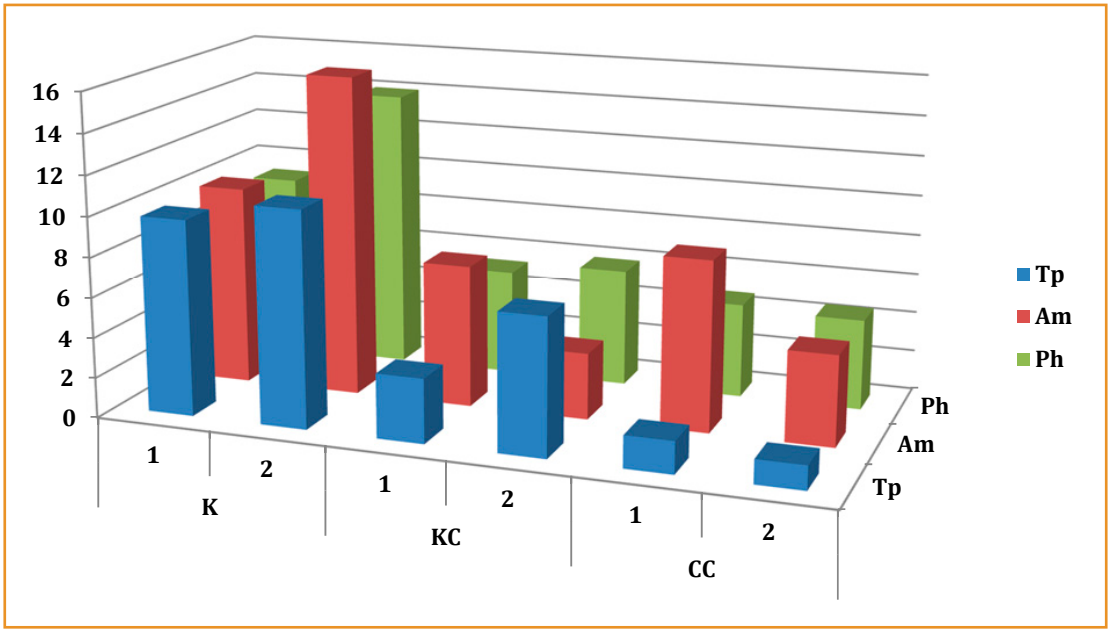

Рисунок 2 Влияние разных видов подсветки узкоспектральным светом на проницаемость клеточных мембран растений (выход ионов калия, мкг/мл): варианты опыта – К (контроль); КС (красный свет); СС (синий свет)
1 – показатель на момент завершения подсветки; 2 – показатель после воздействия холодового стресса. Тр – тагетис (*Tagetes patula* L.), сорт Karmen; Ам – львиный зев (*Antirrhinum majus nanum* L.), сорт Flora Shower White; Ph – петуния (*Petunia hybrida* L.), сорт Mambo blue

Figure 2 The influence of different types of illumination by LED light on the permeability of plant cell membranes (yield of potassium ions, µg/ml)
experimental options – K (control); KC (red light); CC (blue light). 1 – Indicator at the time of completion of exposure; 2 – Indicator after exposure to cold stress. Tp – tagetes (*Tagetes patula* L.), cultivar Karmen; Am – snapdragon (*Antirrhinum majus nanum* L.), cultivar Flora Shower White; Ph – petunia (*Petunia hybrida* L.), cultivar Mambo blue

У всех растений воздействие низкими положительными температурами привело к нарушению полупроницаемости мембран, но в контроле оно было максимальным. Наиболее устойчивыми к кратковременному холодовому стрессу оказались растения петунии, подсвеченные КС и СС: в тканях их листьев выход электролитов снизился на 51–52 % по сравнению с контролем, тогда как у тагетиса снижение составило 12–18 %, а у львиного зева – 9–37 % (Рисунок 2). При воздействии КС и особенно СС у всех растений достоверно снизился выход ионов калия. Следует отметить, что при досветке узкоспектральным светом восстановление растений после окончания холодового стресса шло быстрее, особенно в варианте КС, тогда как контрольные растения не только медленнее восстанавливались, но и снижалась их декоративная оценка.

Подсветка рассады декоративных растений узкоспектральным светом значимо не повлияла на содержание водорастворимых углеводов в тканях листьев – у всех трех декоративных растений данный показатель оставался на уровне контроля (Рисунок 3). Однако воздействие холодового стресса привело к увеличению содержания моносахаров у тагетиса, львиного зева и петунии в обоих вариантах (КС и СС) по сравнению с контролем, при этом наиболее существенно возросло (на 37 % у петунии и на 21 и 20 % у львиного зева и тагетиса, соответственно) содержание водорастворимых углеводов под влиянием СС. Известно, что моносахара, не только служат энергетическим ресурсом, но и играют существенную протекторную роль при сохранении гомеостаза клеток при стрессе (Trunova, 2007). Вероятно, в нашем опыте они способствовали формированию реакции растений, обеспечивающей их выживание в стрессовых условиях гипотермии.

Одним из триггеров протекторного сигнального пути служит салициловая кислота. Ее роль в запуске и регуляции адаптационного механизма неоднозначна (Yuan and Lin, 2014). Недостаток или избыток СК может вызвать усиление стрессового воздействия, так как количества СК и АФК коррелируют, а с содержанием СК связана инициация каскада защитных реакций (Mateo et al., 2006), который светозависим и действует в комплексе с другими протекторными механизмами (Bechtold et al., 2005). У всех растений после окончания подсветки количество СК возросло по сравнению с контролем, наиболее существенно – под влиянием КС у тагетиса (на 223 % по сравнению с контролем) (Рисунок 4). По-видимому, в этом случае начал меняться гормональный баланс в тканях листьев и, как следствие, перестраивался весь метаболизм растений. После холодового стресса содержание СК у растений тагетиса в обоих вариантах снизилось как по сравнению с контролем (на 29–61 %), так и относительно показателей до воздействия холодового стресса: в варианте КС – в 2,3 раза, СС – в 2,0 раза. У растений львиного зева и петунии после окончания подсветки КС и СС содержание СК значимо не изменилось по сравнению с контролем. Воздействие холодового стресса на подсвеченные КС и особенно СС растения обоих видов привело к увеличению содержания СК – на 9 и 14 % (вариант КС) и на 21 и 37 % (вариант СС) соответственно у львиного зева и петунии.

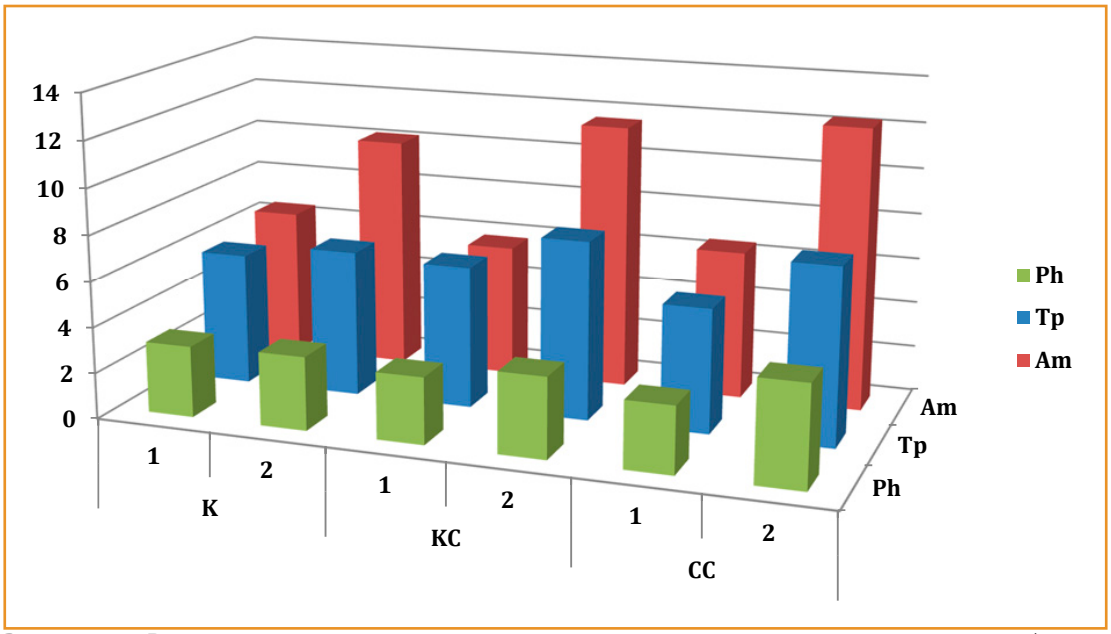

Рисунок 3 Влияние разных видов подсветки узкоспектральным светом на содержание совободных моносахаров (мкг/мг сухого вещества)
варианты опыта – К (контроль); КС (красный свет); СС (синий свет). 1 – показатель на момент завершения подсветки; 2 – показатель после воздействия холодового стресса. Tp – тагетис (*Tagetes patula* L.), сорт Karmen; Am – львиный зев (*Antirrhinum majus nanum* L.), сорт Flora Shower White; Ph – петуния (*Petunia hybrida* L.), сорт Mambo blue

Figure 3 The influence of different types of illumination by LED light on on the content of free monosugars (μg/mg dry matter)
experimental options – K (control); KC (red light); CC (blue light). 1 – indicator at the time of completion of exposure; 2 – indicator after exposure to cold stress. Tp – tagetes (*Tagetes patula* L.), cultivar Karmen; Am – snapdragon (*Antirrhinum majus nanum* L.), cultivar Flora Shower White; Ph – petunia (*Petunia hybrida* L.), cultivar Mambo blue

Следует отметить, что растения тагетиса при подсветке синим светом после воздействия холодового стресса быстро потеряли тургор, началось повреждение краев листьев. Растения в этом варианте опыта долго восстанавливались от повреждений, поздно зацвели, почти треть из них погибла. Менее выраженные признаки повреждений были у контрольных растений, они перенесли охлаждение лучше, но были менее декоративны (слабое ветвление, мелкие бутоны). Растения, подсвеченные красным светом, имели хороший тургор листьев, у них отмечали раскрытие цветочных бутонов. Возможно, изменение гормонального статуса тканей тагетиса при подсветке красным светом способствовало включению протекторного каскада реакций, которые нивелировали негативные последствия от охлаждения. Изменение соотношения гормонов под воздействием синего света не дало положительного эффекта. Растения львиного зева и петунии быстро восстановились после холодового стресса в обоих вариантах опыта (КС и СС): их тургор, надземная масса и габитус почти не изменились. При этом в контроле восстановление после холодового стресса у львиного зева и петунии шло медленно, снизилась масса надземных (на 15 %) и подземных (на

10 %) органов, декоративная оценка неконтрольных растений была ниже, чем при подсветке.

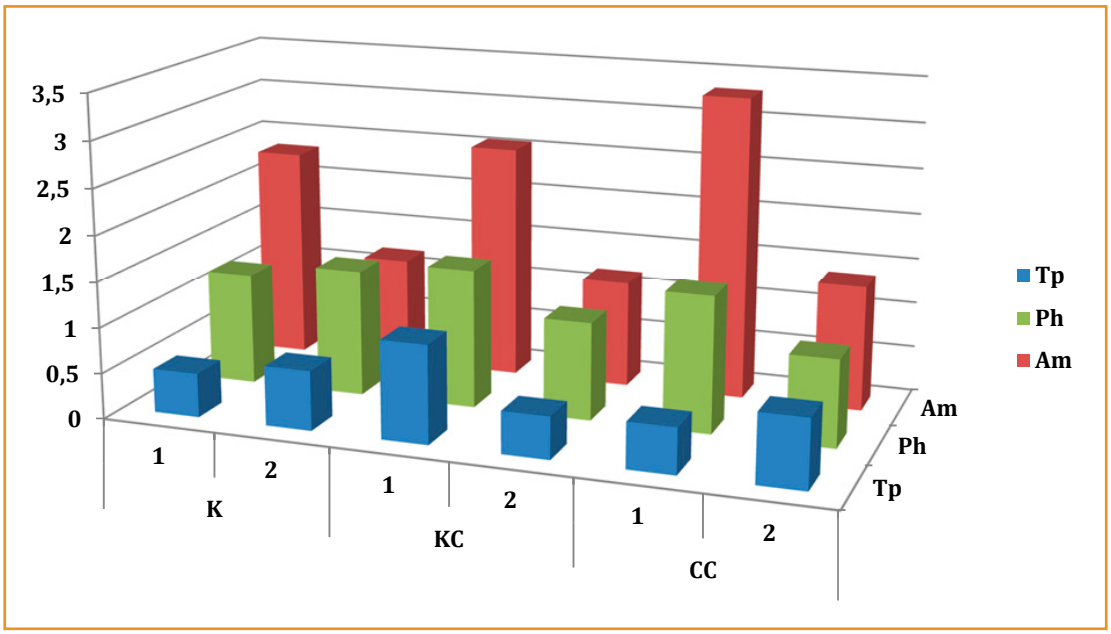

Рисунок 4 Влияние разных видов подсветки узкоспектральным светом на содержание салициловой кислоты (мкг/г сырого вещества)
варианты опыта – К (контроль); КС (красный свет); СС (синий свет). 1 – показатель на момент завершения подсветки; 2 – показатель после воздействия холодового стресса. Тр – тагетис (*Tagetes patula* L.), сорт Karmen; Ам – львиный зев (*Antirrhinum majus nanum* L.), сорт Flora Shower White; Ph – петуния (*Petunia hybrida* L.), сорт Mambo blue

Figure 4 The influence of different types of illumination by LED light on the content of salicylic acid (μg/g of flabby matter)
experimental options – K (control); KC (red light); CC (blue light). 1 – indicator at the time of completion of exposure; 2 – indicator after exposure to cold stress. Tp – tagetes (*Tagetes patula* L.), cultivar Karmen; Am – snapdragon (*Antirrhinum majus nanum* L.), cultivar Flora Shower White; Ph – petunia (*Petunia hybrida* L.), cultivar Mambo blue

В инициацию каскадных реакций, формирующих ответ на абиотический стресс, также вовлечена абсцизовая кислота (Shi and Yang, 2014). Этот гормон определяли только в тканях растений тагетиса. После окончания подсветки содержание АБК в тканях растений возросло под влиянием КС (до 0,191 ±0,02 мкг/г) и несколько снизилось (до 0,037 ±0,005 мкг/г) в случае СС по сравнению с контролем (0,043 ±0,003 мкг/г). После воздействия холодового стресса количество АБК в тканях листьев в варианте СС существенно увеличилось по сравнению с исходным (0,066 ±0,008 мкг/г), в контроле – почти не изменилось (0,048 ±0,004 мкг/г), а при подсветке красным светом – снизилось в 5 раз (до 0,038 ±0,004 мкг/г). То есть эти результаты также свидетельствуют в пользу нашего предположения об изменении гормонального баланса в тканях листьев тагетиса при подсветке и, как следствие, о перестройке всего метаболизма растений.

Возможно, свет различного спектрального состава включал разные пути активации протекторных механизмов. Триггерную функцию в такой активации могут выполнять и СК, и АБК, их сигнальные пути частично перекрываются. Формируется информационная сеть с антагонистическими и синергическими звеньями (Trunova, 2007). Возможно, в случае СС салициловая кислота способствовала быстрому переключению метаболических процессов на адаптационный режим, а при подсветке красным светом этот механизм мог включаться после выброса АФК, и салициловая кислота не вовлекалось в экспрессию защитных генов.

Выводы

Совокупность физиологических и биохимических изменений в тканях листьев декоративных растений (габитус, морфология, накопление биомассы, выход электролитов, содержание ионов калия, салициловой и абсцизовой кислот, суммы свободных моносахаров) позволяет сделать вывод, что подсветка спектральным светом способствует началу перестройки метаболических процессов и активации неспецифических протекторных механизмов, сохраняющих ионный и окислительно-восстановительный гомеостаз клеток. Показано, что подсветка рассады узкоспектральным светом позволила этим растениям успешно адаптироваться к кратковременному воздействию низких температур. При пересадке в открытый грунт (в условиях ночной температуры 2–5 °С) их приживаемость после подсветки составила 80–100 % против 60–70 % в контроле. Следовательно, добавление узкоспектрального света от светодиодных панелей к естественному освещению при выращивании рассады декоративных растений позволит снизить их гибель от резкой смены условий обитания при использовании для озеленения территорий.

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COMPARATIVE CHARACTERISTICS OF MORPHOMETRIC PARAMETERS OF ACHENES (SEEDS) FOR *ADENOCAULON ADHAERESCENS* MAXIM. (ASTERACEAE) IN NATIVE AND SECONDARY DISTRIBUTION RANGES

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Adenocaulon adhaerescens Maxim. is the potentially medicinal species with high antioxidant activity. Morphometric characteristics of achenes (seeds) for the species have been studied in four populations of native distribution range in Primorsky Krai (Russia). The distance from the southernmost (Popova Island) to the northernmost (buffer zone of the Ussuri Nature Reserve) of the seed collection point is about 100 km, and this, of course, is not enough to identify correlation between geographical latitude and seed size. The environmental conditions in all four habitats are also the same – shaded paths in the forest along streams or wet plots, so that light and moisture do not seem to influence the size of the seeds. Studied plants in the fruiting phase had an average height of 83.9 cm, with 70% of the main axis being the inflorescence and the number of lateral axes of the inflorescence ranging from 10 to 28. The size of the leaf at the base of the panicle was, on average, 8.8 × 10.6 cm. Plants on Russky Island have reliably smaller achenes (5.4 × 1.9 mm) than in other studied populations (6.7 × 2.3 mm). It was noted that the length and diameter of the achene vary in the native distribution range at a low level (*CV* = 10–12%). However, we were surprised to compare our data with ones in the Moscow: in the secondary distribution range, which is almost 9 thousand kilometers away from natural habitats, the average size of seeds of this species has not changed over 70 years of introduction. In the native distribution range, seed productivity of one plant is two to three times higher than in the secondary distribution range formed in the Moscow. This is explained not by the large number of seeds in a head (5–7 seeds), but by the large number of heads formed on the plant (46–77 vr. 25–30 heads).

Keywords: *Adenocaulon adhaerescens*, seed, achenes, morphometric characteristic, native distribution range, Primorsky Krai

Введение

Дальневосточный вид прилипало пристающее (*Adenocaulon adhaerescens* Maxim.) имеет необычные для семейства Asteraceae Bercht. & J.Presl морфологические

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признаки. На семянках отсутствует паппус, но есть крупные железки (Karrfalt and Kreitner, 1980). Краевые цветки женские, а срединные – мужские, тогда как в норме для семейства характерно как раз наличие женских цветков в центре корзинки. Род Adenocaulon относится к особой трибе Mutisieae (Funk et al., 2016).

Род *Adenocaulon* включает всего 6 видов: *A. bicolor* Hook., *A. chilense* Less., *A. lyratum* S.F. Blake, *A. nepalense* Bittmann, *Adenocaulon adhaerescens* Maxim. и *A. himalaicum* Edgew. Наиболее хорошо изучен последний вид, поскольку он обладает хозяйственно-ценными признаками. Экстракты растений *A. himalaicum* могут использоваться в качестве химиопрепаратов для профилактики и/или лечения рака человека (Yun et al., 2013). Его воздушные части применялись для лечения абсцессов, кровоизлияний и воспалений в корейской народной медицине, а ростки съедобны (Kwon and Lee, 2001).

Близкородственный американский вид *Adenocaulon bicolor* не используется столь широко, напротив, нежелателен как источник корма для оленей. Он является важным компонентом травянистого яруса в лесах из секвойи и служит растением-индикатором освещенности в лесном сообществе (Pfitsch and Pearcy, 1989a,b; Pearcy and Yang, 1998).

У всех видов рода *Adenocaulon* семенная продуктивность и параметры семянок не изучены. В России произрастает единственный вид – *Adenocaulon adhaerescens*. Его ареал занимает Приморье, Приамурье и острова Сахалин и Кунашир.

Этот вид успешно адаптировался к условиям московского климата и сформировал устойчивую инвазионную популяцию в Главном ботаническом саду Российской академии наук (Москва) и в ряде московских лесопарков. Выявлено, что этот вид может стать потенциальным источником биологически активных веществ для улучшения системы антиоксидантной защиты человека (Vinogradova et al., 2019). Ссылка на источники Наиболее высокой антиоксидантной активностью обладают молодые листочки, собранные в течение 1 – 2 недель после таяния снега, а также соцветия в стадии начала цветения (Vinogradova et al., 2019).

Во вторичном ареале морфо-биологические признаки *A. adhaerescens* изучены достаточно хорошо (Vinogradova and Rykhlikova, 2006; Vinogradova, 2010; Vinogradova, 2013). Одно растение формирует за вегетационный сезон около 25 – 30 корзинок, каждая из них содержит 6 – 8 цветков, и в ней образуется 5 – 7 семянок размером 6.8 ±0,1 × 2.5 ±0,0 мм, что составляет порядка 250 семян на одном растении (Ganina and Vinogradova, 2019). Однако в естественном ареале изменчивость биологических признаков этого вида до сих пор практически не изучена.

Цель настоящего исследования – оценить потенциальную семенную продуктивность и вариабельность размеров семянок *A. adhaerescens* в естественном ареале и провести сравнение с аналогичными параметрами во вторичном ареале на основе наших предыдущих исследований.

Материалы и методы

Материал исследования

Семянки *Adenocaulon adhaerescens* для анализа собирали в сентябре 2019 г. в четырех местообитаниях юга Приморского края:

A) остров Русский N 42.9712 E1 31.8858;

B) остров Попова N 42.9622 E 131.7249;

C) г. Владивосток, лесная территория ботанического сада N 43.2226 E 131.9932;

D) окрестности г. Уссурийска, буферная зона Уссурийского заповедника N 43.6323 E1 32.2651.

Каждый из четырех образцов семян включал все семянки, собранные с 10 растений из каждого пункта сбора. Семена хранили при комнатной температуре в течение недели. Проба для определения морфометрических признаков содержала не менее 70 семянок.

Методы исследования

На цифровом микроскопе Keyence VHX-1000 определяли длину (*L*) и диаметр семянок в самой широкой их части (*D*). Средний объем семянки вычисляли по формуле эллипсоида $4/3\ \pi\ ld^2$, где $l = 1/2$ L, $d = 1/2$ D; форму семянок – по соотношению L/D.

Статистическая обработка

Данные обрабатывали в программе PAST 3.17.

Результаты

Семянки *A. adhaerescens* обратнояйцевидные, зеленого или коричневого цвета, покрыты железками с клейким экссудатом, ось соцветия также опушена железистыми волосками (Рисунок 1).

На побережье острова Русский на мысе Тобизина N 42.9574 E 131.8768 мы обнаружили еще одну популяцию *A. adhaerescens*. Однако в этой популяции растения отличалась меньшей длиной соцветия (57 см), более мелкими листьями (7,3 × 8,9 см) и немногочисленными корзинками (в среднем 46 шт.) с мелкими семянками. Семянки, собранные в этой популяции, мы в анализ не включали, стараясь сравнивать семенную продуктивность растений средней для вида виталитетности.

В изученных четырех популяциях вторичного ареала растения в фазе плодоношения имели в высоту в среднем 83,9 см, причем 70 % главной оси приходилось на соцветие, а число боковых осей соцветия колебалось от 10 до 28. Размер листа в основании метелки составлял в среднем 8,8 × 10,6 см.

Размеры семянок варьировали незначительно, только образец из центральной части острова Русский достоверно отличался более мелкими (Рисунок 2) семянками. Длина семянок с острова Русский составляла 3,8 – 6 ,7 (5,4 ±0,0) мм, диаметр – 1,2 – 2,6 (1,9 ±0,0) мм, объем – 11,0 ±0,4 мм^3, отношение длины семянки к ее диаметру составляло 2,2 – 3,5 (в среднем 2,8). В этой же популяции одно растение в среднем

формировало меньшее число корзинок (от 7 до 96, в среднем 46), но все равно семенная продуктивность одного растения в полтора-два раза выше, чем во вторичном ареале (276 против 162 семянок).

Рисунок 1 *Adenocaulon adhaerescens* Maxim
1 – общий вид растения; 2 – плоды; 3 – пункты сбора семян для анализа; A – остров Русский; B – остров Попова; C – Ботанический сад, г. Владивосток; D – Уссурийский заповедник

Figure 1 *Adenocaulon adhaerescens* Maxim
1 – habitus of the plant; 2 – fruits; 3 – collection points for seed's analysis; A – Russky Island; B – Popova Island; C – Botanical Garden, Vladivostok; D – Ussurian Reserve

Рисунок 2 Семянка (слева) и ось соцветия (справа) *Adenocaulon adhaerescens* Maxim

Figure 2 Achene (left) and rachis of inflorescence (right) of *Adenocaulon adhaerescens* Maxim

Семянки у растений, произрастающих в остальных трех местообитаниях, были более крупными, но достоверного различия между этими тремя пробами не выявлено (Таблица 1). Длина семянок составляет 4,2 – 8,1 (6,7 ±0,0) мм, диаметр – 1,2 – 3,1 (2,3 ±0,0) мм, объем – 3,0 – 32,5 (18,5 ±0,4) мм^3, отношение длины семянки к ее диаметру составляло 2,1 – 3,9 (в среднем 3,0). Метелка содержала в среднем 77 корзинок (22 – 240), т.е. в три раза больше, чем во вторичном ареале в Москве, а максимальное число корзинок на растении достигало 240 шт.! Семенная продуктивность одного растения составляла в среднем 462 семянки, что также в три раза выше, чем во вторичном ареале.

Таблица 1 Морфометрические признаки семянок *Adenocaulon adhaerescens* Maxim.
Table 1 Morphometric characteristics of achenes for *Adenocaulon adhaerescens* Maxim.

Местообитание	***L* (mm)**			***D* (mm)**		
	$\bar{x} \pm S_x$	min	max	$\bar{x} \pm S_x$	min	max
A	5,4 ±0,1	3,8	6,7	1,9 ±0,02	1,2	2,6
B	6,5 ±0,1	5,4	7,4	2,3 ±0,01	1,8	3,1
C	6,6 ±0,1	5,0	8,1	2,3 ±0,01	1,7	2,9
D	7,0 ±0,1	4,2	8,0	2,2 ±0,01	1,2	2,8

Местообитание	***L/D***			***V* (mm^3)**		
	$\bar{x} \pm S_x$	min	max	$\bar{x} \pm S_x$	min	max
A	2,8 ±0,03	2,2	3,5	11,0 ±0,4	3,1	21,5
B	2,8 ±0,02	2,1	3,4	18,9 ±0,6	8,8	31,7
C	2,9 ±0,02	2,4	3,8	19,2 ±0,08	8,8	31,8
D	3,2 ±0,03	2,8	3,9	17,6 ±0,06	3,0	32,5

Примечание: A – остров Русский; B – остров Попова; C – Уссурийский заповедник; *D* – Ботанический сад, г. Владивосток; *L* – длина; *D* – ширина; *L/D* – отношение длины семянок к ширине; *V* – объем семянки; min – минимальное значение; max – максимальное значение; $\bar{x}$ – среднее значение; S_x – стандартное отклонение
Следовательно, размер семянок у растений из вторичного ареала (6,8 ±0,1 × 2,5 ±0,0) не имеет значительных отличий от размеров семянок растений, произрастающих в естественном ареале.

У всех четырех образцов изменчивость морфометрических признаков была низкой: коэффициент вариации составлял для длины семянок 9 %, диаметра семянок – 12 %, отношения *L/D* – 11 %. Объем семянок варьировал на среднем уровне: у образца с острова Русский 28 %, у сборного образца из остальных трех местообитаний – 31 %. По форме семянок образцы также не различались (Рисунок 3).

Следовательно, размер семянок у растений из вторичного ареала (6,8 ±0,1 × 2,5 ±0,0) не имеет значительных отличий от размеров семянок растений, произрастающих в естественном ареале.

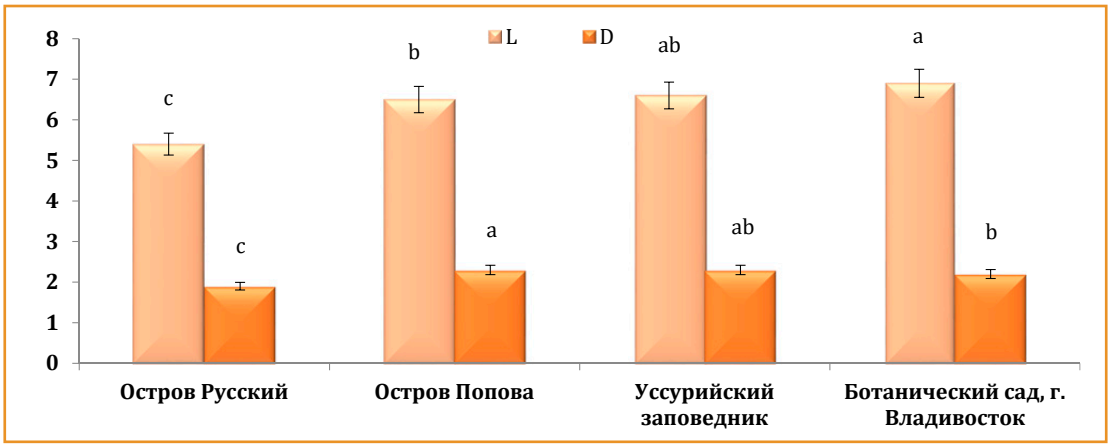

Рисунок 3 Морфометрические признаки семянок *Adenocaulon adhaerescens* Maxim
L – длина, мм; *D* – диаметр, мм

Figure 3 Morphometric characters of achenes of *Adenocaulon adhaerescens* Maxim
L – lenght, mm; *D* – diameter, mm

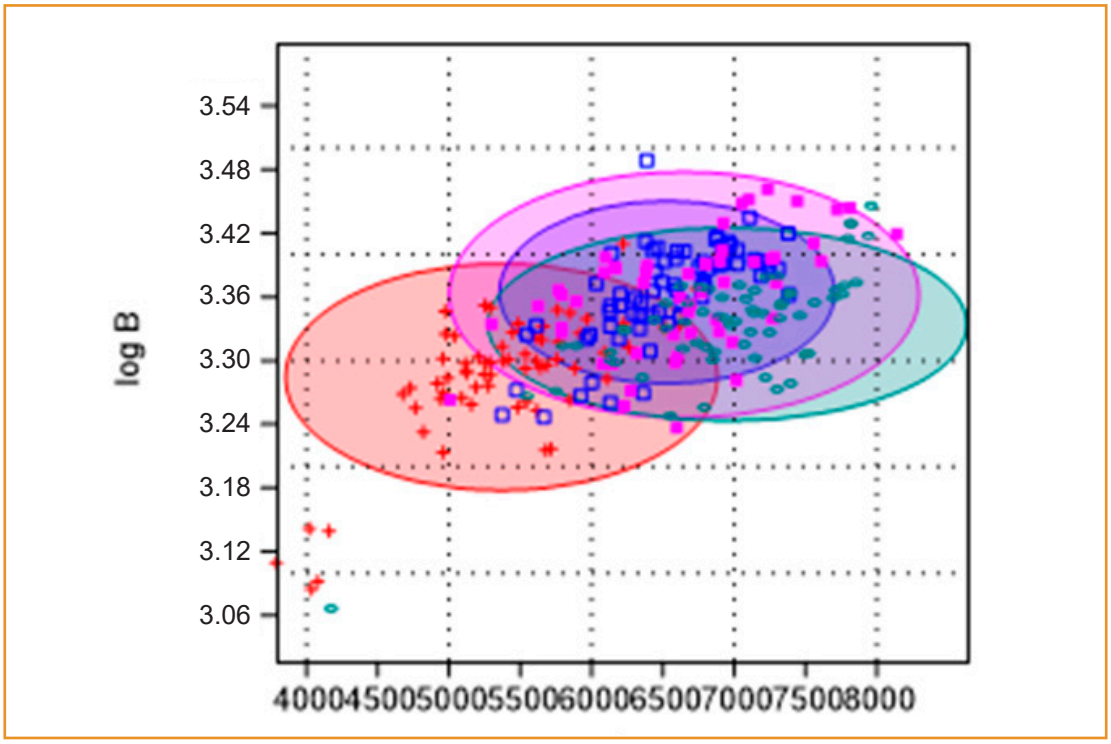

Рисунок 4 Форма семянок *L*/*D* (ось *y*) и средний объем семянок *V* (ось *x*, мм^3) *Adenocaulon adhaerescens* Maxim в различных местообитаниях Приморского Края
красный цвет – остров Русский; синий цвет – остров Попова; фиолетовый цвет – Уссурийский заповедник; зеленый цвет – Ботанический сад, г. Владивосток

Figure 4 Shape of achenes (*L*/*D*, *y*-axis) and average *V*, mm^3 (4/3 π*LD*^{2}, *x*-axis) of *Adenocaulon adhaerescens* Maxim in the different habitats in Primorsky Krai
red – Russky Island; blue – Popova Island; violet – Ussurian Reserve; green – Botanical Garden, Vladivostok

Обсуждение

Подобно другим видам рода *Adenocaulon*, семянки *A. adhaerescens* имеют железистые волоски и поэтому легко рассеиваются, прилипая к одежде человека или шерсти животных (Redonda-Martínez et al., 2020). К сожалению, нет литературных данных о размерах семянок близкородственных видов *Adenocaulon*, однако для множества других видов отмечено значительное варьирование размеров семянок в зависимости от экологических факторов (Massimi, 2018; Pinto et al., 2018). Что касается оценки характеристик природных и инвазионных популяций в полевых условиях, то исследований здесь довольно много. Интерес к этому вопросу возрос после появления гипотезы увеличения конкурентной способности вида EICA, которая утверждает, что после избавления от природных врагов, во вторичном ареале эволюция заносных видов ускоряется. Если это происходит в результате распределения ресурсов между ростовыми и защитными механизмами, естественный отбор должен идти в сторону создания менее защищенных, но более конкурентоспособных генотипов в условиях вторичного ареала (Blossey and Nötzold, 1995). Основной путь проверки этой гипотезы состоит в сравнении потомков природных и интродукционных популяций в устойчивых условиях среды. Однако работы, в основном, касаются размеров популяций и высоты растений, а полученные результаты противоречивы. Тем не менее, семь из девяти сравнений размеров и плодовитости растений показывают, что эти параметры выше во вторичном ареале (Khoroon, 2014).

Расстояние от самого южного (остров Попова) до самого северного (буферная зона Уссурийского заповедника) пункта сбора семян *A. adhaerescens* составляет по прямой около 100 км, и это, разумеется, недостаточно, чтобы уловить связь между географической широтой местности и размером семянок. Экологические условия произрастания растений во всех четырех местообитаниях также однотипны – это затененные тропинки в лесу вдоль ручьев или топких мест, так что на размер семянок свет и влага, по-видимому, не оказывают влияния. Отличие популяции с острова Русский по наиболее мелким семянкам мы объясняем расположением популяции недалеко от морского побережья и сильным антропогенным воздействием (уплотнением грунта многочисленными туристами).

Однако удивительным для нас оказался факт, что и в Москве, во вторичном ареале, удаленном от естественных местообитаний почти на 9 тыс. километров, средний размер семянок этого вида за 70 лет интродукции не изменился.

Выводы

Размеры семянок у растений *A. adhaerescens* в естественном ареале варьируют в зависимости от местообитания: на острове Русский семена достоверно мельче (5,4 × 1,9 мм), чем в остальных изученных популяциях (6,7 × 2,3 мм). В естественном ареале семянки *A. adhaerescens* не различаются по параметру L/D, а также по коэффициенту вариации параметров L и D. Растения *A. adhaerescens* во вторичном ареале в Москве отличаются от растений естественного ареала не по размерам семянок

и не по числу семянок в корзинке, а по числу формирующихся на генеративном побеге корзинок: в Москве число корзинок в среднем 25 – 30, тогда как в естественном ареале 46 – 77. Таким образом, семенная продуктивность одного растения в естественном ареале в два-три раза выше, чем во вторичном ареале.

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METAL RESISTANCE AND TOLERANCE TO TEMPERATURE STRESS OF PLANTS INOCULATED WITH ENDOPHYTE *CYLINDROCARPON MAGNUSIANUM* WOLLENW.

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Inoculations of *Cylindrocarpon magnusianum* Wollenw. endotrophic micromycete and inoculant reactions to the action of heavy metal salts in the substrate (for *Solánum lycopérsicum* L.) and the effect of high temperatures (for *Poa pratensis* L.) were studied. The experimental design included inoculation with the culture of the fungus and populations of the fungus adapted to the action of the stress factor. Then, inoculated plants were grown under control conditions and on substrates with the addition of different concentrations of zinc, copper, lead and chromium salts, or under conditions of temperature stress.We have not revealed a stimulating effect that increases the resistance of plants to the action of salts of heavy metals during inoculation of plants with a control population of the fungus. When using non-biogenic chemical elements, adaptive plant reactions associated with the content of photosynthetic pigments in the leaves and the formation of plant biomass were significantly manifested when plants were inoculated with adapted populations of the fungus *C. magnusianum* on substrates with the addition of chromium and lead salts. Under these conditions, a more intense development of fungal infection in plant roots was observed, in contrast to the use of the control fungal population. High temperatures led to significant changes in the content of ascorbic acid, photosynthetic pigments in the leaves, the distribution of plastic material between the aerial part and the root system of plants in inoculated plants. These reactions are adaptive. The facts obtained indicate the most effective partnership of the fungus *C. magnusianum* and the root system of plants under conditions extreme for plant life.

Keywords: *Cylindrocarpon magnusianum*; fungi; heavy metals; inoculation; biochemical indices

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Введение

В настоящее время в научном сообществе повысился интерес к изучению роли консортивных связей растений с корневыми микромицетами. Определенные успехи достигнуты в изучении роли эндомикоризы и ее самой распространенной формы – арбускулярной микоризы (АМ), которая характерна для большинства современных филогенетических групп растений, представлена во всех биомах земного шара (Wilkinson, 2001). Она формируется грибами, принадлежащими к подотделу *Glomeromycotina* отдела *Mucoromycota* (Yurkov et al., 2018). Но использование АМГ в растениеводстве ограничено, что является следствием их облигатной симбиотрофии (Ijdo et al., 2011).

В связи с этим особый интерес вызывает изучение роли других групп корневых микромицетов эндофитов и их отдельных представителей в формировании механизмов устойчивости у высших растений. Исторически, были выделены две группы эндофитов (*Clavicipitaceous* (C) и *Nonclavicipitaceous* (NC)) на основе филогении и признаков жизненного цикла (Rodriguez et al., 2009; El-Samad and El-Hakeem, 2019). В целом эта разнородная группа грибов может оказывать сильное воздействие на растительные сообщества посредством обеспечения устойчивости растений к абиотическому и биотическому стрессам. Особый интерес представляют исследования роли эндофитов в формировании металлрезистентности растений, включая сельскохозяйственные культуры (Rodriguez et al., 2009; Ikram et al., 2018; El-Samad and El-Hakeem, 2019; Surbhi et al., 2019; Bilal et al., 2020), причем в отношении особо опасных для растений химических элементов (Ali et al., 2019; Bilal et al., 2019; Li et al., 2019; Sharma et al., 2019; Hou et al., 2020). Ряд исследований направлен на исследование возможности применения микромицетов в качестве гербицидов (Boyette et al., 2016; Boyette et al., 2018; Meepagala et al., 2019; Sobowale, 2019).

Одним из перспективных микромицетов является эндофит *Cylindrocarpon magnusianum* Wollenw. (Sogonov and Velikanov, 2004; Amaral et al., 2009; Bukharina and Islamova, 2016; Bukharina et al., 2019; Bukharina et al., 2019a). Установлено, что его метаболиты могут быть использованы в борьбе с нематодами (Amaral et al., 2009), он способен расти в условиях высокого содержания нефтепродуктов в почве (Sogonov and Velikanov, 2004; Amaral et al., 2009). В серии авторских экспериментов, проведенных с *C. magnusianum*, установлено, что культура этого гриба способна выдерживать действие высокого осмотического давления, сохраняя рост культурального мицелия. А опыты с инокулированными данным грибом растениями показали возможность его использования в качестве агента повышения солеустойчивости и термостойкости растений (Bukharina and Islamova, 2016; Bukharina et al., 2019; Bukharina et al., 2019a).

Целью наших исследований являлось изучение влияние инокуляции культурой гриба *C. magnusianum* на формирование адаптивных реакций растений к действию солей тяжелых металлов в субстрате (на примере тестовой культуры томата *Solánum lycopérsicum* L.) и к действию температурного стресса (на примере мятлика лугового (*Poa pratensis* L.).

Материал и методика

Материал исследования

Культура *C. magnusianum* выделена из корневой системы древесных растений (*Acer negundo* L. хорошего жизненного состояния), длительно произрастающих в условиях городских почв с высоким содержанием солей тяжелых металлов (примагистральные посадки, санитарно-защитная зона ОАО „Ижсталь", г. Ижевск, Удмуртия). Гриб культивируется на питательной среде вне корневой ситемы растений. Видовая принадлежность гриба установлена методами микроскопирования и молекулярного анализа ДНК в лаборатории Лейбницкого института овощных и декоративных культур (г. Берлин) (Bukharina et al., 2016).

Методы исследования

Согласно схеме эксперимента подготовлены популяции гриба, адаптированные к субстратам с разными концентрациями солей тяжелых металлов (ТМ), мг/л: А0 – контрольный; А1 – на субстрате с Zn_{100}; А2 – Cu_{50}; А3 – Cu_{100}; А4 – Cu_{150}; А5 – Pb_{10}; А6 – Pb_{50}; А7 – $Cr_{2,5}$; А8 – Cr_{10}. Мицелиальные диски культуры гриба (⌀ = 5 мм) были перенесены на пентозо-декстрозную агаризированную среду (PDA medium) с внесенными, согласно расчетным концентрациям, солями ТМ и инкубировались в течение двух недель в климатической камере «BinderKBWF720» при температуре + 25 °С. Затем были подготовлены суспензионные культуры этих популяций (содержание спор – 3 млн. шт./мл; фрагментов мицелия – 200 шт./мл) и проведена инокуляция растений методом полива сеянцев в период пикировки. Для приготовления суспензионных культур гриба в стерильный картофельный бульон с декстрозой (Potato Dextrose Broth) были внесены мицелиальные диски адаптированных популяций гриба и инкубировались в течение 10 дней в термо-шейкере-инкубаторе (температура +25 – 27 °С, вращение 60 оборотов/мин (Решение РОСПАТЕНТа о выдаче патента на изобретение, заявка №2019112511/10(024247) от 2.04.2020г .).

Опыт включал варианты:

1. инокулированные томаты (инокуляция контрольным изолятом А0) выращивались на субстратах с разным содержанием солей тяжелых металлов, мг/л: В0 – контрольный – без ТМ; В1 – Zn_{100}; В2 – Cu_{50}; В3 – Cu_{100}; В4 – Cu_{150}; В5 – Pb_{10}; В6 – Pb_{50}; В7 – $Cr_{2,5}$; В8 – Cr_{10};
2. томаты, инокулированные популяциями грибов, адаптированными к тяжелым металлам (А1 – А8), выращивались на субстратах без внесения (В0) и с внесением солей ТМ (В1 – В8).

Повторность вариантов опыта четырехкратная. Субстрат представлял собой смесь торфа низкой зольности и песка 1 : 2. Растения выращивали в климатической камере «BinderKBWF720» при соблюдении оптимальных условий культуры томата (влажность субстрата 75 %, освещенность 20 000 лк (16 ч в сутки), температура воздуха днем – 23 °С, ночью – 19 °С). Использовали карликовый сорт томата

«Балконное чудо». Растения выращивали в течение 3 месяцев до стадии начала плодоношения.

Для изучения влияния инокуляции на устойчивость газонных культур к температурному стрессу помимо использования культуры гриба были подготовлены популяции гриба, предварительно адаптированные к действию высоких температур (33 – 37 °C). Опыт включал варианты: инокулированные растения (А1 – культура гриба, А2 – адаптированная популяция гриба, А0 – контроль, без инокуляции) выращивались в условиях оптимального режима температур (В0) и при высоких температурах (В1). Растения выращивались в климатической камере: освещенность 20 000 лк 16 ч в сутки; температура воздуха днем – 25 °C, ночью – 22 °C (оптимальный режим температур); температура воздуха днем – 37 °C, ночью – 33 °C (экстремальный режим температур). Повторность вариантов опыта четырехкратная. Субстрат представлял собой смесь торфа низкой зольности и песка 1:2.

По завершении эксперимента была проведена оценка развития грибов эндофитов в корнях методом световой микроскопии (Shtark and Labutova, 2014). Оценка устойчивости растений проведена на основе: содержания нитратов в листьях – ионометрическим методом (ГОСТ 29270-95); биомассы и процентного содержания сухого вещества в надземной части и корневой системе растений весовым методом (ГОСТ 28561-90); фотосинтетических пигментов в листьях среднего яруса (хлорофиллы *a* и *b*, каротиноиды) спектрофотометрическим методом в ацетоновых экстрактах (поглощение 662, 644 и 440,5 нм, соответственно), расчет концентрации пигментов проведен по уравнениям Холма-Веттштейна.

Статистическая обработка

Математическую обработку материалов осуществляли с применением статистического пакета «Statistica 6.0» методами описательной статистики. Достоверные различия установлены при p <0,05.

Результаты и их обсуждение

Анализ результатов показал (Рисунки 1 – 3), что во всех вариантах с внесением цинка содержание пигментов в листьях растений имело общие закономерности: инокуляция растений контрольной популяцией (А0) при выращивании на субстрате с цинком не повлияла на содержание фотосинтетических пигментов; инокуляция растений адаптированными популяциями при выращивании на контрольном субстрате (В0) вызвала достоверное увеличение содержания хлорофиллов *a* и *b*, каротиноидов, а при выращивании на субстрате с цинком, наоборот, наблюдалось значительное, почти в два раза, снижение содержания пигментов. Что касается других изучаемых показателей (Таблица 1), то инокуляция контрольной популяцией при выращивании растений на субстрате с цинком привела к достоверному снижению содержания сухого вещества в корневой системе растений. Инокуляция адаптированными популяциями гриба вызвала достоверное снижение надземной биомассы растений (при выращивании на контрольном субстрате) и не повлияла

на изучаемые параметры растений при культивировании на субстрате с внесением цинка.

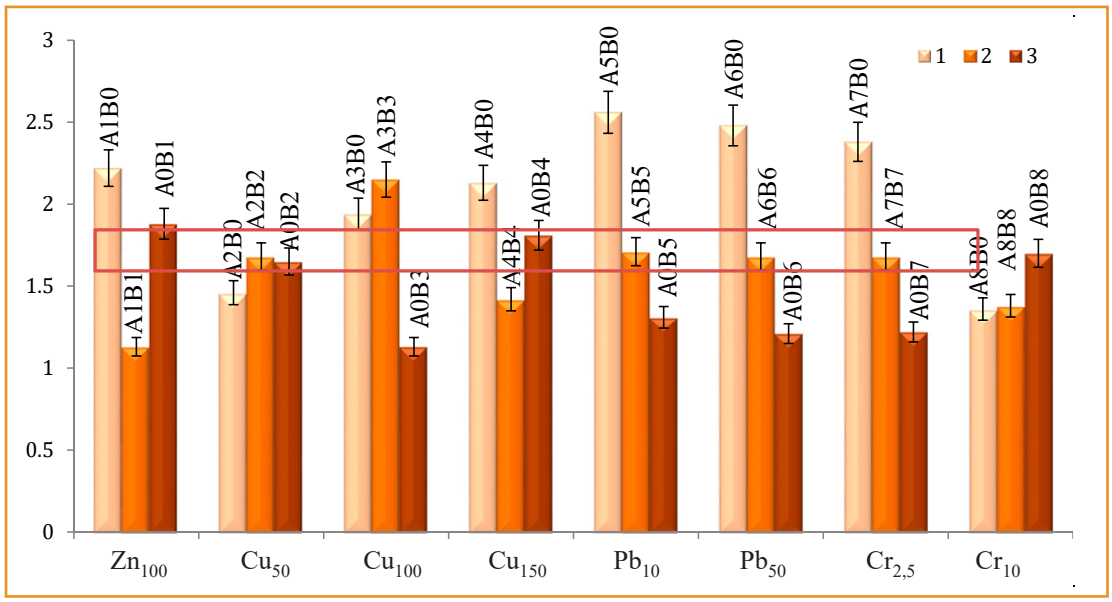

Рисунок 1 Содержание хлорофилла *a* в листьях инокулированных растений томата в условиях разных концентраций тяжелых металлов в субстрате

1 – популяция гриба (A1 – Zn_{100}; A2 – Cu_{50}; A3 – Cu_{100}; A4 – Cu_{150}; A5 – Pb_{10}; A6 – Pb_{50}; A7 – $Cr_{2,5}$; A8 – Cr_{10}) + субстрат без тяжелых металлов (B0); 2 – популяция гриба (A1 – Zn_{100}; A2 – Cu_{50}; A3 – Cu_{100}; A4 – Cu_{150}; A5 – Pb_{10}; A6 – Pb_{50}; A7 – $Cr_{2,5}$; A8 – Cr_{10}) + субстрат с внесением солей тяжелых металлов, мг/л (B1 – Zn_{100}; B2 – Cu_{50}; B3 – Cu_{100}; B4 – Cu_{150}; B5 – Pb_{10}; B6 – Pb_{50}; B7 – $Cr_{2,5}$; B8 – Cr_{10}); 3 – контрольная популяция (A0) + субстрат с внесением солей тяжелых металлов мг/л (B1 – Zn_{100}; B2– Cu_{50}; B3– Cu_{100}; B4– Cu_{150}; B5 – Pb_{10}; B6 – Pb_{50}; B7– $Cr_{2,5}$; B8 – Cr_{10}); A0B0 – контрольная популяция гриба на субстрате без внесения тяжелых металлов (красным прямоугольником обозначен доверительный интервал средних значений показателя для данного варианта)

Figure 1 The content of chlorophyll *a* in the leaves of inoculated tomato plants under conditions of different concentrations of heavy metals in the substrate

1 – fungus population (A1 – Zn_{100}; A2 – Cu_{50}; A3 – Cu_{100}; A4 – Cu_{150}; A5 – Pb_{10}; A6 – Pb_{50}; A7 – $Cr_{2,5}$; A8 – Cr_{10}) + substrate without heavy metals (B0); 2 – fungus population (A1 – Zn_{100}; A2 – Cu_{50}; A3 – Cu_{100}; A4 – Cu_{150}; A5 – Pb_{10}; A6 – Pb_{50}; A7 – $Cr_{2,5}$; A8 – Cr_{10}) + substrate with heavy metals, mg/l (B1 – Zn_{100}; B2 – Cu_{50}; B3 – Cu_{100}; B4 – Cu_{150}; B5 – Pb_{10}; B6 – Pb_{50}; B7 – $Cr_{2,5}$; B8 – Cr_{10}); 3 – control population fungi (A0) + substrate with heavy metals (B1 – Zn_{100}; B2 – Cu_{50}; B3 – Cu_{100}; B4 – Cu_{150}; B5 – Pb_{10}; B6 – Pb_{50}; B7 – $Cr_{2,5}$; B8 – Cr_{10}); A0B0– control population fungi + substrate without heavy metals (the red box indicates the confidence interval of the average values of the indicator for this option). On the *Y*-axis – Content of chlorophyll *a*, mg/g"; on the *X*-axis – Content of heavy metals in the substrate, mg/l"

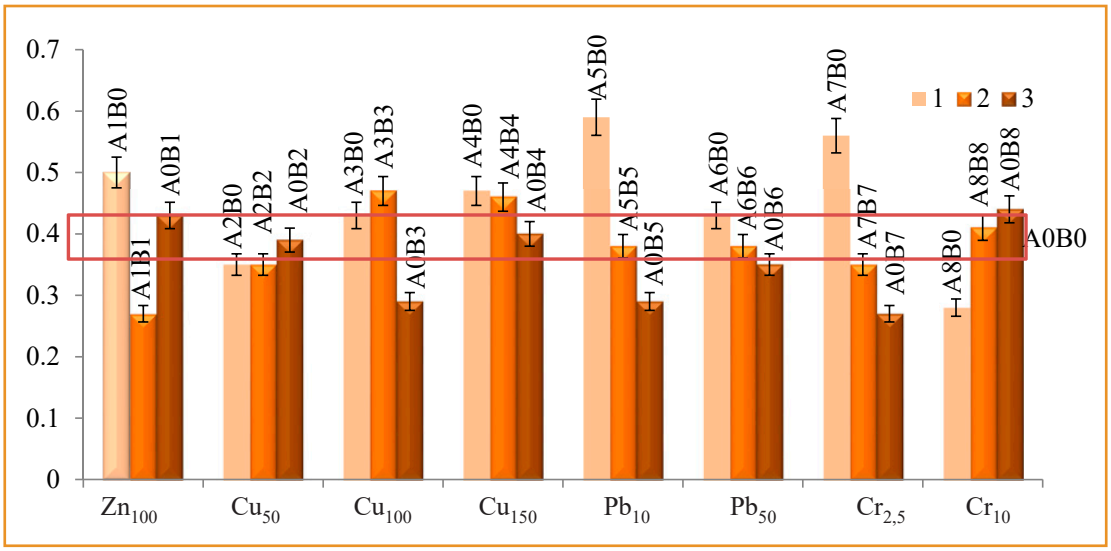

Рисунок 2 Содержание хлорофилла *b* в листьях инокулированных растений томата в условиях разных концентраций тяжелых металлов в субстрате

1 – популяция гриба (A1 – Zn_{100}; A2 – Cu_{50}; A3 – Cu_{100}; A4 – Cu_{150}; A5 – Pb_{10}; A6 – Pb_{50}; A7 – $Cr_{2,5}$; A8 – Cr_{10}) + субстрат без тяжелых металлов (B0); 2 – популяция гриба (A1– Zn_{100}; A2 – Cu_{50}; A3 – Cu_{100}; A4 – Cu_{150}; A5 – Pb_{10}; A6 – Pb_{50}; A7 – $Cr_{2,5}$; A8 – Cr_{10}) + субстрат с внесением солей тяжелых металлов, мг/л (B1 – Zn_{100}; B2 – Cu_{50}; B3 – Cu_{100}; B4 – Cu_{150}; B5 – Pb_{10}; B6 – Pb_{50}; B7 – $Cr_{2,5}$; B8 – Cr_{10}); 3 – контрольная популяция (A0) + субстрат с внесением солей тяжелых металлов мг/л (B1 – Zn_{100}; B2 – Cu_{50}; B3 – Cu_{100}; B4 – Cu_{150}; B5 – Pb_{10}; B6 – Pb_{50}; B7 – $Cr_{2,5}$; B8 – Cr_{10}); A0B0 – контрольная популяция гриба на субстрате без внесения тяжелых металлов (красным прямоугольником обозначен доверительный интервал средних значений показателя для данного варианта)

Figure 2 The content of chlorophyll *b* in the leaves of inoculated tomato plants under conditions of different concentrations of heavy metals in the substrate

1 – fungus population (A1 – Zn_{100}; A2 – Cu_{50}; A3 – Cu_{100}; A4 – Cu_{150}; A5 – Pb_{10}; A6 – Pb_{50}; A7 – $Cr_{2,5}$; A8 – Cr_{10}) + substrate without heavy metals (B0); 2 – fungus population (A1 – Zn_{100}; A2 – Cu_{50}; A3 – Cu_{100}; A4 – Cu_{150}; A5 – Pb_{10}; A6 – Pb_{50}; A7 – $Cr_{2,5}$; A8 – Cr_{10}) + substrate with heavy metals, mg/l (B1 – Zn_{100}; B2 – Cu_{50}; B3 – Cu_{100}; B4 – Cu_{150}; B5 – Pb_{10}; B6 – Pb_{50}; B7 – $Cr_{2,5}$; B8 – Cr_{10}); 3 – control population fungi (A0) + substrate with heavy metals (B1 – Zn_{100}; B2 – Cu_{50}; B3 – Cu_{100}; B4 – Cu_{150}; B5 – Pb_{10}; B6 – Pb_{50}; B7 – $Cr_{2,5}$; B8 – Cr_{10}); A0B0– control population fungi + substrate without heavy metals (the red box indicates the confidence interval of the average values of the indicator for this option). On the *Y*-axis – Content of chlorophyll *b*, mg/g"; on the *X*-axis – Content of heavy metals in the substrate, mg/l"

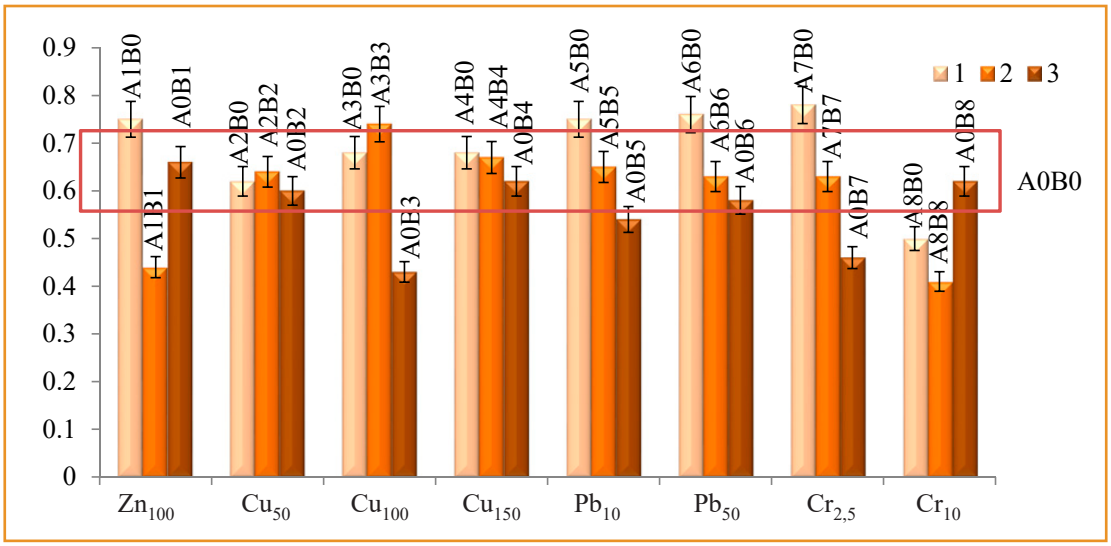

Рисунок 3 Содержание каротиноидов в листьях инокулированных растений томата в условиях разных концентраций тяжелых металлов в субстрате

1 – популяция гриба (A1 – Zn_{100}; A2 – Cu_{50}; A3 – Cu_{100}; A4 – Cu_{150}; A5 – Pb_{10}; A6 – Pb_{50}; A7 – $Cr_{2,5}$; A8 – Cr_{10}) + субстрат без тяжелых металлов (B0); 2 – популяция гриба (A1 – Zn_{100}; A2 – Cu_{50}; A3 – Cu_{100}; A4 – Cu_{150}; A5 – Pb_{10}; A6 – Pb_{50}; A7 – $Cr_{2,5}$; A8 – Cr_{10}) + субстрат с внесением солей тяжелых металлов, мг/л (B1 – Zn_{100}; B2 – Cu_{50}; B3 – Cu_{100}; B4 – Cu_{150}; B5 – Pb_{10}; B6 – Pb_{50}; B7 – $Cr_{2,5}$; B8 – Cr_{10}); 3 – контрольная популяция (A0) + субстрат с внесением солей тяжелых металлов мг/л (B1 – Zn_{100}; B2 – Cu_{50}; B3 – Cu_{100}; B4 – Cu_{150}; B5 – Pb_{10}; B6 – Pb_{50}; B7 – $Cr_{2,5}$; B8 – Cr_{10}); A0B0 – контрольная популяция гриба на субстрате без внесения тяжелых металлов (красным прямоугольником обозначен доверительный интервал средних значений показателя для данного варианта)

Figure 3 The content of carotenoids in the leaves of inoculated tomato plants under conditions of different concentrations of heavy metals in the substrate

1 – fungus population (A1 – Zn_{100}; A2 – Cu_{50}; A3 – Cu_{100}; A4 – Cu_{150}; A5 – Pb_{10}; A6 – Pb_{50}; A7 – $Cr_{2,5}$; A8 – Cr_{10}) + substrate without heavy metals (B0); 2 – fungus population (A1 – Zn_{100}; A2 – Cu_{50}; A3 – Cu_{100}; A4 – Cu_{150}; A5 – Pb_{10}; A6 – Pb_{50}; A7 – $Cr_{2,5}$; A8 – Cr_{10}) + substrate with heavy metals, mg/l (B1 – Zn_{100}; B2 – Cu_{50}; B3 – Cu_{100}; B4 – Cu_{150}; B5 – Pb_{10}; B6 – Pb_{50}; B7 – $Cr_{2,5}$; B8 – Cr_{10}); 3 – control population fungi (A0) + substrate with heavy metals (B1 – Zn_{100}; B2 – Cu_{50}; B3 – Cu_{100}; B4 – Cu_{150}; B5 – Pb_{10}; B6 – Pb_{50}; B7 – $Cr_{2,5}$; B8 – Cr_{10}); A0B0– control population fungi + substrate without heavy metals (the red box indicates the confidence interval of the average values of the indicator for this option). On the *Y*-axis – Content of carotenoids, mg/g"; on the *X*-axis – Content of heavy metals in the substrate, mg/l"

Отмечены высокие показатели развития грибной инфекции *C. magnusianum* в корневой системе растений в варианте контрольная популяция/Zn_{100} (Таблица 1), при использовании же адаптированных популяций грибная инфекция была менее развита, особенно в варианте Zn_{100}/Zn_{100}.

В вариантах с Cu_{100} установлено увеличение содержания хлорофиллов *a* и *b* при использовании адаптированных популяций, в то время как инокуляция контрольной популяцией, наоборот, привела к достоверному резкому снижению содержания пигментов. В вариантах с максимальным содержанием меди (Cu_{150}) мы не выявили изменений в содержании каротиноидов, но использование адаптированных популяций привело к увеличению содержания хлорофиллов.

Таблица 1 Биологические показатели инокулированных растений томата в условиях эксперимента
Table 1 Biological indicators of inoculated tomato in experimental conditions

Варианты опыта: популяция (А)/ субстрат (В)	Показатели					Развитие грибной инфекции	
	биомасса (г)		содержание сухого вещества (%)		содержание нитратов (мг/100 г)	частота встречаемости (%)	интенсивность (%)
	надземная часть	корни	надземная часть	корни			
Контроль/Zn_{100}	29,37 ±2,23*	4,70 ±0,28	12,83 ±0,67	7,85 ±0,33↓	3 890,43 ±159,98	86,7	4,3
Контроль/Cu_{50}	25,54 ±0,80↓**	3,81 ±0,24	13,01 ±1,99	5,32 ±1,96↓	4 327,69 ±144,6 ↑	80,0	4
Контроль/Cu_{100}	24,31 ±1,86	4,40 ±0,30	12,64 ±0,02	13,79 ±3,80↑	5 326,66 ±110,4↑	86,7	4,3
Контроль/Cu_{150}	27,60 ±0,70	3,63 ±0,24	12,14 ±0,89	13,29 ±1,13↑	4 308,72 ±298,07	53,6	2,7
Контроль/Pb_{10}	24,51 ±1,28	3,88 ±0,35	8,85 ±0,50↓	10,58 ±2,01	4 321,20 ±258,40	93,3	4,7
Контроль/Pb_{50}	28,81 ±0,39	3,81 ±0,07	10,02 ±0,86↓	8,75 ±1,38	5 014,62 ±466,07	93,3	4,7
Контроль/$Cr_{2,5}$	26,87 ±0,35	3,30 ±0,14↓	11,74 ±1,87	8,89 ±1,79	4 415,13 ±331,23	40,0	2
Контроль/Cr_{10}	25,58 ±0,45↓	4,72 ±0,28	11,59 ±0,98	7,75 ±0,18↓	3 213,16 ±96,82↓	40,0	2
Zn_{100}/контроль	25,58 ±0,73↓	3,84 ±0,12	14,92 ±2,32	9,35 ±1,41	3 476,33 ±325,75	60,0	3
Zn_{100}/Zn_{100}	27,80 ±0,64	4,72 ±0,45	14,95 ±1,23	11,19 ±2,20	3 585,72 ±606,07	33,3	1,7
Cu_{50}/контроль	23,96 ±1,63	2,16 ±0,18↓	10,99 ±1,14	14,78 ±2,82	3 365,41 ±72,51	100,0	5
Cu_{50}/Cu_{50}	29,68 ±1,05	2,13 ±0,23	14,10 ±1,64	15,22 ±2,97	4 638,21 ±346,8↑	66,7	3,3
Cu_{100}/контроль	19,82 ±0,40↓	2,30 ±0,15↓	10,91 ±1,64	13,17 ±2,43	4 837,86 ±206,82	93,3	4,7
Cu_{100}/Cu_{100}	35,29 ±0,25↑	2,39 ±0,69	12,67 ±0,82	12,68 ±2,45	3 534,60 ±99,78	100,0	5
Cu_{150}/контроль	27,99 ±0,81	1,93 ±0,04↓	9,44 ±1,79	16,10 ±3,80	3 058,14 ±25,50↓	86,7	4,3
Cu_{150}/Cu_{150}	24,16 ±1,12	2,23 ±0,18	12,26 ±1,21	13,25 ±2,73	4 487,60 ±103,3↑	86,7	4,3
Pb_{10}/контроль	32,66 ±2,01	2,98 ±0,15 ↓	13,67 ±1,92	10,24 ±0,65	3 356,96 ±241,51	73,3	3,4
Pb_{10}/Pb_{10}	26,30 ±0,87	2,36 ±0,22	11,41 ±1,09	11,71 ±1,01	4 488,58 ±102,6↑	66,7	3,3
Pb_{50}/контроль	21,88 ±1,31↓	1,55 ±0,10↓	12,39 ±1,36	10,98 ±1,16	3 986,02 ±82,59	86,7	4,3

Продолжение таблицы 1
Continuation of table 1

Варианты опыта: популяция (А)/ субстрат (В)	Показатели					Развитие грибной инфекции	
	биомасса (г)		содержание сухого вещества (%)		содержание нитратов (мг/100 г)	частота встречаемости (%)	интенсивность (%)
	надземная часть	корни	надземная часть	корни			
Pb_{50}/Pb_{50}	28,16 ±1,30	2,49 ±0,36	12,92 ±1,16	10,01 ±1,17	4 229,96 ±177,36	86,7	4,3
$Cr_{2,5}$/контроль	21,59 ±2,04↓	1,92 ±0,08↓	12,24 ±0,26	11,38 ±1,85	4 384,27 ±195,22	73,3	3,4
$Cr_{2,5}/Cr_{2,5}$	29,54 ±0,09↑	2,50 ±0,01↑	13,16 ±0,61	9,52 ±1,49	4 161,79 ±494,02	73,3	3,4
Cr_{10}/контроль	16,36 ±0,94↓	1,56 ±0,15↓	13,12 ±1,98	14,17 ±2,00	5 188,76 ±622,04	80,0	4
Cr_{10}/Cr_{10}	27,30 ±0,26↑	2,06 ±0,22	14,23 ±2,73	11,90 ±1,12	3 583,89 ±471,03	80,0	4
Контроль/ контроль	29,30 ±0,70	5,44 ±0,63	15,33 ±2,02	9,46 ±0,15	3 693,55 ±87,76	60,0	3

Примечания. * – указано среднее значение показателя ± стандартное отклонение; ** показано достоверное отличие от контроля: увеличение ↑ или уменьшение ↓ показателя (p <0,05). Контроль – исходная, неадаптированная к ТМ популяция (соответствует А0 на рисунках 1 – 3) и субстрат без металлов (соответствует В0 на рисунках 1 – 3). А – адаптированные популяции гриба, выращенные на агаровых субстратах с внесением разных концентраций солей тяжелых металлов (мг/л) (соответствует А1 – А8 на рисунках 1 – 3). В – субстраты с разным содержанием солей тяжелых металлов (мг/л) (соответствует В1 – В8 на рисунках 1 – 3)

Результаты анализа других изучаемых параметров растений показали, что инокуляция растений контрольной популяцией гриба привела к росту содержания нитратов в листьях в вариантах субстратов Cu_{50} и Cu_{100}, а также к увеличению процентного содержания сухого вещества в корневой системе растений в вариантах Cu_{100} и Cu_{150}. Это согласуется с данными о влиянии инокуляции на растения при воздействии ТМ, связанной с изменением архитектуры корневой системы и накоплением общего азота (Hou et al., 2020). Использование адаптированных популяций гриба при культивировании инокулированных растений на контрольном субстрате привело к снижению биомассы корневой системы, а в варианте Cu_{150} – и к снижению содержания нитратов в листьях. При инокуляции адаптированными популяциями гриба на субстратах с внесением Cu_{50} и Cu_{150} отмечен достоверный рост содержания нитратов в листьях, а при Cu_{100} – увеличение надземной биомассы растений.

Наиболее интенсивно грибная инфекция формировалась при использовании адаптированных популяций Cu_{100} и Cu_{150}. Максимальное развитие грибной инфекции отмечено в варианте Cu_{100}/Cu_{100}.

Особый интерес вызвали результаты вариантов опыта с использованием небиогенных химических элементов (хрома и свинца). При инокуляции растений контрольной популяцией гриба и при культивировании на субстрате Pb_{10} наблюдалось достоверное снижение хлорофиллов *a* и *b*, на субстрате Pb_{50} – хлорофилла *a*, при этом достоверного снижения содержания каротиноидов не наблюдалось.

Использование адаптированных популяций гриба при выращивании растений на В0 вызвало увеличение содержания всех изучаемых пигментов, а при выращивании на субстратах с внесением солей свинца достоверных изменений по сравнению с контролем не выявлено.

Инокуляция растений контрольной популяцией гриба привела к достоверному снижению процентного содержания сухого вещества в надземной части растений. При использовании адаптированных популяций и при выращивании растений на контрольном субстрате отмечено снижение биомассы корневой системы, а при культивировании растений на субстратах с Pb_{10} и Pb_{50} биомасса и содержание сухого вещества достоверных изменений не имели, но при этом отмечен рост содержания нитратов в листьях.

Во всех вариантах со свинцом грибная инфекция в корневой системе растений имела высокие показатели развития, наибольшие – в вариантах Контроль/Pb_{10}, Pb_{50} и Pb_{10}, Pb_{50}/контроль.

В вариантах с хромом инокуляция растений контрольной популяцией при культивировании в субстрате с $Cr_{2,5}$ привела к достоверному снижению содержания пигментов в листьях, а в субстрате с Cr_{10} – уже нет.

Инокуляция растений адаптированными популяциями гриба при их культивировании на контрольных субстратах имела различия: при $Cr_{2,5}$ вызвала достоверный рост в содержании фотосинтетических пигментов, а при Cr_{10}, наоборот,

достоверное снижение их содержания. При культивировании растений на субстатх с внесением $Cr_{2,5}$ достоверных изменений не наблюдалось, и лишь при внесении Cr_{10} наблюдалось снижение содержания хлорофилла *a* и каротиноидов, при отсутствии достоверных различий с контролем в содержании хлорофилла *b*.

При инокуляции растений контрольной популяцией гриба и культивировании на субстрате с Cr_{10} мы наблюдали снижение показателей надземной биомассы, процентного содержания сухого вещества в корневой системе растений и нитратов в листьях. Интерес представляют результаты вариантов с использованием инокуляции растений адаптированными популяциями гриба: при культивировании растений на контрольных субстратах мы наблюдали снижение показателей биомассы надземной части и корневой системы растений, но когда растения культивировались на субстратах с внесением хрома, наоборот, отмечен рост биомассы растений.

В вариантах с хромом использование адаптированных популяций гриба привело к наиболее высоким показателям развития грибной инфекции в корне растений, причем максимальным – при наиболее высоком содержании хрома в субстрате (вариант Cr_{10}/Cr_{10}).

Результаты проведенных нами исследований с использованием небиогенных опасных для жизнедеятельности растений химических элементов согласуются с результатами наших исследований, проведенных в течение ряда лет (Bukharina and Islamova, 2016; Bukharina et al., 2019; Bukharina et al., 2019a) и мнением ряда научных публикаций о своеобразной форме партнерства эндотрофных грибов с корневой системой растений (Bukharina et al., 2016; Bilal et al., 2019; Li et al., 2019; Hou et al., 2020): защитное действие грибов наиболее эффективно проявляется в условиях неблагоприятных для жизнедеятельности растений.

Результаты эксперимента по влиянию температурного стресса казали (Таблица 2), что в условиях оптимального режима через 2 недели после начала эксперимента, инокулированные растения отличались более высокими показателями содержания аскорбиновой кислоты в листьях по сравнению с контрольными не инокулированными растениями. У них отмечено достоверное снижение содержания хлорофилла *b* в листьях, существенное перераспределение пластического материала: увеличение надземной биомассы и снижение биомассы корневой системы (p <0,05).

Таблица 2 Показатели биомассы и содержания сухого вещества инокулированных растений мятлика лугового (*Poa pratensis* L.) в условиях разных температурных режимов культивирования

Table 2 Biomass and dry matter of inoculated meadow bluegrass plants (*Poa pratensis* L.) under different temperature conditions of cultivation

Вариант опыта	Биомасса (г)		Содержание сухого вещества в надземной части (%)
	надземная часть	корни	
Температура 22 – 25 °С			
Контроль (В0)	3,30 ±0,05 3,22 – 3,38	28,19 ±0,80 26,92 – 29,45	49,8 ±0,2 49,6 – 50,1
Cylindrocarpon magnusianum	4,80 ±0,01 4,78 – 4,81	25,01 ±0,66 23,96 – 26,07	51,2 ±1,4 49,1 – 53,4
***Cylindrocarpon magnusianum* (АП)**	4,75 ±0,04 4,69 – 4,81	13,98 ±0,38 13,37 – 14,58	51,8 ±1,3 49,7 – 53,9
Температура 33 – 37 °С			
Контроль (В0)	нет данных	нет данных	нет данных
Cylindrocarpon magnusianum	нет данных	15,15 ±0,76 13,93 – 16,36	нет данных
***Cylindrocarpon magnusianum* (АП)**	нет данных	16,13 ±0,48 15,36 – 16,90	нет данных

Примечания: * – указано среднее значение показателя ± стандартное отклонение, доверительный интервал среднего значения показателя (p <0,05); АП – адаптированная популяция

Таблица 3 Биохимические показатели инокулированных растений мятлика лугового (*Poa pratensis* L.) в условиях разных температурных режимов культивирования

Table 3 Biochemical parameters of inoculated meadow bluegrass plants (*Poa pratensis* L.) under different temperature conditions of cultivation

Вариант опыта	Содержание аскорбиновой кислоты (мг/100 г)	Содержание фотосинтетических пигментов (мг/г)		
		хлорофилл *a*	**хлорофилл *b***	**каротиноиды**
Температура 22 – 25 °С				
Контроль (В0)	*143,01 ±2,41 139,19 – 146,84	3,235 ±0,081 3,105 – 3,364	2,961 ±0,269 2,532 – 3,390	0,661±0,022 0,626 – 0,695
Cylindrocarpon magnusianum	161,49 ±2,09 158,16 – 164,81	3,198 ±0,094 3,049 – 3,347	0,977 ±0,066 0,872 – 1,081	0,691 ±0,026 0,649 – 0,733
***Cylindrocarpon magnusianum* (АП)**	165,06 ±3,67 159,21 – 170,90	3,082 ±0,023 3,045 – 3,199	0,615 ±0,017 0,588 – 0,642	0,783 ±0,065 0,680 – 0,886
Температура 33 – 37 °С				
Контроль (В0)	400,72 ±6,23 390,81 – 410,62	4,349 ±0,019 4,319 – 4,379	1,250 ±0,083 1,118 – 1,382	1,589 ±0,014 1,566 – 1,612
Cylindrocarpon magnusianum	218,14 ±0,46 217,41 – 218,87	4,652 ±0,250 4,254 – 5,050	1,042 ±0,063 0,942 – 1,142	1,554 ±0,017 1,526 – 1,581
***Cylindrocarpon magnusianum* (АП)**	383,66 ±5,03 375,66 – 391,66	5,697 ±0,058 5,605 – 5,789	1,673 ±0,076 1,552 – 1,794	1,271 ±0,033 1,219 – 1,323

Примечания. * – указано среднее значение показателя ± стандартное отклонение, доверительный интервал среднего значения показателя (p <0,05); АП – адаптированная популяция

При культивировании в условиях температурного стресса у инокулированных растений по сравнению с контрольными достоверно снижалось содержание аскорбиновой кислоты в листьях, при этом у растений, инокулированных адаптированными популяциями гриба, содержание хлорофиллов было существенно выше, а каротиноидов – ниже по сравнению с контрольными растениями. Инокуляция адаптированными популяциями оказалась наиболее эффективной в условиях температурного стресса. После полутора месяцев культивирования растений в условиях высоких температур контрольные растения погибли, у инокулированных растений также наблюдалось отмирание надземной части, но при этом корневая система растений сохранила свою жизнеспособность.

Выводы

Инокуляция растений контрольной популяцией гриба привела к снижению содержания фотосинтетических пигментов и биомассы, таким образом не способствовала формированию адаптивных реакций у растений. Инокуляция растений адаптированными популяциями имела положительный эффект: для Zn_{100} – при культивировании растений на контрольном субстрате; для вариантов Cu_{100} и Cu_{150} – как на контрольном, так и на субстратах с внесением меди; для всех вариантов с внесением солей хрома и свинца – на субстратах с внесением тяжелых металлов. Инокуляция мятлика лугового адаптированными популяциями гриба при культивировании в условиях температурного стресса, привела к сохранению жизнеспособности корневой системы растений при отмирании надземной части в отличии от гибели не инокулированных растений. Эти факты свидетельствуют о наиболее эффективном партнерстве гриба *C. magnusianum* и растений в условиях стресса. Грибная инфекция в корнях растений во всех вариантах опыта была хорошо развита, а использование адаптированных к действию солей хрома изолятов *C. magnusianum* при дальнейшем культивировании растений на субстратах с внесением солей хрома стимулировало развитие грибной инфекции в корневой системе растений.

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POSSIBILITY OF USING AN INVASIVE SPECIES *ADENOCAULON ADHAERESCENS* MAXIM. (ASTERACEAE) AS A MEDICIN PLANT

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Many alien invasive species are characterized by the accumulation of biologically active substances antioxidants, which are used in the prevention of diseases of the cardiovascular system and many cancers. The purpose of this study was to compare the antioxidant (antiradical) properties of *Adenocaulon adhaerescens* Maxim extracts obtained from plants growing in both natural and secondary distribution ranges. The literature data on the analysis of medicinal properties of closely related species of the genus *Adenocaulon* are summarized. The antioxidant activity was determined by the method using 2.2-diphenyl-1-picrylhydrazil (DPPH) on spectrophotometer Genesis 20, the USA at the Slovak University of Agriculture in Nitra (Slovak Republic). Water and alcohol extracts (methanol and ethanol) were tested. The total antioxidant activity of extracts from leaves of juvenile plants was about 80% (alcoholic extracts) and 34% (water extracts). The total antioxidant activity of leaf extracts collected from flowering plants was 76% (methanol extracts), 59% (ethanol extracts), and 48% (water extracts). The total antioxidant activity of extracts from inflorescences was significantly higher and amounted to 83% (methanol extracts), 85% (ethanol extracts), and 49% (water extracts). Indicators of antioxidant activity of the aboveground part of plants collected in populations from the natural and secondary ranges do not have reliable differences. Methanol extracts from plants collected in the natural range have the antioxidant activity of 77–78%, ethanol extracts of 78–81%, and water extracts of 61–66%. In plants from invasive populations, the antioxidant activity of methanol extracts is 77–80%, ethanol extracts 76–79%, water extracts 32–67%. The studied species demonstrated a significant antioxidant activity comparable to many valuable medicinal plants, such as *Urtica dioica* L., *Bidens pilosa* Linn., *Acacia auriculiformis* A. Cunn, *Salvia officinalis* L., and others. Thus, alien (*A. adhaerescens*) which has successfully adapted to the Moscow climate and formed a stable invasive population, may become a potential source of antioxidants to improve the system of antioxidant protection of humans.

Keywords: *Adenocaulon adhaerescens*, antioxidant activity, invasion, alien species

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Введение

Изучение растений в поисках новых лекарственных веществ является одним из основных прикладных направлений ботаники. Многие соединения, выделенные из растений в последнее время в научных лабораториях, исследуются как химиотерапевтические «кандидаты» (Lee, 2010). С каждым годом, тенденция использования растительного сырья увеличивается, и в связи с повышением спроса на натуральные лекарства во многих странах широко культивируют дикорастущие лекарственные растения. Однако до сих пор только 5% мировой флоры введено в культуру с лекарственными целями, и практически не используется большая группа видов – инвазионные растения, численность которых, а также площадь вторичного ареала во всех регионах мира увеличиваются с каждым годом.

В центре внимания многочисленных исследовательских программ находится разработка противоопухолевых препаратов (Balunas, 2005). Более 60% противоопухолевых лекарственных средств, используемых в настоящее время, получены из природных источников, включая растения (Cragg, 2005). Чужеродные инвазионные виды растений также могут применяться для лечения опухолей, поскольку содержат вещества с высокой антиоксидантной активностью. В этом отношении наше внимание привлек малоизученный вид *Adenocaulon adhaerescens* Maxim. Согласно последней ревизии (Bittmann, 1990), *Adenocaulon* – это небольшой род цветковых растений семейства Астровые, впервые описанный Вильямом Гукером (Hooker, 1829), включающий в себя пять видов и один подвид:

- *A. bicolor* Hook. – Соединённые штаты, Канада (Nash et al., 1976).
- *A. chilense* Less. – Чили, Аргентина (Marticorena and Quezada, 1985).
- *A. lyratum* S.F. Blake – Гватемала (Nash et al., 1976), Чьяпас (Breedlove, 1986).
- *A. nepalense* Bittmann – Непал (Bittmann, 1986a).
- *A. himalaicum* Edgew. – Китай, Индия, Япония, Корея, Непал (Yousheng and Hind, 1829).

При этом группа популяций, занимающая северо-восточную часть ареала *A. himalaicum* auct., чаще рассматривается как отдельный вид *A. adhaerescens* Maxim. Он впервые описан Карлом Максимовичем в его работе по флоре Амура (Maximowicz, 1859).

A. adhaerescens (прилипало пристающее) – многолетнее травянистое растение (Рисунок 1). Побеги прямостоячие с очередным листорасположением, высота растений второго года жизни – до 80 см. Листья простые, черешковые. Нижняя поверхность листа покрыта густым войлочным опушением. Цветение – июнь-октябрь. Плоды – семянки зелёного цвета, с железистыми трихомами (Fahn, 1988; Vinogradova, 2013). В 1953 году *A. adhaerescens* интродуцирован в Главный ботанический сад Академии Наук СССР (Москва). Через 30 лет отдельные особи были отмечены за пределами экспозиции. В настоящее время этот вид встречается массово вдоль дорожек по всему ботаническому саду, внедряется в естественные ценозы и формирует крупные (площадью до 5 м) локальные микропопуляции плотностью 50 растений на м^2. В 2005 г. *A. adhaerescens* обнаружен в парке Останкино и на ВДНХ, которые граничат с ГБС, а в 2007 г. отмечен

в лесопарке возле метро Щукинская. Размножается преимущественно семенным способом (Vinogradova, 2010; Mayorov et al., 2013).

Рисунок 1 *Adenocaulon adhaerescens* Maxim. в естественном ареале в Приморье
Figure 1 *Adenocaulon adhaerescens* Maxim. in the native distribution range in the Primorsky Territory

В качестве источника химических соединений *A. adhaerescens* ранее не использовался, однако, наземная часть близкородственного вида *A. himalaicum* Edgew применяется в районах его естественного произрастания – Китае, Корее и Японии для лечения абсцессов, кровоизлияний и воспалений (Hak, Kang, 2001; Wang et al., 2007).

Основными действующими компонентами *A. himalaicum* являются производные кофеиновой кислоты (Kulesh et al., 1986). Химическое исследование метанольного экстракта этого растения привело к выделению двух новых и восьми известных соединений (ацетилен 1-O-ферулоил-тетрадека-4E, 6E, 12E-триен-8,10-диин, монотерпенгликозид 9-гидроксилиноил-3-0-(4-0-кумароил) -j3-D- глюкопиранозид, фитол, парасорбозид, пруназин, (Z)-3-гексенил/3-D-глюкопиранозид и 9-гидроксиналоилглюкозид). С помощью ТН-ЯМР и ГХ-МС анализа были идентифицированы три известных моноглицерида, 10-(9Z, 12Z, 15Z-октадекатриеноил) глицерин, 1-O-гексадеканоилглицерин и 1-0-(9Z, 12Z-октадекадиеноил) глицерин (Hak, Kang, 2001). Позднее выделен новый трициклический α, β-ненасыщенный кетон (аденокаулон) и новый δ-гексанолактонгликозид (аденокаулолид) (Wang et al., 2007).

Имеются также данные по скринингу антибактериальной активности *A. himalaicum*. В качестве контроля использовался хлорамфеникол. Соединение 2 проявляло антибактериальную активность против *E. coli* и *S. aureus*, а соединение 4 – против *E. coli* (Wang et al., 2007).

Экстракты *A. himalaicum* содержат антипролиферативные соединения, которые протестированы как лекарственные средства. Экстракты оказались активны против аденокарциномы желудка человека (МК-1), рака матки человека (HeLa), меланомы мыши (B16F10) и Т-клеточных человека типа 2 (МТ-2), но не активны против лимфотропных вирусов человека типа 1 (HTLV-1) и МТ-1 (Kinjo, 2016).

В настоящее время исследуются различные варианты фармакологических вмешательств с целью снижения образования активных форм кислорода и обезвреживания уже имеющихся свободных радикалов с помощью антиоксидантов.

По данным корейских ученых, антиоксидантная активность *A. himalaicum* низкая: 20,2 % (метанольные экстракты) и 17,8 % (водные экстракты) (Lee et al., 2011). Однако уровень антиоксидантной активности экстрактов может меняться под влиянием различных факторов: климат, тип почвы, количество солнечных дней, объем выпавших осадков и т.д. (Goriunova, 2009). К тому же *A. adhaerescens*, хотя и близок к *A. himalaicum*, но все же другой вид. Кроме того, он является инвазионным видом, а ранее проведенный нами скрининг других чужеродных инвазионных растений продемонстрировал очень высокую степень выраженности их антиоксидантных свойств (Shelepova et al., 2019; Vinogradova et al., 2019 a,b).

Не менее интересно сравнение естественных и инвазионных популяций одного итого же вида по их биохимическим признакам, в том числе и по антиоксидантной активности. Сравнительный анализ дает возможность прогнозировать успешность

и целесообразность искусственного выращивания (культивирования) вида в том случае, если он является потенциально лекарственным растением.

Цель данного исследования – сравнительная оценка антиоксидантных (антирадикальных) свойств экстрактов *A. adhaerescens*, полученных из растений, произрастающих в условиях как естественного, так и вторичного ареала.

Материалы и методы

Растительный материал

Материалом для исследования антиоксидантной активности различных органов растения послужили растения *A. adhaerescens* из инвазионной популяции, произрастающей в ГБС РАН (Москва, N 55.78 E 36.80). В апреле 2018 г. собраны листочки ювенильных растений (образец 1), в августе – срединные листья (образец 2) и цветущие побеги (образец 3) растений в генеративной фазе развития (начало цветения).

Материалом для сравнения антиоксидантной активности в популяциях различного географического происхождения являлись пробы всей надземной части растений из 3 популяций естественного ареала и 2 инвазионных популяций вторичного ареала. В сентябре 2019 г. растения собирали в естественном ареале в трех местообитаниях юга Приморского края: E1) остров Русский N 42.97 E 131.89; E2) окрестности г. Уссурийска, буферная зона Уссурийского заповедника N 43.63 E 132.27; E3) г. Владивосток, лесная территория ботанического сада N 43.22 E 131.99. В конце сентября того же года материал собрали в двух инвазионных популяциях вторичного ареала: B1) в лесопарке у метро Щукинская, Москва N 55.80 E 37.47 и B2) в Главном ботаническом саду Российской академии наук.

Материал сушили при комнатной температуре и затем мололи до состояния порошка. Тестировали водные и спиртовые (метанол и этанол) экстракты.

Методика приготовления экстрактов

Навеску измельченного образца (1 г) экстрагировали в 25 мл растворителя (дистиллированная вода, этанол и метанол). Экстракция проводилась в течение 12 ч при непрерывном перемешивании. После этого экстракты фильтровали для последующего измерения.

ДФПГ-метод определения антирадикальной активности растительных экстрактов

Антирадикальную активность определяли по методике Brand-Williams et al. (1995) с использованием 2,2-дифенил-1-пикрилгидразила (DPPH) на спектрофотометре Genesis 20 (США). Заранее готовили раствор 2,2-дифенил-1-пикрилгидразила (ДФПГ). Для этого взвешивали 0,025 г радикала, переносили в мерную колбу на 100 мл и доводили до метки метанолом. Данный раствор разбавляли в 10 раз при непосредственном определении плотности исследуемых растворов и хранили

в холодильнике. Плотность рабочего раствора радикала находилась в пределах 0,700–0,800. В кювету добавляли 3,9 мл раствора ДФПГ и измеряли плотность на спектрофотометре. Добавляли 100 мл исследуемого раствора и оставляли в темноте на 10 минут. После этого снова проводили измерения полученных экстрактов на спектрофотометре. Полученные результаты рассчитывали в процентах с использованием следующего уравнения: $\%\, Inh = (A_0 - A_1)/A_0 \times 100$, где: A_0 – поглощение контрольного раствора (раствор радикала); A_1 – оптическая плотность раствра в присутствии образца.

Статистические методы

При анализе полученных данных использовали среднее значение и стандартное отклонение трех повторностей. Статистический анализ выполнен в программе PAST 2.17. Данные проанализированы с помощью теста ANOVA, различия между средними значениями признака проверены по критерию множественных сравнений Тьюки-Крамера (p <0.05).

Результаты и их обсуждение

Группа инвазионных растений, наряду с другими растениями, является потенциальным источником сырья для фармакологических исследований благодаря ряду полезных свойств, которые могут использоваться в медицинских целях (Pappan and Thomas, 2017; Kozuharova et al., 2019). Растения из семейства Астровых представляют научный интерес не только в области ботаники и экологии, а также как источник биологически активных веществ и как сырье с различной биологической активностью, в том числе и антиоксидантной (Bessada et al., 2015). Одним из наиболее известных и широко применяемых методов определения антирадикальной активности растительных экстрактов является реакция обесцвечивания раствора радикала экстрактом исследуемого образца (ДФПГ-метод). Метод отличается простотой, не занимает много времени и относительно экономичен (Marinova and Batchvarov, 2011). Проведенные нами исследования антиоксидантной активности различных органов *A. adhaerescens*, произрастающего во вторичном ареале в ГБС РАН, показали, что все органы растения обладают антиоксидантной активностью, хотя и в разной степени. Общая антиоксидантная активность экстрактов из листьев ювенильных растений, собранных в апреле, была высокой и составила около 80 % (метанольные и этанольные экстракты) и 35 % (водные экстракты). При этом общая антиоксидантная активность экстрактов из листьев, собранных в сентябре с цветущих растений, также оставалась повышенной и составила около 77 % (метанольные экстракты), 60 % (этанольные экстракты) и 48 % (водные экстракты) (Рисунок 2). Но наиболее высокой была общая антиоксидантная активность экстрактов из соцветий, она достигала практически 85 % для этанольных и метанольных экстрактов и практически 50 % для водных экстрактов.

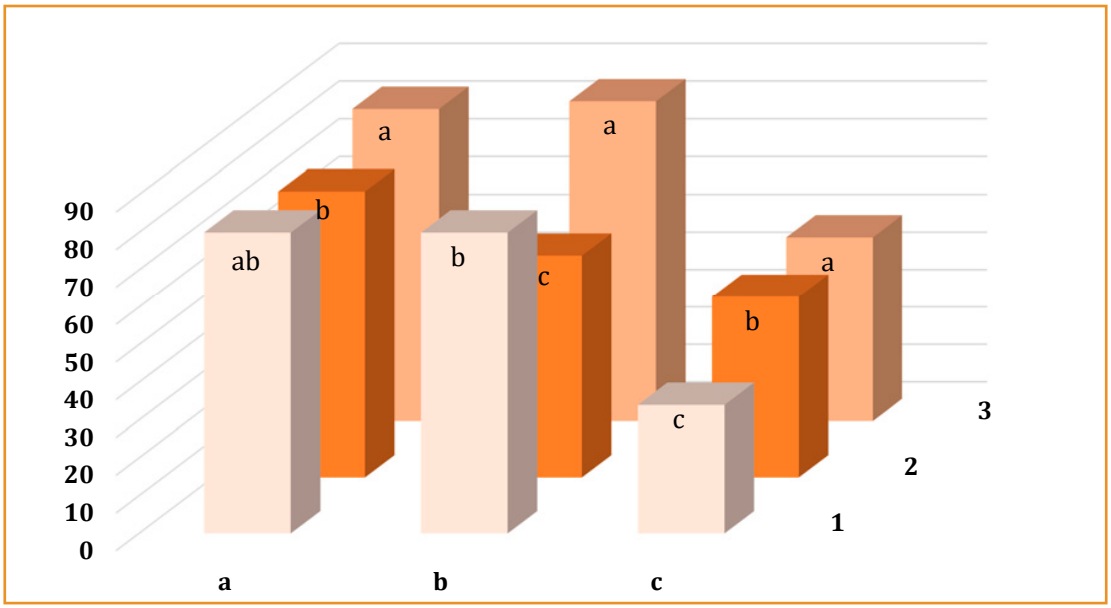

Рисунок 2 Антиоксидантная активность экстрактов из разных органов *Adenocaulon adhaerescens* Maxim методом ДФПГ (%)
1 – листья ювенильных растений; 2 – листья генеративных растений; 3 – соцветия; a – метанольные экстракты; b – этанольные экстракты; c – водные экстракты (средние значения признака в колонке, обозначеннойбуквами, существенно не отличаются, $P \leq 0{,}05$)

Figure 2 Antioxidant activity of extracts of different organs of *Adenocaulon adhaerescens* Maxim by DPPH method (%)
1 – leaves of juvenile plants; 2 – leaves of generative plants; 3 – inflorescences; a – methanol extracts; b – ethanol extracts; c – water extracts (means in each column followed by different letters are not significantly different ($P \leq 0.05$)

Однако в практике фармакопеи экономически более целесообразно собирать и использовать не отдельные органы, а всю надземную часть растения. Это тем более относится к изучаемому виду, у которого соцветие обильно олиственно. Поэтому для сравнения антиоксидантной активности растений из естественного и вторичного ареалов мы брали всю надземную часть растений целиком. Результаты исследований представлены на Рисунке 3.

Показатели антиоксидантной активности спиртовых экстрактов листьев и соцветий исследованных растений выше, чем водных. Это говорит, вероятно, о содержании в растения большей частью спирторастворимых антиоксидантов фенольной природы, которые придают данному виду лекарственные свойства.

Существенных различий по показателям антиоксидантной активности растений из естественного и вторичного ареала не выявлено. Метанольные экстракты из растений, собранных в естественном ареале, обладают антиоксидантной активностью 77 – 78 %, этанольные – 78 – 81 %, водные – 61 – 66 %. У растений из инвазионных популяций антиоксидантная активность метанольных экстрактов составляет 77 – 80 %, этанольных – 76 – 79 %, водных – 32 – 67 %.

Рисунок 3 Антирадикальная активность *A. adhaerescens* Maxim в популяциях различного географического происхождения
E1 – остров Русский; E2 – Уссурийский заповедник; E3 – Ботанический сад-институт ДВО РАН; B1 – Щукинский лесопарк; B2 – Главный ботанический сад РАН (средние значения признака в колонке, обозначенной буквами, существенно не отличаются, $p \leq 0{,}05$)

Figure 3 Antiradical activity of *A. adhaerescens* Maxim in the populations of different origins
E1 – island Russian; E2 – Ussuri Nature Reserve; E3 – Botanical garden-institute FEB of RAS; B1 – Schukin Forest Park; B2 – Main Botanical Garden of RAS (means in each column followed by different letters are not significantly different ($p \leq 0.05$)

Таким образом, наши результаты демонстрируют значительную вариабельность фитохимических характеристик *A. adhaerescens* в течение вегетационного сезона и очень высокую антиоксидантную активность (свыше 80 %) спиртовых экстрактов из молодых листьев и соцветий данного вида. Изученный вид продемонстрировал значительную антиоксидантную активность, сопоставимую со многими ценными лекарственными растениями, такими как *Urtica dioica* L., *Bidens pilosa* Linn., *Acacia auriculiformis* A. Cunn, *Salvia officinalis* L., *Polyalthia cerasoides* (Roxb.) Bedd. и др. (Krishnaiah et al., 2011). Антиоксидантная активность спиртовых экстрактов из молодых листьев и соцветий *A. adhaerescens* оказалась на уровне аналогичных показателей, зафиксированных у воздушно-сухих листьев *Solidago canadensis* L. (84 %) (Shelepova et al., 2019) и существенно выше, чем у листьев *S. virgaurea* L. (64 %) (Demir et al., 2009) и листьев *Conyza bonariensis* (L.) Cronquist (58 %) (Daur, 2015). Другое исследование группы растений из семейства Астровых показало, что антиоксидантная активность водных экстрактов методом ДФПГ составила от 73,34 (*Echinacea purpurea* L.) до 92,56 (*Calendula officinalis* L.) % (Sytar et al., 2018). В этом случае результаты оказались выше тех, что получили исследуя водные екстракты *A. adhaerescens*. Arituluk et al. (2016), изучая антиоксидантные параметры метанольных экстрактов 5 видов *Tanacetum* spp., определил антирадикальную активность исследуемых растворов (400 µг/мл) в пределах 77,61 – 84,41 %, что было практически идентично сравнительно с исследуемыми нами растворами *A. adhaerescens*.

Ресурсный потенциал вида в естественном ареале достаточно высок. Ареал его охватывает практически весь Приморский край, острова Сахалин и Кунашир, а также юг Хабаровского края. Растет этот вид преимущественно вдоль дорог, но в большом количестве. Локальные популяции имеют плотность 50 растений/м^2 и проективное покрытие до 98 %. Практически это одновидовые заросли, что обеспечивает менее трудоемкий сбор сырья. Ресурсы возобновляются примерно через 5 лет, поскольку вид имеет большую семенную продуктивность (одно растение продуцирует ~1,5 тысячи семян; почвенный семенной банк составляет 2,5 – 3,5 тыс. семян/м^2) и способен разрастаться за счет вегетативного размножения, закладывая 1 – 2 зимующие почки (Vinogradova, 2013).

Выводы

Чужеродный *A. adhaerescens*, который успешно адаптировался к условиям московского климата и сформировал устойчивую инвазионную популяцию, может стать потенциальным источником антиоксидантов для улучшения системы антиоксидантной защиты человека. Наиболее высокой антиоксидантной активностью обладают молодые листочки, собранные в течение 1 – 2 недель после таяния снега, а также соцветия в стадии начала цветения. Однако и вся надземная часть растений отличается повышенным содержанием веществ с антиоксидантной активностью: спиртовые экстракты 76–81 %, а водные – 32–67 %. Кроме того, растения из естественного и вторичного ареала не имеют существенных различий по содержанию веществ антиоксидантной активности, следовательно, при намеренной интродукции растений этот показатель не будет изменяться. Необходимо дальнейшее изучение фитохимических характеристик *A. adhaerescens* как в первичном, так и во вторичном ареалах для выявления образцов с высокой антиоксидантной активностью.

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ANTIOXIDANT POTENTIAL OF SOME ASTERACEAE BERCHT. & J. PRESL. REPRESENTATIVES

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Asteraceae Bercht. & J. Presl. is a large family of plants with a different direction of use, among which are medicine, decorative, dietary crops that widely have used in human life. Plant raw material of these plants is a source of biologically active compounds with numerous biological activities such as antioxidant, antimicrobial, anti-inflammatory, etc. This study was aimed to evaluate the antioxidant potential of selected plants from Asteraceae: *Bidens ferulifolia* (Jacq.) Sweet, *Echinacea purpurea* (L.) Moench, *Rhaponticum carthamoides* (Willd.) Iljin, *Silphium asteriscus* L., *S. lacinatum* L., *S. perfoliatum* L., *S. trifoliatum* L. Raw collected from the collection of M.M. Gryshko National Botanical Garden of the NAS of Ukraine (NBG) in the stage of budding and flowering. Determination of the antioxidant activity of investigated plants conducted by DPPH free radical scavenging activity. In this case, 1 g of dried and milled plant raw extracted in methanol and water in volume 25 ml and 100 µl of filtrate mixed with 3.9 ml of a radical solution. The optical density of the plant extracts measured using the spectrophotometer Unico 2800 (Russia) at the wavelength of 515 nm. Results expressed in % of inhibition. The DPPH free radical scavenging activity of methanol extracts of *Bidens ferulifolia* was 72.27–86.69%, *E. purpurea* of 31.19–75.92%, *Rh. carthamoides* of 56.31–73.14% and *Silphium* spp. from 34.86 to 92.51%. This parameter in water extracts was for *Bidens ferulifolia* 14.68–62.07%, *E. purpurea* of 45.47–68.90%, *Rh. carthamoides* of 52.61–79.05%, *Silphium* spp. from 14.88 to 93.47%. Thus, a study of the inhibition ability of different extracts of selected Asteraceae plants from NBG demonstrated the antioxidant potential of investigated plants than can be used for further study. Also, raw of these plants can be recommended for farther pharmacological investigations and as useful forage plants.

Keywords: Asteraceae, 2.2-diphenyl-1-picrylhydrazyl, free radical scavenging activity

Introduction

According to some researchers, most plant species (two-thirds) known as medicinal plants and have appropriate biological activities (Krishnaiah et al., 2011). Among known plant's families should be highlighting the Asteraceae Bercht. & J. Presl., which consists of

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approximately 1,000 genera and 25,000 species some of them use in medicine (Bessada et al., 2015). Family of Asteraceae includes different groups of plants that are important in human life such as medicinal (Vijaylakshmi et al., 2009; Patel, 2012), forage (Rakhmetov et al., 2019), food (García-Herrera et al., 2014), etc.

Raw of these plants has exhibited biological activities such as antioxidant (Vergun et al., 2018; Shelepova et al., 2019), antimicrobial (Babotă et al., 2018), and even invasive representatives have a high biological activity (Kozuharova et al., 2019). Plants from Asteraceae demonstrate the antioxidant activity due to the content of different groups of phenolic compounds in particular. Among them chlorogenic acid, luteolin, quercetin, apigenin, rutin (Bakar et al., 2015). Also, an important group of biologically active compounds isolated from Asteraceae is terpenoids that exhibited cancer-preventive effects, analgesic, anti-inflammatory, antimicrobial, antifungal, antiviral, antiparasitic activities (Sülsen et al., 2017). The investigation of raw showed that Asteraceae plants produced the essential oil with rich biochemical content (Raal et al., 2011). The *Bidens pilosa* oil, for example, demonstrated high inhibition of DPPH radical, and the main constituent of it was α-pinene, ε-caryophyllene, ß-ocimene (Goudoum et al., 2016).

Taking into account previous studies of Asteraceae plants, this work was aimed to evaluate the antioxidant potential of selected species raw as a potential source of antioxidants.

Material and methodology

Biological material

It was investigated some representatives from the Asteraceae Bercht. & J. Presl. such as *Bidens ferulifolia* (Jacq.) Sweet, *Echinacea purpurea* (L.) Moench, *Rhaponticum carthamoides* (Willd.) Iljin, *Silphium asteriscus* L., *S. lacinatum* L., *S. perfoliatum* L., *S. trifoliatum* L. An experiment carried out during 2018 at the laboratory of Cultural Flora Department of M.M. Gryshko National Botanical Garden of the NAS of Ukraine. Plants samples took at the budding and flowering stages and dried at 45 °C for 72 hours. All investigated plants are perennial.

Determination of DPPH scavenging activity

1 g of dried and milled plant raw extracted in the 25 ml of solvent (methanol and water) for 24 hours. After filtration procedure obtained extracts used to determined antiradical activity on a spectrophotometer Unico UV 2800 (Russia). A working solution of 2.2-diphenyl-1-picrylhydrazyl (DPPH) prepared the following way: 25 mg of radical dissolved in 100 ml of methanol. Obtained radical solution dissolved in 10 times till optical density was in the range of 0.700–0.800. The procedure of measuring conducted by Brand-Williams et al. (1995). 3.9 ml of radical solution mixed with 100 µl of plant extract and put for 10 min in the dark. During the procedure of measuring on a spectrophotometer at a wavelength of 515 nm used value of radical solution and value of the radical solution with the sample. Obtained results expressed in percentages.

Statistical analysis

The mean values of three replicates and the standard deviation are given. Data submitted with ANOVA and differences between means compared using the Tukey-Kramer test ($p = 0.05$).

Results and discussion

Use of DPPH scavenging activity method for evaluating the antioxidant potential of plant raw material widespread last decades and helps to find new sources of antioxidants. Plants from Asteraceae, also, not an exception in this relation and wild plants and crops from this plant family are rich in different compounds with antioxidant activity (Jamuna and Paulsamy, 2014; Indradi et al., 2017).

Plants of *Bidens* spp. use in folk medicine and exhibit numerous biological and pharmacological activities such as antioxidant, immunomodulatory, antidiabetic, antimicrobial anti-hypertensive, anti-hyperglycemic, antitumor, immunosuppressive, anti-inflammatory, antimalarial (Bessada et al., 2015). *B. pilosa* also has an essential oil that rich in biologically active compounds and demonstrated high antioxidant activity. In this case, the DPPH scavenging activity showed inhibition of 18.69–77.4% (Goudoum et al., 2016).

DPPH scavenging activity of different organs of *Bidens ferulifolia* was 72.27–86.69% in methanol extracts and 14.68–62.07% in water extracts (Figure 1). The most antiradical activity of extracts noticed for inflorescences and above-ground parts. Also, it should be noted that methanol extracts of *B. ferulifolia* demonstrated higher free radical scavenging activity than water extracts.

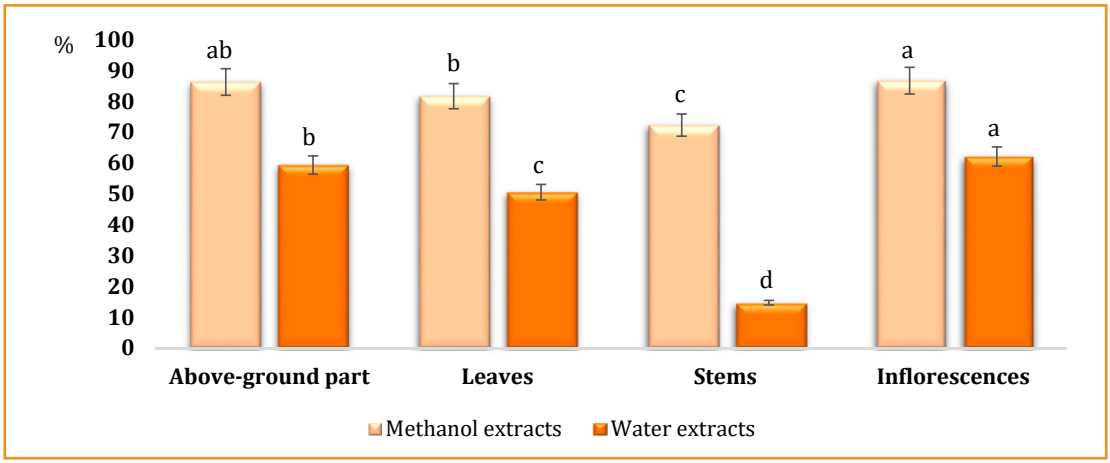

Figure 1 DPPH scavenging activity of *Bidens ferulifolia* (Jacq.) at the stage of flowering (means in columns followed by different letter are different at $p = 0.05$; each value represents the mean of three independent experiments (±SD))

Echinacea purpurea is a well-known medicinal plant with immunomodulatory, anti-inflammatory, antibacterial, antifungal properties and beneficial effects such as antianxiety, antidepression, cytotoxicity, antimutogenicity, etc. Among phenolic compounds, an efficient

effect determined for cichoric acid (Manayi et al., 2015). That is an ethnomedicinal plant that used at cough, respiratory infection, and bronchitis. Leaf and root essential oil of *Echinacea* spp. rich in volatile components (Nyalambisa et al., 2017).

Scavenging activity of radical in methanol and water extracts of *E. purpurea* in our experiment was 31.19–75.92% and 45.47–68.90%, respectively (Figure 2). In this case, most values of this parameter determined for leaves and above-ground parts.

The pharmacological study of *E. purpurea* demonstrated immunomodulatory, anti-inflammatory, antiviral, antifungal, antimicrobial activity, etc. (Barnes et al., 2005). The study of different extracts of *E. purpurea* showed antioxidant activity of 89.2% (Rady et al., 2018). Stanisavljević et al. (2009) found DPPH scavenging activity for ethanol extracts of *E. purpurea* 93.6% and antimicrobial activity.

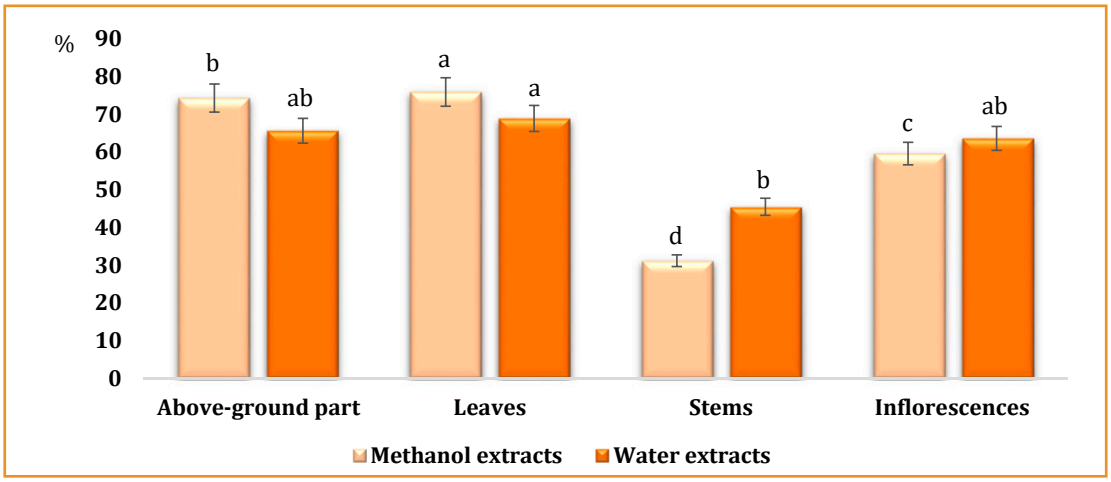

Figure 2 DPPH scavenging activity of *Echinacea purpurea* (L.) Moench. at the stage of flowering (means in columns followed by different letter are different at $p = 0.05$; each value represents the mean of three independent experiments (±SD))

Rhaponticum carthamoides commonly known as maral root or Russian leuzea uses in the folk medicine of some countries because of medicinal properties. The main groups of isolated compounds of this plant are steroids, flavonoids, phenolics (phenolic acids and flavonoids), triterpenoid glycosides, terpenes, etc. (Kokoska and Janovska, 2009). At the stage of flowering, we determined that methanol extracts of *Rh. carthamoides* were 56.31–73.14% and water extracts 52.61–79.05% (Figure 3). Methanol extracts of whole above-ground parts, leaves, and inflorescences had higher values of inhibition than water and stem extracts opposite. The previous study of the leaf ethanol extracts of this species showed that Trolox equivalent capacity by DPPH method was 9 mg TE/g and molybdenum reducing power of extracts was 77.87 mg TE/g DW (Vergun et al., 2019). Roots of *Rh. carthamoides* well-known source of biologically active compounds and exhibited antioxidant activity (Biskup and Lojkowska, 2009; Biskup et al., 2013).

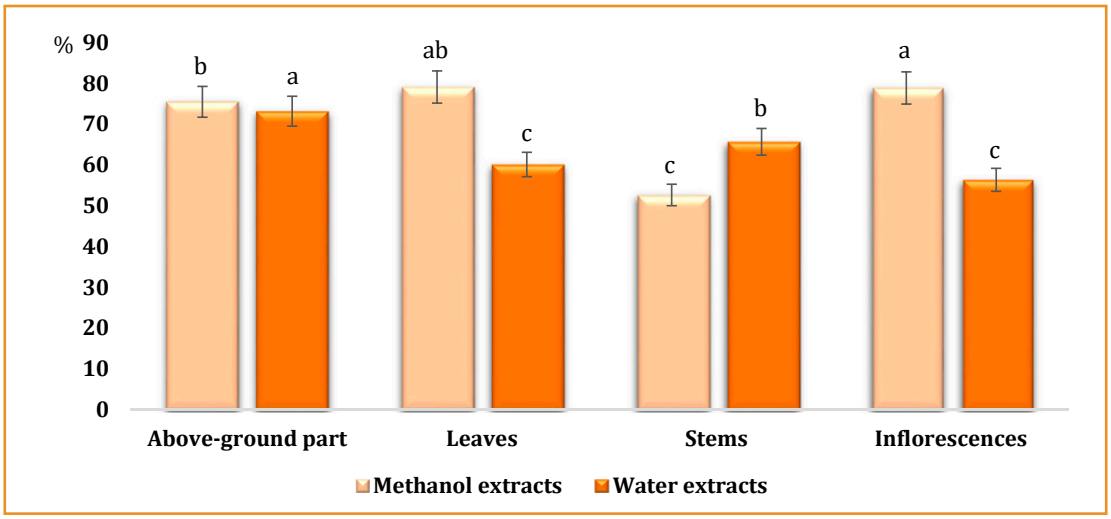

Figure 3 DPPH scavenging activity of *Rhaponticum carthamoides* (Willd.) at the stage of flowering (means in columns followed by different letter are different at $p = 0.05$; each value represents the mean of three independent experiments (±SD))

Different species of *Silphium* genus are a good source of nutrients for forage usage (Rakhmetov et al., 2019).

Plants from genus *Silphium* L. studied primarily at the stage of budding and one species at the stage of flowering (Figure 4). Methanol extracts exhibited results in a range of 34.86–92.51% and water extracts in the range of 14.88–93.47%. If compare generative organs of *S. asteriscus* that values of antiradical activity of both methanol and ethanol extracts increased from budding to flowering period. The study of *S. lacinatum* showed approximately the same values in methanol extracts as well as in water extracts. According to Shang et al. (2017), the scavenging activity of *S. perfoliatum* extracts was 75.71%.

The study of other species from Asteraceae *Tragopogon porrifolius* L. showed DPPH free radical scavenging activity of water and ethanol extracts as 77.3 and 83.2%, respectively (Al-Rimawi et al., 2016). Tandon and Gupta (2020), determined for *Sphaeranthus indicus* Linn. this parameter of whole plant extracts from 27.55 to 87.25%. Mosquera et al. (2009) evaluated 10 species from the Asteraceae family and determined the DPPH scavenging activity of methanol extracts from 4.0 (*Montonoa* spp.) to 33 (*Mikania leiostachya* Benth.) %.

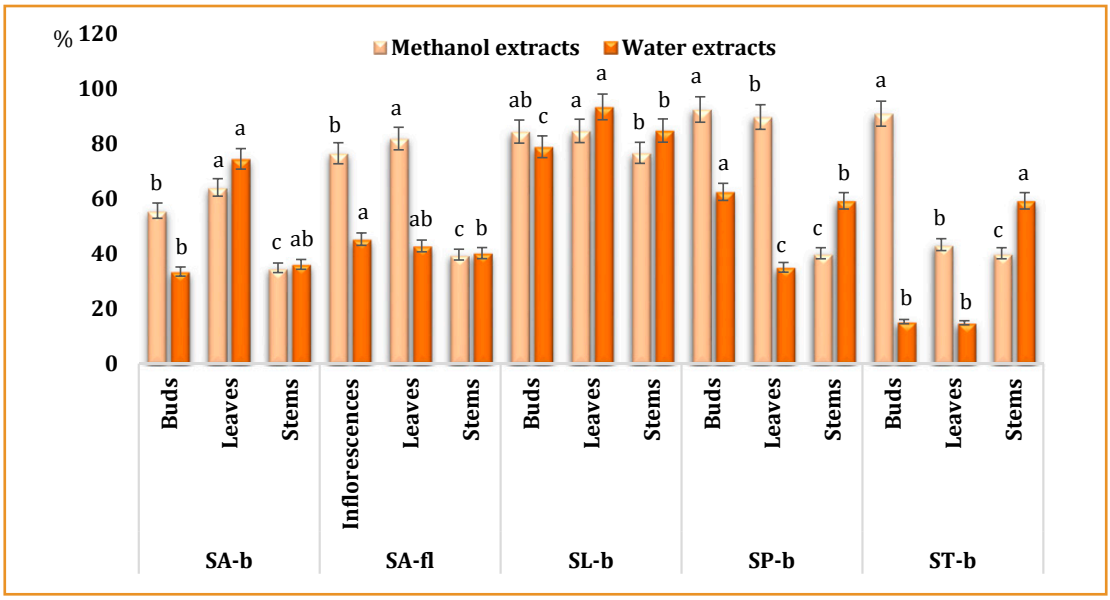

Figure 4 DPPH scavenging activity of *Silphium* spp. at the stage of budding and flowering
SA-b – *Silphium asteriscus* (budding stage), SA-fl – *S. asteriscus* (flowering stage), SL-b – *S. lacinatum* (budding stage), SP-b – *S. perfoliatum* (budding stage), ST-b – *S. trifoliatum* (means in columns followed by different letter are different at $p = 0.05$; each value represents the mean of three independent experiments (±SD))

Conclusions

Thus, all investigated representatives from the Asteraceae family exhibited antioxidant potential through free radical scavenging activity. The plant raw material of *Silphium* spp. showed maximal values of inhibition in methanol as well as in water extracts. It wasn't found strong regularity in peculiarities of inhibition in different organs of plants but in some cases, leaves and generative organs had higher values of scavenging activity than stems. Thereby, obtained data showed that the accumulation of compounds that possess antioxidant activity of plant extracts depends on species, stage of growth, and organ. The screening of new resources of antioxidant compounds among plant species is an important direction of modern science and allows to use of results for deep pharmacological investigations and others.

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CHEMICAL COMPOSITION OF LEAVES OF CHINESE QUINCE (*PSEUDOCYDONIA SINENSIS* (THOUIN) C.K. SCHNEID.)

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Pseudocydonia sinensis (Thouin) C.K. Schneid. less known plant species in the Ukraine conditions, but the fruits were widely used in traditional Chinese medicine for the treatment of asthma, colds, sore throat, mastitis, rheumatoid arthritis, and tuberculosis. In this study, chemical compositions of the leaves of *Pseudocydonia sinensis* were investigated. They contained total protein 6.66%, ash 8.54%, lipids 3.38%, beta carotene 90.30 mg/kg DW. Monosaccharide analysis revealed that the neutral carbohydrate part (fructose, maltose, sucrose, and lactose) was found in low amounts only (<0.5 g/kg). The major quantitative tocopherol in leaves was α-tocopherol (80.73 mg/kg DW). Saturated, monounsaturated and polyunsaturated fatty acids, palmitic acid (C16:0; 53.36 g/100 g DW), oleic acid (C18:1; 12.49 g/100 g DW) and linoleic acid (C18:2; 8.24 g/100 g DW), respectively, were found predominant. Palmitic acid makes up 57.2% of the total amount. The total amount of amino acids found in the leaves was 53.90 g/kg DW, including total essential amino acids (28.60 g/kg DW) and percentage of total essential amino acids (53.06%). Glutamic acid was found of leaves to be the dominant free amino acid (6.5 g/kg DW) followed by aspartic acid (5.4 g/kg DW) and leucine (4.9 g/kg DW DW). The mineral composition of leaves of *P. sinensis* demonstrated the presence of elements in following order: Ca>K>Mg>P>S>Fe>Zn>Na>Mn>Al>Cu>Ni>Cr>Pb>Cd>Hg>As>Se. Studied antioxidant parameters showed that antioxidant activity by DPPH and molybdenum reducing power was 8.76 and 289.73 mg TE/g, respectively. Also, the total content of polyphenols, flavonoids, and phenolic acids amounted to 65.77 mg GAE/g, 22.47 mg QE/g, and 9.06 mg CAE/g, respectively. The obtained data represent that leaves of *P. sisnensis* contain rich mineral composition, amino, and fatty acid composition and biologically active compounds such as polyphenols that can be used in the pharmaceutical study to validate its possible medicinal application. The study of less know and neglected plant species and it's raw can increase possible use in human life beneficial plant products.

Keywords: Chinese quince, leaves, chemical compositions, antioxidant activity

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Introduction

Interest in plants as a source of natural bioactive compounds has prompted researchers to investigate their tissue chemical composition and therapeutic potential. It is many plant species exist in the world have cultivated for food, but still less known, neglected and underutilized not full while they play important role in procuring the food security to improve health and nutrition, ecological sustainability and livelihoods (Mal, 2007; Magbagbeola et al., 2010; Dansi et al., 2012; Chivenge et al., 2015; Klymenko et al., 2017).

Chinese quince (*Pseudocydonia sinensis* (Thouin) C.K. Schneid.) of the family Rosaceae Juss. the only species in the genus *Pseudocydonia* C.K. Schneid., native to eastern Asia in China. It is closely related to the East Asian genus, *Chaenomeles* Lindl. (is sometimes placed in *Chaenomeles* as *C. sinensis*), and to the European genus, *Cydonia* Mill. (Suzuki, 1994).

Fruits of the *Pseudocydonia sinensis* are very fragrant, yellow edible pomes with an elliptical or ovoid shape (Mihara et al., 1987; Monka et al., 2014; Klymenko et al., 2017; Choi et al., 2018). Their fruits have a big size with a height of 98.06–124.48 mm, an average diameter of 62.33–88.64 mm, and an average weight in the range of 197.85–466.38 g (Monka et al., 2014).

The fresh fruit of *Pseudocydonia sinensis* are sour and hard and consumed after processing into spreads, marmalades, jams, fruit jellies, candied pulp, sweetened syrups and juices, wines, liqueurs, and use in preparing of flour products, candies (Hamauzu et al., 2006; Monka et al., 2014; Klymenko et al., 2017).

The fruit of investigated species includes organic acids, both flavonoids rutin and quercetin, procyanidins, and volatile compounds (Hamauzu et al., 2005; 2014). The main volatile compounds in Chinese quince peel are (E,E)-α-farnesene, isobutyl octanoate, ethyl octanoate, isobutyl 7-octanoate, and hexyl hexanoate (Mihara et al., 1987). In the peel, ethyl 2-methylpropanoate, ethyl (E)-2-butenoate, ethyl 2-methyl butanoate, methional, (Z)-3-hexenyl acetate, β-ionone, ethyl nonanoate, and γ-decalactone were found as the potent aroma-active compounds (Choi et al., 2018).

The fruits of the *Pseudocydonia sinensis* were widely used in traditional Chinese medicine for the treatment of asthma, colds, sore throat, mastitis, rheumatoid arthritis, and tuberculosis (Chung et al., 1988a; 1988b; Hamauzu et al., 2005; 2014; Mihara et al., 1987). The pharmacological studies have shown the antibacterial, antihaemolytic (Osawa et al., 1997), anti-inflammatory (Osawa et al., 1999), antipruritic (Oku et al., 2003), antioxidant (Hamauzu et al., 2005; 2007; Monka et al., 2014; Grygorieva et al., 2020), antiviral (Hamauzu et al., 2005; 2007; Sawai et al., 2008; Sawai-Kuroda et al., 2013), anti-ulcerative (Hamauzu et al., 2006), gastroprotective (Hamauzu et al., 2018), antitumor (Chun et al., 2012), and antimicrobial (Essuman et al., 2017; Kabir et al., 2015) properties of *Pseudocydonia sinensis* fruit.

To the best of our knowledge, there is no previously reported study of the phytochemical characteristics of *Pseudocydonia sinensis* leaves and scientific information still not enough. Therefore, this work was carried out to determine the chemical composition of leaves of less known species *Pseudocydonia sinensis* to assess the possibility of using this species in the future.

Material and methodology

Biological material

Pseudocydonia sinensis (Figure 1) leaves were collected in the July 2018 from trees growing in an M.M. Gryshko National Botanical Garden (Kyiv, Ukraine). The concentration of bioactive compounds detected in the dry material.

Figure 1 Leaves *Pseudocydonia sinensis* C.K. Schneid.

Chemicals

All the chemicals used were of analytical grade and were purchased from Sigma-Aldrich (Steinheim, Germany), Merck (Darmstadt, Germany) and CentralChem (Slovakia).

Phytochemical analyses

Determination of dry matter, ash and protein content

Total dry matter, ash, and protein content were determined according to EN method (CSN EN 12145, 1997). Total lipid content was determined according to methods specified in ISO method (ISO 659:1998).

Determination of saccharides

For the determination of saccharides, 1 g of sample was extracted with 10 mL of extraction solution (ultrapure water and ethanol mixed in ration 4 : 1) in a 50 mL centrifugation tube placed on vertical shake table (GFL, Germany). After 1 h of extraction, samples were centrifuged for 4 min at 6,000 rpm in a centrifuge (EBA 21, Hettich, Germany); the supernatant was filtered using a filter with 0.45 mm pore size (Labicom, Czech Republic) and filled up to 50 mL in a volumetric flask with ultrapure water. An Agilent Infinity 1260 liquid chromatography (Agilent Technologies, USA) equipped with ELSD detector was used for the determination of saccharides. A Prevail Carbohydrates ES column (250/4.6 mm) was used as a stationary

phase and acetonitrile (VWR) mixed with water in 75 : 25 volume ratio was used as the mobile phase.

Determination of carotenoid

Total carotenoid content expressed as beta-carotene was analyzed at a wavelength of 445 nm spectrophotometrically (VIS spectrophotometer UV Jenway Model 6405 UV/VIS). Sample (1 g) was disrupted with sea sand and extracted with acetone until complete discoloration. Petroleum-ether was added and then water, in purpose to the separation of phases. After the separation, the petroleum ether-carotenoid phase was obtained and the absorbance was measured (ČSN 560053, 1986).

Determination of mineral contents

Sample for elemental analysis was prepared using the wet ashing method in a microwave oven (Milestone 1200, Milestone, Italy). Total of 0.25 g sample matrix was decomposed in a mixture of nitric acid (6 mL) (Analytika Praha spol. s.r.o., Czech Republic) and hydrochloric acid (2 mL) (Analytika Praha spol. s.r.o., Czech Republic). After the decomposition sample was filtered using a filter with 0.45 mm pore size and filled up to 25 mL in a volumetric flask with ultrapure water. Elemental analysis was performed using ICP-OES (Ultima 2, Horiba Scientific, France) according to the procedure described by Divis et al. (2015).

Determination of amino acids

Amino acids were determined by ion-exchange liquid chromatography (Model AAA-400 amino acid analyzer, Ingos, Czech Republic) using post-column derivatization with ninhydrin and a VIS detector. A glass column (inner diameter 3.7 mm, length 350 mm) was filled manually with a strong cation exchanger in the LG ANB sodium cycle (Laboratory of Spolchemie) with average particles size 12 μM and 8% porosity. The column was tempered within the range of 35 to 95 °C. The elution of the studied amino acids took place at a column temperature set to 74 °C. A double-channel VIS detector with the inner cell volume of 5 μL was set to two wavelengths: 440 and 570 nm. A solution of ninhydrin (Ingos, Czech Republic) was prepared in 75% v/v methyl cellosolve (Ingos, Czech Republic) and in 2% v/v 4 M acetic buffer (pH 5.5). Tin chloride (SnCl2) was used as a reducing agent. The prepared solution of ninhydrin was stored in an inert atmosphere (N2) in darkness at 4 °C. The flow rate was 0.25 mL/min. and the reactor temperature was 120 °C.

Determination of total polyphenol, flavonoid, and phenolic acid content

The total polyphenol content (TPC) was measured by the method of Singleton and Rossi (1965) using the Folin-Ciocalteu reagent. A quantity of 0.1 mL of each sample was mixed with 0.1 mL of the Folin-Ciocalteu reagent, 1 mL of 20% (w/v) sodium carbonate, and 8.8 mL of distilled water. After 30 min in darkness, the absorbance at 700 nm was measured with the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25–300 mg/L; R^2 = 0.998) was used as the standard. The results were expressed in mg/g DM gallic acid equivalent.

The total flavonoid content (TFC) was determined by the modified method described by Shafii et al. (2017). An aliquot of 0.5 mL of the sample was mixed with 0.1mL of 10% (w/v)

ethanolic solution of aluminum chloride, 0.1 mL of 1 M potassium acetate and 4.3 mL of distilled water. After 30 min in darkness, the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (1–400 mg/L; R^2 = 0.9977) was used as the standard. The results were expressed in mg/g DM quercetin equivalent.

Total phenolic acid (TPA) content was determined using the method of Farmakopea Polska (1999). A 0.5 mL of sample extract was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL Arnova reagent (10% $NaNO_2$ + 10% Na_2MoO_4), 0.5 mL of 1 M sodium hydroxide (w/v) and 0.5 mL of water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1–200 mg/L, R^2 = 0.999) was used as a standard and the results were expressed in mg/g DM caffeic acid equivalents.

Determination of antioxidant activity

Free radical scavenging activity

Free radical scavenging activity of samples was measured using the 2.2-diphenyl-1-picrylhydrazyl (DPPH) (Sanches-Moreno et al., 1998). An amount of 0.4mL of the sample was mixed with 3.6mL of DPPH solution (0.025 g DPPH in 100 mL methanol). The absorbance of the reaction mixture was determined with the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2.5.7.8-tetramethylchroman-2-carboxylic acid) (10–100 mg/L; R^2 = 0.989) was used as the standard and the results were expressed in mg/g DM Trolox equivalents.

Molybdenum reducing antioxidant power

Molybdenum reducing (MRP) antioxidant power of samples was determined by the method of Prieto et al. (1999) with slight modifications. The mixture of the sample (1 mL), monopotassium phosphate (2.8 mL, 0.1 M), sulfuric acid (6 mL, 1 M), ammonium heptamolybdate (0.4 mL, 0.1 M) and distilled water (0.8 mL) was incubated at 90 °C for 120 min, then rapidly cooled. The absorbance at 700 nm was detected with the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10–1,000 mg/L; R^2 = 0.998) was used as the standard and the results were expressed in mg/g DMTrolox equivalent.

Statistic analysis

Basic statistical analyses were performed using PAST 2.17. Data were analyzed with ANOVA test and differences between means compared through the Tukey-Kramer test (p <0.05). The variability of all these parameters was evaluated using descriptive statistics.

Results and discussion

Biochemical study of fruit plants, basically, relates to the composition of fruits and few only highlight the accumulation of biochemical compounds in the leaves. But, evidently, that leaves of fruit plants also can be a useful source of nutrients with beneficial biological activities.

Chemical analyses of *Pseudocydonia sinensis* leaves revealed the presence of protein (6.66 wt/wt%), lipids (3.38 wt/wt%) and inorganic material (8.54 wt/wt%) (Table 1).

Table 1 The contents of some phytochemical compounds of *Pseudocydonia sinensis* C.K. Schneid.

Components	$\bar{x} \pm S_x^-$	Components	$\bar{x} \pm S_x^-$
Total dry matter (%)	92.66 ±3.05	**Polyunsaturated fatty acids (g/100 g oil)**	13.40 ±0.11
Total content of protein (%)	6.66 ±0.14	**Fructose (g/kg)**	<0.5
Total content of ash (%)	8.54 ±0.31	**Maltose (g/kg)**	<0.5
Total content of lipids (%)	3.38 ±0.12	**Sucrose (g/kg)**	<0.5
Beta carotene (mg/kg)	90.30 ±1.09	**Lactose (g/kg)**	<0.5
Saturated fatty acids (g/100 g oil)	70.0 ±0.56	**Vitamin A (retinyl acetate) (mg/kg)**	<0.1
Monounsaturated fatty acids (g/100 g oil)	12.95 ±0.16	**Vitamin E (α-tocopherol) (mg/kg)**	80.73 ±2.12

Note: $\bar{x}$ – arithmetic mean; S_x^- – standard error of the mean.

Monosaccharide analysis revealed that the neutral carbohydrate part (fructose, maltose, sucrose, and lactose) was found in low amounts only (<0.5 g/kg). Monosaccharides are primary products of photosynthesis. They are involved in nearly all fundamental processes within plant metabolism, including synthesis of organic and amino acids, polyphenols, pigments and aroma compounds (Halford et al., 2011). It is important to underline that leaves with increased monosaccharide content were found in plants when grown under stressful factors (Wind et al., 2010). Therefore, a lower level of sugars in the leaves indicates optimal environmental conditions for plant growth.

It should be noted that the main attention of researchers is aimed at studying the composition of monosaccharides and polysaccharides of fruits and seeds of *Chaenomeles* species. Thus, the polysaccharide fraction from the seeds of *Chaenomeles sinensis* and *Chaenomeles speciosa* consists of arabinose, glucose, xylose, galacturonic acid and glucuronic acid (Wang et al., 2018; Deng et al., 2020). Hypoglycemic analyzes showed that polysaccharides have good activity in inhibiting α-amylase and α-glucosidase. Therefore, a polysaccharide from the *Chaenomeles* species can be used as a potential natural source to slow down the effects of postprandial hyperglycemia. Also, polysaccharides and monosaccharides from these plants can be used in the food and pharmaceutical industries.

Pseudocydonia sinensis contains beta carotene (90.30 mg/kg). Carotenoids are among the most common natural pigments, and with β-carotene as the most prominent. They are pigments that play a major role in the protection of plants against photooxidative processes. Carotenoids are efficient antioxidants scavenging singlet molecular oxygen and peroxyl radicals. They interact synergistically with other antioxidants (Stahl and Sies, 2003).

The major quantitative tocopherol in *Pseudocydonia sinensis* leaves was α-tocopherol (80.73 mg/kg DWP). Four tocopherols and tocotrienols are collectively referred to as vitamin E. The reactivity with organic peroxyl radicals accounts for their antioxidant activity and is believed to be their major biochemical function (Shahidi and Ambigaipalan, 2015). These reactions are

the basis that vitamin E functions as an antioxidant, protecting tissue lipids from free radical attack.

The oil contents were 3.4% dry weight plant material (Table 1). It is reasonable because the majority of botanical materials (leaves) contain low amounts of lipids. The fatty acids composition was comprised of saturated fatty, monounsaturated, and polyunsaturated fatty acids (70.0; 12.95 and 13.40 g/100 g oil, respectively).

The lipophilic fraction contains of 20 fatty acids (Figure 2); five acids (palmitic acid C-16:0 in quantity 53.4 g/100 g; oleic acid C-18:1 in quantity 12.5 g/100 g; linoleic acid C-18:2 in quantity 8.2 g/100 g; stearic acid, C-18:0 in quantity 8.0 g/100 g; linolenic acid, C-18:3 in quantity 8.0 g/100 g) are dominated. Of these acids, amounted to 87.3% of the total (Figure 2). *P. sinensis* fatty acid profiles showed the presence of high amounts of palmitic acid (53.4%) (Figure 2). Palmitic acid is dominated in fatty acid profiles of leaves of many species such as Lamiaceae species (27.7–60.0%) (Cacan et al., 2018; Kilic, 2018); *Cassia tora* (L.) Roxb. (18.6–38.7%) (Shukla et al., 2018); *Nicotiana* species (13–18%) (Koiwai et al., 1983); *Cistus ladanifer* L. (13.6–17.5%) (Jerónimo et al., 2020). The second quantitatively major compound was oleic acid (12.5%). There are literature reports of insecticidal activity of oleic acid against *Aedesae gyptii* larvae (Kumar et al., 2009).

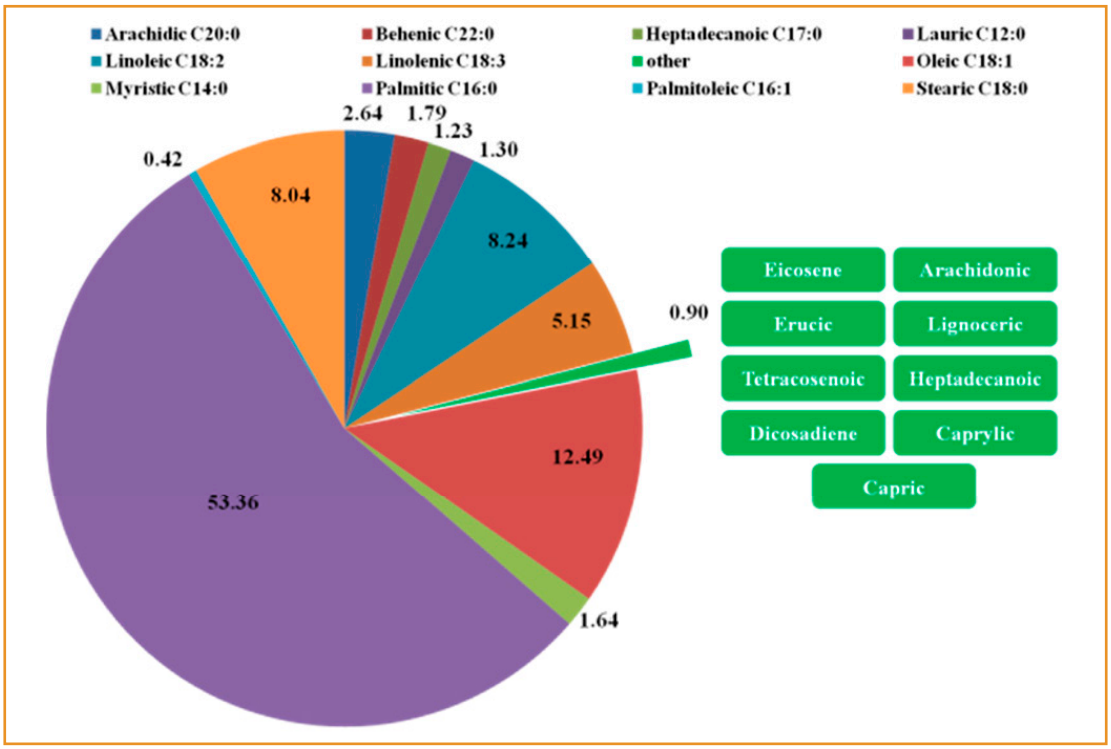

Figure 2 Fatty acid composition from leaves of *Pseudocydonia sinensis* C.K. Schneid. (g/100 g oil). Minor components (<0.1)
Eicosene C20:1; Arachidonic C20:4; Erucic C22:1; Lignoceric C24:0; Tetracosenoic C24:1; Heptadecanoic C17:1; Dicosadiene C22:2; Caprylic C8:0; Capric C10:0 are in the right column, their total amount is 0.9 g/100 g oil

According to the literature, *Pseudocydonia sinensis* fruit was rich in oleanolic acid and ursolic acid (Zhou et al., 2020). In addition, from the twigs of *Pseudocydonia sinensis* isolated five new oxylipins of chaenomic acid (Kim et al., 2014).

Lipids consisted of fatty acids, classified on saturated, monounsaturated, and polyunsaturated fatty acids (Mišurcová et al., 2011). Fatty acids play an important role as nutritious substances and metabolites in living organisms (Cakir, 2004). Many fatty acids are known to have antibacterial and antifungal properties (McGaw et al., 2002; Seidel and Taylor, 2004) and also have an important impact on human health, particularly in the prevention of cardiovascular disease, coronary heart disease, cancer, hypertension, diabetes type two, renal diseases, rheumatoid arthritis, ulcerative colitis, and Crohn's disease (De Caterina et al., 2000; Abedi and Sahari, 2014).

Fatty acids are involved in the formation of plant adaptive capacity to abiotic stresses: extreme positive and negative temperatures, lack of moisture causes a change in the composition of fatty acids (Gigon et al., 2004; Liu and Huang, 2004; Zhon get al., 2011; Li et al., 2017).

Amino acid analysis has shown that the studied *Pseudocydonia sinensis* leaves contained 18 amino acids (10 essential and 8 non-essential) (Figure 3).

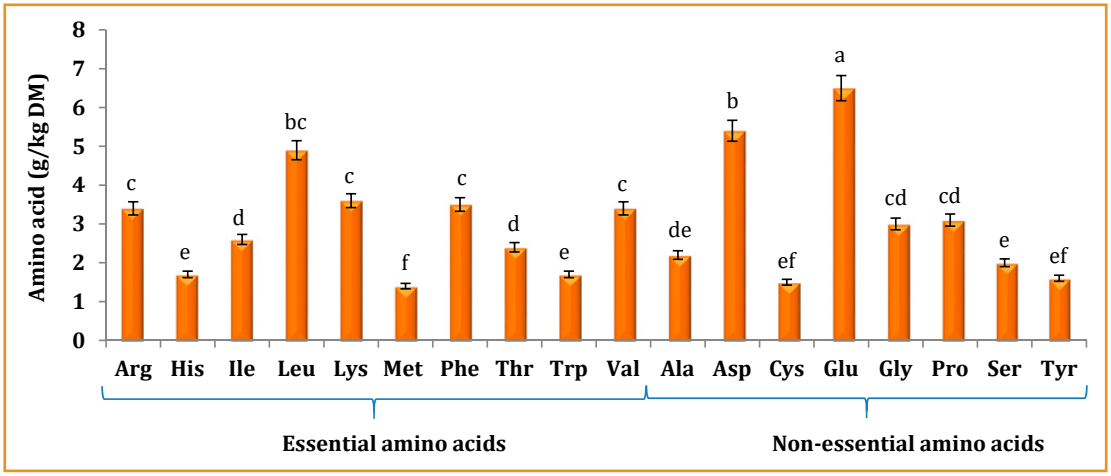

Figure 3 Amino acid composition of *Pseudocydonia sinensis* C.K. Schneid. leaves, g/kg DM (different superscripts in each column indicate the significant differences in the mean at $p < 0.05$)

Total amount of amino acids found in the leaves was 53.90 g/kg DM, including total essential amino acids (28.60 g/kg DM) and percentage of total essential amino acids (53.06%). Glutamic acid was found of leaves to be the dominant free amino acid (6.5 g/kg) followed by aspartic acid (5.4 g/kg) and leucine (4.9 g/kg).

One important factor for the formation of active constituents in plants is the trace elements because they are known to play an important role in plant metabolism and active constituents of medicinal plants are metabolic products of plant cells. In fact, the chemical constituents present in plants are responsible for their medicinal as well as toxic properties which

include vegetable bases comprising of alkaloids and amines, glycosides, essential oils responsible for their characteristic odour, toxic substances known as toxalbumin, resins, and antibiotics. Whereby the trace elements play a very important role in the formation of these compounds.

In addition, leaf analysis for mineral elements is an important guide to sustainable plant nutrition. The mineral composition of plants is influenced by factors such as growing conditions (climate, soil) and the phase of plant development (phenophase) (Penauelas et al., 2001; Erdal et al., 2006; Lipa, 2013; Yildirim et al., 2015).

The results of the elemental analysis of *Pseudocydonia sinensis* leaves are summarized in Table 2.

Table 2 Mineral composition of *Pseudocydonia sinensis* C.K. Schneid. leaves (mg/kg)

Components	$\bar{x} \pm S_x^-$	Components	$\bar{x} \pm S_x^-$	Components	$\bar{x} \pm S_x^-$
P	1,776 ±117	**Mg**	3,113 ±218	**Se**	<0.2
K	10,628 ±321	**Na**	13.0 ±0.7	**As**	<0.3
Ca	24,304 ±450	**Al**	8.9 ±1.4	**Cd**	0.051 ±0.003
S	1,335 ±98	**Cr**	0.20 ±0.08	**Ni**	1.37 ±0.02
Fe	56.0 ±1.6	**Cu**	6.0 ±0.7	**Hg**	0.024 ±0.005
Mn	9.8 ±0.8	**Zn**	32.0 ±1.5	**Pb**	0.100 ±0.003

Note: $\bar{x}$ – arithmetic mean; S_x^- – standard error of the mean.

Concentration of various elements of leaves decreases in the order: Ca>K>Mg>P>S>Fe>Zn>Na>Mn>Al>Cu>Ni>Cr>Pb>Cd>Hg>As>Se. Among the various elements As, Se, Cd, Hg are found to be present at the trace level. Fe, Zn, Mn, Na, Al, Ni, and Cu are at the minor level, and Ca, K, Mg, P, and S are at the major levels. This result confirmed by the data Lewko et al. (2004) and showed that the leaves of quince (*Cydonia oblonga*) contained a large amount of calcium. Calcium is an essential mineral for human health, participating in the biological functions of several tissues (musculoskeletal, nervous and cardiac system, bones and teeth, and parathyroid gland). In addition, Ca can act as a cofactor in enzyme reactions (fatty acid oxidation, mitochondrial carrier for ATP, etc.) and it is involved in the maintenance of the mineral homeostasis and physiological performance in general (Theobald, 2005; Huskisson et al., 2007; Morgan, 2008; Williams, 2008).

Based on a large amount of scientific data proving the beneficial effect of phenolic content in humans, it is appropriate to perform estimation of these compounds content of leaves extracts of *Pseudocydonia sinensis*.

The amount of total phenolic acid, flavonoids, and polyphenols content was 9.06 mg CAE/g DW, 22.47 mg QE/g DW, and 65.73 mg GAE/g DW, respectively (Figure 4).

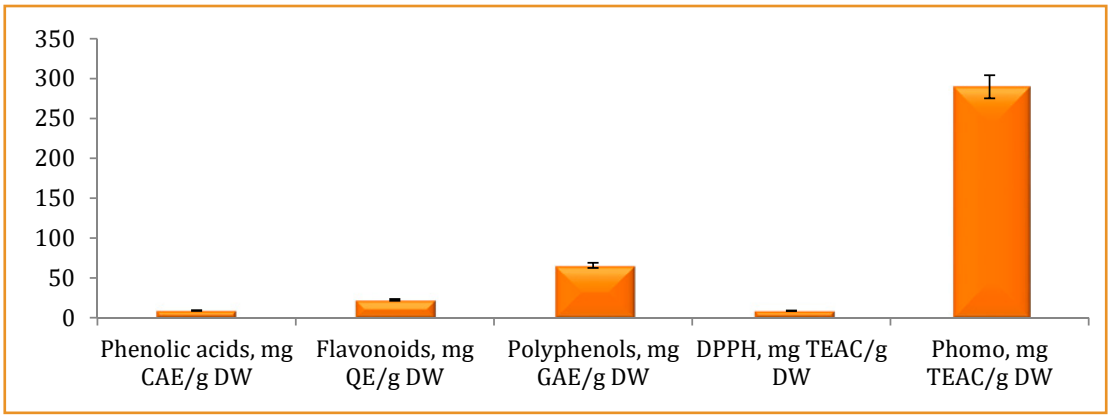

Figure 4 Parameters of antioxidant activity of *Pseudocydonia sinensis* C.K. Schneid. leaves

The leaves of crops and wild plants are a valuable source of antioxidant substances, especially polyphenols. According to published data, the total phenol content for the leaves of *Mangifera indica* L. was 65 mg/g, for *Anacardium occidentale* L. 58.57 mg/g, for *Cymbopogon citratus* (DC.) Stapf 28.30 mg/g, for *Carica papaya* L. 21.80 mg/g (Iyawe and Azih, 2011), for *Euphorbia* spp. 19.10–20.30 mg/g (Gapuz and Besagas, 2018) and for *Azadirachta indica* Juss. 14.43 mg/g (Iyawe and Azih, 2011). Thi and Hwang (2014) found that the total polyphenol content of *Aronia mitschurinii* ranged from 139.3 to 250.8 mg GAE/g DW. The result of another study submitted by Shahin et al. (2019), related to *Aronia melanocarpa*, demonstrated that the total polyphenol content in the dried leaves was 765.63 mg GAE/g. According to Męczarska et al. (2017), *Amelanchier alnifolia* leaves showed a total polyphenol content of 185.23 mg GAE/g DW. As reported by Barreira et al. (2010), the total flavonoid content of *Castanea sativa* Mill. 73.31–90.39 mg/g, for data of Stankovic et al. (2014) *Cornus mas* L. leaves had a total flavonoid content of 22.18 mg/g, according to Al-Saeedi et al. (2016) for *Ziziphus jujuba* Mill. methanol extracts were 90.28 mg/g.

The information about the characterization of *Pseudocydonia sinensis* leaf extract is limited. Zhou et al. (2020) using HPLC reported the phenolic profile of leaves and found 5-compounds. Phthalic acid, di(2.3-dimethylphenyl) ester (3.693%), heptasiloxane, hexadecamethyl (25.425%) and neophytadiene (25.309%) are major compound in the leaves.

Most research on *Cydonia oblonga* leaf characterization is form Portugal (Oliveria et al., 2007). The total phenolic content of *C. oblonga* leaves was very high, varying from 4.9 to 16.5 g/kg dry matter (mean value of 10.3 g/kg dry matter). In the phenolic profile of Portuguese quince leaves were found 9 compounds. The 5-O-caffeoylquinic acid was the major compound (36.2%), followed by quercetin-3-O-rutinoside (21.1%) and kaempferol-3-O-rutinoside (12.5%). The leaves of Tunisian quince variety "Commune" have 9-phenolic acids and flavonoids (Benzarti et al., 2018). Among these polyphenols, 6-were identified, one as caffeoylquinic acid (4-O-caffeoylquinic acid), two as quercetin heterosides (quercetin-3-O-rutinoside and quercetin-3-O-galactoside), and three as kaempferol heterosides (kaempferol-3-O-rutinoside, kaempferol-3-O-glycoside, and kaempferol-3-O-glucoside).

The evaluation of the antioxidant activity of leaf extracts showed that *Pseudocydonia sinensis* leaves have reducing capacity and 2.2-diphenyl-1-picrylhydrazyl (DDPH) radical scavenging activity. Antioxidant activity by DPPH method and molybdenum reducing antioxidant power method 8.76 and 289.79 mg TEAC/g DW, respectively (Figure 4).

Zhou et al. (2020) described *Pseudocydonia sinensis* leaves contain abundant bio-energy components, such as Heptasiloxane, hexadecamethyl-, which has a higher content in ethanol extracts, and the active components of medical components, such as d-alpha-tocopherol, which are contained in both solvent extracts (ethanol and acetone.) Hamauzu et al. (2006) described Chinese quince fruits and leaves comprise a hopeful natural source of bioactive compounds, namely caffeoylquinic acids and epicatechin. The antioxidant and antiproliferative activities described for this material may be indicative of application in nutritional and pharmaceutical fields, in the prevention and treatment of free radical-mediated human chronic pathologies, such as cardiovascular diseases and cancer. Leaves from *Pseudocydonia sinensis* can be used as an immense natural and inexpensive source of bioactive compounds with major antioxidative properties along with other mechanisms of action. By modulating various cardiovascular risk factors such as atherosclerosis, smoking, endothelial dysfunction hypertension, diabetes, and hyperhomocysteinaemia, *Pseudocydonia sinensis* leaf extract may have relevance in the prevention and treatment of different pathological states of ischemic inflammatory and hypertrophic heart disease.

Conclusion

As a result, this study demonstrates that leaves of *Pseudocydonia sinensis* rich source of useful biochemical compounds that conclude mineral components and polyphenol compounds. Among mineral compounds, the high concentrations found for calcium, potassium, magnesium, and phosphorus. Also, plant raw of *Pseudocydonia sinensis* leaves had potential as an antioxidant resource that can be used in further study. The findings of this study support the fact that leaves of *Pseudocydonia sinensis* can be used as a raw material in medical practice as well as the development and production of dietary supplements and cosmetic preparations rich in biologically active compounds.

Acknowledgments

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DETERMINING THE OPTIMAL SEASON FOR DETECTION OF PRUNE DWARF VIRUS AND PRUNUS NECROTIC RINGSPOT VIRUS IN SOUR CHERRY CULTIVARS

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Prune dwarf virus (PDV) and Prunus necrotic ringspot virus (PNRV) are most widespread pathogens in sour cherry orchards in Ukraine. The study is aimed to select the optimal season and tissues of sour cherry plants for the detection of PDV and PNRV in the climatic condition of Ukraine. The experiment was performed by DAS-ELISA using certified test kits manufactured by Loewe Biochemica GmbH (Germany). Trees of cherry cultivars 'Bohuslavka' (PDV infected) and 'Kseniia' (PNRV infected) were selected for testing. Healthy plants of the same cultivars were selected as the negative controls. Samples of various tissue (young leaves, dorman leaves, flower petals, fruits and cambium) were taken at different terms of the vegetation period. The young leaves demonstrated the highest absorbance levels of PDV and PNRV in April when $A_{405\ nm}$ was at least 19 times higher comparing to negative control. Also reliable results were recorded at the beginning of the growing season when flower petals were used. So leaves and flowers were the most reliable source for the detection of these viruses from April till August. Instead, in October there was a high possibility of false-negative results as the results didn't exceed the negative control value more then 2.5 times. This study will contribute to the optimization of DAS-ELISA PDV and PNRV detection in Ukraine.

Keywords: virus, cherry, leaf, flower, absorbance

Вступ

Віруси некротичної кільцевої плямистості (ВНКП) та карликовості сливи (ВКС) – є найпоширенішими патогенами вишні як в Україні, так і поза її межами (Mandic et al., 2007; Perez-Sanchez et al., 2017; Pavliuk et al., 2019). Також вони становлять небезпеку для інших представників роду *Prunus* spp. (персика, аличі, сливи та мигдалю) (Pallas et al., 2012). ВНКП та ВКС можуть викликати на плодових деревах різноманітні симптоми, прояв яких значно залежать від рослини-господаря, ізолята та кліматичних умов (Nemeth, 1986). Інфікування ВКС може спричинювати зміну форми листкової

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пластини – вона може згортатися чи звужуватися, також на листі часто з'являються хлоротичні кільця та плями (Öztürk and Çevik, 2015). В результаті інфікування ВНКП відбувається деформація квітів, листя та плодів. Попри те, що патогени можуть викликати виражені симптоми, інфікування ними часто має латентний характер (Sokhandan-Bashir et al., 2017).

Обидва досліджувані віруси були вперше описані в США. ВКС було виявлено у 1936 році на сливі (Thomas and Hildebrand, 1936), згодом у 1941 році на рослинах персика було описано ВНКП (Cochran and Hutchins, 1941). З того часу розробили ряд методів, включно з ELISA та молекулярними методиками (зт-ПЛР, флуоресцентна зт-ПЛР, ПЛР в режимі реального часу, ізотермічна ампліфікація), для виявлення даних патогенів. Всі ці методи відрізняються за чутливістю та використовуються для різних цілей, але найкращим варіантом для проведення діагностики великої кількості рослинного матеріалу залишається саме ELISA. Однак виявлення вірусів цим методом може мати помилкові результати, наприклад, за зниження вірусних титрів у рослин в період спокою чи під час підвищеного температурного режиму повітря (Huo et al., 2017).

Згідно рекомендацій Європейської організації захисту рослин по сертифікації садивного матеріалу вишні, черешні та їх підщеп PM 4/29 (1) (2000), через здатність ВКС та ВНКП розповсюджуватися з пилком, рекомендується перевіряти базовий матеріал першого покоління у маточних насадженнях сортів та підщеп щорічно. Для якісної перевірки рослинного матеріалу на відсутність вірусних патогенів необхідне визначення оптимальних строків та тканин рослини для проведення діагностики, тому дане дослідження є актуальним завданням.

Матеріали та методи

Рослинний матеріал

В якості досліджуваних зразків було обрано матеріал рослин із насаджень вишні звичайної (*Prunus cerasus* L.), Інституту садівництва НААН (Київська обл.). Зміну концентрацій вірусних титрів ВКС вивчали на прикладі сорту Богуславка, а ВНКП – Ксенії, а в якості негативного контролю використовували здорові рослини тих же сортів. Зразки відбирали з двох інфікованих дерев. Попередньо наявність патогенів у обраних рослин було підтверджено методом зт-ПЛР.

Зразки відбирали протягом 2019–2020 років, з квітня по жовтень, з чотирьох сторін дерева. Переважно у якості досліджуваного матеріалу обирали листя, але в квітні додатково тестували квіти, в червні – плоди, а у вересні та жовтні – камбій.

Імуноферментний аналіз

Для імунодіагностики ВНКП та ВКС використовували сертифіковані тестові набори виробництва Loewe Biochemica GmbH (Німеччина) згідно з рекомендаціями виробника. Постановку ELISA здійснювали за стандартною методикою Clark and Adams (1977). При тестуванні матеріалу листя – відбирали базальну частину листка, при тестуванні квітів використовували пелюстки. Матеріал гомогенізували

в екстракційному буфері у співвідношенні 1 : 20. Кожен зразок тестували у двократному повторі. Інкубацію здійснювали при кімнатній температурі, протягом 60 хвилин після внесення субстратного буфера. Результати реєстрували за допомогою мікропланшетного фотометра ImmunoChem – 2100 Microplate Reader (USA), при довжині хвилі 405 нм ($A_{405\ нм}$). Позитивним вважали зразок, в якому $A_{405\ нм}$ перевищував негативний контроль в 2,5 рази.

Статистичні обрахунки

Статистичну обробку результатів з вирахування середніх показників $A_{405\ нм}$ та стандартної помилки проводили за допомогою програми STATISTICA.

Результати та обговорення

Листя є найбільш поширеним матеріалом, що використовується для тестування на наявність вірусів, оскільки воно доступне протягом тривалого періоду, а також легко піддається гомогенізації. В умовах Правобережної частини Західного Лісостепу (Київська область) вегетація рослин вишні починається в другій декаді квітня, тому дослідження починали проводити в даний період.

При порівнянні рівнів абсорбції ВКС у 2019 – 2020 роках відмічено, що у 2019 році показники були більш стабільними. Найвищий рівень абсорбції у 2019 році спостерігали в квітні (1,015 ±0,03) (Рисунок 1). Незважаючи на підвищення температури в червні 23 ±0,07 °С (Рисунок 2), показники $A_{405\ нм}$ залишалися в цьому місяці досить високими – 0,527 ±0,1, подальше зростання концентрації реєстрували в серпні – 0,711 ±0,03. В наступних місяцях показники $A_{405\ нм}$ знижувалися, але перевищували негативний контроль більше ніж в 2,5 рази, що дозволяло нам вважати результати позитивними.

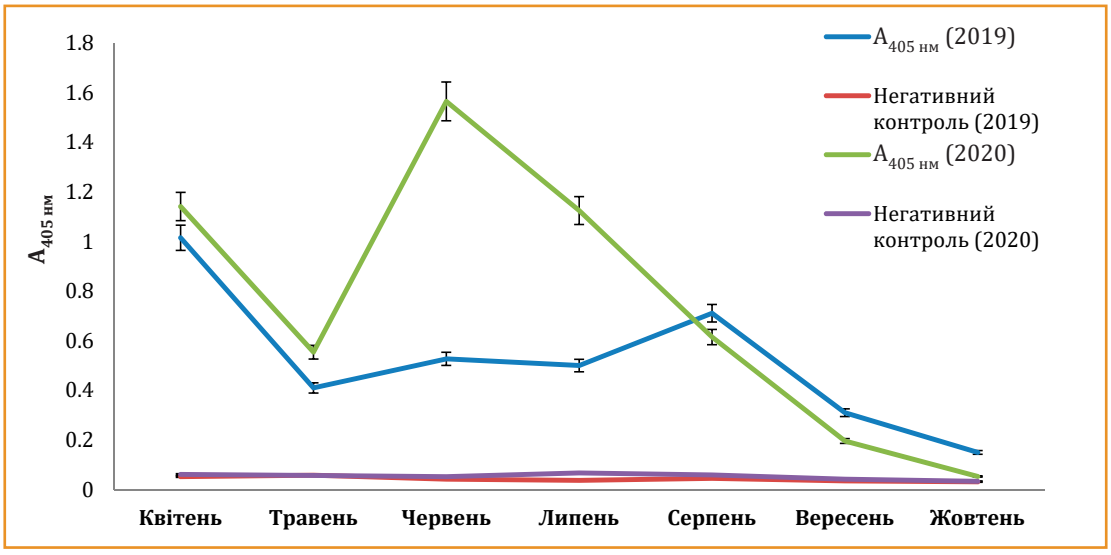

Рисунок 1 Середній показник рівня абсорбції ($A_{405\ нм}$) ВКС у зразках листя (2019 – 2020)
Figure 1 The average absorbance level ($A_{405\ nm}$) of PDV in leaf samples during (2019–2020)

У 2020 р. рівень абсорбції був дещо вищим. Найбільшим він був в квітні (1,141 ±0,01) та червні (1,565 ±0,2). Варто відмітити, що $A_{405\ нм}$, який фіксували в червні, був найвищим за роки проведення досліджень. Це, можливо, пов'язано з помірною середньодобовою температурою протягом кількох тижнів до відбору зразків (16,9 ±0,2) та активним ростом дерева, що сприяє реплікації вірусу. Далі показники поступово знижувалися. В жовтні $A_{405\ нм}$ перевищував негативний контроль лише в 1,6 рази, що не дозволяє вважати отриманий результат позитивним.

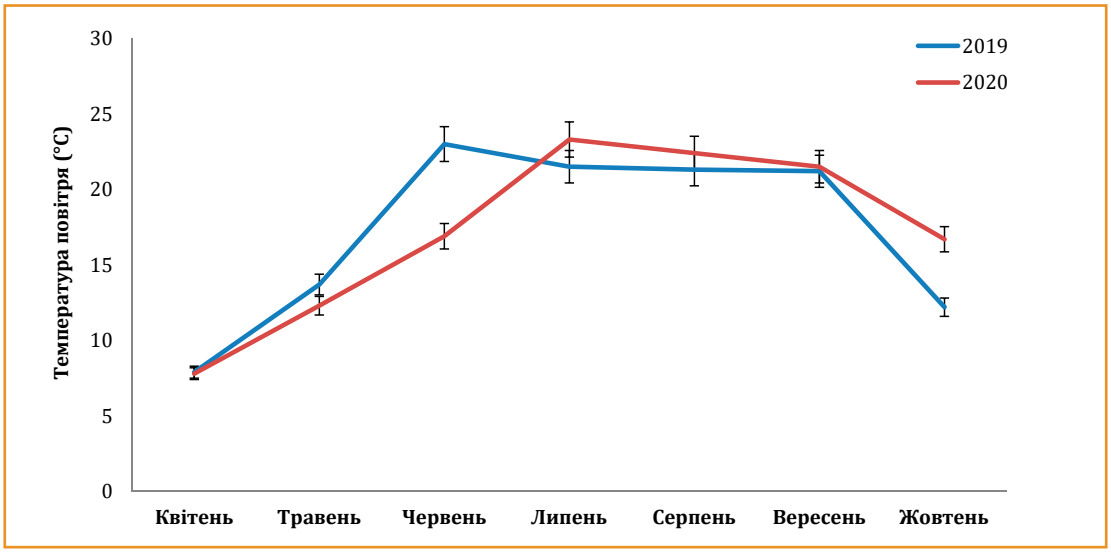

Рисунок 2 Середньодобова температура повітря за 30 днів до відбору зразків (2019 – 2020)
Figure 2 Average air temperature 30 days before sampling during (2019–2020)

Середній показник абсорбції за два роки у зразках квітів складав 1,332 ±0,04. Даний матеріал давав високі результати та легко піддавався гомогенізації, але він доступний лише короткий проміжок часу, тому його тестування є обмеженим. В червні для діагностики додатково брали плоди, середній рівень $A_{405\ нм}$ за два роки становив 0,133 ±0,04 і перевищував негативний контроль в 2,7 рази. Цей показник дозволяв нам вважати зразок позитивним, але титр вірусу знаходився на межі чутливості тестового набору. У вересні, окрім листя, тестували камбіальні тканини, середній показник $A_{405\ нм}$ яких за два роки становив 0,264 ±0,002. Проте вже у жовтні рівень абсорбції знизився і дорівнював лише 0,084 ±0,004, що перевищувало негативний контроль в 2,2 рази.

Отримані дані рівня абсорбції ВНКП у 2019–2020 рр., сильно різнилися в залежності від року проведення досліджень (Рисунок 3). На початку вегетації показники А405н були досить високими і перевищували негативний контроль в 39 – 46 разів. В перший рік дослідження високі показники фіксували протягом всього весняно-літнього періоду із несуттєвим їх зниженням ближче до осені. У вересні та жовтні А405н дорівнював 0,505 ±0,07 та 0,267 ±0,01 відповідно. Осінні результати перевищували негативний зразок більше ніж в 2,5 рази, що вважалося позитивним результатом.

У 2020 р. рівень абсорбції був найвищим в квітні – 2,613 ±0,03 та травні – 1,104 ±0,08. В червні відбулося значне зниження титру вірусу у листі, але даний матеріал все ще був придатний для серологічного тестування та успішного виявлення інфікованих та здорових рослин.

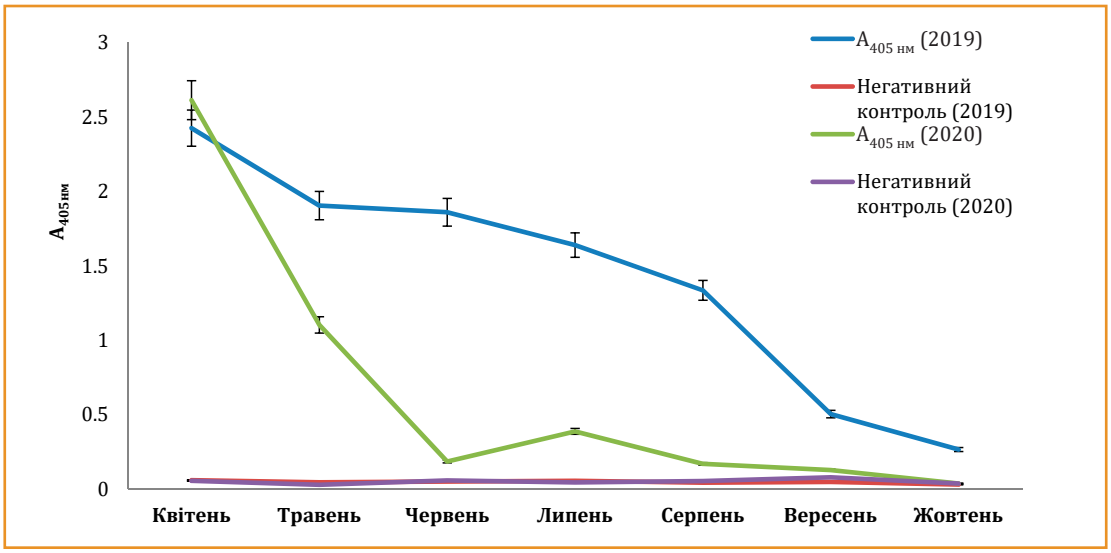

Рисунок 3 Середній показник рівня абсорбції ($A_{405\ нм}$) ВНКП у зразках листя (2019 – 2020)
Figure 3 The average absorbance level ($A_{405\ nm}$) of PNRV in leaf samples during (2019–2020)

В осінні місяці показник $A_{405\ нм}$ інфікованих зразків листя перевищував негативний контроль лише в 1,6 рази у вересні та був однаковим з ним у жовтні.

Варто відзначити, що у 2020 році відбулося загальне зниження концентрації ВНКП в інфікованих рослинах, що може свідчити про задіяння механізмів захисту рослин від вірусів та перехід хвороби у хронічну форму (Verderevskaya and Marinesku, 1985; Honjo et al., 2019).

Додатково тестували квіти, плоди та камбіальні тканини. Показники $A_{405\ нм}$, які фіксували з матеріалу квітів були досить високими, і за два роки становили 2,239 ±0,1. На відміну від попереднього матеріалу, плоди виявилися не придатними для виявлення інфікованих рослин. Середній показник $A_{405\ нм}$ у плодах за два роки становив 0,067 ±0,009, що не перевищувало негативний контроль в 2,5 рази. У матеріалі камбію рівень абсорбції у вересні складав 0,214 ±0,01, а у жовтні – 0,088 ±0,001. Таким чином, не рекомендовано проводити діагностику ВНКП у зразках вишні восени.

В дослідженнях зміни вірусного титру вірусу мозаїки яблуні (ВМЯ), які були проведені на інфікованих рослинах яблуні, були отримані аналогічні результати з нашими. Автори пояснюють, що отримані навесні високі титри ВМЯ пов'язані з тим, що реплікація даного вірусу краще відбувається в прохолодну пору року (Svoboda and Polak, 2010). Інші науковці вказують, що сприятливим часом для реплікації вірусів є період, коли

температура повітря знаходиться в межах 15 – 30 °С та в період активного росту рослини (Uyemoto et al., 1989; Scott et al., 1992; Honjo et al., 2019).

Наші дослідження цілком підтверджують дані твердження, адже найвищі показники $A_{405\ нм}$ ми фіксували навесні, хоча, для нашого клімату, літній період виявився також цілком сприятливим для тестування. Достовірність тестування обох вірусів знижувалася у вересні та жовтні, що може бути пов'язано з зупинкою ростових процесів. Плоди не є оптимальним варіантом у якості тестованого матеріалу вишні на ВКС та ВНКП. До таких висновків дійшли вчені, які успішно тестували матеріали бруньок, листя, квітів, камбію та плодів мигдалю, персика і сливи, проте при тестуванні плодів останньої культури не отримали позитивного результату (Salem et al., 2003). Також є повідомлення про те, що величина показника $A_{405\ нм}$ може змінюватися в залежності від сортових особливостей рослини (Zotto et al.,1999).

Висновки

Дані дослідження дозволили визначити оптимальні строки відбору зразків вишні для проведення серологічної діагностики ВНКП та ВКС в умовах Західного Лісостепу України. Найбільш достовірні результати отримували в квітні – травні, використовуючи при цьому молоде листя або квіти. Плоди є менш надійним джерелом тканини для тестування в порівнянні з листям. В осінні місяці можна отримати хибнонегативний результат, отже діагностику рослин вишні краще не проводити в даний період.

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CONTENT OF NUTRIENTS IN DIFFERENT PARTS OF *IPOMOEA BATATAS* L. (LAM.)

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Ipomoea batatas (L.) Lam. (sweet potato) is a popular food crop that is planted vegetatively and native to the American tropics. It is one of the most economically important crops alongside other that widely used in the world. This study was aimed to investigate the biochemical composition of plant raw material of *I. batatas* in conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine. It was investigated the leaves, stems, and tubers at the end of vegetation. The content of dry matter, lipids, the total content of reducing sugars, tannins, titrable acidity, ascorbic acid, ß-carotene, total ash content, calcium, phosphorus, and the energetic value were detected. At the end of vegetation content of dry matter was from 13.00 to 25.45%, the total content of sugars from 12.32 to 34.43%, the titratable acidity from 1.82 to 5.48%, the tannin content from 0.82 to 6.96%, ascorbic acid content from 34.1 to 53.34 mg%, the content of ß-carotene from 0.238 to 0.641 mg%, lipids content from 1.89 to 6.93%, total ash content from 2.1 to 11.99%, calcium content from 0.53 to 1.86%, phosphorus content from 0.51 to 1.81%. The energetic value was in the range 3,242–3,623 Cal/g. Very strong positive correlation found between total tannin content and ash (r = 0.952), ß-carotene content and phosphorus (r = 0.919), total tannin content and titrable acidity (r = 0.845), ß-carotene content and lipids (r = 0.837). Plant parts of *I. batatas* contain functional and nutrient components that make it useful for the food industry. Obtained data can be used for the deep further biochemical, pharmacological study, and selective work.

Keywords: *Ipomoea batatas*, biochemical composition, correlation.

Introduction

Ipomoea batatas L. (Lam.) (sweet potato) belongs to the Convolvulaceae family is widely grown in tropical, subtropical, and warm temperate regions. It is widespread forage (Ruiz et al., 1980; Nguyen et al., 2004), food (Ababukar et al., 2010), medicinal, ritual, agricultural (Meira et al., 2012) crop in the world.

This plant has different pharmacological activities such as antioxidant, antiviral, anti-inflammatory, hepatoprotective, gastroprotective, and immunomodulatory effects (Panda and

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Sonkamble, 2012). The antidiabetic potential *I. batatas* caused its bioactive compounds and isolated antidiabetic agents have a unique mechanism of action (Kusano et al., 2001; Akhtar et al., 2018). The most common biologically active compounds from *Ipomoea* spp. are ergoline alkaloids, indolizidine alkaloids, nortropane alkaloids, phenolic compounds, coumarins, norisoprenoids, diterpene, isocoumarin, glycolipids, lignan, triterpenes, etc. (Meira et al., 2012).

The study of the biochemical composition of leaves indicated the presence of amino acids (Ravindran et al., 1995), anthocyanin, polyphenols, vitamins, protein (Islam et al., 2002). The study of mineral composition of *I. batatas* tubers demonstrated the presence of iron (0.53–0.73 mg per 100 g), zinc (0.23–0.27 mg per 100 g), calcium (23.04–29.97 mg per 100 g), magnesium (21.30–25.40 mg per 100 g), phosphorus (42.0–46.33 mg per 100 g), potassium (308.67–328.67 mg per 100 g), and sodium (29.0–34.0 mg per 100 g) (Sanoussi et al., 2016a).

According to Ababukar et al. (2010), the protein content in sweet potato leaf decoction was 12.41%. This plant is a good source of dietary antioxidants (Alam et al., 2016). Leaf, stem, and root extracts of *I. procumbens* demonstrated the antioxidant and antimicrobial activity (Batiga et al., 2019). The essential oil from leaves of *I. batatas* contains monoterpenes, sesquiterpenes, diterpenes, abietadiene, β-caryophyllene, abieta-8, 11, 13-triene, *trans*-(Z)-α-bergamotol, *cis*-sabinen, etc. (Ogunmoye et al., 2015). Sweet potato can be used for bioethanol production (Swain et al., 2013). The influence of nitrogen fertilizer on biochemical parameters of *I. batatas* studied and it was indicated increasing of β-carotene and crude protein at 40-80 kg N/ha (Ukom et al., 2009).

This study aimed to determine the peculiarities of biochemical compound accumulation in raw of two varieties of *Ipomoea batatas* (L.) Lam., which were grown in M. M. Gryshko National Botanical Garden of the NAS of Ukraine.

Materials and methodology

Biological material

This study investigated two varieties of *Ipomoea batatas* (L.) Lam. (f. 1 and f. 3). Plants collected from the experimental collection of the Department of Cultural Flora in M.M. Gryshko National Botanical Garden of the NAS of Ukraine (NBG) at the end of vegetation (at the beginning of October) during 2018–2019. Biochemical analyses were conducted in the laboratory of Department Cultural Flora of NBG. All investigated plants are annual.

Biochemical analyses

Dry matter determination

Plant samples were dried in a drying oven at 105 °C till constant weight in aluminum boxes. Results are given in percentages (Yermakov et al., 1972).

The total content of sugars determination

The total content of sugars was investigated by Bertrand's method in water extracts. 4 g of fresh mass mixed and homogenized with distilled water (approximately 50 ml) in the 100 ml test-tubes and heated in the water bath at 70 °C during 15–20 min. After cooling in the obtained mixtures added 1 ml of the phosphate-oxalate mixture. After this was added 1.5 ml of lead acetate. The obtained mixture brings to the mark (100 ml) with water. After filtration from the obtained solution took 50 ml and mixed with 8 ml of 20% HCl (at the 70 °C in a water bath for 5 min) and after cooling was neutralized by 12% NaOH and brought to the mark by distilled water (100 ml). 3 ml of obtained solution mixed with 6 ml of Fehling's solution reagent (6 min boiling in the water bath). Obtained mixture analyzed for the total content of sugars. Results are given by percentages (Hrytsajenko et al., 2003).

The total content of ascorbic acid

Determination of ascorbic acid content conducted by method offered by K. Murri. 2 g of fresh mass mixed with 50 ml of 2% oxalic acid. Obtained mixture put into the dark for 20 min. Content of ascorbic acid of obtained extracts determined by a 2.6-dichlorophenol-indophenol method that is based on the reduction properties of ascorbic acid. Obtained results were expressed in the mg% DW (Hrytsajenko et al., 2003).

The total content of ß-carotene

The concentration of total carotene determined according to Pleshkov (1985) using extraction with rubber solvent (petrol). 1 g of absolutely dried raw mixed with 20 ml of Kalosha petrol for 2 hours. After this obtained filtrate measured spectrophotometrically at the wavelength 440 nm at the Unico spectrophotometer. Obtained results expressed in mg% DW. This parameter is determined in the leaves and stems.

The total content of tannins

The content of tannins was determined with indigo carmine as an indicator (Yermakov et al., 1972). 5 g of fresh mass mixed with distilled water (approximately 50 ml) in 100 ml taste-tubes. Obtained mixture heated in the water bath at 70 °C for 2 hours. After cooling, adding water to the 100 ml, and following filtration 10 ml of filtrate used for the determination of the total content of tannins. This procedure used 700 ml distilled water and 25 ml of 1% solvent of indigo carmine. Obtained results expressed in %.

The total content of organic acids

The total content of organic acids is determined with phenolphthalein and results calculated with a malic acid coefficient (Krishchenko, 1983). 10 ml of filtrate (the same procedure described for the determination of total content of tannins) titrated with 1 N solvent of NaOH in presence of phenolphthalein. Obtained results expressed in percentages.

The total ß-content of lipids

The total content of lipids is determined using the Soxhlet extractor. Dried raw was extracted with petroleum ether (boiling temperature 40–60 °C) according to Yermakov et al. (1972).

The total content of ash, calcium, and phosphorus

The total content of ash is determined by combustion in the muffle-oven at 200–500 °C for 3 days considering the mass before and after combustion (Hrytsajenko et al., 2003). After the combustion procedure ash was used to determining calcium and phosphorus content. Total calcium content was detected by Trilon-B titration (Hrytsajenko et al., 2003). Phosphorus content determined dissolving ash in the nitric acid (1 : 5), after the routing procedure with adding molybdenum reactive, mixtures were titrated with sodium hydroxide (Pochinok, 1976).

Energetic value

The procedure of caloricity measurement conducted using calorimeter IKA C-200 (Germany). 0.1–0.2 g of dried plant raw material was combusted in an oxygen bomb for approximately 15 minutes.

Statistical analysis

The mean values of three replicates and the standard deviation are given. Data submitted with ANOVA and differences between means compared using the Tukey-Kramer test ($\alpha = 0.05$). Correlation analysis performed using Pearson's criterion.

Results and discussion

The biochemical composition study of crops is a very important part of investigations to evaluate nutrition value. Among other parameters, should be highlighted dry matter content (Shipley and Vu, 2002) and the total content of sugars that also play a significant role in plant tolerance to stress factors (Sami et al., 2016). The content of dry matter was from 13.00 to 25.45% depending on the plant part (Figure 1).

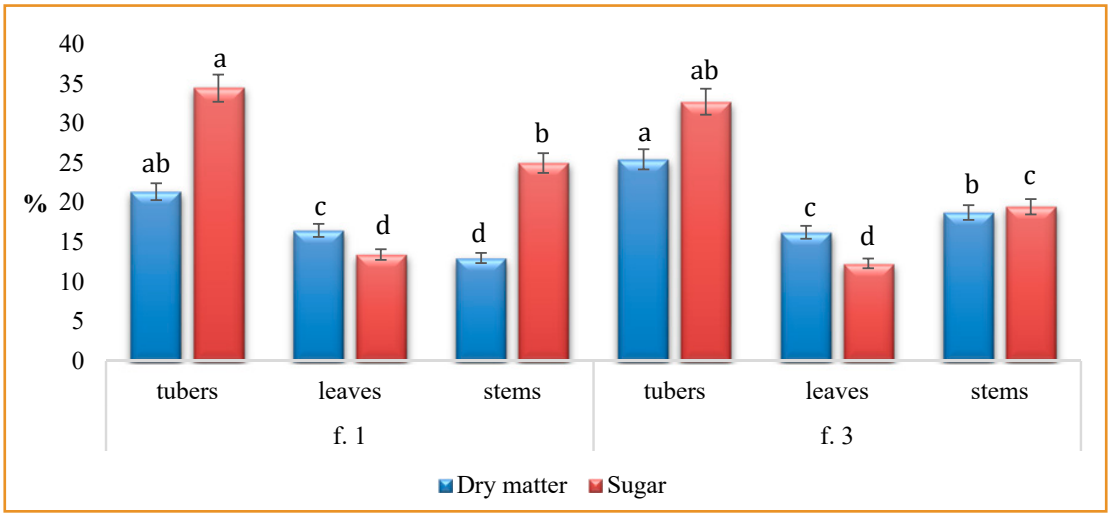

Figure 1 The total content of sugars and dry matter of the plant raw material of *Ipomoea batatas* (L.) Lam. at the end of vegetation (means in columns followed by different letters are different at $p < 0.05$. Each value represents the mean of three independent experiments (±SD))

The most value of dry matter found in the tubers of *I. batatas*. Also, tubers accumulated the most sugars comparing with other plant parts, while leaves had the least content of it. At all, the total content of sugars was 12.32–34.43%.

A study of the chemical composition of sweet potato dishes showed that the content of moisture in leaf soup varied from 35.15 to 70.54% and carbohydrate content was 25.74–70.00% (Ababukar et al., 2010). According to Dinu et al. (2018), the dry matter content of sweet potato blades and petioles were 16.0–18.5 and 12.3–16.0%, respectively. Also, it was reported that reducing sugars content was 23.80–51.91%, depending on the cultivar and plant part. As reported Hossain et al. (2019), the dry matter content of three *I. batatas* varieties detected from 30.3 to 34.5%, sugars from 10.61 to 11.82%. Nascimento et al. (2015) reported that total carbohydrates content in tubers was 41.08–73.43%. According to Ravindran et al. (1995), dry matter content of *I. batatas* cultivars was in a range of 30.6–37.2%.

The ascorbic acid content one of the important parameters to evaluate the nutritive quality of plant raw material. Ascorbic acid as a biochemical compound acts as an enzyme cofactor, as a radical scavenger, and as a donor/acceptor on electron transport either at the plasma membrane or in the chloroplasts (Davey et al., 2000). The raw of f. 1 ascorbic acid accumulated in the range 34.1–53.34 mg% and f. 3 in a range of 18.86–64.03 mg% (Figure 2). Carotenoids are natural pigments, including ß-carotene, lycopene, lutein, etc. Among the carotenoids, the ß-carotene one of the most widespread groups of secondary metabolites with high bioactivity (Bogacz-Radomska and Harasym, 2018). The content of ß-carotene in our study was 0.238–0.641 mg% for both varieties.

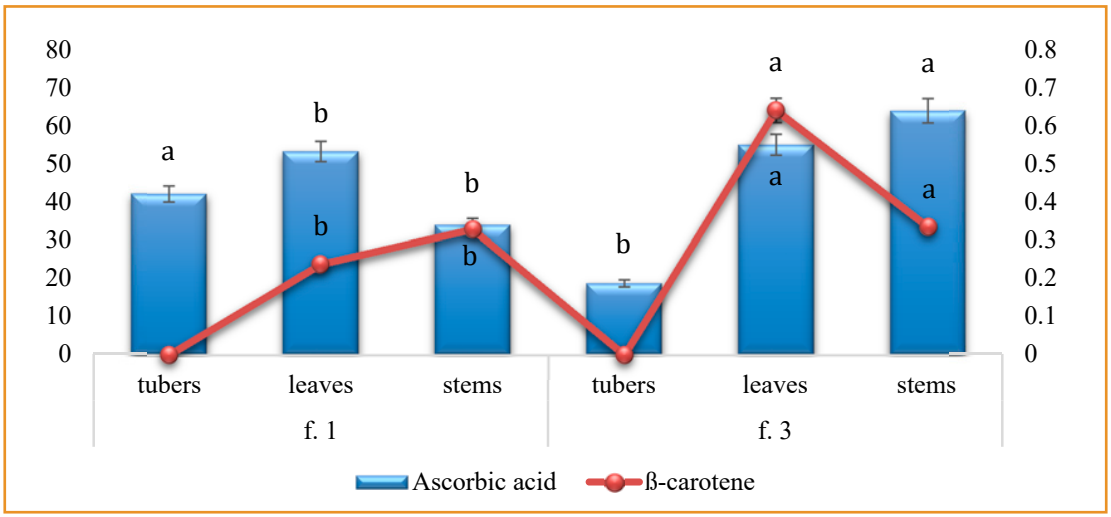

Figure 2 The content of ascorbic acid and ß-carotene in the plant raw material of *Ipomoea batatas* (L.) Lam. at the end of vegetation, mg% (means in columns followed by different letters are different at p <0.05. Each value represents the mean of three independent experiments (±SD))

Alam et al. (2016) reported the total content of carotenoids in different *I. batatas* varieties from Bangladesh was from 0.38 to 7.24 mg%. According to Dinu et al. (2018), carotene content in the blade and petiole extracts was 23.80–25.05 and 16.39–16.60 mg%, respectively. Sweet

potato leaves accumulated ascorbic acid content of 3.56–5.96 mg% that is approximately 10 times less than our study. As reported Nascimento et al. (2015), the total carotenoid content in tubers was 3182.0 µg per 100 g. Sri Lankan cultivars had an average sugars content of 6 g per 100 g DW (Ravindran et al., 1995).

The most content of tannins in the raw of investigated *I. batatas* identified in the leaves and the least in the tubers % (Figure 3). At all, tannins accumulated in a quantity of 0.82–6.96%. Titrable acidity is determined from 1.82 to 5.48%, depending on the plant part and variety. Li et al. (2017) found that tannin content for different cultivars of sweet potato was 2.28–4.46 mg per 100 g.

The total lipid content in different parts of *I. batatas* plants was in the range of 1.89–6.93% (Figure 3).

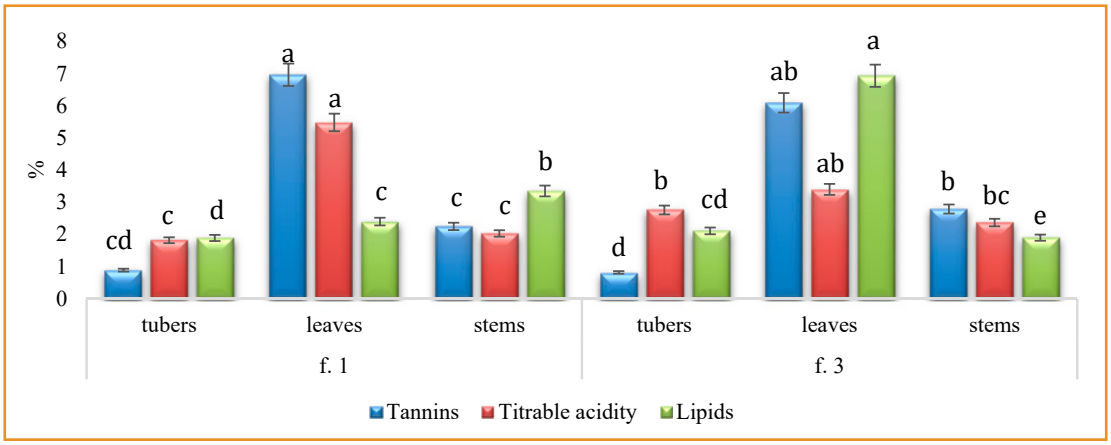

Figure 3 The content of tannins, titrable acidity, and lipids in the plant raw material of *Ipomoea batatas* (L.) Lam. at the end of vegetation (means in columns followed by different letters are different at p <0.05. Each value represents the mean of three independent experiments (±SD))

The highest content of it is identified in the leaves of f. 3 plants. Lipids are important biochemical compounds that play numerous biological functions and accumulated in the seeds (Vergun et al., 2017), leaves, stems, rhizomes (Andrushchenko et al., 2018), tubers (Ramadan and Oraby, 2016), etc.

The fat content in tubers of 16 cultivars of Sri Lankan sweet potato was in a range of 1.07–2.14 g per 100 g DW (Ravindran et al., 1995). The fat content of *I. batatas* dishes ranged from 0.30 to 3.88% and tannin content from 0.22–0.86 mg per 100 g (Ababukar et al., 2010). According to Nascimento et al. (2015), fat content in the sweet batatas tubers was 0.19–4.50% and proteins 0.58–2.53%.

The most content of ash among investigated varieties of *I. batatas* found in the leaves and the least in the tubers (Figure 4). At all, the total content of ash was from 2.1 to 11.99%. The concentration of calcium was from 0.53 to 1.86% and phosphorus from 0.51 to 1.81%. As reported Ravindran et al. (1995), the calcium content in tubers of these plants was 89–239 mg

per 100 g DW and phosphorus 148–226 mg per 100 g DW. According to Ababukar et al., 2010, the ash content of sweet potato dishes varied from 1.13 to 8.83%, and the concentration of calcium was determined from 19.19 to 27.99 mg per 100g. Ash content in tubers, as reported Nascimento et al. (2015), was 0.85–1.29% depending on cultivar.

The energetic value was identified in the leaves and stems and was from 3242 to 3623 Cal/g.

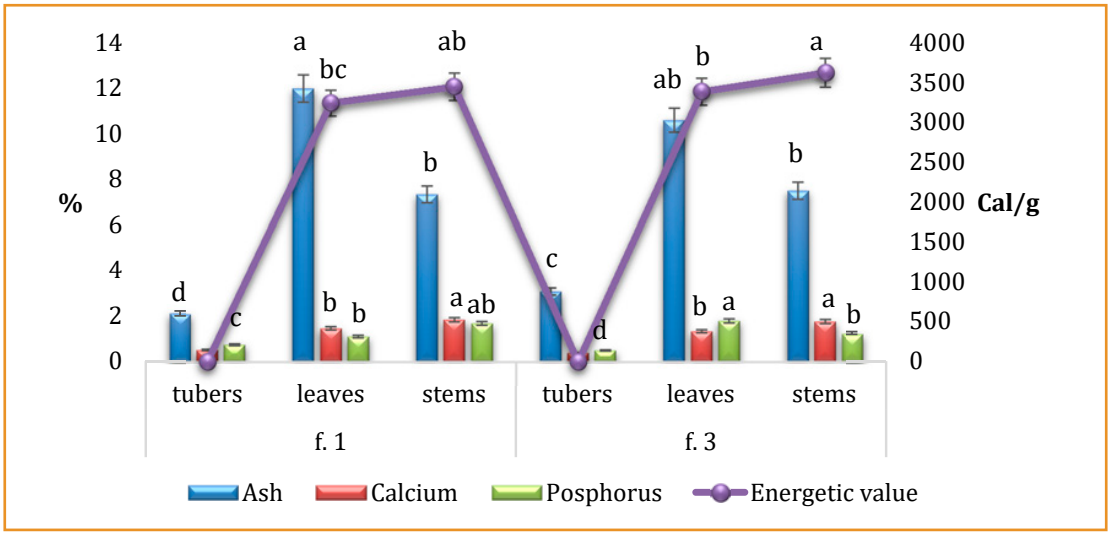

Figure 4 The content of ash, calcium, phosphorus, and energetic value in the plant raw material of *Ipomoea batatas* (L.) Lam. at the end of vegetation (means in columns followed by different letters are different at p <0.05. Each value represents the mean of three independent experiments (±SD))

Very strong positive correlation found between carotene content with lipids (r = 0.837), and with phosphorus (r = 0.919). Total tannin content correlated with titrable acidity (r = 0.845) and with ash content (r = 0.952). Strong correlation found between dry matter and sugars content (r = 0.637), titrable acidity and ash (r = 0.750), lipids and phosphorus (r = 0.740). Ascorbic acid content correlated strongly with carotene content (r = 0.600), total tannin content (r = 0.614), and ash content (r = 0.620), etc. Also, strong relations determined between ash content and its components.

According to Sanoussi et al. (2016b), dry matter content of *I. batatas* positively correlated with carbohydrate content and energy value. Total sugar content is also positively correlated with energy value. Li et al. (2017) found negative correlation between tannin content and ß-carotene (r = -0.214) content while in our study correlation was determined as strong (r = 0.676). Analise of data concerning biochemical composition of *I. batatas* petiole and leaf showed a very strong positive correlation between total phenolic compounds and reducing sugars, and their extracts exhibited the antioxidant activity that correlated with phenolic compounds (Dinu et al., 2018). Biochemical composition of *I. batatas* depending on geographical location, climate, weather, heat treatment, and terrestrial factors as seen from the previous report (Shariff et al., 2017).

Table 1 Correlation analysis between investigated parameters of *Ipomoea batatas* (L.) Lam.

Parameter	DM	TSC	AA	Car	TTC	TA	LC	Ash	Ca	P
TSC	0.637**	1								
AA	-0.459	-0.730**	1							
Car	-0.705**	-0.832**	0.600**	1						
TTC	-0.559	-0.940	0.614**	0.676**	1					
TA	-0.195	-0.683	0.297	0.216	0.845**	1				
LC	-0.447	-0.573	0.228	0.837**	0.539**	0.150	1			
Ash	-0.709	-0.974**	0.620**	0.761**	0.952**	0.750**	0.509*	1		
Ca	-0.868**	-0.691	0.597**	0.693**	0.493*	0.174	0.237	0.718**	1	
P	-0.896**	-0.691	0.480*	0.919**	0.543**	0.064	0.740**	0.682**	0.797**	1
EV	0.339	0.541**	0.271	0.066	-0.810**	-0.835**	-0.237	-0.862**	0.640**	0.062

Note: **– correlation is significant at the 0.01 level; * – correlation is significant at the 0.05 level; DM – dry matter content; TSC – total sugar content; AA – ascorbic acid content; Car – carotene content; TTC – total tannin content; TA – titrable acidity; LC – lipid content; Ca – calcium content; P – phosphorus content; EV – energetic value

Conclusions

Thus, in M.M. Gryshko National Botanical Garden two varieties of *I. batatas* investigated for selected biochemical parameters and it was indicated that plant raw material accumulated a high content of nutrients. The highest content of dry matter and sugars found in tubers, ascorbic acid, ß-carotene, tannin content, lipids, ash in leaves and stems depending on the variety. Between some investigated parameters detected a very strong positive correlation such as carotene content and lipids, carotene and phosphorus, total tannin content with titrable acidity, and ash content. Strong correlation found between dry matter and sugars content, titrable acidity, and ash, lipids, and phosphorus. Obtained data can be used for the deep further biochemical, pharmacological study, and selective work.

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COMPARISON OF OLD AND LANDRACES VARIETIES OF THE APPLE TREE (*MALUS DOMESTICA* BORKH) IN THE VARIABILITY OF SOME MORPHOLOGICAL CHARACTERS OF LEAVES AND FLOWERS

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Old and landraces varieties of cultivated plant species selected from natural populations adapted to long-term cultivation, which represent a rich genetic potential for the development of agroecosystems and agriculture under specific conditions, resources for an environment aestheticization, landscaping and development of cultural traditions. The research focused on determining the economic value of a selected collection of old and landraces varieties and fruit-bearing seedlings of apple tree (*Malus domestica* Borkh), widespread in Slovakia for their practical use in organic farming or as genetic resources for breeding new varieties for organic food production. For experimental evaluation, we used 73 old and landraces varieties of apple trees concentrated and preserved ex-situ in a clone repository in the village Bacúch and 77 fruit-bearing seedlings, which originated from free pollination and grown in-situ form around Nitra, Levice, Nové Zámky, Šaľa, Galanta, Hlohovec, Piešťany, Prievidza, Partizánske, Zlaté Moravce. By morphological analysis we determined for old and landraces varieties/fruit-bearing seedlings the range for the length of leaf blade 37.41–97.50 mm/57.54–107.93 mm, width of the leaf blade 27.00–66.44 mm/30.08–62.18 mm, the diameter of flowers 35.43–54.85 mm/32.55–62.08 mm, length of petals 15.48–24.41 mm/14.63–28.06 mm. The results document that in both collections of old and landraces varieties and fruit-bearing seedlings, we detected genotypes suitable for organic cultivation, such as germplasm for apple breeding for agroecological usage.

Keywords: *Malus domestica*, genetic resources, clone repository, morphometric analysis, variability

Úvod

Na Slovensku má pestovanie ovocných drevín dlhoročnú tradíciu. Dominantné postavenie v ovocinárstve majú jablone. Rod *Malus* z čeľade Rosaceae a podčeľade Pomoideae ako príklad ovocných stromov jablone domácej (*Malus domestica* Borkh.) je jedným z najdôležitejších, najviac rozšírených a najlepšie prispôsobených ovocných druhov mierneho pásma z hľadiska produkcie a zaujíma ústredné miesto vo folklóre, kultúre a umení (Juniper and Mabberley, 2006). Pestuje sa v oblastiach s vysokou zemepisnou šírkou, kde môžu teploty dosahovať -40 °C až do vysokých nadmorských výšok v trópoch, kde je možné pestovať dve plodiny

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v jednom roku (Janick, 1974), avšak najlepšie klimatické podmienky sú pre tento ovocný druh medzi 35 – 50°. Jabloň má rozsah výskytu severnejší než iné ovocné druhy z dôvodu relatívne neskorého kvitnutia a vyššej mrazuvzdornosti (Hancock et al., 2008). V súčasnosti pestovatelia najmä z ekonomického hľadiska využívajú výkonné odrody s novými pestovateľskými tvarmi, s úzkymi intenzívnymi sponmi, pestovaním na slabo rastúcich podpníkoch atď (Forsline et al., 2003; Shakhnovych and Melnychuk, 2015). Popri intenzifikácii ovocinárstva sa aktívne rozvíja aj ekologické ovocinárstvo, v ktorom sa vyžaduje pestovať odrody vysoko tolerantné proti biotickým a abiotickým faktorom pestovateľského prostredia (Cornille et al., 2013; Margitay, 2016). Do pozornosti sa dostávajú staré a krajové odrody kultúrnych druhov rastlín, ktoré vznikli ako súčasť dlhodobého procesu domestikácie rastlín človekom. Boli vyselektované z prírodných populácií a pestované pre využitie vo výžive alebo pre iné účely. S ohľadom na ich dlhodobé pestovanie v rôznych oblastiach sa adaptovali na určité špecifické pestovateľské podmienky, čím nadobudli vysoký stupeň tolerancie proti nepriaznivým faktorom prostredia. Predstavujú výrobné prostriedky pre rozvoj agroekosystémov a poľnohospodárstva v špecifických podmienkach, zdroje pre estetizáciu životného prostredia, krajinotvorbu a rozvoj kultúrnych tradícií (Brindza, 2001; Tóth et al., 2004; Ganopoulos et al., 2017). Zakladanie klonových repozitórií s cieľom záchrany ohrozeného genofondu rastlín má u nás uplatnenie pri mnohých ovocných druhoch akými sú hruška, čerešňa, slivka, gaštan atď. (Bolvanský a Užík, 2012; Paprstein et al., 2013; Benediková et al., 2016).

Okrem krajových odrôd možno za významné genotypy pre ekologické ovocinárstvo využiť aj voľne rastúce semenáče jabloní. Je všeobecne známe, že mnohé krajové odrody ako aj pestované odrody boli vyselektované zo semenáčov (Boček, 2008). Semenáče jablone domácej – rastliny dopestovane zo semien sa využívali a využívajú ako východiskový materiál pre šľachtenie alebo ako genetické zdroje pre podpníky (Sedov et al., 2013; Solomatin et al., 2017). Ontogenetický vývoj semenáčov jabloní a ich význam pre šľachtenie jabloní experimentálne rozpracoval I.V. Mičurin (Sedov et al., 1966; Boček, 2008).

Uvedené problematika sa stala hlavným objektom experimentálneho štúdia genetických zdrojov jablone domácej pre ekologické poľnohospodárstvo v kolekcii krajových a starých odrôd ako aj voľne rastúcich semenáčov rozšírených v podmienkach Slovenska.

Materiál a metodika

Biologický materiál

Pre experimentálne účely sa použili dva súbory biologického materiálu ako genetických zdrojov:

a) staré a krajové odrody sústredené z rôznych oblastí Slovenska a uchovávané v klonovom repozitóriu formou *ex situ* v obci Bacúch – 73 vybraných genotypov. V experimentoch sme ich označovali skratkou R;
b) voľne rastúce hybridy – semenáče z rôznych lokalít (Nitra, Levice, Nové Zámky, Šaľa, Galanta, Hlohovec, Piešťany, Prievidza, Partizánske, Zlaté Moravce) udržiavané formou *in situ* – 77 vybraných genotypov. V experimentoch sme ich označovali skratkou V.

Kvety so stopkami boli odoberané zo stromov a krov v máji, listy so stopkou z letorastov, typické, nepoškodené v auguste 2010 a prenesené do morfometrického laboratória na Inštitúte ochrany biodiverzity a biologickej bezpečnosti v Nitre na analýzy. Fotodokumentácia pochádza z exteriéru (habitus) aj interiéru (púčiky, kvety, listy); obrazové záznamy o genotype boli vyhotovené digitálnymi fotoaparátmi Fuji FinePix S 7000, Panasonic DMC FZ50.

Morfometrická analýza

Hodnotili sa nasledujúce znaky:

- šírka listovej čepele (mm), *n* = 30, z každého genotypu boli hodnotené znaky na 30 listoch;
- dĺžka listovej čepele (mm), *n* = 30;
- index tvaru listovej čepele (mm), *n* = 30;
- priemer kvetu (mm), *n* = 10, z každého genotypu boli hodnotené znaky na 30 kvetoch;
- šírka korunného lupienka (mm), *n* = 30 ;
- dĺžka korunného lupienka (mm), *n* = 30;

Kvety a listy boli merané posuvným meradlom s presnosťou na 0,01 mm.

Štatistická analýza

Základné štatistické analýzy boli vykonané s použitím PAST 2.17; Variabilitu testovaných súborov v jednotlivých znakoch sme hodnotili pomocou deskriptívnej štatistiky. Stupeň variability sme určovali podľa hodnôt variačných koeficientov. Daný ukazovateľ je nezávislý na meranej jednotke hodnoteného znaku. Teoreticky môžu nadobúdať ľubovoľné hodnoty (Stehlíková, 1998). Na vzájomné zistenie rozdielov medzi hodnotenými znakmi sme použili analýzu rozptylu ANOVA v programe STATISTICA 1.10.

Výsledky a diskusia

Hodnotenie a identifikácia genotypov na základe morfologických znakov je dôležitá z hľadiska detekcie a selekcie jedincov, ktoré sú vhodným genetickým materiálom pre hybridizáciu a šľachtenie nových odrôd, čo prispieva k celkovému zachovaniu biologickej diverzity (Monka et al., 2014; Grygorieva et al., 2017, 2018; Ivanišová et al., 2017; Vinogradova et al., 2017).

Morfometrická analýza listovej čepele

Priemernú šírku listovej čepele sme určili v kolekcii krajových a starých odrôd v rozsahu 27,00 mm (R41/4) – 66,44 mm (R28/6) a v kolekcii voľne rastúcich semenáčov v rozsahu 30,08 mm (V44) – 62,18 mm (V30), čo dokumentujú údaje prezentované v Tabuľke 1. Zo vzájomného porovnania genotypov dosahujúcich nízke a vysoké hodnoty znaku a variačných rozpätí hodnoteného znaku vyplýva, že v oboch kolekciách boli určené genotypy s rôznou šírkou listovej čepele. Medzi kolekciami sme nezistili významné rozdiely. Hodnoty variačných koeficientov potvrdzujú nízky až vysoký stupeň variability daného znaku.

Möllerová (2008) a Walia et al. (2012) uvádzajú hodnoty daného znaku v rozsahu 3,5 – 6,5 cm, zatiaľ čo Chuanromanee et al. (2019) prezentujú nižšie priemerné hodnoty šírky listov

2,7 – 3,0 cm. Z porovnania údajov našich experimentov s autormi Möllerová, (2008) a Walia et al. (2012) sme určili významnú zhodu.

Tabuľka 1: Variabilita listovej čepele genotypov starých a krajových odrôd a rodiacich semenáčov jablone domácej (*Malus domestica* Borkh)
Table 1: Variability of leaf blade of genotypes of old and landraces varieties and fruit-bearing seedlings of apple tree (*Malus domestica* Borkh)

Šírka listovej čepele (mm)													
	genotypy voľne rastúce							**genotypy rastúce v repozitóriu Bacúch**					
	n	**min**	**max**	$\bar{x}$	**V**	**TH**		***n***	**min**	**max**	$\bar{x}$	**V**	**TH**
Genotypy s nízkymi hodnotami znaku													
V44	30	26,15	32,58	30,08	5,97	j	**R41/4**	30	21,34	31,8	27,00	9,27	jk
V17	30	28,21	42,96	33,58	12,07	ij	**R3/2**	30	23,62	36,66	27,30	11,14	jk
Genotypy s vysokými hodnotami znaku													
V30	30	40,21	68,78	62,18	10,77	a	**R28/6**	30	58,14	79,26	66,44	8,46	a
V35	30	52,32	69,43	61,83	10,33	a	**R35/3**	30	60,43	71,22	65,34	5,32	a
Dĺžka listovej čepele (mm)													
	genotypy voľne rastúce							**genotypy rastúce v repozitóriu Bacúch**					
	n	**min**	**max**	$\bar{x}$	**V**	**TH**		***n***	**min**	**max**	$\bar{x}$	**V**	**TH**
Genotypy s nízkymi hodnotami znaku													
V44	30	46,76	66,11	57,54	11,21	k	**R33/5**	30	33,13	43,20	37,41	7,24	kl
V9	30	51,12	68,40	58,37	8,71	k	**R3/2**	30	31,82	49,12	39,29	12,60	k
Genotypy s vysokými hodnotami znaku													
V28	30	95,07	120,59	107,93	6,61	a	**R15/5**	30	84,54	108,25	97,50	6,59	a
V76	30	77,27	128,41	104,19	13,30	a	**R5/4**	30	86,94	112,4	96,89	7,03	a
Index tvaru listovej čepele													
	genotypy voľne rastúce							**genotypy rastúce v repozitóriu Bacúch**					
	n	**min**	**max**	$\bar{x}$	**V**	**TH**		***n***	**min**	**max**	$\bar{x}$	**V**	**TH**
Genotypy s nízkymi hodnotami znaku													
V9	30	1,20	1,56	1,24	6,97		**R33/5**	30	1,01	1,19	1,09	4,77	
V59	30	1,00	1,57	1,33	11,30		**R7/6**	30	1,11	1,29	1,18	3,92	
Genotypy s vysokými hodnotami znaku													
V76	30	1,73	3,06	2,19	14,90		**R18/9**	30	1,73	2,64	2,17	11,48	
V22	30	1,83	2,85	2,18	13,20		**R5/4**	30	1,75	2,36	1,93	8,88	

Poznámky: *n* – počet meraní; min, max – minimálna a maximálna nameraná hodnota; $\bar{x}$ – aritmetický priemer; V – variačný koeficient (%); TH – test homogenity podľa LSD pri preukaznosti $P_{0,05}$

Pri hodnotení priemernej dĺžky listovej čepele sme určili v kolekcii krajových a starých odrôd rozsah daného znaku 37,41 mm (R33/5) – 97,50 mm (R15/5) a v kolekcii voľne

rastúcich semenáčov rozsah 57,54 mm (V44) – 107,93 mm (V28), čo dokumentujú údaje prezentované v tabuľke 2. Zo vzájomného porovnania genotypov dosahujúcich nízke a vysoké hodnoty znaku a variačných rozpätí hodnoteného znaku vyplýva, že v oboch kolekciách boli určené genotypy s rôznou dĺžkou listovej čepele. Medzi kolekciami sme nezistili významné rozdiely. Hodnoty variačných koeficientov potvrdzujú nízky až stredný stupeň variability daného znaku. Möllerová (2008) a Walia et al. (2012) uvádzajú hodnoty daného znaku v rozsahu 6 – 12 cm, zatiaľ čo Chuanromanee et al. (2019) prezentujú nižšie priemerné hodnoty dĺžky listov 4,5 – 5,3 cm. Z porovnania nami získaných výsledkov s literárnymi poznatkami vyplýva určitá zhoda.

Tabuľka 2 Analýza rozptylu hodnotených znakov listov genotypov starých a krajových odrôd a rodiacich semenáčov jablone domácej (*Malus domestica* Borkh)

Table 2 Analysis of variance of evaluated leaf traits of genotypes of old and landraces varieties and fruit-bearing seedlings of apple tree (*Malus domestica* Borkh)

Faktory	f	S	MS	F	P	LSD	
Voľne rastúce semenáče (*in situ*)							
Šírka listovej čepele (mm)							
Medzi súbormi	9	2 238,625	248,736	8,677	0,000	0,05	5,255
V rámci súborov	190	5 446,454	28,665			0,01	6,081
Celkom	199	7 685,078					
Dĺžka listovej čepele (mm)							
Medzi súbormi	9	19 049,380	2 116,597	33,746	0,000	0,05	7,774
V rámci súborov	190	11 916,972	62,720			0,01	8,996
Celkom	199	30 966,347					
Krajové a staré odrody v klonovom repozitóriu Bacúch (ex situ**)**							
Šírka listovej čepele (mm)							
Medzi súbormi	9	6 366,700	707,411	69,948	0,000	0,05	4,575
V rámci súborov	90	910,203	10,113			0,01	3,210
Celkom	99	7 276,906					
Dĺžka listovej čepele (mm)							
Medzi súbormi	9	23 792,970	2 643,663	113,657	0,000	0,05	6,939
V rámci súborov	90	2 093,394	23,259			0,01	8,174
Celkom	99	25 886,363					

Vysvetlivky: f – počet stupňov voľnosti; S – súčet štvorcov; MS – priemerný štvorec; F – hodnota testu Fischera; P – štatistická preukaznosť hodnoty Fischera; LSD – najmenšie preukazné rozdiely pre rôzne stupne pravdepodobnosti

Pomer strán dĺžky k šírke je hlavným ukazovateľom zmien v tvare listu. Rozdielne pomery strán vedú k neúmernému zväčšeniu alebo zmenšeniu dĺžky v pomere k šírke, čo má za následok alometrické odchýlky v listoch (Gurevitch, 1992; Chitwood a kol., 2013). Aj keď sú užitočné lineárne merania ako dĺžka a šírka listu, nedokážu zachytiť celý rozsah tvarovej rozmanitosti listov. Na zachytenie genetickej dedičnosti komplexnej morfometrie fenotypu

listov (Migicovsky et al., 2019), pri ktorej nepostačujú iba lineárne merania sa používa technika persistent homology (PH) a eliptické Fourierove deskriptory (EFD).

Index tvaru listovej čepele sme určili v kolekcii krajových a starých odrôd v rozsahu 1,09 (R33/5) – 2,17 (R18/9) a v kolekcii voľne rastúcich semenáčov v rozsahu 1,24 (V9) – 2,19 (V76), čo dokumentujú údaje prezentované v Tabuľke 3. Zo vzájomného porovnania genotypov dosahujúcich nízke a vysoké hodnoty znaku a variačných rozpätí hodnoteného znaku vyplýva, že v oboch kolekciách boli určené genotypy s rôznou hodnotou indexu tvaru listovej čepele. Medzi kolekciami sme nezistili významné rozdiely. Hodnoty variačných koeficientov potvrdzujú nízky až stredný stupeň variability daného znaku. Michálek et al. (2003) uvádza hodnoty tvarového indexu (pomer dĺžky k šírke listovej čepele) v rozmedzí 1,20 až 2,0 pričom najčastejšie sú listy oválneho až vajcovitého tvaru. V porovnaní s výsledkami Michálека et al. (2003) sme zaznamenali hodnoty znaku so širším variačným rozpätím.

V hodnotenej kolekcii krajových a starých odrôd ako aj voľne rastúcich semenáčov sme určili aj významnú fenotypovú variabilitu v tvaroch a farbe listov (Obrázok 1).

Obrázok 1 Porovnanie vybraných genotypov z kolekcie voľne rastúcich semenáčov jablone domácej (*Malus domestica* Borkh) v tvare listov (Foto: M. Hulin, 2010)

Figure 1 Comparison of selected genotypes from the collection of wild seedlings of apple tree (*Malus domestica* Borkh) in the shape of leaves (Photo: M. Hulin, 2010)

Na listoch genotypov sme hodnotili dĺžku a šírku listovej čepele. Výsledky z analýzy rozptylu hodnotených znakov (Tabuľka 2) potvrdzujú štatisticky preukazné rozdiely medzi hodnotenými genotypmi.

Morfometrická analýza kvetu

Kvety sa posudzujú hlavne podľa veľkosti (v mm, pri kvetoch naplno rozvinutých a otvorených), zafarbenia korunných lístkov (najintenzívnejšie je v dobe rozvíjajúceho sa puku, po rozvinutí blednú), celkového tvaru kvetu, i jednotlivých korunných lístkov, ktoré majú u rôznych odrôd taktiež rozdielny povrch. Sú buď rovné, článkovité alebo nepravidelne zvlnené a pod. Taktiež postavenie plátkov v kvetoch je rôzne. Buď je kvet zovretý alebo plochý a pod.

Dobrým určovacím a dôležitým hospodárskym znakom je skorosť a dĺžka obdobia trvania kvetu, ktorými sa jednotlivé odrody líšia. Doba kvetu je skorá, poloskorá, poloneskorá, a neskorá. Neskoro kvitnúce odrody majú spravidla kratšiu dobu trvania kvetu, pretože kvitnú pri teplejšom počasí, kedy kvet už tak dlho nevydrží. Dlhšia doba trvania kvetu má význam hlavne pri nepriaznivom počasí, kedy je obmedzené lietanie včiel. Skorosť a trvanie doby kvetu nie sú v jednotlivých rokoch vždy rovnaké a závisia na stanovišti a priebehu počasia, ktoré býva každým rokom iné. Zvláštny význam má citlivosť kvetu proti namŕzaniu a nepriaznivým prírodným podmienkam, hlavne proti dažďu.

Hodnotu znaku priemeru kvetu sme určili v kolekcii krajových a starých odrôd v rozsahu 35,43 mm (R26/3) – 54,85 mm (R16/12) a v kolekcii voľne rastúcich semenáčov v rozsahu 32,55 mm (V21) – 62,08 mm (V67), čo dokumentujú údaje prezentované v tabuľke 3. Zo vzájomného porovnania genotypov dosahujúcich nízke a vysoké hodnoty znaku a variačných rozpätí hodnoteného znaku vyplýva, že v oboch kolekciách boli určené genotypy s rôznym priemerom kvetu. Medzi kolekciami sme nezistili významné rozdiely. Hodnoty variačných koeficientov potvrdzujú nízky stupeň variability daného znaku. Möllerová (2008) uvádza hodnoty daného znaku v rozsahu 4,0 – 5,0 cm, nami zaznamenané hodnoty znaku vykazujú širšie variačné rozpätie.
Priemernú šírku korunného lupienka sme určili v kolekcii krajových a starých odrôd v rozsahu 11,42 mm (R31/10) – 19,85 mm (R16/12) a v kolekcii voľne rastúcich semenáčov v rozsahu 10,58 mm (V65) – 21,80 mm (V67), čo dokumentujú údaje prezentované v tabuľke 3. Zo vzájomného porovnania genotypov dosahujúcich nízke a vysoké hodnoty znaku a variačných rozpätí hodnoteného znaku vyplýva, že v oboch kolekciách boli určené genotypy s rôznou šírkou korunného lupienka. Medzi kolekciami sme nezistili významné rozdiely. Hodnoty variačných koeficientov potvrdzujú nízky až stredný stupeň variability daného znaku. Variabilitu hodnotených genotypov v znaku šírky korunného lupienka dokumentuje aj obrázok 3. Möllerová (2008) uvádza šírku 15–20 mm, nami zaznamenané hodnoty majú širšie variačné rozpätie znaku.

Priemernú dĺžku korunného lupienka sme určili v kolekcii krajových a starých odrôd v rozsahu 15,48 mm (R31/10) – 24,41 mm (R16/12) a v kolekcii voľne rastúcich semenáčov v rozsahu 14,63 mm (V26) – 28,06 mm (V66), čo dokumentujú údaje prezentované v tabuľke 3. Zo vzájomného porovnania genotypov dosahujúcich nízke a vysoké hodnoty znaku a variačných rozpätí hodnoteného znaku vyplýva, že v oboch kolekciách boli určené genotypy s rôznou

dĺžkou korunného lupienka. Medzi kolekciami sme nezistili významné rozdiely. Hodnoty variačných koeficientov potvrdzujú nízky až stredný stupeň variability daného znaku. Variabilitu hodnotených genotypov v danom znaku dokumentuje aj porovnanie vybranej skupiny genotypov na obrázku 3. Möllerová (2008) uvádza hodnoty daného znaku v rozsahu 16 – 25 mm. Z porovnania nami získaných výsledkov s literárnymi poznatkami vyplýva zhoda.

Tabuľka 3 Variabilita v znakoch kvetu genotypov krajových a starých odrôd z klonového repozitória a voľne rastúcich semenáčov jablone domácej (*Malus domestica* Borkh)
Table 3 Variability of flower of genotypes of old and landraces varieties and fruit-bearing seedlings of apple tree (*Malus domestica* Borkh)

Priemer kvetu (mm)													
	genotypy voľne rastúce							**genotypy rastúce v repozitóriu Bacúch**					
	n	**min**	**max**	**$\bar{x}$**	**V**	**TH**		***n***	**min**	**max**	**$\bar{x}$**	**V**	**TH**
Genotypy s nízkymi hodnotami znaku													
V21	30	29,39	35,81	32,55	5,76	h	**R26/3**	30	33,89	37,18	35,43	2,99	h
V26	30	31,06	38,5	34,22	7,11	gh	**R23/3**	30	32,74	39,08	35,56	5,85	h
Genotypy s vysokými hodnotami znaku													
V67	30	59,77	66,74	62,08	3,25	a	**R16/12**	30	50,66	58,27	54,85	4,45	a
V66	30	57,36	65,36	60,83	4,35	a	**R16/14**	30	50,09	57,96	52,88	4,80	a
Šírka korunného lupienka (mm)													
	genotypy voľne rastúce							**genotypy rastúce v repozitóriu Bacúch**					
	n	**min**	**max**	**$\bar{x}$**	**V**	**TH**		***n***	**min**	**max**	**$\bar{x}$**	**V**	**TH**
Genotypy s nízkymi hodnotami znaku													
V65	30	9,77	11,46	10,58	5,17	d	**R31/10**	30	10,10	12,17	11,42	5,81	f
V25	30	10,07	12,40	10,89	6,01	cd	**R24/13**	30	11,02	12,75	11,79	4,94	ef
Genotypy s vysokými hodnotami znaku													
V67	30	19,36	25,08	21,80	10,22	a	**R16/12**	30	19,05	21,53	19,85	4,47	a
V74	30	18,45	24,05	21,74	8,48	a	**R20/8**	30	16,61	19,47	17,79	5,30	ab
Dĺžka korunného lupienka (mm)													
	genotypy voľne rastúce							**genotypy rastúce v repozitóriu Bacúch**					
	n	**min**	**max**	**$\bar{x}$**	**V**	**TH**		***n***	**min**	**max**	**$\bar{x}$**	**V**	**TH**
Genotypy s nízkymi hodnotami znaku													
V26	30	12,99	16,04	14,63	6,40	g	**R31/10**	30	13,16	16,95	15,48	7,67	d
V25	30	14,78	18,78	16,15	7,28	f	**R4/15**	30	13,45	18,80	15,50	9,92	d
Genotypy s vysokými hodnotami znaku													
V66	30	26,98	28,95	28,06	2,58	a	**R16/12**	30	22,47	26,13	24,41	5,76	a
V67	30	25,56	28,22	26,86	3,23	a	**R16/14**	30	20,11	24,57	22,78	6,68	a

Poznámky: *n* – počet meraní; min, max – minimálna a maximálna nameraná hodnota; $\bar{x}$ – aritmetický priemer; V – variačný koeficient (%); TH – test homogenity podľa LSD pri preukaznosti $P_{0,05}$

V hodnotenej kolekcii krajových a starých odrôd ako aj voľne rastúcich semenáčov sme určili aj významnú fenotypovú variabilitu v tvaroch a farbe kvetných púčikov, kvetov, kvetných lupienkov a kalichov (obrázok 2).

Obrázok 2 Porovnanie genotypov z kolekcie voľne rastúcich semenáčov jablone domácej (*Malus domestica* Borkh) v znakoch púčikov (Foto: M. Hulin, 2010)

Figure 2 Comparison of genotypes from the collection of wild seedlings of apple tree (*Malus domestica* Borkh) in bud traits (Photo: M. Hulin, 2010)

Obrázok 3 Porovnanie genotypov z kolekcie voľne rastúcich semenáčov jablone domácej (*Malus domestica* Borkh) v znakoch kvetov (Foto: M. Hulin, 2010)

Figure 3 Comparison of genotypes from the collection of wild seedlings of apple tree (*Malus domestica* Borkh) in flower traits (Foto: M. Hulin, 2010)

Získaná dokumentácia z hodnotenia kvetov v oboch experimentálnych skupinách potvrdzuje pomerne významnú variabilitu kvalitatívnych ako aj kvantitatívnych znakov. Na kvetoch

genotypov sme hodnotili znaky priemer kvetu (mm), šírka korunných lupienkov (mm), výška korunných lupienkov (mm). Výsledky z analýzy rozptylu hodnotených znakov (Tabuľka 4) potvrdzujú štatisticky preukazné rozdiely medzi hodnotenými genotypmi.

Tabuľka 4 Analýza rozptylu hodnotených znakov kvetov v testovanej kolekcii genotypov krajových a starých odrôd z klonového repozitória a voľne rastúcich semenáčov jablone domácej (*Malus domestica* Borkh)

Table 4 Analysis of variance of evaluated flower traits of genotypes of old and landraces varieties and fruit-bearing seedlings of apple tree (*Malus domestica* Borkh)

Faktory	f	S	MS	F	Preukaznosť	LSD	
Voľne rastúce semenáče (*in situ*)							
Priemer kvetu (mm)							
Medzi súbormi	9	10,109	1,123	15,295	0,000	0,05	0,389
V rámci súborov	90	6,609	0,073			0,01	0,459
Celkom	99	16,719					
Šírka korunných lupienkov (mm)							
Medzi súbormi	9	236,517	26,279	13,868	0,000	0,05	1,980
V rámci súborov	90	170,546	1,895			0,01	2,333
Celkom	99	407,063					
Výška korunných lupienkov (mm)							
Medzi súbormi	9	547,601	60,844	20,567	0,000	0,05	2,474
V rámci súborov	90	266,243	2,958			0,01	2,915
Celkom	99	813,845					
Krajové a staré odrody v klonovom repozitóriu Bacúch (*ex situ*)							
Priemer kvetu (mm)							
Medzi súbormi	9	2 402,594	266,95	47,681	0,000	0,05	3,404
V rámci súborov	90	503,885	5,598			0,01	4,010
Celkom	99	2 906,479					
Šírka korunných lupienkov (mm)							
Medzi súbormi	9	469,980	52,220	70,302	0,000	0,05	1,240
V rámci súborov	90	66,851	0,742			0,01	1,460
Celkom	99	536,831					
Výška korunných lupienkov (mm)							
Medzi súbormi	9	506,574	56,286	39,429	0,000	0,05	1,719
V rámci súborov	90	128,476	1,427			0,01	2,025
Celkom	99	635,050					

Vysvetlivky: f – počet stupňov voľnosti; S – súčet štvorcov; MS – priemerný štvorec; F – hodnota testu Fischera; P – štatistická preukaznosť hodnoty Fischera; LSD – najmenšie preukazné rozdiely pre rôzne stupne pravdepodobnosti.

Závery

Na základe morfologickej analýzy listov a kvetov kolekcie krajových odrôd a voľne rastúcich semenáčov sme určili v oboch skupinách hodnotených genotypov významnú fenotypovú variabilitu vo všetkých znakoch a v kombinácií znakov. Pri vzájomnom porovnaní určených variačných rozpätí pri všetkých hodnotených znakoch sme zistili významný stupeň zhodnosti. Uvedený výsledok dokumentuje, že aj v kolekcii voľne rastúcich semenáčov je možné detekovať genotypy s významnými hospodárskymi a pomologickými znakmi, vhodné pre priame praktické využitie alebo ako potenciálne genetické zdroje pre využitie v šľachtení.

Poďakovanie

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DPPH FREE RADICAL SCAVENGING ACTIVITY OF SOME FABACEAE LINDL. SPECIES

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Searching for new plant raw material with antioxidant activity is still actual. This study was aimed to evaluate the antioxidant potential of selected plants from Fabaceae: *Coronilla varia* L., *Desmodium canadense* (L.) DC., *Glycyrrhiza glabra* L., *Lathyrus grandiflorus* Sibth. & Smith, *Lespedeza bicolor* Turcz., *Onobrychis arenaria* (Kit.) D.C., *O. grandis* Lip., *Trifolium ambiguum* Bieb., *T. pannonicum* Jacq., *T. rubens* L. cv. Skif-1 at the flowering stage. Raw material was collected from the experimental collections of M.M. Gryshko National Botanical Garden of the NAS of Ukraine (NBG) in the stage of flowering. Determination of the antioxidant activity of investigated plants conducted by DPPH free radical scavenging activity according to Brand-Williams et al. (1995). The total antioxidant activity of plant whole above-ground part of methanol extracts was from 62.78 (*Coronilla varia*) to 89.99 (*Glycyrrhiza glabra*) % and water extracts from 51.28 (*Onobrychis grandis*) to 90.35 (*Lespedeza bicolor*) %. Leaf extracts exhibited DPPH scavenging activity in methanol extracts from 63.17 (*Trifolium ambiguum*) to 93.08 (*Onobrychis arenaria*) % and water extracts from 57.62 (*Coronilla varia*) to 95.36 (*Lathyrus grandiflorus*) %. Stem extracts of investigated plants showed inhibition in methanol extracts from 35.04 (*Coronilla varia*) to 90.51 (*Glycyrrhiza glabra*) % and water extracts from 20.35 (*Coronilla varia*) to 77.52 (*Lathyrus grandiflorus*) %. DPPH scavenging activity of these plants depended on species and part of plants. Thus, plants from Fabaceae family are a potential source of antioxidant activity that can be used for deep pharmacological investigations for food science.

Keywords: Fabaceae, 2.2-diphenyl-1-picrylhydrazyl, free radical scavenging activity

Introduction

The study of the antioxidant capacity of plants and finding new sources is an important direction in biological science. Natural antioxidants are widely distributed in the plant world and are represented by different classes of compounds with numerous biological activities (Xu et al., 2017). Screening of new natural sources provides an opportunity to expand the range of new products with antioxidant activity. In this case, the Fabaceae Lindl. family has numerous representatives with a spectrum of biological activities that can be used.

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Many species from Fabaceae plants (Legumes) are known as high-quality feed for livestock such as *Medicago sativa* L. and *Trifolium pratense* L. also, a rich source of phytoestrogens, nonsteroidal compounds with beneficial effect (Tucak et al., 2018). Legumes are a good source of nutrients such as proteins, carbohydrates, dietary fibers, micronutrients, vitamins, fatty acids (Guttiérez-Uribe et al., 2016). Some representatives from Fabaceae well-known forage plants with a high content of polyphenol compounds and antioxidant activity (Shymanska et al., 2018; Vergun et al., 2020). The screening of some legumes plants determined the antimicrobial activity against gram-positive bacteria, *Candida albicans*, *Aspergillus niger* and *Pseudomonas aeruginosa* (Rosado-Vallado et al., 2000). Most of the Fabaceae plants are used as medicinal plants against numerous diseases such as asthma, gonorrhea, ulcer, diabetes, kidney disease, rheumatism, stomach trouble, colic, etc. (Mahbubur and Parvin, 2014). There are numerous pharmacological properties of these plants studied among that contraceptive (Sethi et al., 2017), antiallergic, antimalarial, anticancer, analgesic, aphrodisiac, anti-rheumatic, anthelmintic, diuretic, antiseptic, insecticidal, toxic, etc. (Jahan et al., 2019).

Important chemical components from the plants of this family are flavonoids, coumarins, alkaloids, tannins (Ferreira Macêdo et al., 2018). Some plants, such as *Lathyrus* species, can be considered as ornamental plants (Güneş, 2019). The antioxidant activity of some Fabaceae species such as *Lathyrus binatus*, *Trifolium pannonicum* (Gođevac et al., 2008), *Glycyrrhiza glabra* (Hussein and Iqbal, 2019) was studied.

This study aimed to evaluate the antioxidant potential of selected Fabaceae plants and to compare this parameter in different parts of plants. The results of this assessment are essential for further pharmacological study.

Materials and methodology

Biological material

It was investigated some representatives from the Fabaceae Lindle.: *Coronilla varia* L., *Desmodium canadense* (L.) D.C., *Glycyrrhiza glabra* L., *Lathyrus grandiflorus* Sibth. & Smith, *Lespedeza bicolor* Turcz., *Onobrychis arenaria* (Kit.) D.C., *O. grandis* Lip., *Trifolium ambiguum* Bieb., *T. pannonicum* Jacq., *T. rubens* L. cv. Skif-1. An experiment carried out during 2018–2019 at the laboratory of Cultural Flora Department of M.M. Gryshko National Botanical Garden of the N.A.S. of Ukraine. Plants samples took at the flowering stages and dried at 45 °C for 72 hours. All investigated plants are perennial.

DPPH scavenging activity determination

1 g of dried and milled plant raw extracted in the 25 ml of solvent (methanol and water) for 24 hours. After the filtration procedure obtained extracts used to determined antiradical activity on a spectrophotometer Unico UV 2800 (Russia). A working solution of 2.2-diphenyl-1-picrylhydrazyl (DPPH) prepared the following way: 25 mg of radical dissolved in 100 ml of methanol. Obtained radical's solution dissolved in 10 times till optical density was in the range of 0.700–0.800. The procedure of measuring was conducted by Brand-Williams et al. (1995). 3.9 ml of radical solution mixed with 100 µl of plant extract and put for 10 min in the

dark. During the procedure of measuring on a spectrophotometer at a wavelength of 515 nm used value of radical solution and value of the radical solution with the sample. Obtained results expressed in percentages.

Statistical analysis

The mean values of three replicates and the standard deviation are given. Data submitted with ANOVA and differences between means compared using the Tukey-Kramer test ($\alpha = 0.05$).

Results and discussion

There are numerous methods to determine the antioxidant activity, among which the DPPH scavenging activity has been found as simple, inexpensive, and allows to evaluate of the antioxidant potential at the screening process. However, it is necessary to consider that one method can't be universal for all plants (Kedare and Singh, 2011). This method is based on the reaction discoloration of radical (2.2-diphenyl-1-picrylhydrazyl) solvent by plant extract (Alam et al., 2013). In this study, different plant parts of selected plants were used to extract two solvents.

Coronilla varia is a perennial legume used for cardio-tonic, diuretic, prostate diseases. Coumarins and glycosides were isolated from this species and raw demonstrated antimicrobial and insecticidal effects (Al-Snafi, 2016).

The free radical scavenging activity of *Coronilla varia* methanol extracts was from 35.04 (stems) to 87.05 (leaves) % (Figure 1). In the water extracts, this parameter was from 20.35 (stems) to 65.54 (inflorescences). Renda et al. (2019) determined in methanol extracts (25–100 µg/ml) of *C. varia* the DPPH scavenging activity from 9.89 to 39.40% and in water extracts from 8.64 to 27.31% depending on initial concentration. Also, in this study detected for methanol extracts of *C. orientalis* the DPPH antioxidant activity from 12.59 to 41.91% and for water extracts from 8.14 to 20.45%.

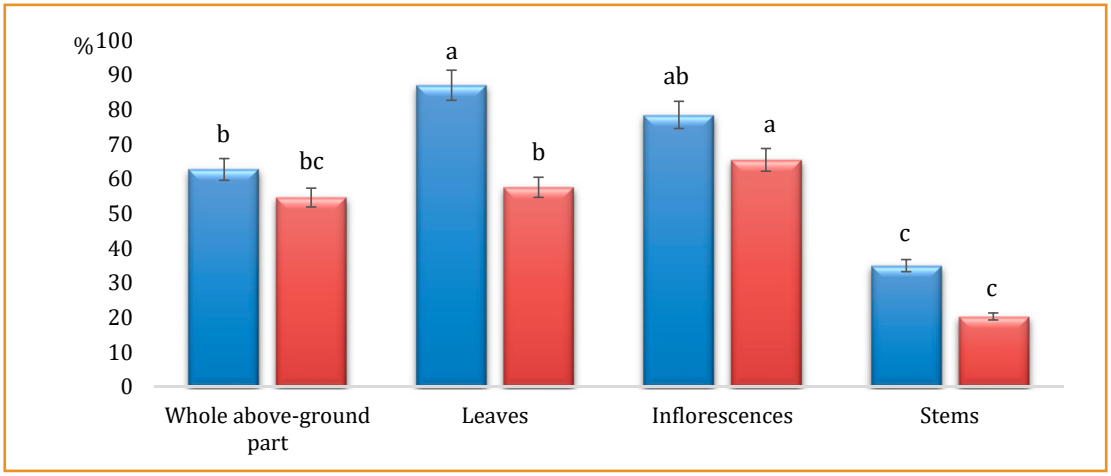

Figure 1 DPPH scavenging activity of *Coronilla varia* L. at the stage of the flowering (means in columns followed by different letters are different at $p < 0.05$. Each value represents the mean of three independent experiments (±SD))

The methanol extracts of *Desmodium canadense* demonstrated inhibition of radical solution from 60.01 (stems) to 85.66 (whole above-ground part) % (Figure 2). Tsai et al. (2011) investigated ten species of *Desmodium* genus and found a high content of phenolic compounds in some species (*D. sequax*) that correlated with antioxidant activity. Venkatachalam and Muthukrishnan (2012) determined the DPPH scavenging activity of different concentration extracts of *D. gangeticum* from 25.12 to 54.14%. Ayoola et al. (2018) identified in leaf extracts radical scavenging activity of 3.17–91.3%, and in stem extracts of 2.87–61.65%. *D. canadense* leaf extracts are a source of polyphenol compounds (flavonoids and phenolic acids) with high antioxidant activity by Trolox equivalent methods (Vergun et al., 2019).

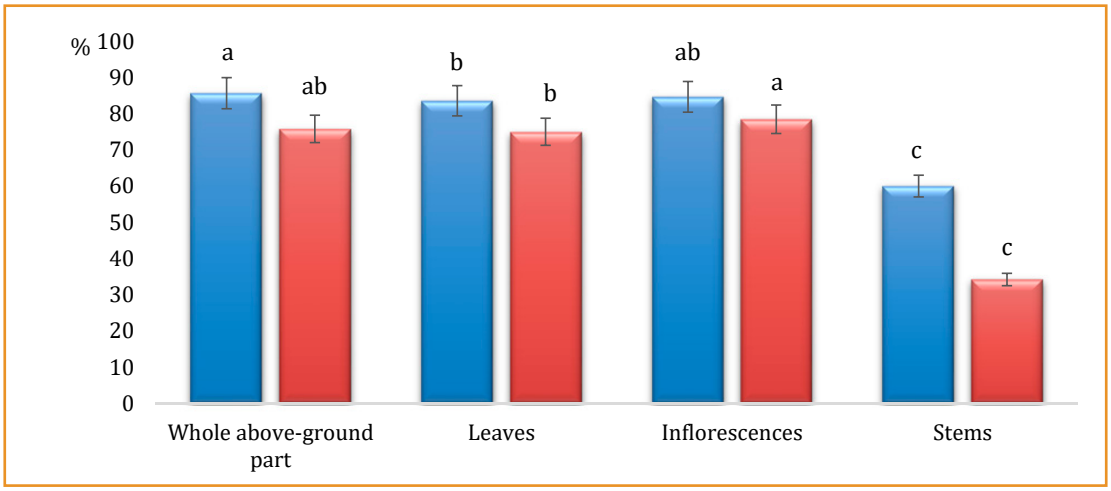

Figure 2 DPPH scavenging activity of *Desmodium canadense* (L.) D.C. at the stage of the flowering (means in columns followed by different letters are different at $p < 0.05$. Each value represents the mean of three independent experiments (±SD))

The study of *Glycyrrhiza glabra* showed the presence of alkaloids, glycosides, carbohydrates, phenolic compounds, proteins, etc. Plant raw exhibited antidepressant, antimicrobial, anticancer, antioxidant, anti-inflammatory, antidiabetic, hypolipidemic, etc. (Al-Snafi, 2018). Traditionally, this plant is recommended as prophylaxis of gastric and duodenal ulcers, dyspepsia (Harwansh et al., 2011), effective against vitiligo (Herath et al., 2018). Pharmacological activity appeared against various microorganisms, parasites, viruses (Batiha et al., 2020). Biological activities of this species have studied basically with roots as useful raw (Chopra et al., 2013). The essential oil of this plant also characterized by numerous biological activities such as antioxidant, anticancer, antifungal, etc. (Ali, 2013).

The free radical scavenging activity of methanol extracts of *Glycyrrhiza glabra* was from 89.99 (whole above-ground part) to 91.58 (leaves) % (Figure 3). In the water extracts, this activity was from 75.79 (stems) to 91.61 (inflorescences) %. Al-Snafi (2018) determined in methanol extracts of roots the inhibition of DPPH radical 67.22%.

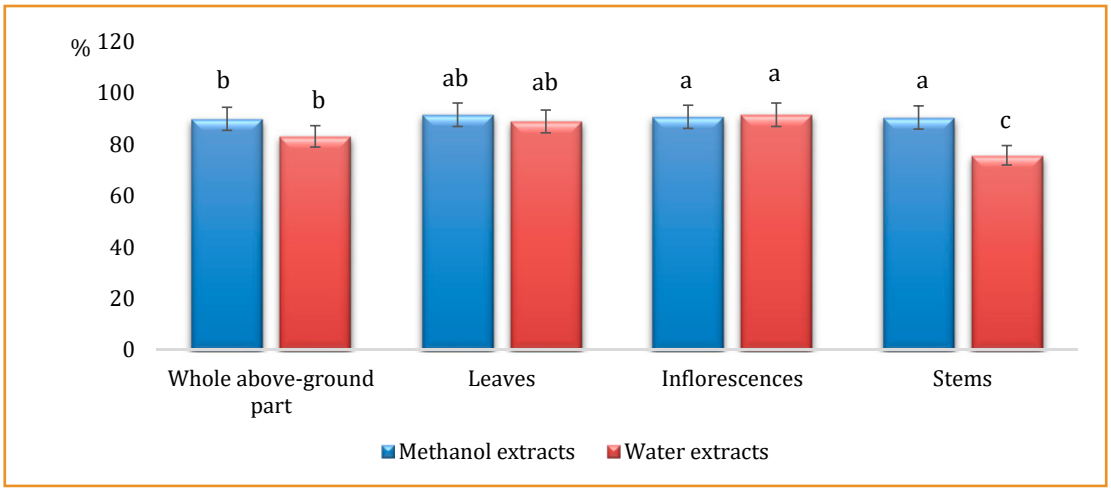

Figure 3 DPPH scavenging activity of *Glycyrrhiza glabra* L. at the stage of the flowering (means in columns followed by different letters are different at p <0.05. Each value represents the mean of three independent experiments (±SD))

Lathyrus species are known as a source of protein, but few of them are cultivated as food plants. Raw of them also contains flavonoids, fatty acids, proanthocyanidins, triterpene saponins, etc. (Heydary et al., 2015).

The free radical scavenging activity of methanol extracts of *Lathyrus grandiflorus* was from 40.07 (stems) to 84.81 (leaves) % (Figure 4). In the water extracts, this activity was from 77.52 (stems) to 95.36 (leaves) %. Heydari et al. (2015) investigated five species of *Lathyrus* and in some cases, DPPH scavenging activity was up to 90%.

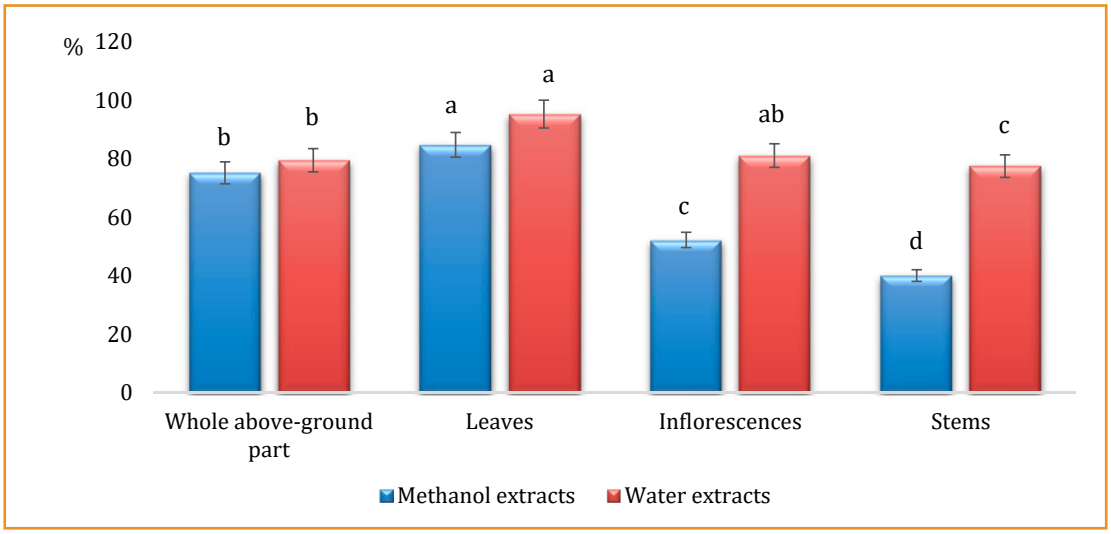

Figure 4 DPPH scavenging activity of *Lathyrus grandiflorus* Sibth. & Smith at the stage of flowering (means in columns followed by different letters are different at p <0.05. Each value represents the mean of three independent experiments (±SD))

The free radical scavenging activity of methanol extracts of *Lespedeza bicolor* was from 53.54 (stems) to 92.07 (inflorescences) % and water extracts from 70.06 (stems) to 92.55 (leaves) % (Figure 5). Lee et al. (2016) investigated extracts of this plant and established the anti-inflammatory, depigmentation, and antioxidant activity effect. Besides the antioxidant effect, *L. bicolor* extracts demonstrated antifungal and antimicrobial activities (Sami, 2017).

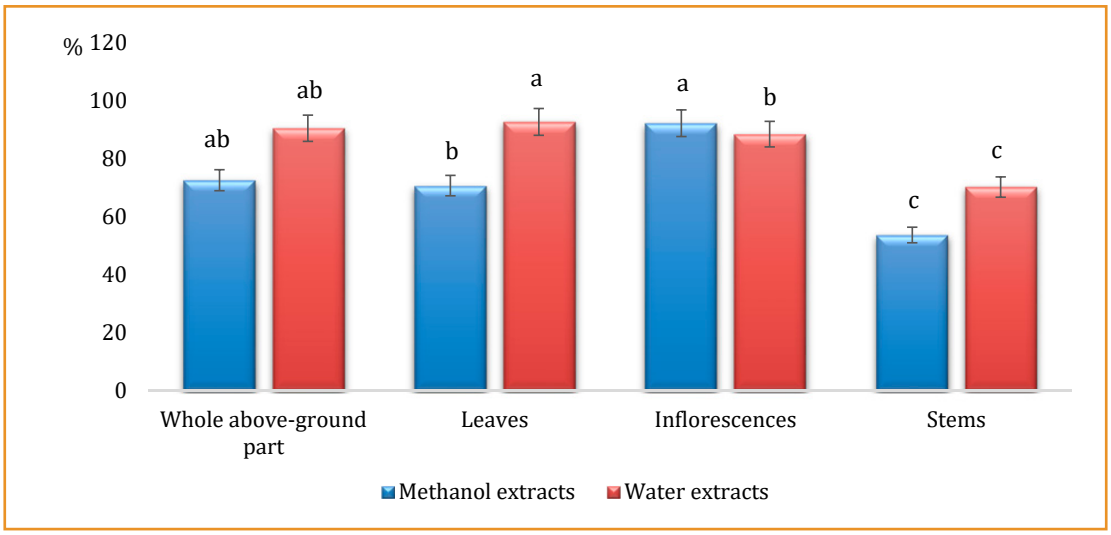

Figure 5 DPPH scavenging activity of *Lespedeza bicolor* Turcz. at the stage of flowering (means in columns followed by different letters are different at p <0.05. Each value represents the mean of three independent experiments (±SD))

The free radical scavenging activity of methanol extracts of *Onobrychis arenaria* was from 63.08 (stems) to 93.08 (leaves) % (Figure 6). In the water extracts, this activity was from 59.85 (stems) to 89.85 (leaves) %. In methanol extract of *O. grandis* determined DPPH scavenging activity from 37.39 (stems) to 86.31 (inflorescences) %, in water extracts from 31.31 (stems) to 86.01 (leaves) %. Karamian and Asadbegy (2016) detected in methanol extracts of *O. viciifolia*, *O. melanotria* and *O. sosnovskyi* inhibition of DPPH as 92.70, 92.27 and 91.38%, respectively.

Al-Snafi (2018) determined in methanol extracts of roots the inhibition of DPPH radical 67.22%.

The methanol extracts of *Trifolium ambiguum* exhibited the DPPH scavenging activity from 28.02 (stems) to 94.07 (inflorescences) % and water extracts from 41.84 (stems) to 93.33 (inflorescences) % (Figure 7). *T. pannonicum* showed scavenging activity in methanol extracts from 75.48 (stems) to 88.24 (inflorescences) % and in water extracts from 38.76 (stems) to 88.24 (whole above-ground part). In methanol extracts of *T. rubens* determined DPPH scavenging activity from 24.85 (stems) to 84.45 (inflorescences) % and in water extracts from 47.56 (stems) to 74.0 (whole above-ground part) %.

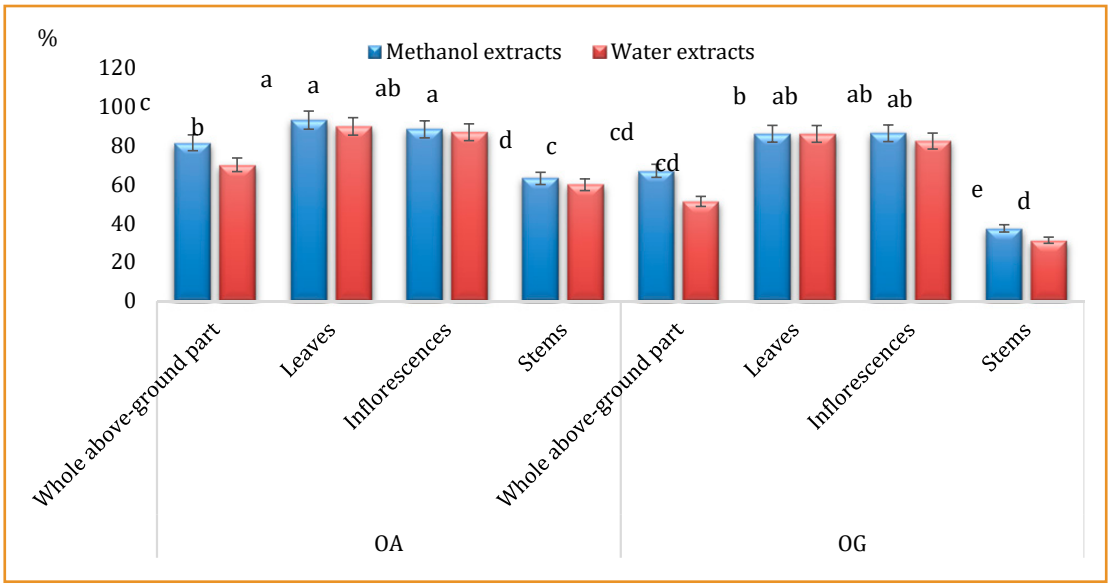

Figure 6 DPPH scavenging activity of *Onobrychis arenaria* (Kit.) D.C. (OA) and *O. grandis* Lip. (OG) at the stage of flowering (means in columns followed by different letters are different at p <0.05. Each value represents the mean of three independent experiments (±SD))

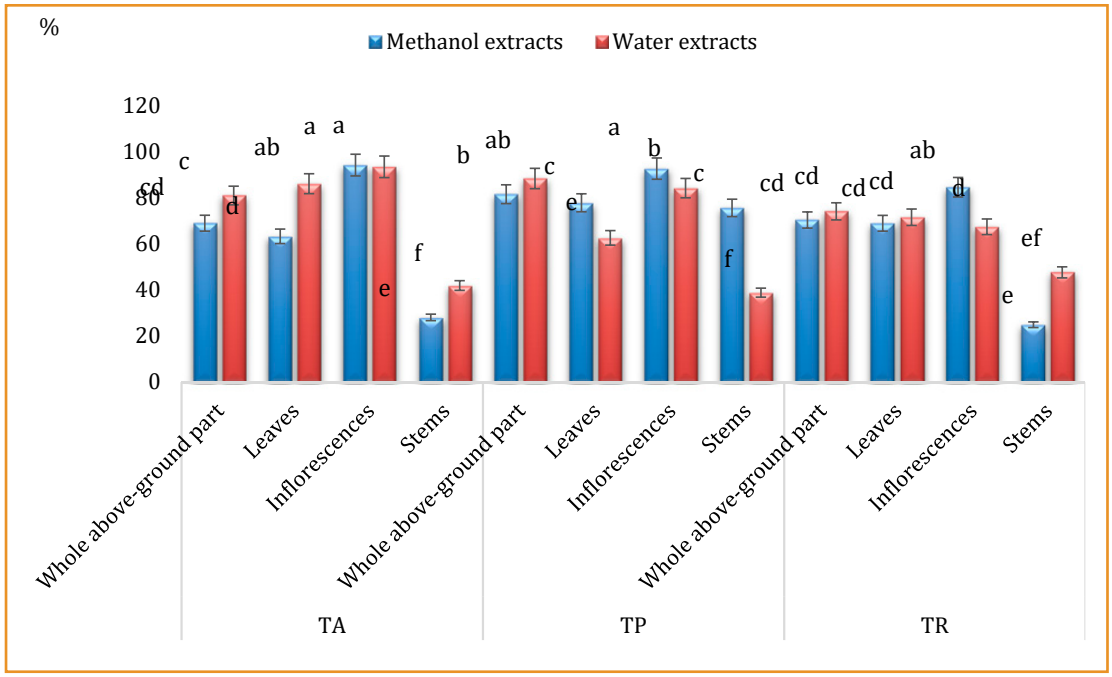

Figure 7 DPPH scavenging activity of *Trifolium ambiguum* Bieb. (TA), *T. pannonicum* Jacq. (TP) and *T. rubens* L. cv. Skif-1 (TR) at the stage of flowering (means in columns followed by different letters are different at p <0.05. Each value represents the mean of three independent experiments (±SD))

The study of other Fabaceae species *Indigofera tinctoria* L. showed the antioxidant activity by DPPH of water extracts was 52.08% (Srinivanasan et al., 2016).

Conclusions

Different extracts of crops from the Fabaceae family collected in the M.M. Gryshko National Botanical Garden exhibited an antioxidant potential. It's should be concluded that the scavenging activity of these plants depended on species and part of plants. The total antioxidant activity of plant whole above-ground part was lowest in methanol extracts of *Coronilla varia* and highest in *Glycyrrhiza glabra* and the minimal result showed water extract of *Onobrychis grandis* and maximal *Lespedeza bicolor*. Leaf extracts exhibited a minimal value of DPPH scavenging activity in methanol extracts of *Trifolium ambiguum* and maximum in *Onobrychis arenaria* and water extracts *Coronilla varia* and *Lathyrus grandiflorus*, respectively. Stem extracts of investigated plants showed the lowest inhibition in methanol extracts for *Coronolla varia* and highest for *Glycyrrhiza glabra* and in water extracts *Coronolla varia* and *Lathyrus grandiflorus*, respectively. Plants from the Fabaceae family are a potential source of antioxidant activity that can be used for deep pharmacological investigations for food science.

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PRO-HEALTH AND FUNCTIONAL PROPERTIES OF GOJI BERRY (*LYCIUM* SPP.)

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Goji (*Lycium* L.) fruit has been an important element of traditional Chinese medicine for centuries. In Asian countries, they are used as an essential component of a healthy diet, a source of many nutrients. Due to their health-promoting properties and chemical composition (phenolic acids, flavonoids, proanthocyanidins, coumarins, tannins, carotenoids, anthocyanins), they deserved the term superfruit. In recent years, goji berries have also become very popular in Europe and America. The fruit is used primarily after drying and is available in the form of various supplements. Two species of *Lycium barbarum* L. and *Lycium chinense* Mill. are cultivated on a larger scale. These species are closely related to each other. They differ slightly in morphological features. *L. chinense* leaves are longer and wider than *L. barbarum*. *L. chinense* fruits are slightly smaller and more elongated. There are several less specific species and botanical varieties in natural sites in central and western China, such as *L. barbarum* var. *aurantiocarpum*, *L. chinense* var. *potaninii*, *L. ruthenicum*, *L. truncatum*. Not only fruits contain biologically active substances, but also other parts of plants, especially leaves. This review highlights the healing properties of the fruits and leaves of these species. The most valuable and most interesting component of goji berries is the water-soluble bioactive polysaccharide complex LBP (*Lycium* Barbarum Polysaccharides) playing an important therapeutic role. The LBP complex has a beneficial effect on the functions of the immune system, inhibits the growth of cancer cells, has antioxidant properties, improves the function of the digestive tract, well-being and sleep quality. Due to the presence of LBP, goji fruit extracts have a hypoglycemic effect, lowering the content of lipids in the blood serum. The diversity of their use as food, medicinal and cosmetic agents was shown.

Keywords: goji berries, *Lycium barbarum*, *Lycium chinense*, health benefits

Introduction

Nowadays, underutilized and less-known species, such as *Pseudocydonia sinensis* Schneid. (Monka et al., 2014; Grygorieva et al., 2020), *Morus nigra* L. (Kucelova et al., 2016), *Ziziphus jujuba* Mill. (Ivanišová et al., 2017), *Diospyros virginiana* L. (Grygorieva et al., 2018), *Sambucus nigra* L. (Horčinová Sedláčková et al., 2018), *Asimina triloba* L. (Brindza et al., 2019),

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Chaenomeles japonica (Thunb.) Lindl. (Klymenko et al., 2019) ect., are becoming more and more well-known for scientific and laic public (Klymenko et al., 2017). One of them is the genus *Lycium* L., which belongs to the family nightshade (Solanaceae) includes about 88(97) species found in temperate and subtropical climates (Levin et al., 2011; Barboza et al., 2016). Several of them: *Lycium barbarum* L., *L. chinense* Mill., *L. ruthenicum* Murray have been recognized in eastern medicine for over 4,000 years and are popularly called Gouqi in China, and Goji berries, Wolfberry, Christmas berry, Desert-thorn, Matrimony vine, Duke of Argyll's tea tree in other countries of the world. There are also a few less popular botanical species and varieties of natural sites in central and western China, e.g. *L. barbarum* var. *aurantiocarpum*, *L. chinense* var. *potaninii*, *L. truncatum* (Zhang et al., 2001). In South Europe there are *L. europaeum* L., *L. intricatum* Boiss., Sounth Africa (*L. afrum* L.), and South and Central America (*L. chinense* Bert., *L. horridum* Thunb., *L. pallidum* Miers. (Seneta and Dolatowski, 2012; Yao et al., 2018).

The first *L. barbarum* plantations were established over 600 years in China, in Ningxia Hui Autonomous Province, mainly in Zhongning. Fruits from this region are considered to be of a particularly high quality due to optimal soil and climatic conditions, such as sunshine, the sum of effective temperatures, large amplitude between day and night temperatures. An excellent substrate is flood plains with a lot of humus (Wang et al., 2015).

Currently, from the beginning of the 21st century, the cultivation of Goji berries has become very popular due to the numerous beneficial properties of compounds contained in fruits, leaves, root and bark. These compounds delay the aging processes, improve eyesight, liver and kidney function, and generally have a positive effect on the well-being and immunity of the body (Dong et al., 2009; Amagase and Farnsworth, 2011). In search of ways to increase the profitability of farms, growers decide to cultivate new plant species. In European countries: Italy, Serbia, Bulgaria, Poland, there is a growing interest in commercial cultivation. Bulgarian cultivars of *L. barbarum* (JB1, JB2, JBX, and JB4) are recommended. Also outside Europe, there is an interest in the wider cultivation of Goji berries. In Korea, when examining the suitability for the cultivation of some natural forms and selected cultivars, differences in growth, the habit of shrubs, yield, and fruit shape were observed. Cultivar Yosong 2 (*L. chinense*) turned out to be the most promising. *L. barbarum* cultivars are bred in the USA and Canada: Crimson Star, Phoenix Tears and Sask Wolfbery. Plants, due to their high frost resistance, tolerate even large, down to -30 °C temperature drops in winter. They have low requirements concerning the position – they grow well and bear fruit, when planted in weaker sandy and permeable soils. They grow worse on heavy, loamy and wet soil (Marosz, 2017).

Description of species and their natural range

The species from which the raw materials for the production of the currently popular "Superfood Goji" are obtained, are those found in the natural flora of China: *L. barbarum*, *L. chinense*, and *L. ruthenicum* (Wanh et al., 2015). The Goji berries occurs in Tibetan valleys and the Himalayas, in areas not contaminated with agricultural chemicals and civilization pollution. Currently, it is also commonly grown in countries with milder and subtropical climate, e.g. in Japan, Korea, but the raw material obtained there has a lower nutritional and medicinal value (Qian et al., 2004). Wolfdogs are shrubs up to three meters high, arched twigs armed with

Figure 1 *Lycium chinense* Mill. (Foto: M. Zhurba, I. Szot)

spikes (the common name boxthorn), small inconspicuous purple flowers, and orange to red fruits (black for *L. ruthenicum*), which reach up to 2 cm in length. The first two species are very phylogenetically similar. They grow in the same environment, are harvested at the same time (usually from August to October), and have been used in traditional medicine throughout East Asia for thousands of years (Potterat, 2010). Wetters et al. (2018) showed that the structure of the flower, and specifically the appearance of the crown, is helpful for the morphological differentiation of *L. barbarum* and *L. chinense*. The flower tube of *L. barbarum* is elongated and significantly longer than the petals, L. chinensis crown tube is twice as short as the hairy at the edges petals. The calyx of *L. barbarum* flowers generally consists of two sepals and in the case of L. chinensis four sepals, however, there are also flowers with only two sepals. Moreover, the seeds of *L. barbarum* are brownish-yellow and have almost twice as much surface area as

Figure 2 *Lycium barbarum* L. (Foto: M. Zhurba, I. Szot)

the yellow seeds of *L. chinense*. *L. chinense* leaves are up to 4 cm longer and twice as wide as *L. barbarum* leaves. These species can also be distinguished by the shape of the fruits. The fruits of *L. barbarum* and *L. chinense* are oblong or ovate, the former being red or red-yellow in color and the latter red. The fruits of *L. ruthenicum* are spherical, purple-black (Wang et al., 2015). Within *L. barbarum* there are many varieties, in Tibet alone there are 40 (Amagase et al., 2009).

Nutritional properties and bioactive substances found in fruits

Modern research has confirmed the high nutritional content of goji berries. The dried fruit contained 5.5% protein (18 amino acids), 26.7% carbohydrates (8 polysaccharides and 6 monosaccharides) including 3.7% crude fiber and 1% fat (including 5 unsaturated fatty acids from the omega-6 group), responsible for the production of hormones and the proper functioning of the brain and nervous system (Qian et al., 2004; Amagase et al., 2009; Bogacz, 2009). Poterańska and Ochmian (2015) report that *L. barbarum* fruits contain from 12.2–17.6% of the extract and 1.12–1.3 acids (g/100 g) Consuming *L. barbarum* fruits provides many health benefits, mainly due to the vitamins they contain. They have a high content of vitamin C, which is fresh fruit is 42 mg/100 g, which is comparable to fresh lemon fruits (Toyoda-Ono et al.,

2004). It is assumed that the consumption of only 7 goji berries covers the daily requirement of this vitamin (Amagase and Farnsworth, 2011). A large group of metabolic compounds are carotenoids, the amount of which increases with the ripening of the fruit. They are represented mainly by zeaxanthin and ester derivatives, the content of which in fruits ranges from 0.03 to 0.5% of dry weight (Inbaraj et al., 2008; Amagase and Farnsworth, 2011). Zeaxanthin is a very important component of the human diet, also provided by some vegetables, the presence of which is particularly important in the macula for the improvement of visual acuity (Cheng et al., 2005). Goji berries are also a source of vitamins B_1, B_2, B_6, and E and minerals (phosphorus, calcium, iron, copper, zinc, selenium) (Qian et al., 2004). In the chemical structure of *L. barbarum* fruits, the most known are water-soluble polysaccharides, the amount of which is estimated at 5–8% of the dry weight of the fruit, and the molecular weight ranges from 24 to 241 kDa (Peng et al., 2005). In nature, polysaccharide complexes occur naturally as glycoconjugates, e.g. as combinations of glycans (structures made up of sugar units) with proteins or fats. Luo et al. (2004) found 6 monosaccharides in the goji polysaccharide complex: rhamnose, galactose, glucose, arabinose, mannose, and xylose. The analysis showed the presence of 17 amino acids, and the total amino acid content was 8.46% (Peng et al., 2001). The LBP polysaccharide complex (*Lycium* Barbarum Polysaccharides) inhibits the growth of cancer cells (He et al., 2012). In studies with experimental animals, the antitumor activity of goji berries against gastric cancer and colon cancer was found (Mao et al., 2010; Mao et al., 2011). During chemotherapy, they play a significant role in protecting the liver cells. In addition, the polysaccharide complex contained in goji fruit has a strong antioxidant activity, which allows inhibiting the aging process (Mao et al., 2011). Due to the presence of LBP, goji berry extracts show a hypoglycemic effect in rabbits with experimental diabetes, while they did not cause such an effect in the organisms of healthy mice. Also, a significant reduction in serum lipids and an increase in HDL cholesterol were found in the organisms of hyperlipidemic rats (Luo et al., 2004). Similar observations were made by Ming et al. (2009) experimenting on mice fed a high-fat diet. They observed a significant reduction in the level of triglycerides, total cholesterol, and LDL fraction, as well as an increase in HDL cholesterol. They also found a drop in blood glucose levels.

The fruit should be eaten regularly but in moderate amounts. Consumption of unripe fruits may lead to poisoning, which is manifested by disturbance of the functions of the digestive tract and the nervous system. In addition, the fruits is are recommended for people taking anticoagulants, pregnant women, and nursing mothers. Side effects of excessive fruit consumption, such as nosebleeds, have been reported (Bogacz, 2009). Increased consumption of goji fruits in Europe has resulted in the emergence of people showing allergic reactions after their consumption (Carnés et al., 2013; Uasuf et al., 2020). Potterat (2010) presented a review based on 150 different reports and does not mention any serious intoxication caused by the consumption of *L. barbarum* or *L. chinense*. However, cases or other gastrointestinal complaints may occur in susceptible individuals. In fully ripe goji berries, traces of atropine were found, without any toxicity, up to 19 ppb (Adams et al., 2006).

Health-promoting properties of leaves and other parts of plants

L. chinense leaves are considered in traditional Chinese medicine as herbs for eternal youth and long life (Soga, 1985), as a nutrient and tonic reducing the risk of arteriosclerosis and

essential hypertension (Mizobuchi et al., 1964). Among the bioactive substances in the leaves are betaine (Hansel et al., 1992; No et al., 1995) and substances used to reduce the risk of fatness or as a digestive aid for people with insufficient stomach acid production. The leaves contain vitamin C and anti-aging tocopherols (Park, 1995), a group of antioxidant compounds such as rutin, quercetin (Aubert and Kapetanidis, 1989; Duke, 1992; Han et al., 2002), chlorogenic acid (Terauchi et al., 1997). *L. barbarum* and *L. chinense* leaves are a valuable source of flavonoids with important antioxidant and antimicrobial properties. Duan et al. (2010) report that the leaves of *L. barbarum* contain gentistic acid (2.5-dihydroxybenzoic acid) with antioxidant properties and enhancing the fungicidal effect of fludioxonil. Grygorieva et al. (2020) studied the content of phenolic compounds in the leaves of several non-traditional crops. The leaves of *L. barbarum* had the highest content of polyphenols and flavonoids (95.84 mgGAE/g and 54.61 mgQE/g, respectively). Phytochemical studies by Mocan et al. (2014) indicate that the leaves of both species are important sources of flavonoids and chlorogenic acid. *L. chinense* leaf extract was characterized by a higher antioxidant activity than *L. barbarum*. Based on antimicrobial tests, it was shown that *L. chinense* leaf extract was more active than *L. barbarum* against strains of Gram-positive and Gram-negative bacteria. *L. chinense* extract against *Bacillus subtilis* had the best antibacterial activity. Terauchi et al. (1998) noted in *L. chinense* leaves the presence of lyciumoside I, a methanol extract showing antimicrobial activity against Gram-positive bacilli. Yeh et al. (2008) showed that *L. chinense* leaf extract stimulates the growth of *Pedicoccus bacteria* – *P. acidilactici*, widely used in the dairy industry in the production of yogurt. Dong et al. (2009) compared the chemical composition of *L. barbarum* from crops to wild plants. They proved that the total content of flavonoids (21.25 mg/g) in the leaves of the cultivated plants was much higher than in the wild (17.86 mg/g). They therefore, found that the leaves of the crops are more suitable for consumption in salads and as a tea. Liu et al. (2012) investigated the content of polysaccharides in *L. barbarum* leaves. They found that leaves, like fruits, are a rich source of polysaccharides with immunostimulating properties and should be used more widely in the production of dietary supplements.

Possibilities of use in the food industry

The fruits of edible wolfberry (*L. barbarum*, *L. chinense*) are very delicate. Poterańska and Ochmian (2015), comparing the fruits quality of two *L. barbarum* cultivars showed that their firmness (172–182 G/mm) is comparable to that of the honeysuckle berry cv. Wojtek (176 G/mm). The taste of goji fruits is very specific, perceived by some as delicious, similar to licorice (Velder, 1999). Others compare their taste to cranberries with a hint of tomato and herbal flavor, very tart and moderately sweet (Stobnicka et al., 2011). Some cultivars have a less bitter taste. The taste of the fruits is determined by the ratio of the extract to the acidity. The amount of the extract in the fruits is positively correlated with the sugar content. There is more extract, less sour taste is felt. According to the studies by Poterańska and Ochmian (2015), the ratio of extract to acidity of *L. barbarum* cv. New Bing fruits was 14.7. The fruits are usually used after processing. Most of the fruit is dried immediately after harvesting. Less commonly, they are available frozen. In Western countries, dried goji berries are eaten as a snack, similar to raisins or other dried fruits (Bogacz, 2009). The fruits are also used to make juices and jams. Oil is pressed from the seeds. In Chinese cuisine, dried goji berries are

boiled before consumption. They are added to rice, soups, chicken, and pork, and mixed with vegetables. The fruits are also cooked as herbal tea, often with the addition of chrysanthemum flower or red tea (Bogacz, 2009). Powdered dried goji berries have a beneficial effect on the physical organoleptic properties, including the flavor and aroma of the bread. Ziemichód and Różyło (2018) showed that the addition of goji berries improved the colour of the bread. With an increase in the addition of dried blueberries in the range of 3–15%, the hardness of gluten-free bread decreased, and the flexibility increased significantly. For some time, when the term "Superfoods" came up, Goji berries quickly became an established product around the world. Due to the fact that the name Goji is a term that covers a wide variety of closely related plant species, mislabeling and adulteration (unknowingly or intentionally) is possible. In China, dried "Goji" berries are generally *L. barbarum*, but are sold in different grades: super, "king", "special" and "Grade A" (Wang et al., 2015). This is due to the differences in the size of the berries. The various uses of goji berries in the production of traditional and new products, with the use of innovative technologies, are described by Ye and Jiang (2020). The food industry in China uses the young shoots and leaves of *L. barbarum* and *L. chinense* to make salads. In the East, leaves have been used for 2,000 years as an endurance, calming, and thirst-quenching tea (Kim et al., 1997). In addition, the leaves are added to soups and sauces or chopped and fried with eggs (Velder, 1999).

In the pharmaceutical industry

The herbal industry uses fruits (Lycii Fructus), flowers (Lycii flox), bark (Lycii Cortex), leaves (Lycii Folium), and roots (Lycii radix). The individual parts of the plant, such as leaves, flowers, fruits, and roots, should be harvested at different times of the year, i.e. in spring, summer, autumn, and winter, respectively. In the last decade, goji fruits have been prescribed by dieticians in the form of natural products as a powerful supplement to help lose weight (Carnés et al., 2013). It is available to choose the least processed goji berry products. Unfortunately, the standard procedure in the production of pharmaceuticals using goji powder is the addition of chemical excipients, which are inactive micronutrients devoid of therapeutic effects. They are added to give such products a taste and protect them against the undesirable influence of microorganisms (Vasconcelos et al., 2012). Preservatives, dyes, flavors, sweeteners, thickeners, emulsifiers, and stabilizers are commonly used in pharmaceutical laboratories (Balbani et al., 2006). Moura et al. (2018) used the root meristem of *Alliym cepa* L. (onion) as an efficient bioassay for the initial screening of the genetic toxicity. They found that industrialized goji berries at all concentrations, including those indicated for use by pharmaceutical companies, had significant potential for toxicity.

In the cosmetics industry

Cosmetic preparations are applied to the external tissue of our body, which is the skin. They have a nurturing and anti-aging effect, due to their anti-inflammatory properties and protection against excessive water loss. The following active substances of plant origin are used in cosmetics, such as antioxidants, exfoliating substances, moisturizing, brightening or protecting against UV rays. In each part of the plants of the described *Lycium* species, there are antioxidants, which, according to the free radical theory of aging, are also important in

skincare. The dominant component with antioxidant properties in the case of *L. barbarum* are peptidoglycans, also called LB polysaccharides (Zhang, 1993; Qiu et al., 2014), and also vitamin B, C, taurine, and carotenoids in fruits, and rutin in leaves (Jin et al., 2013). Carotenoids are a group of plant pigments that are lyophilic. Due to the fact that these pigments are fat-soluble, they perfectly penetrate the epidermis. Apart from the fact that they neutralize free radicals, they give cosmetics a yellow shade. B vitamins have been used externally to relieve dermatitis, acne, oily hair, and anti-wrinkle creams. Vitamin C shows anti-radical activity because it reduces the tocopheryl radical and reduces the consumption of vitamin E. Vitamin C after penetrating the stratum corneum, regulates the collagen biosynthesis. It brightens the skin by inhibiting the activity of tyrosine and reducing melanin synthesis. Zhao et al. (2005) applied LB polysaccharides topically to full-thickness human skin explants by a selectively inhibiting metalloproteinase. One of the five major LBPs, LBGp5, was applied to fibroblasts grown under suboptimal conditions. It has been found to stimulate the production of type I collagen and promote cell viability. Plant substances can also have a beneficial effect on wound healing. Several experiments have demonstrated the stimulating effect of LBP on human skin fibroblasts and the role of directional fibroblast migration during wound healing has been explained (Wang et al., 2011; Song et al., 2013; Zhao and Bojanowski, 2015). These experiences justify the advisability of developing the production of cosmetics based on LBP. The seeds contained in the goji fruit contain a cosmetically valuable oil with anti-inflammatory properties and improving the skin's protective barrier by reducing epithelial water loss. However, until now cosmetics based on substances derived from *Lycium* species have not developed on a large scale, mainly due to the lack of registration as a cosmetic raw material in countries such as China and Japan. Another limitation is the high cost of extracting the oil from the fruit due to its low content.

Conclusion

The fruit, popularly referred to as goji, have been well-established for centuries as a medicine for many ailments: in treatment heart, liver, lung, kidney diseases, and above all as a means of human longevity. Thanks to the content of various bioactive substances such as vitamins, flavonoids, carotenoids, anthocyanins and the so-called LBP (*Lycium* Barbarum Polysaccharides) has strong antioxidant, anti-inflammatory properties and regulates carbohydrate and lipid metabolism. The fruit is usually used as a dried raw material. Apart from fruit, other parts of plants, especially leaves, are a rich source of health-promoting ingredients. Currently, there is an intense interest in consumption, and hence in the production of these fruits. Although the highest quality fruit is obtained in the Chinese province of Ningxia, its commercial cultivation is also beginning to develop in other countries. The climatic and soil requirements of the two most important species, *L. barbarum* and *L. chinense*, enable their cultivation also in European countries. Of course, it is necessary to select the appropriate variety, which fruits are high quality, i.e. with a high extract-acidity ratio, less tart, yet rich in bioactive substances. As in the case of elderberry and viburnum, goji berries intended for consumption must be fully ripened. Sensitive people, allergy sufferers should consume them in moderation.

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OPEN ACCESS

ASSESSMENT OF SELECTED MORPHOLOGICAL TRAITS OF SWEET CHESTNUT (*CASTANEA SATIVA* MILL.) FRUITS FROM REPOSITORY PRÍBELCE (SLOVAKIA)

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Sweet chestnut (*Castanea sativa* Mill.) as an introduced species in Slovakia is the object of our research in terms of morphometric analysis of fruits from randomly selected 30 genotypes from the repository Príbelce with presence of old varieties, biennial seedlings of intraspecific and interspecific hybrids and individuals from free pollination from the Arboretum Mlyňany and M.M. Gryshka National Botanical Garden of National Academy of Sciences of Ukraine in Kyiv. In the characteristics and evaluation of fruit characters, we determined high variability in all quantitative and qualitative traits. The collection recorded significant differences in fruit weight in the range of 2.08–13.21 g with a coefficient of variation in the interval 7.12–34.26%, average fruit height 15.50–30.37 mm (3.62–14.79%), fruit width 17.80–34.51 mm (3.62–15.10%), hilum length 13.31–29.20 mm (6.22–16.75%), hilum width 7.12–15.87 mm (6.49–17.50%) and a nut hairiness of 9.81–22.39 mm (6.75–20.65%). According to the fruit shape index, we determined a triangular, spherical, transversely elliptical and transversely broad-elliptical shape in the collection of genotypes. The results of the research point to the fact that the *Castanea sativa* genetic material from the clone repository is a rich source of genetic diversity and can be used in the selection to create new varieties and cultivars.

Keywords: *Castanea sativa*, fruits, genetic plant resources, morphological characteristic

Úvod

Rod Gaštany (*Castanea* Mill.) pozostáva z lesných stromov s výnimočným ekologickým, socioekonomickým a kultúrnym významom. *Castanea*, patriace do čeľade Fagaceae Dumort., sú prirodzene rozšírené v listnatých lesoch Severnej Ameriky, Európy a Ázie (Fei et al., 2012). Oblasť výskytu sa pohybuje od južnej Európy (Pyrenejský polostrov, Taliansko, Balkán, Stredozemné ostrovy) a severnej Afriky (Maroko), po severozápadnú Európu (Anglicko,

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Belgicko) a na východ po západnú Áziu (severovýchodné Turecko, Arménsko, Gruzínsko, Azerbajdžan, Sýria), s nadmorskou výškou medzi 200 až 1 800 m.n.m, v závislosti od zemepisnej šírky a aspektu lokality. V Európe sa gaštan jedlý rozprestiera na ploche viac ako 2,5 milióna hektárov. Väčšina územia (89 %) je sústredená iba v niekoľkých krajinách (Francúzsko, Taliansko, Španielsko, Portugalsko a Švajčiarsko) s dlhoročnou tradíciou gaštanu (Conedera et al., 2016).

Rod zahŕňa štyri ekonomicky dôležité druhy s hojným výskytom sladkých orechov a produkcie dreva: gaštan čínsky (*Castanea mollissima* Blume), gaštan japonský (*Castanea crenata* Siebold & Zucc.), gaštan európsky (*Castanea sativa* Mill.) a gaštan americký (*Castanea dentata* (Marshall) Borkh.). Literatúra naznačuje, že druhy *Castanea* majú bohatú škálu genetickej diverzity a morfologickej a ekologickej variability (Aravanopoulos et al., 2001; Furones-Pérez et Fernández-López, 2009; Benedetti et al., 2018; Stojanović et Magazin, 2020).

V našich podmienkach je drevinou introdukovanou s obmedzeným výskytom v pahorkatinách juhozápadného až juhovýchodného Slovenska. Oblasť rozšírenia gaštana na Slovensku možno vymedziť Malackami na západe a Petrovcami na východe územia. Južný okraj tvorí približne čiara Bratislava – Šahy – Lučenec – Slanec. Na severe vybieha dolinou Váhu až k Lednickému Rovnému, dolinou rieky Nitry do Bojníc, dolinou Hrona do Badína, dolinou Ipľa až do Ipeľského Potoka, dolinou Rimavy až do Kokavy a Hnúšte a na východnom Slovensku dolinou Torysy až po Uzovský Šalgov a dolinou rieky Topľa ku Giraltovciam (Bolvanský et al., 2008). Výskum tejto dreviny sa nielen u nás sústredí na variabilitu reprodukčných orgánov (Benčať, 1967), morfologických znakov kvetov, plodov, listov (Bolvanský, 1989; Furones-Pérez et Fernández-López, 2009; Grygorieva et al., 2016, 2017, 2018), rastových vlastností (Benčať a Tokár, 1967), kvalitu drevnej produkcie (Tokár, 1991), štúdium chorôb a škodcov (Juhásová a Hrubík, 1984; Juhásová, 1999; Dar et Rai, 2015), tiež na výber hospodársky cenných genotypov, ktoré by sa mohli stať odrodami pre produkciu plodov (Solar et al., 2005; Stojanović et Magazin, 2020). Pástor et al. (2017) skúmal biokultúrnu hodnotu gaštana jedlého v krajinných typoch s tradičným využívaním krajiny, kde gaštan je cennou črtou hospodárskej krajiny. Obrovské (pozoruhodné) gaštany sa pestovali ako samostatné stromy, ktoré zvyčajne stoja na pastvine alebo poľnohospodárskej pôde vo vzťahu k určitým prvkom vytvoreným človekom, ako sú osady, hranice nehnuteľností a chodníky (Krebs et al., 2012).

Ochrana a rast biologickej diverzity má strategický význam pre trvalo udržateľný rozvoj spoločnosti (IPGRI, 2002). Potreba výskumu venovať sa problematike variability plodov gaštana jedlého v repozitóriu Príbelce je dôsledkom strategického plánu medzinárodnej biodiverzity, ktorá zabezpečuje a udržuje ochranu a stabilitu ekosystémov.

Materiál a metodika

Biologický materiál

Pre experimentálne účely sme vybrali jedince *Castanea sativa* z gaštanového sadu v obci Príbelce. Sad Príbelce – klonové repozitórium sa nachádza na východnom okraji obce Príbelce s nadmorskou výškou 270 – 300 m n. m., so súradnicami 48° 12’ 10’’ s. z. š. a 19° 15’ 40’’ v. z. d. Sad bol založený v roku 1998 na ploche s rozlohou 2,7 ha s ojedinelým výskytom starých

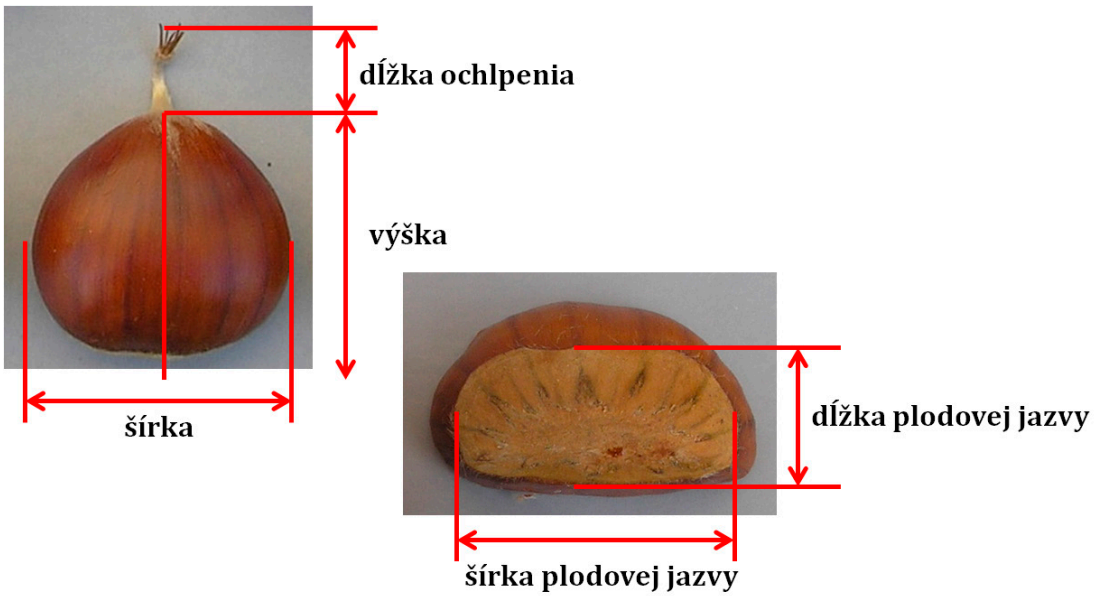
jedincov gaštana jedlého, ktoré boli ponechané a zakomponované do novej výsadby dvojročných semenáčikov pochádzajúcich z voľného opelenia medzidruhových a vnútrodruhových hybridov a výsadby jedincov z voľného opelenia z Arboréta Mlyňany a z Národnej Botanickej záhrady M.M. Grishka pri Národnej akadémii vied Ukrajiny v Kyjeve introdukovaných na naše podmienky.

Morfometrická analýza

Hodnotili sme 30 náhodne vybraných zdravých genotypov (C01–C30). Z každého genotypu sme zabezpečili morfometrickú analýzu z 30 náhodne vybraných plodov. Pri plodoch sme hodnotili nasledovné znaky:

a) hmotnosť plodov (g);
b) výška a šírka plodov (mm);
c) dĺžka a šírka jazvy plodov (mm);
d) dĺžka ochlpenia (mm);
e) index tvaru plodov a index tvaru jazvy.

Hmotnosť plodov sme stanovili použitím analytických váh (Kern ADB-A01S05, Germany) s presnosťou na 0,01 g a dĺžku a šírku plodov, dĺžku a šírku jazvy plodov a dĺžku ochlpenia posuvným digitálnym meradlom.



Obrázok 1 Nákres meraní: výška a šírka plodu, dĺžka a šírka jazvy plodu a dĺžka ochlpenia
Figure 1 Illustration of measuring process: fruit height and width, hilum length and width, length of nut hairiness

Obrazová analýza

Obrazová dokumentácia sa zabezpečila digitálnymi fotoaparátmi Canon PowerShot G5, Panasonic DMC FZ50.

Pre komplexnejšie zhodnotenie sme na plodoch hodnotili aj kvalitatívne znaky:

a) plody – farba a tvar;
b) priečny rez plodov – farba.

Pri hodnotení kvantitatívnych a kvalitatívnych znakov sme vychádzali z deskriptora pre genetické zdroje gaštana na Slovensku zostaveného Bolvanským (Bolvanský et al., 2008).

Štatistická analýza

Základné štatistické analýzy boli vykonané s použitím PAST 2.17; Variabilitu testovaných súborov v jednotlivých znakoch sme hodnotili pomocou deskriptívnej štatistiky. Stupeň variability sme určovali podľa hodnôt variačných koeficientov. Daný ukazovateľ je nezávislý na meranej jednotke hodnoteného znaku. Teoreticky môžu nadobúdať ľubovoľné hodnoty (Stehlíková, 1998). Na zistenie vzájomnej závislosti medzi jednotlivými znakmi sme použili korelačnú analýzu a klastrovú analýzu v programe STATISTICA 1.10.

Výsledky a diskusia

Medzi najvýznamnejšie ukazovatele veľkosti plodov patrí priemerná hmotnosť plodov. V hodnotenej kolekcii 30 genetických zdrojov gaštana jedlého sme zistili priemernú hmotnosť plodov v rozsahu od 2,08 (C18) do 13,21 g (C25). V kolekcii sme zaznamenali tri genotypy s priemernou hmotnosťou plodov nad 10 g. Hodnoty variačných koeficientov zistené v intervale 7,12 (C02) – 34,26 % (C18) dokumentujú v rámci hodnotených genotypov nízky až vysoký stupeň variability daného znaku, tzn. že vytvorené plody môžu dosahovať rôznu hmotnosť. Podľa deskriptora pre hmotnosť plodov (Bolvanský et al., 2008) možno hmotnosť plodov v našej kolekcii kategorizovať ako veľmi malé až stredne veľké plody. Pri porovnaní našich výsledkov s literárnymi údajmi z ďalších oblastí Slovenska (Benčať, 1968; Bolvanský et al., 2008; Bolvanský et al., 2012) sme určili významnú zhodu. Určité odlišnosti pri tomto znaku zaznamenali autori z iných krajín. Grygorieva et al. (2017) na Ukrajine stanovila rozsah meraní v intervale 1,70 – 20,0 g s vysokým variačným koeficientom (45,92 %), Mujić et al. (2010) v Bosne a Hercegovine v rozsahu 4,32–6,67 g, Pandit et al. (2011) v Indii v rozsahu 5,23 – 16,37 g. V tabuľke 1 sú prezentované minimálne a maximálne hodnoty všetkých hodnotených znakov 30 genotypov z repozitória Príbelce.

Medzi významné agrofyzikálne parametre veľkosti plodov patria aj výška a šírka plodov. Uvedené znaky sú dôležitým ukazovateľom veľkosti plodov ako aj technický parameter pri triedení plodov gaštana jedlého do kvalitatívnych kategórií. V hodnotenej kolekcii 30 genetických zdrojov gaštana jedlého sme zistili priemernú výšku plodov v rozsahu od 15,50 (C18) do 30,37 mm (C09) a šírku v rozsahu od 17,80 (C18) do 34,51 mm (C25). V kolekcii sme zaznamenali 1 genotyp s priemernou výškou plodov nad 30 mm a 9 genotypov s priemernou šírkou plodov nad 30 mm. Podľa deskriptora pre hodnotené znaky plodov (Bolvanský et al., 2008) možno výšku a šírku plodov v hodnotenej kolekcii kategorizovať ako veľmi malé až veľké. Hodnoty variačných koeficientov určené v rozsahu 3,62 (C21) – 14,79 % (C29) pre výšku plodov a hodnoty 3,62 (C25) – 15,10 % (C16) pre šírku plodov dokumentujú, že v rámci hodnotených genotypov je nízky až stredný stupeň variability pre oba znaky. Ako doplnkový

parameter sa môže použiť šírka plodu, ktorá je v tesnej, kladnej korelácii s hmotnosťou plodu. Klasifikácia na základe šírky plodu sa odporúča najmä pri vysušených plodoch. Pri porovnaní našich výsledkov s literárnymi údajmi z ďalších oblastí Slovenska (Benčať, 1968; Bolvanský et al., 2008; Bolvanský et al., 2012) sme určili významnú zhodu, naproti tomu Benedetti et al. (2018) v Chile určil širšie rozpätie pre výšku (17,10 – 36,7 mm) a šírku plodov (17,5 – 44,5 mm), rovnako tiež Grygorieva et al. (2017) na Ukrajine pri oboch znakoch (8,07 – 33,39 mm; 16,34 – 40,95 mm).

Tabuľka 1 Variabilita vybraných znakov na plodoch gaštana jedlého (*Castanea sativa* Mill.) v hodnotenej kolekcii genotypov z klonového repozitória

Table 1 Variability of selected fruit traits of sweet chestnut (*Castanea sativa* Mill.) in the evaluated collection of genotypes from the clone repository

	Hmotnosť plodov (g)							Dĺžka ochlpenia (mm)					
	n	**min**	**max**	**$\bar{x}$**	**V**	**TH**		***n***	**min**	**max**	**$\bar{x}$**	**V**	**TH**
Genotypy s nízkymi hodnotami znaku													
C18	30	1,10	3,60	2,08	34,26	r	**C30**	30	7,61	11,92	9,81	10,61	n
C30	30	1,70	3,10	2,20	18,41	p	**C03**	30	8,60	12,44	10,06	8,54	n
Genotypy s vysokými hodnotami znaku													
C25	30	11,70	15,90	13,21	7,12	a	**C13**	30	17,40	25,76	22,39	8,24	a
C08	30	5,40	18,30	11,09	29,70	ab	**C21**	30	18,92	25,43	22,19	7,42	ab
	Výška plodov (mm)							**Šírka plodov (mm)**					
	n	**min**	**max**	**$\bar{x}$**	**V**	**TH**		***n***	**min**	**max**	**$\bar{x}$**	**V**	**TH**
Genotypy s nízkymi hodnotami znaku													
C18	30	13,63	18,61	15,50	7,72	n	**C18**	30	12,19	22,36	17,80	11,87	m
C30	30	15,09	18,17	16,65	4,32	m	**C30**	30	16,84	21,41	18,78	5,91	l
Genotypy s vysokými hodnotami znaku													
C09	30	26,07	33,26	30,37	6,46	a	**C25**	30	32,13	36,46	34,51	3,62	a
C08	30	23,75	34,11	28,54	8,89	a	**C13**	30	27,83	35,35	31,99	6,73	a
	Dĺžka jazvy (mm)							**Šírka jazvy (mm)**					
	n	**min**	**max**	**$\bar{x}$**	**V**	**TH**		***n***	**min**	**max**	**$\bar{x}$**	**V**	**TH**
Genotypy s nízkymi hodnotami znaku													
C18	30	9,77	17,97	13,31	13,58	o	**C30**	30	5,32	9,11	7,12	13,72	l
C30	30	13,33	18,84	16,21	7,12	n	**C18**	30	5,23	11,14	7,66	16,50	l
Genotypy s vysokými hodnotami znaku													
C25	30	23,00	32,56	29,20	6,69	a	**C08**	30	11,30	21,57	15,87	17,07	a
C28	30	21,84	36,65	26,46	10,76	ab	**C25**	30	14,08	19,53	15,85	7,76	ab

Poznámky: *n* – počet meraní; min, max – minimálna a maximálna nameraná hodnota; $\bar{x}$ – aritmetický priemer; V – variačný koeficient (%); TH – test homogenity podľa LSD pri preukaznosti P0,05

Pri hodnotení plodov gaštana jedlého má svoje praktické opodstatnenie aj dĺžka a šírka plodovej jazvy. V hodnotenej kolekcii 30 genetických zdrojov gaštana jedlého sme zistili priemernú dĺžku plodovej jazvy od 13,31 (C18) do 29,20 mm (C25) a priemernú šírku plodovej jazvy v rozsahu od 7,12 (C30) do 15,87 mm (C08). V kolekcii sme zaznamenali osem genotypov s priemernou dĺžkou plodovej jazvy nad 25 mm a dva genotypy s priemernou šírkou plodovej jazvy nad 15 mm. Hodnoty variačných koeficientov zistené v rozsahu 6,22 (C01) – 16,75 % (C09) pre dĺžku jazvy a v rozsahu 6,49 (C01) – 17,50 % (C16) pre šírku jazvy dokumentujú nízky až stredne vysoký stupeň variability pri oboch znakoch. Pri porovnaní našich výsledkov s literárnymi údajmi z ďalších oblastí Slovenska (Benčať, 1968; Bolvanský et al., 2008; Bolvanský et al., 2012) sme určili významnú zhodu. Podľa deskriptora pre veľkosť plodovej jazvy – dĺžka jazvy/šírka plodu (Bolvanský et al., 2008) možno v hodnotenej kolekcii kategorizovať znak ako malá až stredná veľkosť jazvy.

Pri hodnotení plodov sa aplikuje aj priemerná dĺžka a šírka ochlpenia plodov. V hodnotenej kolekcii 30 genetických zdrojov gaštana jedlého sme zistili priemernú dĺžku ochlpenia plodov v rozsahu od 9,81 (C30) do 22,39 mm (C13), tzn. že vytvorené plody môžu dosahovať rôznu dĺžku ochlpenia plodov. V kolekcii sme zaznamenali 3 genotypy s priemernou dĺžkou ochlpenia plodov nad 20 mm. Podľa deskriptora pre ochlpenie plodov (Bolvanský et al., 2008) možno dĺžku ochlpenia v hodnotenej kolekcii kategorizovať ako malú až veľkú. Hodnoty variačných koeficientov pre dĺžku ochlpenia zistené v rozsahu 7,11 (C23) – 20,65 % (C26) dokumentujú nízky až mierne vysoký stupeň variability. Pri porovnaní našich výsledkov s literárnymi údajmi (Benčať, 1968; Bolvanský et al., 2008; Bolvanský et al., 2012) sme určili významnú zhodu.

Výsledky našich meraní sme porovnali s inými autormi zaoberajúcimi sa rôznymi populáciami *Castanea sativa* (tabuľka 2) prispôsobených určitým regionálnym a klimatickým podmienkam v Grécku (Aravanopoulos et al., 2001), Slovinsku (Solar et al., 2005), Bosne a Hercegovine (Mujić et al., 2010), Indii (Pandit et al., 2011), Turecku (Ormeci et al., 2016), Ukrajine (Grygorieva et al., 2017), Čile (Benedetti et al., 2018), Čiernej Hore (Odalovic et al., 2013; Stojanović et Magazin, 2020).

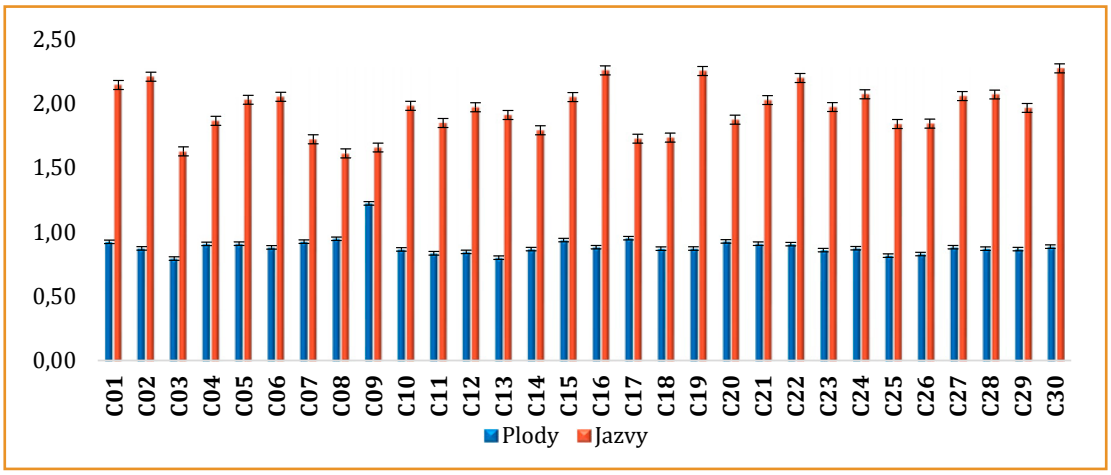
Index tvaru plodov sme stanovili ako pomer výšky plodu k šírke plodu v intervale 0,79 (E03) – 1,22 (L08) a index tvaru jazvy sme stanovili ako pomer dĺžky k šírke jazvy v rozsahu 1,61 (L07) – 2,28 (I07), čo dokumentuje obrázok 2. Pri porovnaní výsledkov s autormi Grygorieva et al. (2017), ktorí stanovili index tvaru plodov v intervale 1,48 – 2,03 a index tvaru jazvy v intervale 0,81 – 0,98 sme zaznamenali významné rozdiely.

Tabuľka 2 Variabilita niektorých morfologických znakov plodov gaštana jedlého (*Castanea sativa* Mill) podľa autorov z iných krajín

Table 2 Variability of some morphometric characteristics on sweet chestnut (*Castanea sativa* Mill) fruits according to the authors from different countries

Autori	Plody			Jazva	
	hmotnosť (g)	dĺžka (mm)	šírka (mm)	dĺžka (mm)	šírka (mm)
Aravanopoulos et al. (2001)	2,98–6,07	19,10–24,90	18,80–23,80	12,90–14,50	6,00–7,00
Solar et al. (2005)	3,50–18,60	20,00–37,00	12,00–39,00	12,00–32,00	7,00–16,00
Mujić et al. (2010)	4,32–6,67	20,45–24,89	23,45–27,10	–*	–*
Bolvanský et al. (2012)	2,94–13,40	16,41–27,75	19,81–34,17	–*	–*
Odalovic et al. (2013)	4,80–10,60	19,60–30,60	23,70–34,90	19,00–31,00	11,00– 16,00
Ormeci et al. (2016)	10,26–22,32	27,74–39,73	26,80–42,47	–*	–*
Grygorieva et al. 2017	1,70–20,0	8,07–33,39	16,34–40,95	6,62–31,30	6,50–19,99
Stojanović et Magazin 2020	5,67–11,70	24,75–29,73	25,85–32,74	16,87–26,41	8,60–14,78

Poznámky: * nemerané

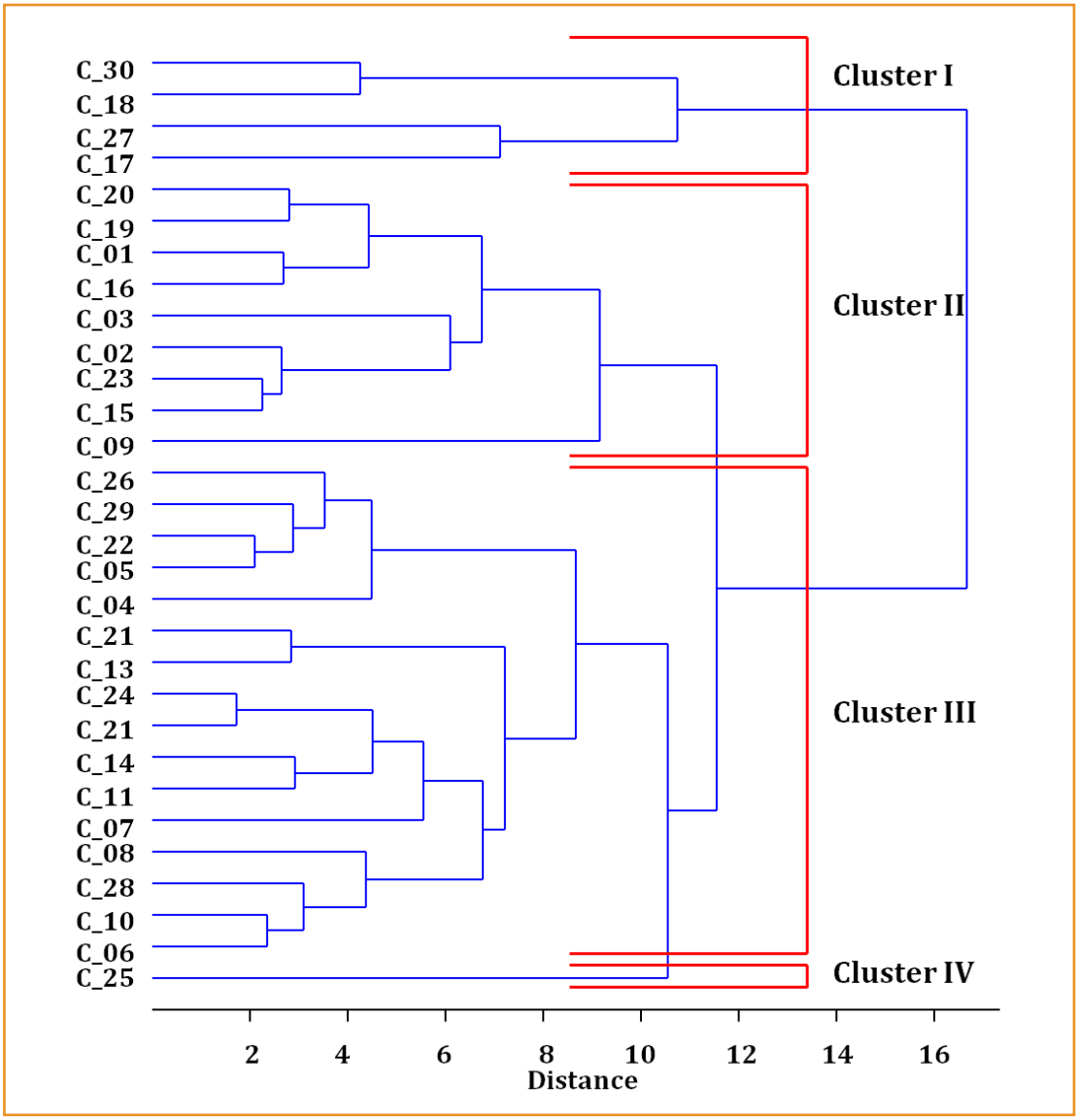


Obrázok 2 Porovnanie vybraných genotypov gaštana jedlého (*Castanea sativa* Mill.) v indexe tvaru plodov a jaziev

Figure 2 Comparison of selected genotypes of sweet chestnut (*Castanea sativa* Mill.) in the shape index of fruit and hilum

Genetický vzťah medzi 30 genotypmi sa skúmal klastrovou analýzou. Analýza preukázala významné rozdiely medzi testovanými genotypmi gaštana jedlého. Dendrogram identifikoval štyri hlavné skupiny (obrázok 3). Štyri z 30 genotypov sú zahrnuté do skupiny I, 9 genotypov v skupine II, 16 genotypov v skupine III a 1 genotyp v skupine IV. Skupina I dosahovala najnižšie priemerné hodnoty morfologických charakteristík (hmotnosť plodu, dĺžka plodu, šírka plodu, dĺžka plodovej jazvy, šírka plodovej jazvy), ktoré sa výrazne líšili od ostatných skupín. Skupinu IV, ktorú tvoril jeden genotyp, naopak, dosahovala najvyšší

priemer morfologických parametrov. Obrázok 3 potvrdzuje výsledky hodnotenej variability morfometrických charakteristík (tabuľka 1).



Obrázok 3 Dendrogram zostavený na základe morfometrických znakov plodov 30 genotypov gaštana jedlého (*Castanea sativa* Mill.)

Figure 3 Dendrogram of 30 genotypes of sweet chestnut (*Castanea sativa* Mill.) based on morphometric characteristics of fruits

Pri všetkých hodnotených znakoch sme zaznamenali kladnú koreláciu (tabuľka 3). Korelačná analýza preukázala úzky vzťah najmä medzi znakmi hmotnosť plodov a výška plodov, kde bola zistená tesná kladná korelácia (r = 0,877), a medzi hmotnosťou a šírkou plodov (r = 0,955).

Tabuľka 3 Korelačná analýza hodnotených znakov plodov gaštana jedlého (*Castanea sativa* Mill.)
Table 3 Correlation analysis of evaluated traits of sweet chestnut (*Castanea sativa* Mill.)

Parametre	Hmotnosť plodu (g)	Výška plodu (mm)	Šírka plodu (mm)	Dĺžka jazvy (mm)	Šírka jazvy (mm)
Výška plodu	0,877**	1			
Šírka plodu	0,955**	0,865**	1		
Dĺžka jazvy	0,905**	0,799*	0,920**	1	
Šírka jazvy	0,948**	0,855**	0,885**	0,869**	1
Dĺžka ochlpenia	0,302*	0,248	0,363*	0,222	0,244

Poznámky: ** korelácia je významná na úrovni 0,01; * korelácia je významná na úrovni 0,05

Tvar plodu gaštana jedlého je baňatý vo vrchnej časti zakončený zaschnutým kratším semenníkom. Plody sú matné alebo lesklé. Vonkajšie osemenie je tenšie svetlo-hnedé. Plody dokonale vypĺňajú celý priestor (Benčať, 1968; Bolvanský et al., 2008). Dané poznatky

Obrázok 4 Porovnanie vybraných genetických zdrojov gaštana jedlého (*Castanea sativa* Mill.) v tvare a farbe plodov (Foto V. Horčinová Sedláčková, 2020)
Figure 4 Comparison of selected genetic resources of sweet chestnut (*Castanea sativa* Mill.) in the shape and colour of fruits (Photo V. Horčinová Sedláčková, 2020)

sme potvrdili aj pri štúdiu genetických zdrojov z klonového repozitória, čo dokumentuje aj obrázok 4. V našej kolekcii genotypov sme určili tvar plodov trojuholníkový, guľovitý, priečne elipsovitý až priečne široko-elipsovitý.

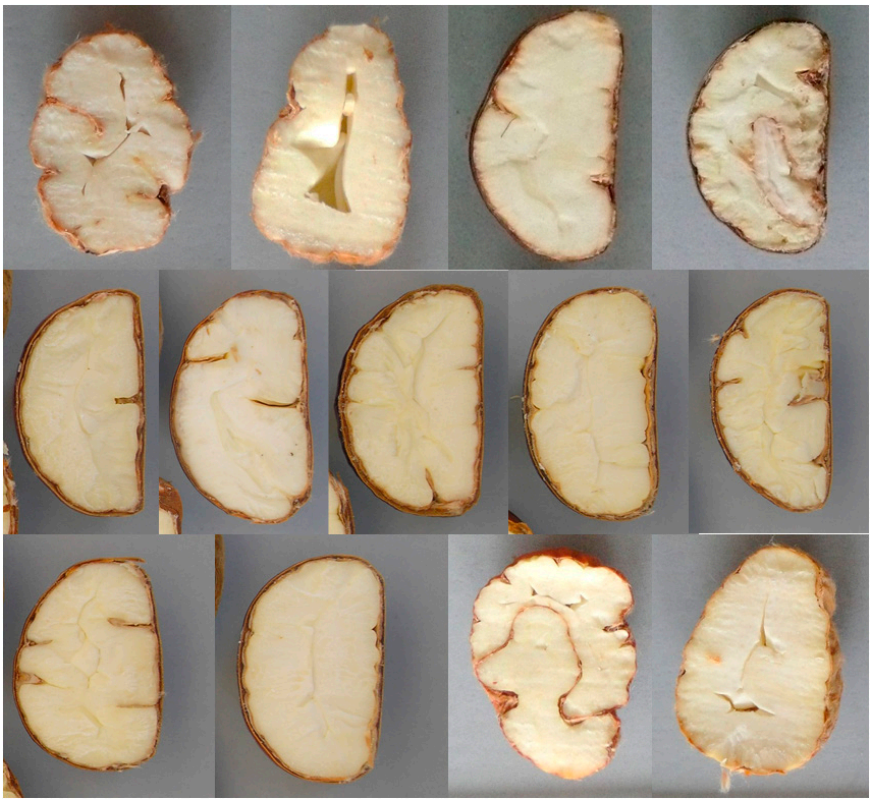
Na obrázku 4 je prezentované porovnanie vybraných genotypov vo farbe plodov. Prezentované porovnanie dokumentuje významné rozdiely medzi genotypmi aj v danom znaku. Podľa deskriptora pre klasifikáciu znakov (Bolvanský et al., 2008) sa pri plodoch vyskytuje sfarbenie od svetlo hnedej, cez hnedú, červeno-hnedú, tmavo-hnedú až po čierno-hnedú, čo sme určili aj v kolekcii hodnotených genotypov.

Významné rozdiely sme určili aj v tvare, veľkosti a ochlpení pútok plodov, čo dokumentuje porovnanie genotypov na obrázku 5.

Obrázok 5 Variabilita v tvare, veľkosti a ochlpení pútok plodov genotypov gaštana jedlého (*Castanea sativa* Mill.) (Foto V. Horčinová Sedláčková, 2020)

Figure 5 Variability in the shape, size and nut hairiness of genotypes of sweet chestnut (*Castanea sativa* Mill.) (Photo V. Horčinová Sedláčková, 2020)

Povrch jadra plodov gaštana jedlého je smotanovo-bielej farby. Jadro je na priereze prevažne biele, svetlo krémové alebo krémové. Na obrázku 6 je prezentované porovnanie vybraných genotypov vo sfarbení jadra plodov. Prezentované porovnanie dokumentuje určité rozdiely medzi genotypmi aj v danom znaku.



Obrázok 6 Porovnanie vybraných genetických zdrojov gaštana jedlého (*Castanea sativa* Mill) v priečnom reze plodov (Foto V. Horčinová Sedláčková, 2020)

Figure 6 Comparison of selected genetic resources of sweet chestnut (*Castanea sativa* Mill.) in cross-section of fruits (Photo V. Horčinová Sedláčková, 2020)

Závery

Na základe uskutočnených experimentálnych prác pri riešení problematiky morfologických znakov plodov genetických zdrojov *Castanea sativa* z repozitória Príbelce sme určili významnú fenotypovú variabilitu vo všetkých znakoch a v kombinácií znakov. Je dôležité spoznať ďalšie biologické zvláštnosti a špecifiká uchovávaných genetických zdrojov v repozitóriu, informovať odbornú a ostatnú verejnosť o pestovaní a využívaní gaštana jedlého v podmienkach Slovenska ako zdroja hospodársky významných plodov, a významnej lesníckej dreviny využiteľnej v agrolesníctve, alebo aj ako genetického materiálu pre rozširovanie a pestovanie v okolitých obciach.

Poďakovanie

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MORPHOLOGICAL FEATURES OF FRUITS OF VARIOUS SPECIES OF CHILLI PEPPERS

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The research focused on *Capsicum* spp. collection (*C. annuum* L., *C. baccatum* L., *C. chinense* Jacq.) cultivated under green-house conditions and examined in the term of morphological characteristics of quantitative (6) and qualitative (4) traits. Aim of the study was to determine the variability in the morphological fruit characters of 28 cultivars. The results revealed considerable morphological variability between average weight of fruits (0.32–25.94 g), weight of peduncle (0.01–0.41 g), weight of exocarp (0.24–24.60 g), weight of seed (0.04–2.14 g), weight of ovary (0.17–4.92 g) and number seeds per fruit (14.2–185.8 pieces). A cluster analysis was carried out, and a dendrogram was established. With quantitative variables, 6 groups were obtained. Correlations between morphological variables were also estimated. There were highly significant differences for most quantitative characters. The study showed that the weight of fruits parameters is correlated with the variables related to the weight of exocarp and weight of ovary. High diversity based on qualitative traits was detected for the shape of fruits, the colour of fruits ect. Obtained results confirmed that chilli peppers are suitable germplasm for their cultivation and distribution as ornamental plants, their potential uses and benefits to mankind cover many areas processing into food and nutrition, cosmetics, plant-based insecticides, pharmaceutical or medicine products.

Keywords: *Capsicum* spp., fruits, cultivars, morphometric characteristic

Introduction

Chilli pepper (*Capsicum* spp.) is a solaneceous plant, whose centre of origin in Middle America and Mexico is centre of genetic diversity and domestication. In the world, several hundred types of peppers are cultivated. The five major cultivated and economically most important species of *Capsicum* are *C. annuum* L., *C. chinense* Jacq., *C. frutescens* L., now widely cultivated throughout Europe, the southern United States, Africa, India, and China, and *C. baccatum* L. and *C. pubescens* Ruiz & Pav., cultivated predominantly in South America (Basu and De, 2003; Scaldaferro et al., 2018). The genus comprises approximately 42 described

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species (Barboza et al., 2019), with wide range of morphological variability, mainly in different shapes, sizes, colours and sensory attributes of its fruits (Nwachukwu et al., 2007; Barbosa et al., 2010; Ballina-Gómez et al., 2013; Wahua et al., 2014; Bicikliski et al., 2018).

Capsicum terminology is very confusing with pepper, chilli, chile, chilli, aji, paprika, and capsicum all used interchangeably to describe the plant (DeWitt and Bosland, 2009). Csillery (2006) indicates that the first component description of *Capsicum* was given in Hungarian herbal by Dioszegi and Fazekas (1807) cited by Bozokalfa et al., (2009). Chilli pepper is an important agricultural crop, not only because of its economic importance, but also for the nutritional value of its fruits (Aliu et al., 2017), mainly due to the fact that they are an excellent source of natural colours and antioxidant compounds (Rodríguez-Maturino et al., 2011; Medina-Juárez et al., 2012).

The *Capsicum* genus includes the sweet and hot chilli peppers, which have been popular from ancient times and at present are of great commercial interest, not only for the taste and colour of their fruits (Shaha et al., 2013), but also because of their essential oils and the presence of capsaicin (Pruthi, 2003; Nadi et al., 2020). Capsaicin is the pungent principle of hot chilli peppers, which are commonly used as spices and as medicines (Saleh et al., 2018), mainly applied topically to relieve neuropathic pain and itching (Papoui and Yosipovitch, 2010). The number of studies report that hot pepper seeds are rich in minerals content (Jarret et al., 2013; Zou et al., 2015). The content of vitamin C in the pepper fruit is higher than in Citrus (Finger et al., 2010). The pepper fruit is a rich source of vitamin A, E, C and P in green chilli (Howard et al., 2000; Marin et al., 2004). They have a high level of vitamins C and E as well as the total of antioxidants is completed by phenolic compounds, which occur in peppers in connection with sugars (Shotorbani and Jamei, 2013; Batiha et al., 2020).

Preservation of such plant genetic resources is extremely important for plant breeding as well as for society as a whole (Moreira et al., 2018). In view of the cultural and economic importance of the *Capsicum*, the development of new and improved cultivars carrying characteristics that meet the needs of farmers and the consumers is primordial. To achieve this goal, plant breeders are dependent on plant genetic resources and need access to the widest genetic diversity available. For these reasons, the preservation of wild species, local varieties and traditional genotypes in collections or germplasm banks is very important (Nass et al., 2012). In addition, the characterization of these materials is essential information for the conservation and use in plant breeding programs (Murillo-Amador et al., 2015; Sarobo, 2019).

Variability study of agro-morphological characteristics of various *Capsicum* species in conditions of Botanical Garden (Slovakia) due to biodiversity conservation and plant breeding program was aim of our examination.

Material and methodology

Biological material

We experimentally studied 28 cultivars of peppers from the three *Capsicum* species (*C. annuum*, *C. baccatum* and *C. chinense*) (Table 1). All 28 cultivars of chilli peppers that were

the subject of the study were grown under green-house conditions in the Botanical Garden, which is located on the area of the Slovak University of Agriculture in Nitra.

Table 1 List of 28 cultivars of *Capsicum* spp.

No.	Species	Cultivars
1	*Capsicum annuum* L.	Black Kobra
2	*Capsicum annuum* L.	Chocho
3	*Capsicum annuum* L.	Jalapeno
4	*Capsicum annuum* L.	Kilian
5	*Capsicum annuum* L.	Medusa
6	*Capsicum annuum* L.	Pepperoncini Greek
7	*Capsicum annuum* L.	Tabasco
8	*Capsicum annuum* L.	Trinidad Hot Cherry
9	*Capsicum annuum* var. *nigrum*	Black Prince
10	*Capsicum baccatum* L.	Aji Amarilo
11	*Capsicum baccatum* L.	Aji fantasy sparkly white
12	*Capsicum baccatum* L.	Aji Rojo
13	*Capsicum baccatum* L.	Bishops Crown Red
14	*Capsicum baccatum* L.	El Oro De Ecuador
15	*Capsicum baccatum* L. var. *pendulum*	Escabeche
16	*Capsicum chinense* Jacq.	Aji Charapita
17	*Capsicum chinense* Jacq.	Carolina Reaper
18	*Capsicum chinense* Jacq.	Citron
19	*Capsicum chinense* Jacq.	Fatali Red
20	*Capsicum chinense* Jacq.	Fidalgo Roxa
21	*Capsicum chinense* Jacq.	Habanero Chocolate
22	*Capsicum chinense* Jacq.	Habanero Peach
23	*Capsicum chinense* Jacq.	Haba nero Red
24	*Capsicum chinense* Jacq.	Habanero Red Savina
25	*Capsicum chinense* Jacq.	Jolokia White
26	*Capsicum chinense* Jacq.	Peter´s Orange
27	*Capsicum chinense* Jacq.	Pimenta De Neyde
28	*Capsicum chinense* Jacq.	Trinidad Scorpion Peach

Morphometrical analysis

The following quantitative and qualitative properties were evaluated by morphometrical analysis:

a) fruit weight (g); stem weight (g); weight of exocarp (g); ovary weight (g); seed weight (g); number of seeds in fruit (pcs);
b) shape of fruits; fruit shape at peduncle; fruit shape at blossom end; colour of the fruits.

The weights were evaluated by analytical balance (Kern ADB-A01S05, Germany), accurate to 0.01 g.

Statistical analysis

It was evaluated the variability of the test files in each character using descriptive statistics. For the characteristics of the files, it was used the basic descriptors of variability: average, minimum measured value, maximum measured value, the coefficient of variation (%). The degree of variability was determined by the coefficient of variation values. The given parameter is independent of the unit of the evaluated character. Theoretically, they can acquire different values (Stehlíková, 1998). Cluster dendrogram were performed in the free software for scientific data analysis PAST 2.17. We used correlation analysis in the program STATISTICA 1.10 to determine the dependence between individual characters.

Results and discussion

The researched morphological diversity among pepper populations is helpful for breeding programs aimed in selecting superior genotypes. Local or introduced pepper populations is included in broader genetic analyses and considered as a source of new genetic variability used for the development of inbred lines in breeding program.

The evaluation of plant genetic resources has been considered of prime importance, especially in those species having economic importance (Dibuz et al., 1998; Grygorieva et al., 2014, 2017, 2018a, b; Monka et al., 2014; Lima et al., 2017; Ivanišová et al., 2017; Vinogradova et al., 2017; Fatrcová-Šramková et al., 2019; Brindza et al., 2019).

The weight of fruits of chilli pepper cultivars is presented in Table 2. In the evaluated collection was determined the average weight of fruits in the range from 0.318 g (Aji Charapita) to 25.94 g (Trinidad Hot Cherry). For the complete, we determined the weight of fruits up to 10 g for 18 cultivars, from 10.1 to 20 g for 7 cultivars, and the weight of fruits above 20 g for 3 cultivars. The values of the coefficient of variation were in the interval 9.166–42,020%, which documented low up to high degree of variability of the character within the collection.

In agreement with our description, similar fruit weight was found by Bianchi et al., (2020) for *C. chinense* from four Brazilian region and one from Peru (1.04–18.61 g), for cultivated species from West Africa *C. annuum* (5.14 ±0.42–20.97 ±2.69) and *C. frutescens* (1.83 ±0.25) by Olatunji and Afolayan (2019). Jarret and Berke (2008) characterized *C. chinense* from USDA/ARS *Capsicum* germplasm collection and determined mean fruit weight in a range of 0.18 to 22.7 g.

Table 2 presents the weights of peduncle, which were determined as the average values in the range from 0.017 g (Black Prince) to 0.410 g (Trinidad Hot Cherry). For the complete, we determined the weight of peduncles to 0.10 g for 16 cultivars, from 0.10 to 0.20 g for 8 cultivars, and the peduncle weights above 0.20 g and up for 4 cultivars. The values of coefficients of variation document a medium up to high degree of variability.

Table 2 Statistical characteristics of variability of fruits in the collection of chilli pepper genotypes

Cultivars	Fruit weight (g)				Peduncle weight (g)			
	min	max	$\bar{x}$	V (%)	min	max	$\bar{x}$	V (%)
Habanero Red	7.95	11.95	9,76	17.21	0.07	0.17	0.12	34.48
Peter´S Orange	12.65	17.81	14.66	15.40	0.27	0.41	0.33	19.24
Tr. Hot Cherry	21.48	29.98	25.94	12.88	0.04	0.78	0.41	68.96
Kilian	1.43	3.51	2.32	32.38	0.01	0.06	0.02	74.06
Tabasco	1.02	2.23	1.79	27.28	0.11	0.30	0.18	42.53
Escabeche	4.86	8.05	7.05	18.63	0.03	0.06	0.04	24.69
Pepper. Greek	4.71	11.36	6.53	42.02	0.03	0.13	0.09	38.69
Fidalgo Roxa	3.39	5.30	3.89	20.80	0.01	0.14	0.05	96.55
Jalapeno	11.43	20.92	15.67	22.09	0.09	0.13	0.12	15.00
Fatali Red	5.69	7.32	6.66	12.39	0.06	0.07	0.07	3.89
Black Kobra	3.08	4.70	3.73	18.97	0.02	0.07	0.05	41.53
Aji Rojo	16.71	27.87	23.18	19.48	0.23	0.42	0.32	22.93
Carolina Reaper	5.61	8.25	6.67	16.39	0.04	0.06	0.04	19.44
Tr. Scorp. Peach	4.95	8.52	6.74	18.90	0.02	0.04	0.03	22.02
Aji Charapita	0.26	0.44	0.32	22.49	0.01	0.14	0.05	100.54
Bishops Cr. Red	15.90	26.87	22.60	20.11	0.07	0.16	0.10	38.74
Black Prince	0.62	1.02	0.87	17.92	0.01	0.02	0.01	18.10
El Oro De Ecuador	3.93	8.08	6.08	25.66	0.07	0.17	0.12	33.54
Chocho	9.52	22.08	14.29	37.17	0.27	0.45	0.38	20.44
Habanero Peach	11.14	16.83	14.37	17.66	0.04	0.15	0.11	37.69
Medusa	1.72	2.28	2.10	10.67	0.07	0.08	0.07	7.20
Jolokia White	6.69	8.66	7.75	9.77	0.06	0.08	0.07	17.20
Habanero Chocolate	12.31	19.28	15.39	18.85	0.03	0.08	0.06	28.69
Pimenta De Neyde	3.81	5.55	4.72	15.75	0.03	0.06	0.05	25.17
Habanero Red S.	7.49	13.35	10.50	26.19	0.03	0.10	0.06	41.13
Citron	2.08	2.63	2.30	9.16	0.04	0.07	0.05	21.30
Aji Amarilo	4.08	6.53	5.42	17.49	0.08	0.15	0.11	24.77
Aji F. Sparkly W.	10.20	14.70	12.40	14.27	0.15	0.18	0.17	7.22

Note: n – the number of measurements; min, max – minimal and maximal measured values; $\bar{x}$ – arithmetic mean; V – coefficient of variation (%)

The exocarp weight of the evaluated chilli pepper cultivars is presented in Table 3. It was determined the average exocarp weight in the range from 0.244 g (Aji Charapita) to 24.601 g (Trinidad Hot Cherry). For the complete, we determined the weight of exocarp up to 10 g in 20 cultivars, from 10.1 to 20 g in 5 cultivars and the weight of the exocarp from above 20 g upwards in 3 cultivars. The values of coefficients of variation document a medium up to high degree of variability.

Table 3 Statistical characteristics of variability of exocarp weight and number of seeds per fruit in the collection of chilli pepper genotypes

Cultivar	Number Of Seeds Per Fruit (Pcs)				Number Of Seeds Per Fruit (Pcs)			
	min	max	$\bar{x}$	V (%)	min	max	$\bar{x}$	V (%)
Habanero Red	7.85	11.69	9.62	16.86	35	57	43.80	20.06
Peter´s Orange	12.33	17.41	14.31	15.65	55	86	67.20	18.98
Tr. Hot Cherry	20.50	28.33	24.60	12.67	158	243	185.80	18.34
Kilian	0.99	2.71	1.76	34.60	18	49	35.40	39.07
Tabasco	0.89	2.00	1.58	28.09	20	72	46.60	47.44
Escabeche	3.62	7.19	5.55	23.84	2	52	43.60	27.36
Pepper. Greek	3.99	10.62	5.76	47.73	116	170	139.60	16.75
Fidalgo Roxa	3.16	5.10	3.70	21.72	10	40	23.00	52.17
Jalapeno	11.20	20.57	15.38	22.22	37	106	74.80	33.14
Fatali Red	5.46	7.05	6.44	12.77	12	41	26.00	47.73
Black Kobra	2.70	4.24	3.33	20.22	46	72	60.40	18.68
Aji Rojo	15.49	25.94	21.70	19.31	52	139	100.20	33.81
Carolina Reaper	4.96	7.53	6.02	17.56	14	44	28.00	44.67
Tr. Scorp. Peach	4.81	8.32	6.58	19.01	4	23	14.20	62.07
Aji Charapita	0.22	0.27	0.24	8.49	15	17	16.00	6.25
Bishops Cr. Red	13.49	25.45	20.69	25.31	34	136	81.40	44.72
Black Prince	0.52	0.88	0.76	18.86	15	41	31.80	33.23
El Oro De Ecuador	3.85	7.80	5.94	25.30	6	37	27.20	46.16
Chocho	2.00	15.54	9.14	53.44	75	166	115.40	35.91
Habanero Peach	11.00	16.61	14.22	17.61	43	76	52.60	26.19
Medusa	1.14	1.62	1.43	12.77	38	78	60.80	24.76
Jolokia White	6.60	8.56	7.64	9.88	16	56	28.20	57.85
Habanero Chocolate	12.01	18.88	15.05	18.95	38	76	59.20	23.47
Pimenta De Neyde	2.89	4.73	3.87	18.94	19	64	39.20	42.12
Habanero Red S.	6.90	12.24	9.66	26.39	25	63	37.20	43.36
Citron	1.50	2.00	1.74	13.09	36	47	39.60	10.94
Aji Amarilo	4.00	6.37	5.31	17.30	30	131	91.20	40.64
Aji F. Sparkly White	10.05	14.48	12.17	14.20	25	102	70.60	41.01

Note: n – the number of measurements; min, max – minimal and maximal measured values; $\bar{x}$ – arithmetic mean; V – coefficient of variation (%)

Table 3 presents the number of seeds per fruit. We determined the average number of seeds in the range from 14.2 pieces (Trinidad Scorpion Peach) to 185.8 pieces (Trinidad Hot Cherry). For the complete, we determined the number of seeds up to 50 pieces for 13 cultivars, from 51 to 100 pieces for 9 cultivars and the number of seeds over 100 pieces for 4 cultivars. The values of the coefficients of variation document a medium up to high degree of variability. Comparison our values with other authors showed similar results, e.g. by Zhigila et al. (2013) seeds per fruit were determined in five varieties *C. annuum* L. in Nigeria (39–44 and 97–122 pcs), Carvalho et al. (2017) studied Brazilian *C. frutescens* L. fruit features and determined seeds below 20 pcs per fruit.

The ovary weight of the evaluated chilli pepper cultivars is presented in Table 4. We determined the average ovary weight in the range from 0.174 g (Aji Charapita) to 4.920 g (Trinidad Hot Cherry). For the complete, we determined the ovary weight up to 1 g for 16 cultivars, from 1.1 to 2 g for 5 cultivars and the ovary weight over 2 g for 7 cultivars. The values of coefficients of variation document a medium up to high degree of variability.

Table 4 presents the seed weight. We determined the average seed weight in the range from 0.046 g (Aji Charapita) to 2.146 g (Chocho). For the complete, we determined the seed weight up to 1 g for 24 cultivars, from 1 to 2 g for 3 cultivars and the seed weight above 2 g for 1 cultivar. The values of coefficients of variation document a medium up to high degree of variability.

Dias et al. (2013) studied weight of 1000 seeds (g) for *C. chinense* (6.57 g), *C. annuum* (5.18 g), *C. baccatum* (5.91 g) and *C. frutescens* (4.29 g) in Brazil. Nsabiyera et al. (2012) characterized weight of 300 seeds *C. annuum* of 10 local (1.5 g) and 27 exotic introduced genotypes (1. g) in Uganda regions. Results from West Africa by Olatunji and Afolayan (2019) showed for two cultivated species *C. annuum* and *C. frutescens* number of seeds per fruit values 18.70 ±2.54–118.50 ±14.91 and 24.10 ±2.20, respectively.

For a more complex assessment of the issue was determined the linear dependence between the weight of fruit and weight of exocarp and other traits. In both evaluated variants was determined a positive statistically high dependence between fruit weight and exocarp weight, with one exception. A similar dependence was also found between exocarp weight and ovary weight, with few exceptions. The correlation linear dependence between some features of the evaluated chilli pepper cultivars is presented in Table 5.

Table 4 Statistical characteristics of variability of ovary weight and seed weight in the collection of chilli pepper genotypes

Cultivar	Ovary weight (g)				Seed weight (g)			
	min	max	$\bar{x}$	V (%)	min	max	$\bar{x}$	V (%)
Habanero Red	0.80	1.23	0.99	17.65	0.27	0.42	0.36	16.78
Peter´s Orange	1.12	1.68	1.32	17.44	0.55	0.85	0.67	18.75
Tr. Hot Cherry	4.15	5.77	4.92	14.15	1.60	1.91	1.75	8.61
Kilian	0.31	0.58	0.45	25.13	0.15	0.38	0.25	39.30
Tabasco	0.28	0.84	0.62	37.34	0.13	0.57	0.33	52.16
Escabeche	0.50	0.81	0.66	18.65	0.19	0.40	0.33	25.51
Pepper. Greek	1.61	2.01	1.84	10.05	0.94	1.09	1.02	6.16
Fidalgo Roxa	0.35	0.71	0.49	29.98	0.06	0.28	0.13	62.93
Jalapeno	1.42	3.71	2.21	39.64	0.45	1.45	0.82	46.90
Fatali Red	0.57	3.04	1.69	69.34	0.11	0.36	0.22	42.83
Black Kobra	0.65	1.14	0.90	20.08	0.23	0.61	0.42	34.10
Aji Rojo	1.48	3.83	2.58	33.62	0.58	1.55	1.04	34.30
Carolina Reaper	0.28	0.94	0.55	45.01	0.13	0.54	0.30	49.91
Tr. Scorp. Peach	0.26	1.05	0.57	52.28	0.01	0.58	0.19	119.65
Aji Charapita	0.05	0.60	0.17	137.30	0.04	0.05	0.04	11.90
Bishops Cr. Red	1.56	3.31	2.31	28.11	0.42	1.41	0.85	41.72
Black Prince	0.12	0.30	0.22	27.71	0.07	0.17	0.14	25.46
El Oro De Ecuador	0.21	0.72	0.54	36.65	0.06	0.38	0.27	45.11
Chocho	1.82	3.62	2.74	31.58	0.85	5.74	2.14	95.90
Habanero Peach	1.38	2.71	2.25	22.84	0.43	0.70	0.51	22.12
Medusa	0.23	0.41	0.35	20.42	0.17	0.29	0.24	18.40
Jolokia White	0.72	3.58	1.99	70.45	0.12	0.47	0.23	60.31
Habanero Chocolate	1.71	3.14	2.15	27.23	0.48	0.93	0.75	22.98
Pimenta De Neyde	0.44	0.76	0.61	18.47	0.16	0.32	0.27	23.23
Habanero Red S.	0.53	1.20	0.77	39.48	0.22	0.54	0.32	41.70
Citron	0.54	0.62	0.56	6.18	0.33	0.42	0.36	9.86
Aji Amarilo	0.38	1.24	0.85	36.76	0.24	0.95	0.64	40.06
Aji F. Sparkly White	1.21	2.16	1.54	24.11	0.21	0.81	0.53	40.57

Note: n – the number of measurements; min, max – minimal and maximal measured values; $\bar{x}$ – arithmetic mean; V – coefficient of variation (%).

Table 5 Correlation linear dependence between fruit traits

Cultivar	Fruit weight (g)					Exocarp weight (g)				
	WEX	WOV	WSE	THF	NSF	WOV	WSE	THF	NSF	LEF
Habanero Red	0.999	0.548*	0.461	0.110	0.019	0.562	0.477	0.099	0.039	0.953**
Peter´S Orange	0.999	0.553*	0.397	0.734*	0.472	0.546	0.386	0.726*	0.458	0.652*
Tr. Hot Cherry	0.999	0.805**	0.934**	0.555	0.508	0.811**	0.943**	0.544	0.523	0.787*
Kilian	0.996**	0.750*	0.637*	0.912**	0.651*	0.723*	0.587	0.910**	0.607*	0.584
Tabasco	0.989**	-0.012	0.203	-0.564	0.068	-0.096	0.157	-0.595	0.007	0.488
Escabeche	0.927**	0.921	0.864**	0.783*	0.842**	0.977**	0.832**	0.835**	0.781*	0.735*
Pepper. Greek	0.995**	0.401	-0.083	0.952**	-0.197	0.450	-0.009	0.962**	-0.118	-0.834
Fidalgo Roxa	0.999**	0.746*	0.874**	0.575	0.663*	0.714*	0.855**	0.580	0.628	-0.618
Jalapeno	0.999	0.956**	0.879**	0.044	0.947**	0.956**	0.880**	0.049	0.946**	-0.271
Fatali Red	0.999	0.775*	0.783*	0.662*	0.759*	0.793*	0.795*	0.668	0.777*	0.947**
Black Kobra	0.999**	0.730*	-0.164	0.929**	0.515	0.753*	-0.134	0.927**	0.548	0.510
Aji Rojo	0.996**	0.924**	0.422	-0.468	0.619*	0.933**	0.405	-0.444	0.597	0.949**
Carolina Reaper	0.997**	0.974**	0.923**	0.361	0.869**	0.978**	0.918**	0.384	0.847**	0.079
Tr. Scorp. Peach	0.999	0.435	-0.766	0.353	-0.553	0.428	-0.769*	0.347	-0.552	0.728*
Aji Charapita	0.020	-0.079	0.249	-0.063	-0.489	0.374	-0.924**	0.066	0.241	-0.358
Bishops Cr. Red	0.958**	0.649*	0.545	0.558	0.616*	0.798*	0.710*	0.683	0.748*	0.681*
Black Prince	0.996**	0.630*	0.865**	0.918**	0.691*	0.693	0.896**	0.894**	0.746*	0.757*
El Oro De Ecuador	0.999**	0.761*	0.775*	0.800**	0.894**	0.766*	0.787*	0.779*	0.905**	0.854**
Chocho	-0.339	0.520	0.365	0.889**	0.258	0.331	0.741*	-0.537	0.546	0.216
Habanero Peach	0.999	0.093	0.726*	0.585	0.733*	0.095	0.713*	0.601	0.720*	0.459
Medusa	0.977**	0.931**	0.813**	0.106	0.794*	0.841**	0.716*	0.290	0.683*	0.344
Jolokia White	0.999	0.662*	0.300	-0.097	0.323	0.658	0.289	-0.097	0.313	0.313

Continuation of table 5

Cultivar	Fruit weight (g)					Exocarp weight (g)				
	WEX	WOV	WSE	THF	NSF	WOV	WSE	THF	NSF	LEF
Habanero Chocolate	0.999	0.859**	0.529	0.902**	0.526	0.857**	0.522	0.900**	0.520	-0.309
Pimenta De Neyde	0.983**	0.421	0.097	0.643	-0.229	0.250	-0.082	0.694	-0.346	0.597
Habanero Red S.	0.996**	0.638*	0.655	-0.133	0.606	0.564	0.590	-0.080	0.535	0.511
Citron	0.759*	0.683*	0.876**	-0.910**	0.874**	0.181	0.389	-0.789*	0.580	0.694*
Aji Amarilo	0.999	0.981**	0.958**	0.497	0.891**	0.981**	0.959**	0.506	0.893**	0.779*
Aji F. Sparkly W.	0.999	0.738*	0.693*	-0.043	0.694	0.754*	0.701*	-0.023	0.697*	0.798*

Note: WEX – weight of exocarp; WOV – weight of ovary; WSE – weight of seed; THF – thickness of flesh; NSF – number seeds per fruit; LEF – length of fruit; **– correlation is significant at the 0.01 level; * – correlation is significant at the 0.05 level

Shape and colour of fruits

In Figure 1 we present the shape and colour of the fruits of the evaluated chilli pepper cultivars. The comparison shows a significant variability of both traits and extreme differences between cultivars, which are also important distinguishing features of individual varieties. The shape of fruits is characterized as campanulate, blocky, ovoid, round, almost round, linear, triangular, rectangular, elongate etc. Fruit shapes at peduncle attachment were described as lobate, cordate or truncate, fruit shapes at blossom end were described as sunken or pointed (Figure 1). The colour of the fruit can also be misleading, as each variety has a different length of vegetative period and sometimes varieties with a longer vegetative period do not have enough time to reach full technological maturity. Mature fruit colours included red, orange, yellow, brown (chocolate), and cream. In many cases the colouring of fruits with the presence of natural dyes in the flesh also determines the fruit antioxidant activity (Rodríguez-Maturino et al., 2011; Medina-Juárez et al., 2012).

Figure 1 Shape and colour of fruits of evaluated cultivars of chilli pepper (Photo: V. Horčinová Sedláčková, A. Oravec, 2018)

Figure 2 presents a longitudinal section of the fruits of chilli pepper cultivars, in which the number of seeds per fruit was evaluated. The comparison shows significant variability in the given trait and large differences between cultivars. The number of seeds per fruit has a significant effect on the multiplications of individual cultivars.

Figure 3 presents a cross-section of the fruits of the evaluated cultivars of chilli pepper, in which we evaluated the width of the fruit and the thickness of the flesh. The comparison shows a relatively high variability in both features. The fruit width and the flesh thickness determine the total weight of the fruit and thus the amount of dry matter in the fruit.

Figure 2 Comparison of evaluated cultivars of chilli pepper in longitudinal section of fruits (Photo: V. Horčinová Sedláčková, A. Oravec, 2018)

Figure 3 Comparison of the evaluated cultivars of chilli pepper in the cross section of the fruit (Photo: V. Horčinová Sedláčková, A. Oravec, 2018)

Examine of variability for fruit-related traits to select the suitable genetic material has been also shown in other *Capsicum* spp. studies (Carvalho et al., 2017; Bicikliski et al., 2018; Olatunji and Afolayan, 2019; Bianchi et al., 2020). Genetic diversity of characters of chilli peppers based on phenotypic and molecular descriptors have been studied by several authors (Bozokalfa et al., 2009; Dias et al., 2013; Moreira et al., 2018). However, most of them focused in morphological descriptors of fruit and fewer ones have examined a broad range

of morpho-agronomic descriptors including both qualitative and quantitative traits (Orobiyi et al., 2018; Andrade et al., 2020; Bianchi et al., 2020).

The documentation obtained from the evaluation of chilli pepper fruits confirms the significant variability of qualitative as well as quantitative features. Results from the correlation analysis (Table 5) and the cluster analysis (Figure 4) confirm the statistically significant differences between the cultivars.

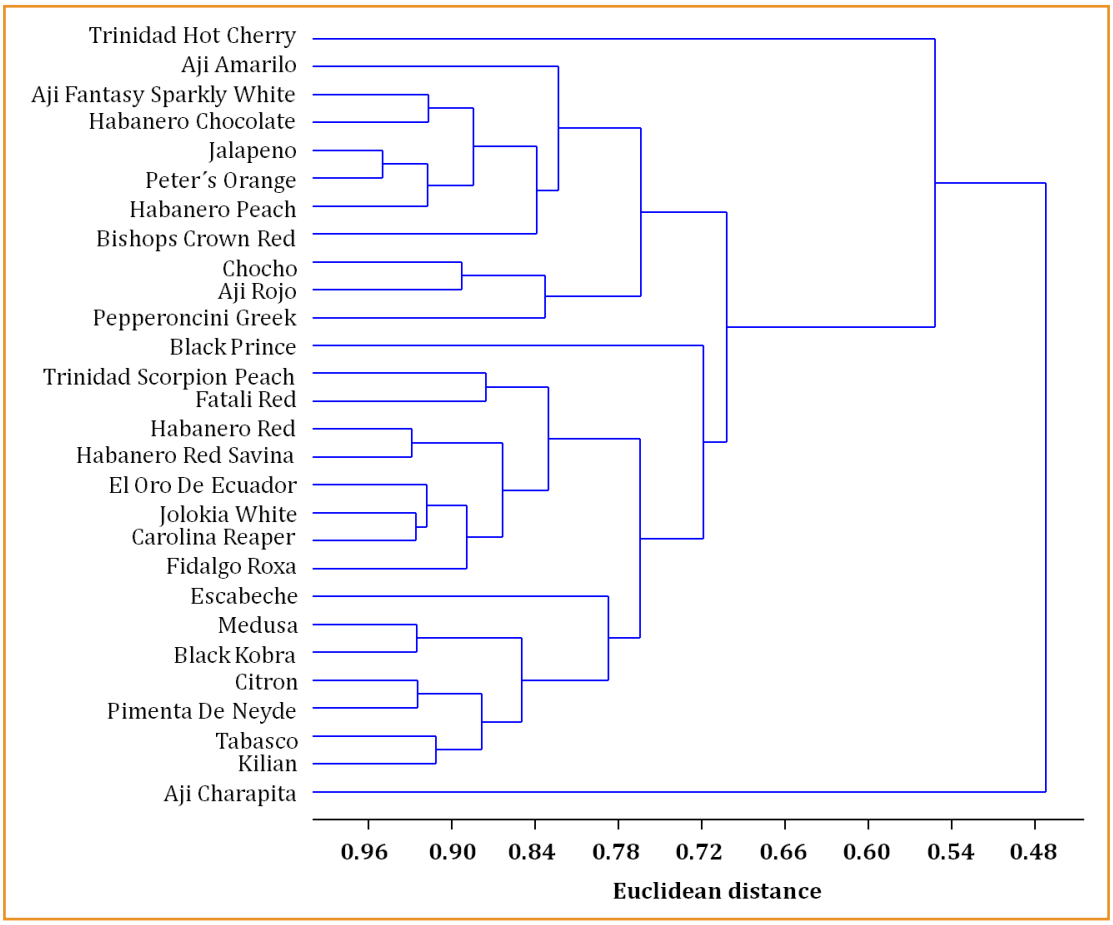

Figure 4 Dendrogram of 28 cultivars of chilli peppers based on morphometric characteristics of fruits

The information gathered from cluster analysis are useful to identify genetic variability among plants. Clustering of genotypes signifies close genetic affinity between/among species and can be used in resolving taxonomic complexities (Olatunji and Afolayan, 2019).

The result from the cluster analysis indicated that there was considerable variability among the cultivars of three *Capsicum* species which allowed them to be separated into distinct groups (Figure 4). The cluster analysis from 6 quantitative morphological traits examined that *Capsicum* spp. cultivars were divided into six clusters. Cluster II, IV and cluster V contained the

largest number of genotypes. Cluster I (Trinidad Hot Cherry with the highest mean values) and cluster III (Black Prince) and Cluster VI (Aji Charapita with the lowest mean values) contained only cultivar, which differ from other genotypes of collection by all parameters.

Many other authors have used cluster analysis to study morphological characters of chilli peppers (Nsabiyera et al., 2013; Orobiyi et al., 2018; Olatunji and Afolayan, 2019; Andrade et al., 2020). Based on the distance between species of different clusters, contrasting parents may be identified and used in hybridization program for generating wider variability for selection and crop improvement (Sarobo, 2019).

Conclusions

Our results clearly confirmed statistically significant differences between the evaluated pepper cultivars in all examined traits. The results contribute to the expansion of theoretical and practical knowledge about some basic morphometric and production characteristics of chilli pepper fruits in the conditions of Slovakia. Many tested cultivars of chilli peppers are promising for their cultivation, distribution as ornamental plants and processing into food products, especially for small, young and family farms, which can significantly contribute to their socio-economic development and environmental improvement.

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