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Amephana dalmatica (Rebel, 1919) (Lepidoptera: Noctuidae) FOUND IN THE NATURE PARK "GOLIJA" − A NEW SPECIES IN THE FAUNA OF THE REPUBLIC OF SERBIA

ABSTRACT: Inventorying the diversity of entomofauna is the first stage in a consistent approach to its conservation. The diversity of Lepidoptera in Serbia, although impressive in terms of the number of inventoried species, is far from complete. This paper describes the first finding of the species *Amephana dalmatica* (Rebel, 1919) in the Republic of Serbia. Moreover, for the first time, a species from the genus *Amephana* Hampson, 1906 is recorded in the fauna of Lepidoptera of Serbia. *A. dalmatica* is a Ponto-Mediterranean species. It occurs in the Southern Europe (western and southern parts of the Balkans, north to Dalmatia), as well as in the western and southwestern parts of European Turkey. Here are presented the basic anatomical-morphological and taxonomic characteristics of the species *A. dalmatica*, its distribution and flight period. The three out of the eight research objectives on the Lepidoptera fauna in the Republic of Serbia were implemented in this research, presented in the "General Plan of Lepidopterological Research for the Achievement of Defined Goals in the Republic of Serbia with a Focus on Climate Change, Allochthonous and Migratory Species": Golija is one of the highest mountains in the Republic of Serbia, it is a protected area and *A. dalmatica* is a rare Lepidoptera species in the Republic of Serbia, which speaks of the importance of this study and the research area.

KEYWORDS: *Amephana dalmatica*, diversity, entomofauna, Lepidoptera, Noctuidae, Nature Park "Golija"

INTRODUCTION

The genus *Amephana* Hampson, 1906 consists of two distinct phyletic lines which are interpreted as subgenera (*Amephana* and *Trigonephra*). It contains only four species, three of them from the southern parts of Europe. Within the

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subgenus *Amephana*, the species *anarrhini* (Duponchel, [1840]) is present in Europe. Within the subgenus *Trigonephra* Berio, 1980, the species *aurita* (Fabricius, 1787) and *dalmatica* (Rebel, 1919) are known in Europe. Beshkov (2000), Hacker (1989), Karsholt & Razowski (1996), Kornosor & Lödl (1990), Leraut (2019), Ronkay & Ronkay (1995), gave the data and records of *A. dalmatica* in Croatia, Montenegro, Greece, Albania, Macedonia, Bulgaria and western and southwestern parts of Turkey. Beshkov & Nahirnić (2016) announced that species *A. dalmatica* might exist in southern Serbia. In their research, Fibiger & Sarto and Monteys (2003) point out the differences between the sister species *A. dalmatica* and *A. aurita* (Fabricius, 1787) and confirm the finding of *A. aurita* in Spain (Balearic Islands).

In the Republic of Serbia, *A. dalmatica* was not recorded in the previous studies (Vasić, 2002; Zečević, 1996; Zečević, 2002). *A. dalmatica* was also not registered in the more contemporary long-term research (Stojanović, 2012; Stojanović & Šumarac, 2020; Stojanović & Šekler, 2022). Furthermore, *A. dalmatica* is neither presented in the comprehensive study on diversity of noctuid moths in Serbia (Stojanović & Ćurčić, 2011), nor in the Red List of Noctuidae of Serbia (Stojanović et al., 2013).

A. dalmatica is a Ponto-Mediterranean species. A xerophilous species is typical for Mediterranean maquis and sclerophyllous forests of the lower and medium altitude zones of the Balkan Peninsula and Asia Minor, absent or very rare at higher altitudes. It is local but numerous and strongly attracted to light. The flight period is the end of May and June. The early stages of development and the host plant are unknown (Ronkay & Ronkay 1995).

MATERIAL AND METHODS

The research was carried out in the Nature Park "Golija", in the location of the Monastery Gradac (UTM square DP 60; Figures 1 and 2) during June, 2022. The altitude of the locality is 550 m a.s.l.

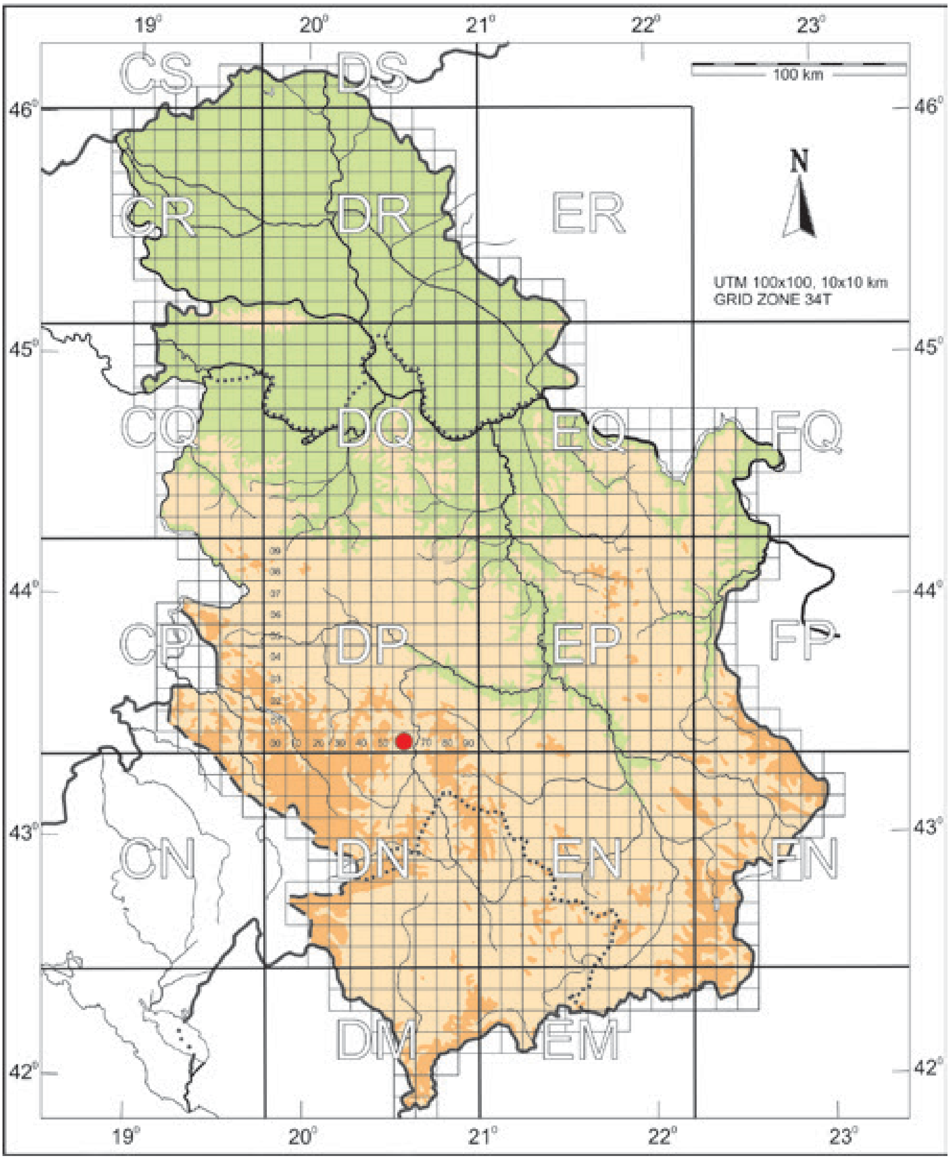


Figure 1. Map with locality of finding of species *Amephana dalmatica* in Serbia with UTM grids

Figure 2. Map with locality of finding of species *Amephana dalmatica* in the “Golija” Nature Park with UTM grids

The samples were collected by the author with a light source (250 and 400 W Philips Ml mercury lamps) behind which a cotton cloth was placed.

The collected adults were prepared, labeled, determined, photographed and stored as dry specimens in entomological boxes in the author’s private collection. The chitinous armatures of their genital apparatus were dissected, identified, photographed and permanent microscopic preparations were made from them, stored in the collection of microscopic preparations, located in the author’s private collection. Identification was carried out according to Ronkay & Ronkay (1995) and Fibiger & Sarto and Monteys (2003).

Photographs of the site were taken using a Canon EOS 5D Mark III digital camera, equipped with a CANON EF 50 mm, 1:1.8L lens. Photographs of insect specimens and genital armature were taken with the same digital camera, equipped with a CANON macro lens EF 100 mm, 1:2.8L. The photographs of the prepared specimens given below were taken in the laboratory of the Institute for Lowland Forestry and the Environment of the University of Novi Sad.

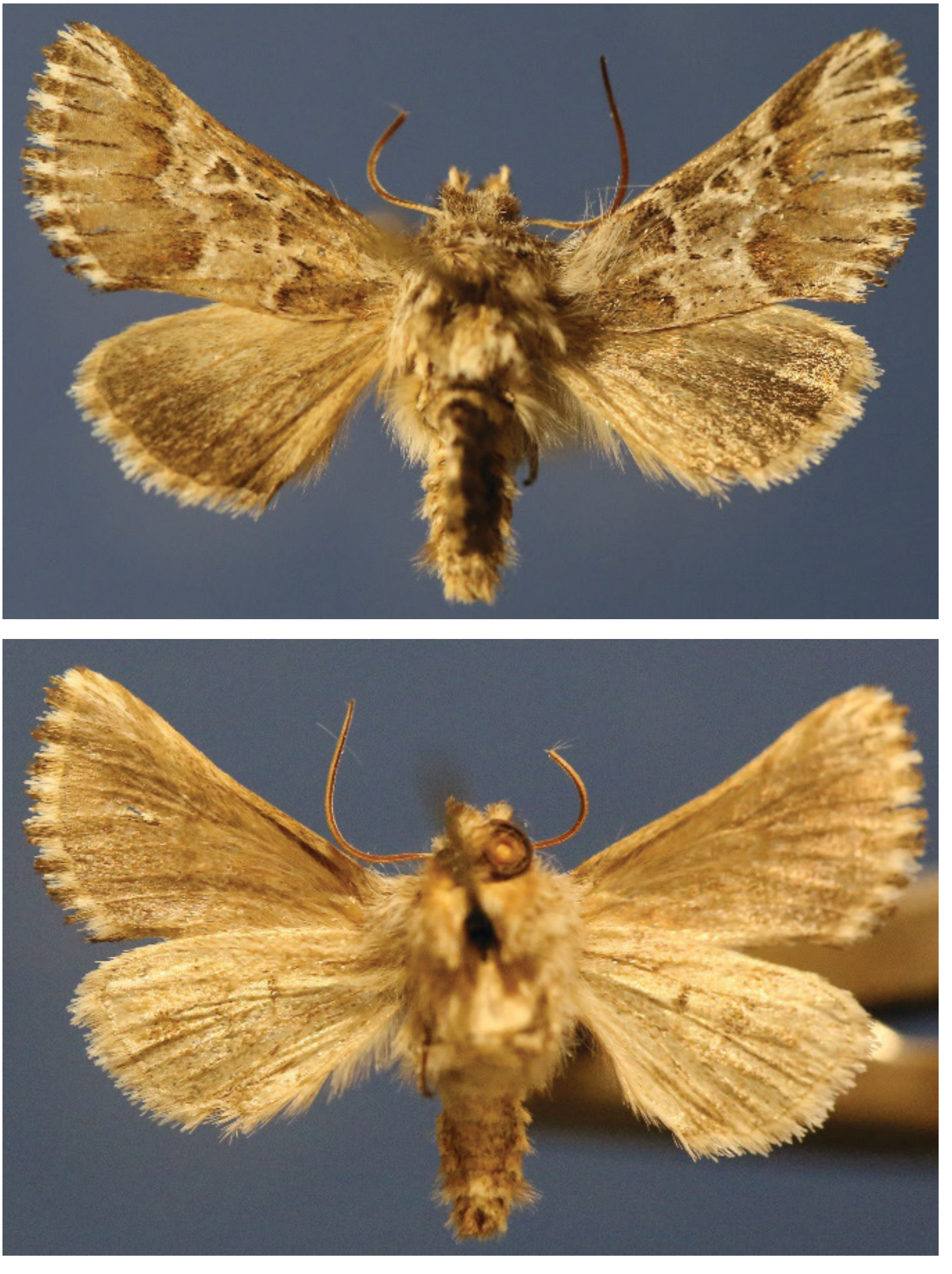
RESULTS AND DISCUSSION

Two males of *A. dalmatica* were found on 23rd of June, 2022. The species was discovered in the Nature Park "Golija", in the locality the Monastery Gradac (Figure 3), that is under the protection of 3rd level regime.

Figure 3. Locality of finding, Monastery Gradac (photo: Stojanović, DV)

Wingspan of *A. dalmatica* is 19–27 mm. It is highly similar to *A. aurita* but distinguished by the antemedial line which is much less sinuous, without deeper angles below cell and at inner margin. Therefore, the median zone is broader and less constricted at its lower third (Ronkay & Ronkay, 1995). Habitus of male is shown on Figures 4–5.

Male genital armature (Figures 6–7): The chitinous armatures of the male genital apparatus differ from the sister species *A. aurita* by the clasper, which is spatulate at the tip. Harpa is thickened at base, less pointed at apex. Juxta is convex at top. Aedoeagus is large, with thickened cornuti. Vesica is bent at an angle of 120 degrees, like an Aboriginal boomerang.



Figures 4–5. *Amephana dalmatica*, habitus of male; 4 – with spread wings (dorsal view); 5 – with spread wings (ventral view) (photo: Stojanović, DV)

Figures 6–7. Amephana dalmatica male genital apparatus; 6 – male genital armature; 7 – aedoeagus (photo: Stojanović, DV)

There are eight objectives of Lepidoptera fauna research in the Republic of Serbia presented in the "General plan of lepidopterological research for the achievement of defined objectives in the Republic of Serbia with reference to climate changes, alien and migratory species" (Stojanović & Konjević, 2023). Three out of them were realized in this research: Golija belongs to the highest mountains in the Republic of Serbia, it is a protected area and *A. dalmatica* is a rare species of Lepidoptera in the Republic of Serbia, which speaks of the importance of this study and the researched area.

CONCLUSION

The discovery of *A. dalmatica* in the "Golija" Nature Park (southwestern part of the Republic of Serbia) represents the first finding of this species in the Republic of Serbia.

Public Company "JP Srbijašume" manages the sustainable protection of natural values in the third level of protection of the "Golija" Nature Park, where *A. dalmatica* was found. Due to the importance of the discovery, it is necessary to establish monitoring, population control and conservation measures for the very rare species, *A. dalmatica* in the "Golija" Nature Park.

It is necessary to determine the probability of endangerment and undertake appropriate, practical conservation measures aimed to protect this rare species as well as its habitat. It is recommended to establish and present *A. dalmatica* as a national treasure of the Republic of Serbia, a tourist attraction and a kind of brand of the "Golija" Nature Park.

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Amephana dalmatica (Rebel, 1919) (Lepidoptera: Noctuidae) ПРОНАЂЕНА У ПАРКУ ПРИРОДЕ „ГОЛИЈА" − НОВА ВРСТА У ФАУНИ СРБИЈЕ

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РЕЗИМЕ: Инвентаризација диверзитета ентомофауне је прва фаза у конзистентном приступу његовог очувања. Диверзитет Lepidoptera у Србији, иако импресиван по броју инвентарисаних врста, далеко је од потпуне истражености. У раду је описан први налаз врсте *Amephana dalmatica* (Rebel, 1919) у Републици Србији. Штавише, први пут је забележена врста из рода *Amephana* у фауни Lepidoptera Србије. *A. dalmatica* је понто-медитеранска врста. Јавља се у јужној Европи (западни и јужни делови Балкана, северно до Далмације), као и у западним и југозападним деловима европске Турске. Овде су представљени основни дијагностички карактери, дистрибуција и период лета ове врсте. Од осам циљева истраживања фауне Lepidoptera у Републици Србији који су представљени у „Општем плану лепидоптеролошких истраживања за остваривање дефинисаних циљева у Републици Србији са освртом на климатске промене, алохтоне и мигранторне врсте", у овом истраживању реализована су три: Голија спада у највише планине у Републици Србији, у питању је заштићено подручје, а *A. dalmatica* је ретка врста Lepidoptera у Републици Србији, што говори о значају ове студије и истраживаног подручја.

КЉУЧНЕ РЕЧИ: *Amephana dalmatica*, диверзитет, ентомофауна, Lepidoptera, Noctuidae, Парк природе „Голија"

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Thymus praecox subsp. *polytrichus:* ANTIMICROBIAL AND ANTIDIABETIC ACTIVITY OF ETHANOL EXTRACTS

ABSTRACT: *Thymus praecox* is one of the most widespread species in the Euro-Siberian region. This study was conducted to determine the *in vitro* antimicrobial and antidiabetic activity of its plant extracts prepared with 70% and 96% ethanol, with and without pretreatment with *n*-hexane, using the Soxhlet apparatus. The MICs and MBCs ranged from 0.035 to 0.150 mg/mL and from 0.075 to 0.300 mg/mL, respectively. Both 96% ethanol extracts showed the most promising antibacterial activity, especially against *Bacillus cereus*, *Enterobacter cloacae* and *Salmonella* Typhimurium. The pretreated 96% ethanol extract also showed remarkable antimicrobial activity against *Staphylococcus aureus*. Bacteria *B. cereus*, *S. aureus* and *S. typhimurium* were also sensitive to the pretreated 70% ethanol extract, while 70% ethanol was most effective against *S. aureus* and *Escherichia coli*. The MICs and MFCs varied from 0.017 to 0.30 mg/mL and from 0.035 to 0.70 mg/mL, respectively, with *Aspergillus versicolor* and *Trichoderma viride* being the most sensitive. The IC_{50} values ranged from 0.94 to 1.40 mg/mL for α-amylase and from 76.10 to 610.2 μg/mL for α-glucosidase. The 70% ethanol extract, especially with the hexane pretreatment, showed the highest enzyme inhibitory activity in the α-glucosidase assay (76.10 μg/mL), even higher than the control, acarbose/glucobay (20.46 μg/mL). The highest α-amylase inhibitory effect was obtained for the 96% ethanol extract. The results show that the extracts of *T. praecox* subsp. *polytrichus* have significant antimicrobial and antidiabetic potential and can be used for food preservation and phytopharmacy.

KEYWORDS: *Thymus praecox* subsp. *polytrichus,* extracts, antimicrobial activity, antidiabetic activity

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INTRODUCTION

Today's lifestyle, characterised by constant stress, limited mobility and inadequate nutrition, causes oxidative stress and leads to the development of numerous chronic diseases: cardiovascular, neurodegenerative, inflammation, cancer and diabetes (Kangralkar et al., 2010; Chikezie et al., 2015).

Diabetes mellitus (DM) is a metabolic disease characterised by various symptoms: an elevated level of glucose in the blood (hyperglycaemia) and insufficient production or action of insulin produced by the pancreas (Maritim et al., 2003; Moussa, 2008). Diabetics are considered immune compromised patients and are therefore more affected by microbial infectious diseases and have a higher risk of developing serious complications as a result (Uzunović, 2016). On the other hand, many microorganisms become resistant to existing antibiotics over time due to their excessive and inappropriate use. Therefore, it is necessary to search for new effective medicines and herbs that have antimicrobial effects or could be a good source of them.

The genus *Thymus* L., which belongs to the Lamiaceae family, is widespread in the Old World and has been used in traditional medicine for numerous centuries due to its antioxidant, anti-inflammatory, enzyme-inhibitory and antimicrobial properties (Stahl-Biskup, 2002; Jarić et al., 2007; Kindl et al., 2015; Marković et al., 2020; Şener et al., 2021). For instance, *T. praecox* ssp. *polytrichus* (A. Kern. Ex Borbas) Jalas distributed in south-eastern and western Serbia and Kosovo, in meadows, pastures, on rocks, on limestone in the subalpine and alpine zones (Diklić, 1974), has various biological activities and is therefore used in folk medicine (Jarić et al., 2007, Kindl et al., 2015; Marković et al., 2020).

Considering the traditional use of species of the genus *Thymus*, our idea was to obtain different extracts from *T. praecox* ssp. *polytrichu*s using the Soxhlet apparatus and to investigate their antimicrobial and antidiabetic potential *in vitro*, with the ultimate goal of their possible commercialisation in various applications, in medicine, as food additives or for food preservations.

MATERIALS AND METHODS

Plant material

The aerial parts of *T. praecox* subsp. *polytrichus* were collected in July 2022 on Pasjački Vis (mountain Pasjača), Serbia, in full inflorescence and air-dried in the shade. A voucher specimen, no. 16881, was deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade, Serbia (BEOU).

Preparation of plant extracts

Aerial plant parts of *T. praecox* subsp. *polytrichus* were pulverized and 15 g of plant material was extracted using Soxhlet apparatus with 250 mL of *n*-hexane for 1 h. The solvent was removed by rotatory vacuum evaporator (Büchi rotavapor R-114). Then, the plant material was dried and extracted again in Soxhlet with 70% and 96% ethanol for 4 h, filtered with filter paper Whatman no. 1 and the solvent was removed by rotatory vacuum evaporator. The other two samples (15 g of aerial plant parts of each) were extracted without previous treatment with *n*-hexane. Prepared extracts were kept in the fridge at +4 °C.

Evaluation of antimicrobial activity using micro-well dilution assay

The antibacterial and antifungal activity of *T. praecox* subsp. *polytrichus* extracts was analyzed using the microdilution method according to Soković et al. (2009) and Kostić et al. (2017). For this purpose, 4 gram(+) (*Bacillus cereus* (clinical isolate), *Microccocus luteus* ATTC10240, *Staphylococcus aureus* ATCC6538, *Listeria monocytogenes* NCTC 7973), 4gram(-) bacterial strains (*Escherichia coli* ATCC35210, *Pseudomonas aeruginosa* IBRS P001, *Enterobacter cloacae* ATCC 35030, *Salmonella* Typhymurium ATCC 13311) and 8 mycromycetes (*Aspergillus fumigatus* (clinical isolate), *Aspergillus versicolor* ATTC 11730, *Aspergillus ochraceus* ATTC 12066, *Aspergillus niger* ATCC 6275, *Trichoderma viride* IAM 5061, *Penicillium funiculosum* ATCC 36839, *P. ochrochloron* ATCC 9112, *P. verrucosum* var. *cyclopium* (food isolate)) were used. The working solutions of the studied extracts of *T. praecox* subsp. *polytrichus* were prepared in 30% ethanol. Serial dilutions for testing antibacterial/antifungal activity in tryptic soy broth/malt broth were prepared in 96-microtiter plates. Microbial inoculum was added to each well, except the negative control, at a concentration of 1×10^5 CFU/mL to obtain the desired concentrations of the mixture. The microtiter plates containing bacterial inoculum were incubated at 37 °C for 24 hours, while those containing mycotic inoculums were incubated at 28 °C for 72 hours to measure the minimum inhibitory concentrations (MICs). Serial re-inoculations with 10 µL / 2µL of bacterial/fungal inoculum in 100 µL of appropriate sterile broth were performed to estimate the minimum bactericidal/fungicidal concentrations (MBCs/MFCs). The antibiotic ampicillin and the antifungal ketoconazole served as positive controls, and 30% ethanol served as a negative control.

Estimation of enzyme-inhibitory activity in vitro

The inhibitory activity of *α*-amylase was estimated according to Apostolidis et al. (2011). The amount of 50 µL of the plant extracts concentrations (25–1,000 µg/mL) were mixed with 50 µL of 0.5 mg/mL *α*-amylase/20 mM phosphate buffer (pH 6.9). The mixture was incubated at 37 °C for 10 min and then 50 µL of 1% starch/20 Mm phosphate buffer was added. After 10 min of incubation at 37 °C, 100 µL of coloring reagent (1% DNS in 0.4 M NaOH/ddH_2O of 12% $KNaC_4H_4O_6 \times 4H_2O$/dd$H_2O$) was added and incubated at 100 °C for 15 min. Finally, the total volume was made up to 1.25 mL with ddH_2O, and the absorbance was measured at 405 nm. The *α*-glucosidase inhibitory activity was estimated as described by Wan et al. (2013). Different concentrations of the sample in a final volume of 120 µL were pre-incubated with 20 µL of a 0.6 U/mL *α*-glucosidase solution in 0.1 M phosphate buffer (pH 6.8), in 96-well microtiter plates at 37 ºC for 15 min. Twenty µL of a 3.5 mM *p*-nitrophenyl-*α*-D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) was added to initiate the reaction. After 20 min of incubation at 37 ºC, the reaction was stopped by the adding 0.2 M sodium carbonate (Na_2CO_3) and the absorbance was measured at 405 nm. The results obtained in both tests were expressed as IC_{50} values (mg/mL). Glucobay, an official medicine, was used as a positive control.

RESULTS AND DISCUSSION

Antimicrobial activity of *Thymus praecox* subsp. *polytrichus* extracts

The results of the antibacterial activity of the ethanol extracts of *T. praecox* subsp. *polytrichus* are presented in Table 1. The examined extracts were active against the gram(+) and gram(-) bacteria tested, even more than the commercially used antibiotics, streptomycin/ampicillin. The MICs and MBCs were ranged from 0.035 to 0.150 mg/mL and from 0.075 to 0.300 mg/mL, respectively. The 96% ethanol extracts were most active against *B. cereus*, *E. cloacae* and *S. typhimurium*. Pretreated 96% ethanol extracts exhibited also notable antimicrobial effects against *S. aureus*. Bayoub et al. (2010) previously proved susceptibility of *L. monocytogenes* to *Thymus* sp. ethanol extracts. Moreover, *T. serpyllum* and *T. vulgaris* ethanol extracts affected growth of other pathogenic bacterial strains such as *S. aureus*, *E. cloacae*, *E. coli* and *Acinetobacter baumannii*. Lauk et al. (2015) also reported antimicrobial effects of methanol and hexane extracts of *Thymus spp*. against 17 pathogenic bacteria. The *T. quinquecostatus* ethyl acetate fraction was active against *Kocuria rhizophila* and *S. epidermidis* (Hyun et al., 2014), while the acetone extract of *T. praecox* subsp. *grossheimii* var. *grossheimi* was most potent against *Bacillus cereus* (Ozkan et al., 2016). The methanol extract of *T. praecox* subsp. *skorpilii* var *skorpilii* also showed strong antibacterial effects against the *S. epidermidis* strain, which was explained by high phenolic content (Taşkin et al., 2019). *Thymus praecox* showed antibacterial activity, especially against *S. aureus*, which supports the results presented herein (Şener et al., 2021). Petrović et al. (2016b) found the hexane/ethanol extract less effective against examined bacterial strains than essential oil obtained by supercritical extraction due to lower amounts of thymol.

The examined extracts showed antifungal activity against pathogen fungal strains tested, which was slightly lower than the antibacterial activity, but still higher than the positive control, bifonazole/ketoconazole (Table 2). The MICs and MFCs varied from 0.017 to 0.3 mg/mL and from 0.035 to 0.7 mg/mL, respectively. *A. versicolor* and *T. viride* were the most affected by examined extracts among fungal strains tested. Centeno et al. (2010) previously reported notable antifungal activity of *T. vulgaris* extracts against *A. flavus* and *A. ochraceus*. The antifungal effects of *T. capitatus* extracts against phytopathogenic fungi of *Citrus sinensis* were previously confirmed by Tabti et al. (2014). The promising antifungal effects of *Thymus* species extracts were usually explained by the presence of major oxygenated monoterpenes, particularly thymol and carvacrol, but also to synergism with other constituents represented in lower amounts (Centeno et al., 2010; Petrović et al., 2016a).

Table 1. Antibacterial activity of examined *T. praecox* subsp. *polytrichus* ethanol extracts

	Gram(+) bacteria				Gram(-) bacteria			
	B. cereus	*M. luteus*	*S. aureus*	*L. monocytogens*	*E. coli*	*P. aeruginosa*	*E. cloacae*	*S.* Typhimurium
	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC
Ethanol 70%*	0.075/0.15	0.1/0.15	0.075/0.15	0.15/0.3	0.1/0.15	0.15/0.3	0.1/0.15	0.075/0.15
Ethanol 96%*	0.035/0.075	0.075/0.15	0.035/0.075	0.075/0.15	0.075/0.15	0.15/0.3	0.035/0.075	0.035/0.075
Ethanol 70%	0.075/0.15	0.1/0.15	0.038/0.15	0.2/0.3	0.038/0.15	0.2/0.3	0.075/0.15	0.1/0.15
Ethanol 96%	0.035/0.075	0.075/0.15	0.075/0.15	0.15/0.3	0.075/0.15	0.15/0.3	0.035/0.075	0.035/0.075
Streptomicin	0.1/0.2	0.2/0.3	0.05/0.1	0.2/0.3	0.2/0.3	0.2/0.3	0.3/0.5	0.2/0.3
Ampicillin	0.3/0.4	0.3/0.4	0.3/0.4	0.4/0.5	0.3/0.5	0.8/1.25	0.4/0.8	0.3/0.5

* praetreatment with *n*-hexane

Table 2. Antifungal activity of examined *T. praecox* subsp. *polytrichus* ethanol extracts

	A. fumigatus	*A. versicolor*	*A. ochraceus*	*A. niger*	*T. viride*	*P. funiculosum*	*P. ochrochloron*	*P. verrucosum* var. *cyclopium*
	MIC/MFC	MIC/MFC	MIC/MFC	MIC/MFC	MIC/MFC	MIC/MFC	MIC/MFC	MIC/MFC
Ethanol 70%*	0.15/0.70	0.15/0.30	0.15/0.30	0.15/0.30	0.075/0.15	0.075/0.30	0.3/0.7	0.15/0.3
Ethanol 96%*	0.075/0.15	0.035/0.075	0.075/0.15	0.075/0.15	0.017/0.035	0.035/0.075	0.075/0.15	0.075/0.15
Ethanol 70%	0.15/0.3	0.075/0.15	0.075/0.15	0.15/0.3	0.075/0.15	0.15/0.3	0.15/0.3	0.3/0.7
Ethanol 96%	0.15/0.30	0.035/0.15	0.15/0.30	0.15/0.30	0.075/0.15	0.15/0.30	0.3/0.7	0.3/0.7
Bifonazole	0.15/0.2	0.1/0.2	0.15/0.2	0.15/0.2	0.15/0.2	0.2/0.25	0.2/0.25	0.2/0.3
Ketokonazol	0.2/0.5	0.5/1.0	2.5/3.0	0.2/0.5	2.5/2.5	0.2/0.5	1.5/2.0	1.5/2.0

* praetreatment with *n*-hexane

In vitro antidiabetic activity of *T. praecox* subsp. *polytrichus* ethanol extracts

The α-amylase and α-glucosidase inhibitory activities of investigated ethanol extracts of *T. praecox* subsp. *polytrichus* were determined using acarbose (Glucobay), a clinical drug, as a positive control. As shown in Table 3, the IC_{50} values for α-glucosidase inhibitory activity of examined *Thymus* extracts were in a range from 76.1 to 610.16 µg/mL. The 70% ethanol extract, especially those previously treated with hexane (76.1 µg/mL), showed better activity in inhibiting α-glucosidase. On the other hand, the IC_{50} values for α-amylase inhibitory activity were in the range of 0.941–1.398µg/mL. The results of the α-amylase inhibition test show that pretreatment of the samples with hexane do not contribute to an increase in activity. The 70% ethanol extracts also showed the best enzyme inhibitory activity (0.941 µg/mL), while the 96% ethanol extracts, both pretreated with hexane and untreated with hexane, showed close values (1.352 µg/mL and 1.398 µg/mL, respectively).

Despite the increasing interest in medicinal plants, as potential new sources for the development of drugs with fewer side effects than hypoglycemic agents currently used in the treatment of diabetes mellitus, there are no data in the literature on the antidiabetic effects of extracts from *T. praecox* subsp. *polytrichus*. The potential antidiabetic role of other *Thymus* species through inhibition of α-amylase and α-glucosidase, key enzymes involved in the hydrolysis of carbohydrates and their absorption, has been confirmed earlier by several authors (Hyun et al., 2014; Lauk, 2015; Cam et al., 2017; Ekin et al., 2019). The crude methanol extract and its ethyl acetate fraction from *T. serpyllum* possessed strong antidiabetic activity, which the authors attributed to the presence of various phenolic compounds such as catechin, rutin, chlorogenic and rosmarinic acid, while the α-glucosidase inhibitory activity of the hexane fraction of *T. vulgaris* was positively associated with thymol (Hyun et al., 2014). *Thymus vulgaris* extracts significantly reduced the blood glucose levels in diabetic rabbits, and the authors suggested their use alone or in combination with insulin to manage DM and its associated complications (Mushtaq et al., 2016). Similar to our results, Dessalegn et al. (2019) showed higher α-glucosidase than α-amylase inhibitory activity of *T. shimpery* and *T. vulgaris*. They also demonstrated the influence of solvent polarity on the revealed antidiabetic properties.

Table 3. In vitro α-amylase and α-glucosidase inhibitory effects of examined *T. praecox* subsp. *polytrichus* ethanol extracts.

	α-amylase IC_{50} (µg/mL)	α-glucosidase IC_{50} (µg/mL)
Ethanol 70%*	1.300 ± 0.26	76.10 ± 18.35
Ethanol 96%*	1.398 ± 0.58	610.16 ± 28.28
Ethanol 70%	0.941 ± 0.35	121.26 ± 5.12
Ethanol 96%	1.352 ± 0.68	449.26 ± 22.15
Acarbose/Glucobay	3.40 ± 0.92	20.46 ± 2.91

* pratreatment with *n*-heksane

CONCLUSION

In this study, extracts of *T. praecox* subsp. *polytrichus* were found to have valuable antimicrobial and antidiabetic potential, and could therefore be exploited as a new source of bioactive ingredients in antidiabetic supplements, in products for the treatment of infectious diseases and as food preservatives.

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Thymus praecox subsp. *polytrichus*: АНТИМИКРОБНА И АНТИДИЈАБЕТИЧНА АКТИВНОСТ ЕТАНОЛНИХ ЕКСТРАКАТА

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РЕЗИМЕ: *T. praecox* subsp. *polytrichus* једна је од најраспрострањенијих биљних врста у евро-сибирском региону. У овој студији је тестирана *in vitro* антимикробна и антидијабетична активност екстраката који су припремљени у Soxhlet апарату коришћењем 70% и 96% етанола, са и без предтретмана хексаном. Минималне инхибиторне концентрације (МИК) и минималне бактерицидне концентрације (МБК) биле су у опсегу од 0,035 до 0,150 mg/mL и од 0,075 до 0,300 mg/mL, редоследом. Оба 96% етанолна екстракта су показала значајну антибактеријску активност, посебно на бактерије *Bacillus cereus*, *Enterobacter cloaceae* и *Salmonela* Typhymurium. Екстракт 96% етанола који је претходно третиран хексаном, такође је испољио значајну антибактеријску активност на *Staphylococcus aureus*, *B. cereus*, *S. aureus* и *S.* Typhymurum су такође биле сензитивне на 70% етанолни екстракт (са предтретманом хексаном), док је 70% етанолни екстракт без предтретмана хексаном био ефективнији против *S. aureus* и *Escherichia coli*. Минималне инхибиторне (МИК) и минималне фунгицидне концентрације (МФК) биле су у опсегу од 0,017 до 0,300 mg/mL и од 0,035 до 0,700 mg/mL, редом, при чему су *Aspergillus versicolor* и *Trichoderma viride* показале највећу осетљивост. Вредности инхибиторних концентрација (IC_{50}) су варирале између 0,94 и 1,4 mg/mL у *α*-амилаза тесту и између 76,1 и 610,2 μg/mL у *α*-глукозидазном тесту. Седамдесетопроцентни етанолни екстракти, посебно онај предтретиран хексаном показао је изразито високу ензимску инхибиторну активност у *α*-глукозидазном тесту (76,10 μg/mL), бољу чак и у односу на контролу (glucobay 20,46 μg/mL). Највећи инхибиторни ефекат у *α*-амилаза тесту је показао 96% етанолни екстракт. Добијени резултати показују да етанолни екстракти *T. praecox* subsp. *polytrichus* испо-

љавају значајан антимикробни и антидијабетични потенцијал и могу се користити у фитофармацији и презервацији хране.

КЉУЧНЕ РЕЧИ: *T. praecox* subsp. *polytrichus,* екстракти, антимикробна активност, антидијабетична активност

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CERIUM AND HIGHER PLANTS

ABSTRACT: Rare earth elements (REEs) or *terrae rarae* (TR) are a group of 17 heavy metals which include 15 elements belonging to the lanthanide (Ln) group, as well as scandium and yttrium. REEs are very similar in chemical and physical properties. Lanthanides are considered as rare micro elements, which is not entirely true. Namely, the amount of cerium (Ce) in the Earth's crust is slightly higher than the amount of Cu, Pb or Zn. Except for Eu (+2 and +3) and Ce (+3 and +4), REEs usually form trivalent cations. Studies of individual REEs elements are mostly focused on the effects of Ce and La. Cerium is nowadays used in agriculture, as a microfertilizer. However, it is not essential for higher plants. The effects of Ce on life processes of plants range from stimulation to inhibition, depending on its concentration (hormesis), plant species, the stage of development, method of application and ecological factors. Lower concentrations and amounts of Ce can be beneficial for nutrient uptake, seed germination, photosynthesis, growth, dry matter accumulation and for alleviating different kinds of stress in plants. Cerium decreases oxidative stress and increases antioxidative capacity of plants. Higher concentrations of Ce reduce plant growth. Excessive amounts of Ce have cytotoxic and genotoxic effects. The available literature on the influence of lower concentrations of Ce on life processes in plants suggests positive outcomes. However, further fundamental investigations shall lead to better understanding of the physiological mechanisms of the impact of Ce on plant metabolisms.

KEYWORDS: cerium, ion uptake and interactions, photosynthesis, seed germination, growth, phytotoxicity, stress alleviating

INTRODUCTION

Cerium is one of the rare earth elements which are present in small concentrations in all parts of the biosphere (Turra, 2017). The average abundance of Ce in the Earth's crust is 66 μg/g (Tyler, 2004). In the soils worldwide, it ranges from 13 mg/kg to 273 mg/kg (Ramos et al. 2016) and in various terrestrial plants from 250 ppb to 16,000 ppb in dry matter (Bowen, 1979). Concentration of Ce

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in plant species is different. According to Kabata-Pendias (2000), the Ce concentration in plant species and organs is different. In lichens and *Briophytes* it spans from 600 to 5600, cheatgrass (*Bromus tectorum*) 300, vegetables from 2 to 50, rice straw 174, blueberry tops from 210 to 740 and in pine needles 370 ppb in dry matter. Global production of REEs has increased exponentially in the recent decades. REEs found application in numerous areas of human activity (Kovariková et al., 2019), thus Ce enters the biosphere. REEs are also used in agriculture as a mineral fertilizer or stimulator, primarily in China (Ren et al., 2016; Kastori et al., 2023). Mineral phosphorus fertilizers are characterized by a higher content of Ce (Moreira et al., 2019), as a result of which their application can lead to an increase in its concentration in arable soils. Cerium can enter plants via both root and aboveground parts. The transfer factor (soil-plant) for Ce is low (Mesa-Pérez et al., 2018). This fact, as well as the fact that Ce mainly accumulates in the roots (Liu et al., 2012), indicates that its entry into the food chain is limited, since the aerial parts of plants are most often used in nutrition. The exception are hyperaccumulator plants in which it accumulates in the above-ground organs. Cerium has synergisms and antagonisms with essential and other elements (Ramos et al., 2016). In this way, Ce influences the nutrimental status of plants. Low concentrations of Ce can in some cases act as a biostimulant in seed germination and initial growth of seedlings (Ramirez-Olvera et al., 2018) and have positive effects on photosynthesis.

Treatment with higher concentrations and doses of Ce adversely affects the life processes of plants and thus their growth, development and organic production. The phytotoxic concentration and dose of Ce decrease mitotic index, with increased mitotic aberration (Kotelnikova et al., 2019) and provoke leaf yellowing and necrotic damage and some structural alternations in leaves (Rodrigues at al., 2019). Abiotic and biotic stress conditions can cause an increase in accumulation of reactive oxygen species (ROS) in plants. Cerium is able to suppress accumulation of oxidative species and elevate the level of antioxidant enzymes (Liu et al., 2012; Li et al., 2021). Cerium may help to relieve the inhibition under some kinds of stress: potassium deficiency, cadmium toxicity, high level of UV-B radiation, drought, acid rain and salt stress.

The growing application of REEs and their individual elements in industry, agriculture and medicine will encourage further research in the field of rare earth elements. This is indicated, among other things, by a large number of published research and scientific works in recent decades, especially regarding the application of Ce and La.

Uptake, transport and distribution of cerium in plants

The concentration of Ce in plants varies widely depending on the plant species and environmental conditions. In the dry matter of the aerial part of *Brassica oleracea* var. *capitata*, the average concentration of Ce was 0.028 /µg/g (Bibak et al., 1999), in the leaves of *Agrostis capillaries* 150 /µg/g (Tyler and Olsson 2001), and in the leaves of forest species it ranged from 0.25 /µg/g to 0.55 /µg/g

(Markert and Li, 1991). The accumulation of Ce in plants largely depends on its concentration in the nutrient substrate. There is a significant positive correlation between the concentration of Ce in the shoots (leaves and stems) of barley and its total, water soluble and exchange forms in the soil (Kotelnikova et al., 2020). Cerium can enter plants via both root and aboveground parts. The uptake rate of REEs from soil solution is controlled not only by the plants themselves, but it depends of numerous ecological factors – on their concentration, exchangeable fractions and solubility in the soil, soil organic matter content, presence of other elements etc. (Brioschi et al., 2013). The mechanism of Ce uptake by plants is also insufficiently studied, therefore it is discussed here within the framework of the knowledge gained during the studies of uptake and transport of REEs. From the soil solution, the cell wall of the root cells adsorbs REEs, depending on environmental conditions, via precipitation or complexation. The transport of REEs in the root takes place in two ways: by apoplastic and symplastic pathway, where the role of REEs transporting proteins, endocytosis and ion channels is considered (Wang et al., 2019; Wang et al., 2024). Acropetal transport of mineral substances in vascular plants takes place in the xylem. The transpiration stream through the mass flow generally makes possible the acropetal transport of REEs, whereby the role of root pressure cannot be ruled out either. Acropetal transport of REEs can be influenced by numerous factors. In the xylem vessels of soybean, the majority of REEs are combined with ligands (Ding et al., 2007). In the xylem saps of hydroponically grown *Phytolacca americana,* citric acid facilates the migration of heavy RREs (HREEs) rather than light RREs (LREEs) (Yuan et al., 2017). Some REEs were immobilized during migration in xylem by chemical precipitation by phosphate particles and cell wall absorption. The transfer factor (TF) – the ratio of the element concentration in the aboveground part of plants or plants organs to its concentration in the soil – for certain REEs is typically lower than 1. The value of TF depends on numeorous factors – on the concentration of an element in the soil, the mobility of the ellement, plant species and organs. According to Kotenikova et al. (2020), in barley leaves, the Ce concentration did not exceed 1% of its concentration in soil. The TF (soil- to-plant transfer factor) in rice for Ce is 0.42 and only a small portion of taken up Ce reached the grain (Mesa-Pérez et al., 2018).

The distribution of nutrients in plants is specific for certain elements and plant species. To find out about the distribution of elements in plants is important from scientific and ecological point of view, since their presence in the edible parts of cultivated plants can allow their entrance in the food chain and thus may have, in higher concentrations, adverse health consequences. It can generally be said that distribution of REEs in organs of cultivated plants is as follows: root > leaf > stem > flower > fruit/seed (Wen et al., 2001), root > leaf > husk > grain (Mesa-Pérez et al., 2018). Cerium concentrations in rice were higher in roots than in shoots (Ramirez-Olvera et al., 2018) and it is mainly located in the cell wall of the rice roots (Liu et al., 2012). In contrast, in the plants baring capacity to hyperaccumulate RREs, their concentration in the aboveground parts is usually greater than in the roots (Wang et al., 2024). In microalga *Desmodesmus* *quadricauda*, Ce accumulates exclusively in the chloroplast compartment (Rezanka et al. 2016).

Interaction of cerium with other elements

Elements with similar physical and chemical properties can compete with each other for the same binding site in cell. Cerium similarities to other important essential macronutrients such as Ca, as well as its higher charge density, render this element more available for absorption into plant tissues (Thomas et al., 2014). Trivalency gives lanthanides a much higher charge to volumes ratio and therefore they usually have much higher affinities than Ca^{2+} for the given binding sites. Ions of some lanthanides (La^{+}, Nd^{3+}, Ce^{3+}), with ion radius (Ce^{2+} 0.106 nm Ca^{2+} 0.094 nm) and coordinate number close to the Ca^{2+} ions, might partly be able to replace endogenous Ca of plants or interact positively with calcium in various physiological functions. Lanthanides (Ce^{3+}, La^{3+}) could enter the chloroplast, bind easily to chlorophyll molecule and might replace Mg^{2+} ion and coordinate the porphyrin ring in pheophytin to form lanthanide-chlorophyll (Chl)-complex, Ce-chlorophyll (Rezanka et al, 2016). The influence of Ce on concentrations of elements in plants depends on many factors, e.g. concentration, plant species, organs, features of elements etc. Appropriate concentrations of $Ce(NO_3)_3$ had a positive influence on the mineral element content of the leaves of *Cyclocarya paliurus* seedlings. The maximal increase at 0.20 mmol/L Ce treatment caused the contents of K, P and Cu increase by 105.2%, 74.5% and 133.3% respectively. The contents of Mg, Mn and Fe increased with increasing concentrations of $Ce(NO_3)_3$ from 0 to 100 mmol/L by 93.2%, 29.4% and 133.9% compared to the control (Xie et al., 2015). Cerium nitrate treatment (0 to 1.5 mM) affected differently the concentration of nutrients (K, Mg, Ca, Na, Fe, Mn, Zn, Cu and Mo) in the roots and shoots of rice. The concentration of mentioned elements changed in response to the concentration of Ce (Liu et al., 2012). Adverse effect of 0.5–25 mg/L Ce on the contents of Ca, Ng, K, Cu and Zn in wheat seedlings was reported (Hu et al., 2002). Cerium (25, 50, and 100 µM in the form of $CeCl_3$) did not affect the concentration of macro or micronutrients in rice shoots. However, in roots, the high Ce concentration decreased the concentrations of Ca, Fe, Mn, and Zn, while the Mg concentration increased (Ramirez-Olvera et al., 2018). In the rice, the application of 500 mg/kg of CeO_2 nano particles to the soil increased the concentrations of K and Ca in rice, whereas concentrations of S and Fe decreased (Rico et al., 2013a).

Effect of cerium on seed germination

Cerium can influence seed germination and initial growth of the seedlings. There are opinions that the REEs can have positive effects on seed germination by acting synergically with phytohormones that stimulate germination (Ramirez-Olvera et al., 2018). The germination rate, germination index and vigor index of naturally and artificially aged rice seeds were significantly increased with a maximum effect at 10–20 μg/mL $Ce(NO_3)_3$. It is the result of the treatments of aged seed with $Ce(NO_3)_3$ that enhanced respiratory rate and activities of superoxide dismutase, catalase and peroxidase, and decreased superoxide O^- and malondialdehyde contents (Fashui, 2002). When applying 8 μM Ce and 12 μM $CeCl_3$, germination percentage of rice seeds increased by 36.2%, while with 4 μM Ce, there were no significant differences with respect to the control. Seedlings height and root length increased significantly, by over 100%, upon addition of 4 μM, 8 μM and 12 μM Ce compared to the control (Ramirez-Olvera et al., 2018).

In the literature, there are also many papers in which the favorable effect of Ce application on seed germination has not been confirmed. Cerium treatments did not significantly affect the rate of germination, relative seed germination and germination index (Sobarzo-Bernal et al., 2021). Nevertheless, Ce can also reduce seed germination and seedling growth. At a low soil pH (i.e. pH=4), Ce decreases the germination of radish and tomato seeds, which is associated with higher mobility and availability of Ce in the soil (Thomas et al., 2014). Pre-soaking of *Triticum durum* seeds for 2 h and 4 h with low concentrations (0.01 and 0.1 mM) of light REEs (La, Ce, Pr, Nd and Gd) had no effect on seed germination. Higher concentrations (1 mM and 10 mM) induced significant decrease in germination compared to controls (d'Aquino et al., 2009). Treatments by CeO_2 nanoparticles, in the concentration of 2000 mg/L, did not influence germination of seeds or root length in tomato, canola, radish, and cabbage, whereas lettuce was the only species that exhibited a slight decrease in root length upon Ce exposure (Ma et al., 2010). According to Wang et al. (2012), CeO_2 nanoparticles (0.1–10 mg/L) had no significant effect on seed germination of tomato.

Cerium effects on photosynthesis

The effects of Ce on phytosynthesis were studied in detail. There are publications pointing out that positive effects on photosynthesis are associated to several physiological and biochemical indices, including synthesis and content of photosynthetic pigments, chloroplast development and structure, light absorption, transport and conversion, as well as carboxylation activity of Rubisco. The photosynthetic pigments content is one of the important factors that effect plant growth and development through photosynthesis. Not only the total content of photosynthetic pigments is significant, but also their mutual relationship, the ratio of chlorophyll a/b and a+b/carotenoids (Manios et al., 2003). Literature data indicate that application of the lower concentrations of Ce have positive effects, while higher have negative effects on chlorophyll content and photosynthesis. According to Liu et al. (2012) in rice seedlings, content of chlorophyll a, b, and a + b was increased at 0.05 mM and 0.1 mM $Ce(NO_3)_3$, but decreased at 0.5 mM, 1.0 mM and 1.5 mM Ce. In green alga *Desmodesmus quadricauda*, treatment with 10 μM $CeCl_3$ caused increase of total chlorophyll content and decrease of the ratio of chlorophyll a/b from 2.45 in control plants to 1.21 in Ce-treated plants. The untreated algae contained 14.52 mg of chlorophylls/g of DW, while those treated with Ce contained 19.86 mg/g of DW (Rezanka et al., 2016). According to Chu et al. (1996), $CeCl_3$ could accelerate the synthesis of chlorophyll a in *Spirulina platensis*. In barley, no noticeable changes were found in the content of chlorophyll a and b pigments and their ratio under application of up to 200 mg Ce/kg air-dry soil (Kotelnikova et al., 2020). In spinach grown in pot culture experiments, Ce stimulated chlorophyll formation (Hong et al., 2002). The favorable influence of some REEs elements on the content of chlorophyll is explained by the replacement of Mg in the center of the chlorophyll molecule with the REEs element during its formation (Rezanka et al., 2016) and due to that fact more Mg is available for the formation of new chlorophyll molecules. Chlorophyll and lutein in the chloroplasts are able to form chlorophyll a-Ce, chlorophyll a-Pr and other chlorophyll-REEs complexes. In the fern *Dicpetris dichotoma*, the content of Ce-chlorophyll was 19.40% (Wei et al., 2005). Ramirez-Olvera et al. (2018) reported that the application of 100 µM Ce decreased the photosynthetic pigments chlorophyll a and b content by over 60% in rice. Zicari et al. (2018) also reported a decrease in the content of chlorophylls and carotenoids when higher concentrations of Ce (0.5 and 1 mM) were used to treat in *Lemna minor.* Intracellular localizations of lanthanides (Ne, Ce Gd, La) in *Desmodesmus quadricauda* were different: Nd and Ce were localized in the chloroplasts, while La and Gd were in the cytoplasm (Rezanka et al., 2016). Ren et al. (2007) also reported accumulation of Ce in chloroplast. According to Liu et al. (2012) in the rice leaves, exposition to 1.0 mM Ce resulted in disorderly arranged thylacoids and the chloroplast has gained more rounded instead of ellipsoidal shape.

Cerium solution sprayed onto the leaves at a concentration of 400 mg/L can partially alleviate the photoinhibition and during the midday depression it increases the photochemical efficiency (Fv/Fm) and photosynthetic performance index (PIABS) of *Pseudostellaria heterophylla* (Ma et al., 2022). The optimum concentration of $CeCl_3$ in nutrient solution that enhance photosynthesis in wheat seedlings was 0.2 to 0.5 mg/L, for cucumber from 1 to 5 mg/L, and for sunflower 15 m/L (Chu et al., 1996). Liu et al. (2012) found that higher concentrations of Ce have negative, while lower may have positive effects on photosynthesis in rice seedlings.

The effect of cerium on plant growth

In Chinese agriculture, REEs have been used to improve the nutrition status of cultivated plants for more than three decades (Hu et al., 2004) and have been extended to other countries (Wang et al., 2008). The treatment with Ce ($nCeO_2$, Ce(III) and Ce (IV)) has hormotic effect on growth and depends on many factors – plant species and vegetative stage, the applied dose and means of application, management conditions etc. In rice seedlings, in water culture, the total nodal root length (at 0.05 and 0.1 mM Ce) and shoot height (at 0.1 mM Ce) increased significantly compared with the control whereas at 0.5 mM, 1.0 mM and 1.5 mM Ce they decreased (Liu et al., 2012). Fashui et al. (2002a) found that Ce increases chlorophyll content and photosynthetic rate and stimulates the growth of spinach. Root length and height of rice seedling increased by over 100% upon application of 4,8 µM and 12 µM $CeCl_3$ (Ramirez-Olvera et al., 2018). In *Lemna minor* 0.1 mM Ce increased growth, but 1 mM Ce caused oxidative stress (Zicari et al., 2018). In *Arabidopsis thaliana,* Ce promotes floral initiation and reproductive growth in concentrations of about 0.5 to 10 µmol/L (He and Loh, 2000). Low concentrations of Ce increased the growth of cowpeas, with the maximum enhancement (23.91% for shoot and 67.53% for root length) observed at 17.84 µM Ce (Shyam and Acry, 2012). Cerium nitrate at 1–15 mg/L promoted callus growth and formation of adventitious roots on the stem of *Dioscorea zingiberensis* (Wang et al., 2010).

Cerium can also reduce plant growth. According to Hu et al. (2002) application of Ce can reduce roots length and dry matter mass both of the roots and shoots in wheat (Hu et al., 2002). According to Rodrigues et al. (2019) foliar treatments of soybean with Ce did not affect plant height, number of leaves and pods. Salgado et al. (2020) also did not observe a significant effect on the growth of common bean when applying increasing doses of $Ce(NO_3)_2$.

Higher concentrations of REEs can be phytotoxic. Toxicity effects of REEs include a number of endpoints, such as cytogenetic effects, growth inhibition and organ-specific toxicity (Pagano et al., 2015). The phytotoxicological database of individual REEs is mostly confined to Ce and La. Kotelnikova et al. (2019) found that Ce toxicity on onion evinced in reduced root growth and mitotic index, with increased mitotic aberrations at Ce concentration 200 mg/kg soil. Liman et al. (2019) reported that CeO_2 nanoparticles and microparticles (12.5–100 ppm) had cytotoxic and genotoxic effects in onions for 4 h. The Ce^{3+} caused deleterious effects and cell death when sweet potatoes were exposed to 20–80 mg Ce/L, in nutrient solution (Jiang et al., 2017). In hydroponic culture, Ce exerted significant adverse effects on wheat root elongation (Gong et al., 2019). In greenhouse conditions, foliar application of 200 mg/L and 2,000 mg/L Ce on the leaves of the soybean provoked yellowing and necrotic damage, presented collapsed cells and caused structural alteration to the epidermal cells (Rodrigues et al., 2019). Foliar treatment with 80–300 µM Ce reduced the yield of horseradish (Wang et al., 2017). Certain plant species show different sensitivity to the presence of higher concentrations of Ce. Sunflower and radish are highly sensitive to Ce (Moreira et al., 2019) and maize is less sensitive than mungbean (Diatloff et al., 1995).

Cerium role in alleviating stresses in plants

During vegetation season, plants can be exposed to unfavorable abiotic factors, which adversely affect same physiological and biochemical processes and in this way their growth and development. A number of abiotic, ecological factors can cause oxidative stress in plants, i.e. disturbance in the balance that exists between pro-oxidative processes and the antioxidant system of plants. Abiotic stress factors can lead to the creation of reactive oxygen species (ROS), increase permeability of plasma membrane and decrease activities of antioxidant enzymes (Liu et al., 2009). Pathogenic infection can also cause an increase in the formation of ROS as a part of the plant's defense system (Li et al., 2021). Reactive oxygen species are produced by reduction or activation of O_2 and they are very reactive and cytotoxic in all organisms. Reactive oxygen species like hydrogen peroxide (H_2O_2), single oxygen ($^1O_2$), hydroxyl radical ($^{\cdot}OH$) and superoxide anion ($O_2^{\cdot -}$) cause disturbances in the growth and development of plants, lead to damage of cellular components and peroxidation of membrane lipids (Siddiqui et al. 2019). Numerous authors have shown the protective effect of REEs, especially Ce and La, against oxidative stress by increasing antioxidative capacity (Hong et al. 2017, Cui et al., 2019). In addition, Ce^{3+} elevated the activities of superoxide dismutase (SOD), catalase (CAT), ascorbic acid peroxidase (APx), guaiacol peroxidase (POD) and glutathione reductase (GR) (Hong et al., 2017). Cerium nanoparticles at 62.5 mg $nCeO_2$/L reduce the generation of H_2O_2 in rice roots by 75%. At 125 mg $nCeO_2$/L, the roots showed enhanced lipid peroxidation and electrolyte leakage, while at 500 mg/L the $nCeO_2$ increased H_2O_2 generation in roots (Rico et al., 2013). Positive effects of Ce^{3+} on alleviating stresses are attributed to its capacity to enhance the antioxidant potential of plants. Cerium solutions (400 mg/L) sprayed onto the leaves increase the activities of SOD, POD, and CAT by 22.71%, 31.49% and 69,79% respectively, in the leaves of perennial herb *Pseudostellaria heterophylla* (Ma et al., 2022). According to Salgado et al. (2020), this mechanism is believed to be the conversion of $O_2^{\cdot -}$ to (H_2O_2) by Ce^{3+} and further oxidation of Ce^{3+} to Ce^{4+}. Next, Ce^{4+} could oxidate $O_2^{\cdot -}$ to O_2, while it itself is reduced to Ce^{3+}.

Calcium (Ca^{2+}) is an essential element for plant and is involved in many living processes. Chemical properties of Ce^{3+} are similar to Ca^{2+}, therefore Ce^{2+} could occupy a Ca^{2+} position and bind to different components in plants as "supercalcium" (Ni, 2002). Cerium added to Ca-deficient media in the spinach plants could substitute for Ca and improve spinach growth. Fresh weight, dry weight and chlorophyll content of spinach were increased by 39.9%, 45% and 64% compared to those of plants cultivated in Ca-deficient media (Liu et al., 2008). Cerium improves the absorption and transfer of light and converses efficiency of light energy in spinach chloroplasts under Ca deficiency (Huang et al., 2008; Huang et al., 2008a). In Ca-deficiency media, Ce decreased the permeability of plasma membrane, malondialdehyde and ROS (superoxide radicals, hydrogen peroxide) and increased the antioxidative enzymes such as SOD, CAT, APX, GPX and glutathione content (Liu et al., 2009). Cerium could relieve the inhibition of Ca deprivation on nitrogen metabolism in spinach (Liu et al., 2008).

Treatment of maize shoots with 15 μM $CeCl_3$ relieved inhibition of photosynthesis and growth as well as PSI and PSII injury caused by exposure to potassium (K^+) deficiency, salt stress (80 mM NaCl) and combination of K^+ deficiency and salt stress. Chemical differences between K^+ and Ce^{3+} are large, therefore Ce^+ might not improve photosynthesis by substituting K^+, but might improve photosynthesis by preventing oxidative stress or by activating enzymes under K^+ deficiency (Qu et al., 2013).

Some results suggest that Ce could partly substitute Mg. Soaking of spinach seeds in 15 μM $CeCl_3$ and further plant growth under Mg^{2+} deficiency lead to decreased MDA and ROS and increased activities of the antioxidative defense system – the activity of SOD, CAT, APX, GPX, GR, antioxidants (e.g. carotenoids and glutathione), and improved overall spinach growth (Ze et al., 2009). It was found that $CeCl_3$ promotes chlorophyll synthesis, activities of two key enzymes in CO_2 assimilation (Rubisco carboxylase and Rubisco activase) and expression of *rbcL, rbcS* and *rca*, thus leading to the enhancement of spinach growth under Mg-deficient conditions (Ze et al., 2009a). Under Mg deficiency, 20 μM Ce in culture solution in maize can prevent inhibition of synthesis of photosynthetic pigments, improve light energy absorption and conversion, oxygen evolution and the activity of photo-phosphorylation and its coupling factor Ca^{2+}-ATPase (Zhou et al., 2011). Treatment with 15 μmol/L $CeCl_3$ in spinach grown in Mg deficiency significantly promoted the activity of the key enzymes of nitrogen metabolism (NR, NiR, GDA, GS, urease, GPT, and GOT) (Yin et al., 2009).

Manganese (Mn) is an essential plant micronutrient which has important functions in many metabolic processes. Addition of 20 μM Ce in nutrient solution promoted maize growth through the enhancement of chlorophyll synthesis, the activity of Rubisco and Rubisco activase, and the expression of *rbcL*, *rbcS*, and *rca* under Mn^{2+} deficiency (Gong et al., 2011). Manganese deprivation in maize may disturb photochemical reaction of chloroplasts strongly, which could be improved by addition of 15 μM $CeCl_3$. However, it is not clear whether Ce affected the photochemical reaction of Mn-deprived maize seedlings (Qu et al., 2012).

In the last decades, there have been a vast number of reports on the presence and phytotoxicity of heavy metals, such as lead, cadmium, mercury and chromium. Lead (Pb) stress could significantly inhibit photosynthesis (Kastori et al., 1998). The treatment by 15 μmol/L $CeCl_3$ could alleviate harmful effect of exposition of spinach to 100 μmol $PbCl_2$/L. It was found to improve light absorption and distribution of excitation energy in both photosystems FSI and FSII and increase activity of photochemical reaction and oxygen evolution in spinach chloroplasts (Zhou et al., 2009). Cadmium (Cd) stress inhibits the plant growth, chlorophyll contents, photosynthesis parameters, chloroplast development and leads to significant alteration in antioxidant defense in rice seedlings. In a hydroponic experiment, the growth of rice seedlings was markedly inhibited by 100 μM Cd and the inhibition was significantly alleviated by 10 μM Ce. Rice seedlings treated with Ce exhibited higher SOD, POD and CAT activities compared with the Cd-stressed plants, indicating a better O^- and H_2O_2 scavenging ability. The mechanism of Ce action in those rice seedlings is partly related to improved light use efficiency, increased antioxidant activity and decreased oxidative stress (Wu et al., 2010).

Enhanced ultraviolet-B radiation (UV-B) affects some physiological processes in plants (Wu et al., 2010). Exposition of hydroponically grown *Brassica* *juncea* seedlings to two levels of UV-B radiation (0.15 W/m^2 and 0.35 W/m^2) decreased chlorophyll content, net photosynthesis rate, transpiration rate, stomatal conductance and water use efficiency, but concomitantly increased membrane permeability and activities of antioxidant enzymes (SOD, CAT, POD). The protective effect of Ce on seedling exposed to UV-B radiation was demonstrated and it was superior at 0.15 W/m^2 UV-B (Liang et al., 2006). Cerium alleviated the inhibition of the photosynthesis in hydroponically grown soybean seedlings to a certain extent. The changes of photosynthetic rate were mainly influenced by the effect of Ce on the reaction of Hill and apparent quantum yield (AQY) at low level of UV-B radiation (0.15 W/m^2) (Liang et al., 2006a). It has been reported that Ce protected photosynthetic apparatus from UV-B radiation by stimulating the accumulation of UV-B absorbing compounds, such as flavonoids and carotenoids and by eliminating reactive oxygen species.

Global climate change is a present condition and a concern for the future. Drought is a part of the global climate changes. Both the intensity and duration of droughts are expected to increase (Grillakis, 2019). Cerium was shown to alleviate water stress in common bean, increasing its survival rate and growth. Cerium application increases photosynthesis rate, chlorophyll content and water use efficiency under water stress (Salgado et al., 2020). Cerium (20 mg $CeCl_3$/L) could alleviate water stress induced by UV-B radiation (0.15 and 0.45 W/m^2) by regulating the osmotic and metabolic absorption of water, photosynthesis and growth of soybean seedlings (Mao et al., 2012). Cerium also effects chilling resistance of cucumber seedlings (Li, 2010).

Acid rain is not a new phenomenon; it is created by the dissolution of acidic oxides, especially sulfur dioxide, sulfur trioxide and nitrogen oxides. They are the result of atmospheric pollution. In extreme cases, the pH value of acid rain sediments can be 3.0. Acid rains increase the permeability of plant cell membranes, reduce pollen germination, plant resistance to drought and diseases, etc. Cerium can alleviate the inhibitory effects of acid rain on seed germination and growth of barley due to the elimination of excessive free radicals induced by acid rain and improvement of the synthesis of chlorophyll and growth of roots (Huang et al., 2000).

Soil salinity influences plant production in many areas of the world. Salt stress causes adverse effect on photosynthesis, energy production, lipid metabolism and other life processes in plants (Kasim et al., 2016). Exposure to NaCl (150 mM) markedly inhibited the growth on Jerusalem artichoke, declined in the chlorophyll content and increased oxidative stress; this inhibition was significantly alleviated by application of 0.1 mM $CeCl_3$. Authors speculate that reduced water stress and photosynthesis, due to applied Ce, might be associated with its effect on growth in the present stress condition (Li et al., 2017).

CONCLUSION

Cerium is one of the rare earth elements belonging to lanthanides. Out of the rare earth elements, the effects of Ce and La on the living world have been studied in most detail. Cerium is widely distributed in living and dead nature. Thanks to its influence on the physiological and biochemical processes of plants, it has application in agriculture. Cerium is not an essential, biogenic element for higher plants, but at lower concentrations it can stimulate some life processes in them: uptake of ions, seed germination, photosynthesis, growth and development, accumulation of organic matter, chemical composition and tolerance to some unfavorable abiotic stress conditions (higher concentrations of toxic heavy metals, drought, deficiency of some essential elements, UV-B radiation, acid rain, etc.). Higher concentrations of Ce are phytotoxic. Further studies are needed to better understand the mechanisms of action of Ce on plant metabolism in order to understand the stimulating effect of lower and phytotoxic effects of higher concentrations of Ce.

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ЦЕРИЈУМ И ВИШЕ БИЉКЕ

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РЕЗИМЕ: Церијум припада групи елемената ретких земаља (ЕРЗ). Њих чине 17 елемената, 15 из лантаноид серије и скандијум и итријум. Елементи ретких земаља у малим концентрацијама широко су распрострањени у природи. Одликују се сличним хемијским и физичким особинама. Они су нашли примену у бројним областима човекове активности, као и у биљној производњи у виду микрођубрива. Од ЕРЗ у биолошким исраживањима најдетаљније је проучено дејство церијума и лантана на животне процесе виших биљака. Церијум није биогени, неопходни елеменат за нормално растење и развиће биљака. Биљке церијум усвајају преко корена и надземних органа, а у највећој мери се накупља у корену. У бројним истраживањима је утврђено да ниже концентрације церијума могу повољно утицати на одређене физиолошке процесе (усвајање појединих јона, синтезу фотосинтетички активних пигмената, фотосинтезу, клијање семена, толерантност према неповољним еколошким чиниоцима, растење и накупљање органске материје). Веће концентрације церијума делују фитотоксично, изазивају цитолошке и морфолошке промене. Поред бројних резултата истраживања о утицају церијума на животне процесе виших биљака потребна су даља сазнања да би се његово повољно дејство на физиолошке и биохемијске процесе биљака боље разумело и ефекасније искористило.

КЉУЧНЕ РЕЧИ: усвајање и интеракција јона, клијање семена, фотосинтеза, раст, фитотоксичност, ублажавање стреса

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REVIEW ARTICLE
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ANTIMICROBIAL PRINCIPLES IN THE DIAGNOSIS AND THERAPY OF CANINE PYODERMA: A REVIEW

ABSTRACT: Although bacteria are normal inhabitants of the canine skin, they play an essential role in the pathogenesis of canine pyoderma. As this skin disease is commonly presented in the small animal practice, the use of antibiotics in its treatment is on high level, although it is often misused. Consequently, excessive and irrational use of antimicrobials leads to the growth of antimicrobial resistance (AMR) and resistant bacterial strains. Therefore, it is necessary to follow the right therapy guidelines to provide appropriate treatment management which is crucial in any policy for prudent and rational antimicrobial use (AMU). Hence, this review aimed to summarize established evidence-based antimicrobial guidelines in treating pyoderma in order to help veterinarians in the fight against development of AMR and its further growth, as one of the highest threats to the public health and topics of the global concern.
KEYWORDS: antimicrobial guidelines, antimicrobial resistance, dogs, pyoderma, skin

INTRODUCTION

Cutaneous microbiota consists of various types of bacteria that normally live on the skin and within the ear pinna and canal (Bradley et al., 2020) and any disturbance that results as an itch or break of the skin can provide perfect conditions for them to multiply and cause inflammation and infection (Secker et al., 2023). Furthermore, one of the most common inflammatory skin conditions of the bacterial origin is pyoderma. In canine, clinical manifestation of this disease can include a wide range of lesions from erythema, alopecia and pruritus to serious cases such as deep folliculitis, furunculosis, vasculitis (Summers et al., 2014). Moreover, the division of this disease is based on depth of the pathological lesions and implies surface, superficial and deep pyoderma (May, 2006; Guardabassi et al., 2008).

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Antimicrobial drugs are often included in the canine cutaneous infections treatment, but in many cases this is not effective because of the antimicrobial resistance (AMR) occurrence (Ebani et al., 2020). Furthermore, improper and imprudent antimicrobial use (AMU) in the veterinary health care systems may contribute to the AMR evolvement and could be responsible for the loss of drug efficacy (Mateus et al., 2011). Generally speaking, the number of drug-resistant (DR), multidrug-resistant (MDR) and (MR) bacteria strains has grown over the years (Beco et al., 2013). Interestingly, the appearance of MR *Pseudomonas aeruginosa*, specifically those resistant to drug used in humans but not that frequently used in dogs, can also be indicator of the overuse of these antibiotics or might suggest human-to-dog transmission (Dégi et al., 2021). Moreover, the frequent use of antimicrobials in general may be associated with the development of MDR (Zur et al., 2016), which influences on the different results of local susceptibility testing, due to local habits of using certain antibiotic. The migration effect in the modern global world, as one of the recent One Health problems, through the global interchange of goods by human travelers, migrating animals and even through the help of natural phenomena, could cause spreading of AMR, as well (Hernando-Amado et al., 2019). The rational AMU in the veterinary medicine can prevent the development and spread of resistance to antibiotics (especially those used in human medicine). Furthermore, it also contributes to preserving the effectiveness of antimicrobial drugs. Therefore, records on how, why and which antibiotics are used in the common animal practice, and the circumstances of their consumption, are required to establish whether upgrades are needed (Murphy et al., 2012), as well in order to monitor AMR. Hence, the aim of this review article is to present the existing clinical and bacteriological aspects of the pyodermas to guide for the selection of adequate antimicrobials, especially in order to combat AMR.

COMMON BACTERIAL AGENTS OF THE SKIN INFECTIONS

The bacteria from staphylococci group are normal residents of the human and animal skin (Hoffmann, 2017), however they are also known as the most commonly associated pathogens with skin infections, especially to pyoderma (May, 2006). As for the humans, the most frequently isolated pathogen from pyoderma cases is *Staphylococcus aureus* (Venniyil et al., 2016). Furthermore, MR *Staphylococcus aureus* (MRSA) represents a major pathogen in health care management in humans, as well as in animals, primarily because a lot of these isolates manifest MDR (Beck et al., 2012). However, the incidence of MRSA was generally lower among pets than is reported for livestock (Graveland et al., 2011). Moreover, although these bacteria can colonize or infect dogs and cats, they are not considered as a reservoir host of this pathogen. MR *Staphylococcus pseudintermedius* (MRSP) is more substantial in the veterinary field (Binek et al., 2019) and represents an example of how AMR has a contribution in the treatment failure (Bryan et al., 2012). Similarly to MRSA, MRSP tends to be clonally distributed within countries, meaning that certain clones can be isolated from epidemiologically unrelated dogs and even from veterinary hospitals located at the distant geographical areas within the same country (Bannoehr et al., 2007). Frequent isolation of biofilm-producing *S. pseudintermedius* strains and their resistance to antimicrobials can influence the outcome of infection treatment (Naziri and Majlesi, 2023). Therefore, it is not surprising that this causative agent has become one of the main topics in the modern veterinary medicine. Apart form the mentioned, *Staphylococcus schleiferi* has been registered as a frequent causative agent in animals on emerge (Davis et al., 2013). Although it does not appear to own factors of virulence as other staphylococci, it posses the ability to cause serious infections in some patients (May, 2006). The increased number of coagulase-negative staphylococcal infections is in correlation with evolving medical treatments and advances in human and veterinary medicine (May, 2006). Furthermore, *Pseudomonas aeruginosa* is a frequent canine pathogen isolated from chronic otitis externa and media cases (Secker et al., 2023). However, it is uncommonly associated with skin infections and usually has low incidence in pyoderma cases (Guardabassi et al., 2008). Actually, these bacteria are presiding in chronic, suppurative skin infections, isolated independently or in mix infections (Leonard et al., 2022; Bradley et al., 2020). Other causative agents that can be associated with pyoderma are *Streptococcus* spp., *Proteus* spp., *E. coli* and other *Enterobacterales* (Nocera et al., 2021).

UNDERLYING CAUSES OF THE SKIN INFECTIONS

Most skin infections are secondary to some underlying primary cause (May, 2006; Summers et al., 2012; Beco et al., 2013). Primary bacterial infections can happen, but much less frequently (Guardabassi et al., 2008). Therefore, predisposing factor must occur in order for the infection to develop. Usually these factors include allergies, skin injuries, endocrine or keratinous disorders and the presence of inflammation or ectoparasites (Summers et al., 2012; Beco et al., 2013). Conditions such as allergy can cause not only unpleasantness and agitation to the dog, but also stress and disturbance to its owners (Miller et al., 2023). Furthermore, prosperous management demands that these factors are addressed, so it is essential that history and clinical signs are well assessed to determine the underlying processes (Beco et al., 2013). These infections are more likely to happen without proper managing of the underlying causes (May, 2006). It is important to emphasize that repeated pyodermal infections caused by *S. pseudintermedius* isolates have shown significantly higher resistance than those isolated from previous cases, so identification and elimination of the predisposing factors are crucial to avoid recurrence of infections (Holm et al., 2002), as well as to mitigate AMR.

ANTIBIOTIC SUSCEPTIBILITY TESTS – YES OR NO?

When a medical condition exists, it is important to obtain an accurate clinical diagnosis whenever possible, including determining the likelihood of a bacterial infection that warrants AMU. Isolation and identification of microorganisms included in the canine skin diseases are a key and elementary step in both diagnostics and adequate treatment (Beier et al., 2015; Hillier et al., 2006). The incidence and constancy of isolation should be taken into account while distinguishing normal residents from the microorganisms that cause secondary settlement and contamination instead of the infection (May, 2006). This is essential in order to create an efficient treatment plan for a certain causative agents based on culture and susceptibility test results, particularly when various microorganisms are present (May, 2006).

Skin infections of bacterial origin are frequent in the canine and the empiric choice of antimicrobial therapy is a general approach to reduce the clinical evolution (Dégi et al., 2021). In the case of empirical treatment, a rational approach should be chosen, selecting the prudent and economically acceptable drug that is efficient against expected microorganism (May, 2006). On the other hand, as reiterated episodes of empirically prescribed antibiotic therapy have been recognized as one of the most important risk factors for infections caused by MRSA in pets, failure in therapy should always be followed by reassessment, involving culture and antibiotic sensitivity tests, rather than switching to another empirically selected antimicrobial drug (Magalhães et al., 2010). In addition, in recurrent infections or with appearance of newly discovered lesions regardless of the application of adequate antimicrobial, adjustments in treatment should be made based on the culture and susceptibility data (May, 2006). In the severe cases of pyoderma, antimicrobial susceptibility should be considered imperative (Dégi et al., 2021).

THE TREATMENT APPROACHES IN THE CANINE PYODERMA

The treatment approach for canine pyoderma differs with the deepness of the infection (Loeffler et al., 2011). The vast majority of skin infections in the companion animals are associated with coagulase-positive staphylococci, especially with *S. pseudintermedius*. Hence, a broad spectrum of antibiotic groups has been recommended for therapy of pyoderma caused by staphylococci based on their in vitro efficacy testing, experience from in vivo studies and clinical trial results (Summers et al., 2012). Moreover, if inadequate smaller doses of antimicrobials are used or length of time is too brief, staphylococcal cultures are changing so that antibacterial-resistant isolates are selected, guiding to chronic cases of infections (Hnilica and May, 2004).

Although there are plenty of topical antimicrobial pharmaceutical formulations available and licensed for use in the veterinary practice against skin infections worldwide, superficial pyoderma still remains a frequent occurrence in dogs (Loeffler et al., 2011; Mueller et al., 2012). Topical treatment by itself should be taken into consideration, especially in cases where long-term treatment is expected, such as recurrent infection of superficial pyoderma which, due to the underlying cause, were not detected and rectified on time (Loeffler et al., 2011).

Animals which are under antimicrobial therapy can be particularly at risk for acquisition resistant microorganisms, considering the fact that antimicrobial therapy may promote the transmission of microorganisms through antibiotic-induced reduction of the normal inhabitant population of staphylococci (Loyd, 2005). In addition, systemic antimicrobial treatment may not be ideal considering an enlargement of multiresistant organisms, cost and potential side effects (Mueller et al., 2012). Furthermore, conditions such as deep folliculitis, furunculosis and cellulitis usually require extend treatments. While cicatrization can make it even more complicated, these lesions may also be painful and microorganisms, such as *Pseudomonas* spp., *E. coli* or *Proteus* spp. may be included, besides staphylococci (May, 2006). Thus, systemic antimicrobial treatment is generally indicated in cases such as deep pyodermas, as well as in cases of unsuccessful topical therapy or when it can not be applied properly (Loeffler et al., 2011).

Beta-lactams are one of the most common classes of antimicrobials prescribed in pets for systemic administration (Beaudoin et al., 2023). Rantala et al. (Rantala et al., 2004) have established that cephalexin and amoxicillin with clavulanic acid accounted for 60% and 15%, respectively, of all the antibiotics prescribed for pyoderma infections, followed by clindamycin (10%). Beside mentioned antibiotics, fluoroquinolones (enrofloxacin, marbofloxacin, difloxacin and orbifloxacin) are very efficient in pyoderma treatment and are commonly used for empirical treatment in dogs because of their favorable safe profiles and assured clinical efficacy due to proven antimicrobial activity against *S. pseudintermedius* and skin distribution (Guardabassi et al., 2008). Moreover, the "OIE List of Antimicrobial Agents of Veterinary Importance" suggest that fluoroquinolones and the 3rd and 4th generation cephalosporins, as critically important antimicrobials in human and animal health, should not be used as preventive treatment and as the first line treatment unless they are justified, while when used as the second line, they should be based on the bacteriological tests results. The extra-label or off label use of antimicrobials should be restricted and restrained for cases where there are no alternatives available and should be in accordance with the applicable national legislation (OIE, 2021).

The use of antimicrobial drugs in pets should be assessed based on infectious disease treated in the affected population, available regulations and guidelines, current scientific knowledge and licensed drugs available for veterinary use, research-based knowledge and licensed antimicrobials disposed for use in veterinary medicine (Mateus et al., 2011). The Antimicrobial Stewardship Guidelines in pets are designed to assist veterinarian clinicians in selecting the adequate antimicrobial therapy that will best serve their patients while mitigating the AMR growth and other side effects (Frey et al., 2022). For instance, the guidelines for the treatment of canine pyoderma were already created by the different groups of experts in this field (Table 1). These guidelines should be followed when treating this disease. Based on these guidelines, topical treatment is recommended for treatment of superficial pyoderma, whereas for deep ones, there should be used systemic therapy based on sensitivity testing, supported by topical treatment with antiseptics. For mild, surface and/or focal infections, topical antiseptic preparation and topical antibiotics or locally applied antimicrobials are suggested (in case topical antiseptics do not clear the infection) (Frey et al., 2022; Beco et al., 2013). Topical antiseptic solutions can speed up infection resolution or will significantly reduce the need for systemic antimicrobials (Beco et al., 2013). In the study of Borio et al. (Borio et al., 2015) treatment with chlorhexidine products resulted in clearing of clinical signs of superficial pyoderma in all dogs (including those infected with MRSP). Actually, this study indicated that topical therapy with chlorhexidine may be as effective as systemic therapy with amoxicillin with clavulanic acid, which supports the recommendations of existing guidelines to use topical antiseptics alone for the management of superficial pyoderma (Borio et al., 2015).

The common fact for all guidelines is that systemic antimicrobials are classified in three tiers (lines). First-tier usually implies broad-spectrum drugs that are used when diagnosis is clear and risk factors for AMR do not exist, but they are not considered less-efficient than higher-tier drugs in the correct circumstances (Beco et al., 2013; Hillier et al., 2014). Clindamycin is one of the first-tier drugs in all suggested guidelines (Table 1). However, recent study conducted in Netherlands has shown the high level of resistance to clindamycin in *S. pseudintermedius* isolated from dogs with previous antimicrobial exposure, recommending that a bacterial culture and sensitivity test should be carried out before prescribing these drugs and it should be regarded as the preferred treatment option if susceptibility is confirmed (van Damme et al., 2020). These results indicate that clindamycin might be reconsidered as tier-two drugs which should be used when culture evidence exists. Furthermore, tier-three drugs are very important to animal and human health, especially for treatment of MR organisms, so they are reserved for highly resistant infections and their use should be in consultation with specialists (Beco et al., 2013; Hillier et al., 2014). In Table 1, it can also be seen that dissimilarities exist in the distribution of antimicrobials through group of different guidelines. Existence of the variation in the antibiotic susceptibility patterns of pyoderma causative agents on the local level and dissimilarities in accessibility of drugs, legal status and cost, these all can have influence on the drugs efficiency within various clinical populations and geographical area (Summers et al., 2012; Hillier et al., 2014). Therefore, it is difficult to provide adequate evidence to publish ultimate best practice guidelines for the empiric treatment of superficial and deep pyoderma in dogs (Summers et al., 2012). Thus, every country needs to implement their own guidelines for treating pyoderma cases, based on the national and regional records. In addition, surveys that provide these details should be conducted regularly in order to ensure valuable and updated data.

Table 1. Available referenced guidelines for the pyoderma treatment with suggested principles for rational antimicrobial use through their categorization

Guidelines	Category	Antibiotics
Suggested guidelines for using systemic antimicrobials in bacterial skin infections: part 2 – antimicrobial choice, treatment regimens and compliance (Beco et al., 2013)	First-line	cefadroxil, cefalexin, clavulanate-amoxicillin, clindamycin, lincomycin
	Second-line	cefovecin, cefpodoxime, difloxacin, enrofloxacin, marbofloxacin, orbifloxacin, pradofloxacin
	Third-line	aminoglycosides, azithromycin, ceftazidime, chloramphenicol, clarithromycin, florphenicol, imipenem, phosphomycin, piperacillin, rifampin, tiamphenicol and ticarcillin
Australian Veterinary Prescribing Guidelines: Companion Animals Medical Guidelines-Skin	First-line	clindamycin
	Second-line	cephalexin, amoxycillin-clavulanate, trimethoprim-sulphonamide, doxycycline
	Third-line	enrofloxacin, marbofloxacin, cefovecin
2023 AAHA Management of Allergic Skin Diseases in Dogs and Cats Guidelines (Miller et al., 2023)	First-tier empiric	clindamycin, cephalexin, amoxicillin-clavulanate, trimetrorim-sulfadiazine/sulfamethoxazole
	First OR second tier	cefpodoxime, cefovecin
	Second tier ONLY with culture and susceptibility	minocycline, doxycycline, enrofloxacin, marbofloxacin, radofloxacin, chloramphenicol, rifampin
	Do NOT use for *Straphylococcus* spp. infections	amoxicillin, penicillin, nitrofurantoin
Guidelines for the diagnosis and antimicrobial therapy of canine superficial bacterial folliculitis (Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases) (Hillier et al., 2014)	First-tier	clindamycin or lincomycin cefalexin, cefadroxil, amoxicillin–clavulanate, trimethoprim- and ormetoprim-potentiated sulphonamides
	First or second tier	cefovecin, cefpodoxime
	Second tier	doxycycline or minocycline, hloramphenicol fluoroquinolones (such as enrofloxacin, marbofloxacin, orbifloxacin, pradofloxacin and ciprofloxacin), Aminoglycosides (gentamicin, amikacin), first tier drugs (clindamycin, lincomycin and potentiated sulphonamides) may also be considered
	Third tier	linezolid, teicoplanin, vancomycin

FUTURE OPTIONS OF PYODERMA TREATMENT

There are several new approaches under development for the management of skin and ear infections and inflammation. Although more clinical trials are needed to confirm efficacy, early results (especially *in vitro* studies) are promising (Nuttall, 2023). For instance, natural products, such as essential oils (EOs), with antimicrobial properties, could represent a suitable alternative in the treatment of skin infections, mainly when conventional drugs resulted not effective (Ebani et al., 2020). The *in vitro* study conducted by Ebani et al. (Ebani et al., 2020) tested antimicrobial activity of nine EOs against staphylococcal skin isolates resulting in different antimicrobial activity degrees. *Origanum vulgare* and *Thymus vulgaris* EOs have shown the best antimicrobial activity, indicating that pharmaceutical formulation, based on these Eos, could be promising treatment to combat canine cutaneous infections caused by these pathogens.

CONCLUSION

As bacterial skin infections are one of the most common diseases presented in pets, responsible therapy approaches are crucial in order to prevent overuse of antimicrobials. A correct management of antimicrobial policy through the regular implementations of AMR patterns of the frequently isolated microorganisms is crucial to avert needless prescriptions and further emergence of resistant strains. Promoting responsible use of antimicrobial drugs through guidelines will ensure tenable access to the most efficient therapy. Furthermore, implementing best practical guidelines based on the national and regional records will improve human and animal health, while simultaneously providing reduce of emerge and spread of AMR.

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АНТИМИКРОБНИ ПРИНЦИПИ У ДИЈАГНОСТИЦИ И ТЕРАПИЈИ ПИОДЕРМЕ ПАСА: ПРЕГЛЕД

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РЕЗИМЕ: Иако су бактерије нормални становници коже паса, оне имају суштинску улогу у патогенези псеће пиодерме. Како се ова кожна болест често јавља у пракси малих животиња, употреба антибиотика у њеном лечењу је на високом нивоу, а често се може злоупотребити. Сходно томе, прекомерна и нерационална употреба антимикробних средстава доводи до раста антимикробне резистенције (АМР) и резистентних сојева бактерија. Због тога је неопходно пратити исправне смернице за терапију како би се обезбедио одговарајући третман који је кључан у свакој политици за разумну и рационалну употребу антимикробних средстава (АМУ). Стога је овај преглед имао за циљ да сумира утврђене антимикробне смернице засноване на доказима у лечењу пиодерме како би се помогло ветеринарима у борби против развоја АМР-а и његовог даљег раста, као једне од највећих претњи за јавно здравље и најзначајнијих тема од глобалног значаја.

КЉУЧНЕ РЕЧИ: антимикробне смернице, антимикробна резистенција, пси, пиодерма, кожа

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BIOMASSES OF DIFFERENT *Salix* L. CLONES IN THE DECARBOXYLATION PROCESS DURING ENERGY PRODUCTION

ABSTRACT: Biomass is increasingly employed in diverse applications to achieve and enhance energy efficiency, owing to its carbon-neutral nature. This is attributed to the fact that the quantity of CO_2 released during its combustion corresponds precisely to the amount absorbed by biomass during its growth. The objective of this study is to assess the energy efficiency of biomass derived from analysed clones of fast-growing willow species in co-combustion processes with lignite at varying percentage ratios. The primary goal is to enhance the calorific value of lignite, optimize combustion and mitigate the harmful effects of combustion. The obtained results indicate that the calorific value of willow is higher than the calorific value of coal. The calorific value of coal (lignite) depends on the location of the coal deposit (field), while the calorific value of willows depends on the type of willow. Notably, clones 347 and NS 73/6 of white willow (*Salix alba)*, have the highest energy potential compared to clones B-44 of white willow and basket willow (*Salix viminalis*).

KEYWORDS: biomass, willows, decarboxylation, coal, co-combustion, energy potential, willow

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INTRODUCTION

The process of decarbonization, which has global significance, implies an increase in the participation of renewable energy sources (RES) in energy production. Biomass falls within the RES category and represents organic matter that can be of plant or animal origin. Currently, it accounts for approximately 14% of the world's total energy consumption, with developed nations utilizing a quarter of it for air protection initiatives. The remaining portion of primary energy involves the direct application of biomass in underdeveloped countries for heating in households and other purposes. Additionally, waste and residues from the wood processing industry are employed to generate energy in plants (Parrika, 2004).

Biomass is regarded by the energy community as carbon neutral because the amount of CO_2 released during its combustion represents the same amount that biomass absorbs during its growth in an energy plantation (Mann and Spath, 2001; Heller et al., 2003). The implementation of energy plantations in order to produce biomass that would be used in energy production, either independently in power plants, or through co-combustion with lignite in order to increase the calorific value, brings benefits on one hand to energy management, and on the other to ecology.

Co-combustion of biomass and lignite can satisfy both the needs of energy management and ecology, because, at the same time, the calorific value of lignite can be increased, and the emitted CO_2 reduced. Namely, the obligation of business entities is to perform land recultivation after the completion of lignite exploitation in a given area. Different plant species can be used for land recultivation, and the most suitable woody species are exactly those that are fast-growing, such as willows (*Salix* L.) and poplars (*Populus* L.). Willows grow on floodplains, in river valleys, usually along rivers or on marshy ground (Parfenov and Mazan, 1986). There are a large number of willow species, with different forms, and most often it is a tree. It can be extremely tall, up to 15 meters, and up to 1 meter in diameter, but it can also have the form of a bush or a ground plant (Oljača et al., 2017).

Multiple benefits of recultivation would be gained if energy plantations would be formed in such areas using willows. They would solve the problem of phytoremediation of heavy metals and erosion, owing to their strong root system (Ulzen-Appiah, 2002; Volk, 2002; Heller et al., 2003), and at the same time, the given area would continue to bring "gains" through the production of biomass that would be used for the production of energy.

Willows are a species that does not require complicated conditions for growth, as they are highly adaptable to various types of soil. Simultaneously, they yield well, producing a minimum of 30–40 tons per hectare of dry biomass in a very short time. They can survive on floodplains, but also on polluted and relatively degraded soil. These fast-growing woody plants, which are managed according to the short rotation principle, possess a number of characteristics suitable for the phytoremediation process, the most important of which are: a strongly developed and well-branched root system, high biomass productivity, high intensity of transpiration, as well as genetic variability (Arsenov, 2018). Due to all of the above, willows are very rewarding to use on the tailings of mining pits, where, along with the simultaneous remediation of the land, they will also provide biomass for the production of energy. Combustion of willow and coal contributes to the reduction of greenhouse gas emissions. Biomass obtained from willow, compared to coal, has almost no sulphur, contains less ash and trace metals, and depending on the combustion regime and equipment, can result in lower NO_x emissions (Conn and Tillman, 2000; Hughes, 2000; Tharakan et al., 2003a). These plantations are formed using genetically improved cloned material, with a planting density of 15,000 plants per hectare.

The biomass obtained from willows has an exceptional energy potential because the calorific value of willows can reach 19 MJ/kg. Willows belong to the Short Rotation Coppice (SRC) plantations because their harvest from energy plantations is possible every other year, for the period of up to 25 years. This is precisely why willows have been cultivated successfully for economic purposes over an extended period. Their cultivation thrives owing to their widespread geographic prevalence, adaptability to diverse environmental conditions, and robust biomass growth (Rodzkin, 2014). Willows are able to quickly colonize land surfaces without vegetation or places with poorly developed vegetative cover (Morozov, 1950). Biomass produced in such plantations has multiple applications: as fuel for the production of electricity in special generators, for the production of charcoal, for direct burning due to the low content of ash and moisture, as well as alkali metals, or simply as a source of carbon in atmospheric CO_2 (Nixon et al., 2001). Analyses of fossil fuel-based electricity generation show that producers and consumers tend to favor non-renewable energy over renewable energy (Fuchs and Arentsen, 2002; Unruh, 2002). Adopting innovations in any form is only acceptable by energy producers if it does not deviate much from the dominant technology. This is exactly why it is considered that the production of electricity by burning willow biomass in combination with other wood biomass or with coal in existing power plants is the most optimal commercial option, because it does not deviate much from the dominant technology, i.e. it does not require much investment in already existing power plants (Tillman, 2000).

The potential of willows is recognized by many countries that use them extensively for energy production. Co-combustion of biomass in coal-fired power plants, with a share of 5–20%, depending on the technology and type of biomass used, is in many cases a cost-effective option for replacing part of coal with biomass in the production of electricity, while simultaneously reducing CO_2 emissions (Tillman et. al., 2012). The moderate amount of variation in wood specific gravity can be used to select for increased energy content and reduced transportation costs (Tharakan et al., 2003b). The yield of biomass per hectare depends on the type of soil, specifically the method of wetting the soil and the content of dust and clay fractions on the researched systematic units of land (Živanov and Ivanišević, 1986), but the production of biomass from willows and its burning as a raw material for energy production provide both ecological, as well as rural development. Rodzkin et al. (2016) point out that clones of the species *Salix alba* L. and *Salix dasyclados* Wimm., as well as hybrids *S. aurita* L. and *S. dasyclados*, represent good candidates for the production of biomass on degraded lands. Currently, areas of fast-growing crops can be found in almost all EU countries, as well as in the USA and Canada (Rodzkin et al. 2015). In 2011, the area under energy plantations was, for example, in Sweden about 13,000 hectares, in Germany about 4,000 hectares, in Poland about 9,000 hectares (Dimitriou, 2011; Mola-Yudego, 2010; Rosenqvist and Dawson, 2005; Scholz, 2002; Stenhouse, 1999; Meadows et al., 1972; van Doorn, 2006).

The aim of this work is to investigate the energy efficiency of biomass of the analysed clones of fast-growing willow species in co-combustion processes with lignite in different percentage ratios.

MATERIALS AND METHODS

In this work, four willow genotypes were investigated, namely: one clone of *S. viminalis* and three clones (clone B-44, clone 347, clone NS 73/6) of *S. alba*, which are referred to in the following text as clone 1, clone 2, clone 3 and clone 4, respectively.

After three years of cultivation, in 2021, the willows were cut and dried naturally for two months. Calorific values of three lignite samples and four willow clones were determined, as well as values of the mixture of lignite and willow biomass in different proportions (5, 10, 15 and 20% of biomass). The first and second lignite samples (U_1 and U_2) were taken from two localities in the eastern part of the Kolubara MB and represent mixed samples from field B/C and field E. The third coal sample (U_3) was taken from the western part of the Kolubara basin, at the Drobilana-Kalenić loading site, and represents mixed coal from Tamnava west field and field G.

The calorific value of each of the four tested willow clones, as well as the three tested samples of lignite, was determined without correction, using the IKA C 5003 calorimeter in the accredited laboratory at the Prerada organizational unit of Kolubara MB, JSC EPS.

Numerical data obtained by measuring the calorific value of three samples of coal and the biomass of four willow genotypes, as well as by calculating the differences between the calorific value of coal and a mixture of coal with the biomass of four willow genotypes, were processed using descriptive and univariate statistical methods. Statistical analyses were performed in the computer program Statgraphics Centurion v. XVI.I. (2009; Statpoint Technologies, Inc., Warrenton, VA).

RESULTS AND DISCUSSION

Based on the tested data on the calorific value of three samples of coal in co-combustion with biomass of four willow clones (in the proportion of 5, 10, 15 and 20%), an overview was given as to the results on the possibility of improving the calorific value of coal with biomass.

Average calorific values for willow biomass (Table 1) ranged from 17,966.30 kJ/kg (clone 1) to 18,246.80 kJ/kg (clone 4), depending on the examined genotype (clone). According to Mitić (2018), willow stands out as the species that has found the greatest application in the economy due to its wide ecological valence (resistance to extreme habitat conditions), with an average calorific value of 19,300 kJ/kg of dry biomass.

The minimum value was measured for the biomass of clone 1 and was 17,952.0 kJ/kg, and the maximum – for clones 3 and 4 (18,274.0 kJ/kg). Low coefficients of variation (CV) values (0.08–0.11%) were found for the calorific value of the biomass of the studied genotypes. According to the results of the analysis of variance (ANOVA), the mean values determined for the calorific value of the biomass of four willow genotypes are statistically significantly different from each other (p = 0.0000), forming three homogeneous groups (Table 1). Based on this, it can be concluded that the calorific value of willow biomass depends on the genotype. Clone 2 is close to the values of clones 3 and 4, which is expected, given that clones 2, 3 and 4 are white willow clones, while clone 1 is a basket willow clone and is characterized by the lowest calorific value.

We can say that the *S. viminalis* (clone 1) showed the lowest energy potential, while the *S. alba* clones (clone 3 and clone 4) represented the clones with the highest energy potential. Kijo-Kleczkowska et al. (2016) point out that the calorific value of basket willow is 16,824 kJ/kg, while Karampinis et al. (2011) point out that the calorific value of willow without drying on a "dry basis" is 18,410 kJ/kg.

Table 1. Analysis of variance for the calorific value (kJ/kg) of biomass of willow genotypes

Genotype (clone)	$\bar{X}$	$(\bar{\bar{X}})$	MIN	MAX	SD	CV. (%)	F	p
Clone 1	17,966.30 c	**18,124.3**	17,952.0	17,983.0	13.54	0.08	579.79	**0.0000**
Clone 2	18,046.30 b		18,028.0	18,062.0	14.86	0.08		
Clone 3	18,237.60 a		18,209.0	18,274.0	20.35	0.11		
Clone 4	18,246.80 a		18,209.0	18,274.0	20.00	0.11		

Note: Mean values with different letters within a column are statistically significantly different from each other at the 95% confidence level.

Average calorific values for coal samples (Table 2) ranged from 12,138.03 kJ/kg (U_3) to 15,946.00 kJ/kg (U_2). The minimum value was measured for U_3 and it amounted to 12,070.0 kJ/kg, and the maximum for U_2 (15,978.0 kJ/kg). For the calorific value of the examined coal samples, low values of the coefficient of variation were established (CV) (0,21–0,43%).

Analysis of variance (ANOVA) shows that there is a statistically significant difference (p = 0.0000) between the mean values calculated for the calorific value of the coal samples and three homogeneous groups are formed (Table 2).

Table 2. Analysis of variance for calorific value (kJ/kg) of coal samples

Coal sample	$\bar{X}$	($\bar{\bar{X}}$)	MIN	MAX	SD	CV. (%)	F	*p*
U 1	12,975.00 **b**		12,905.0	13,028.0	53.93	0.42		
U 2	15,946.00 **a**	**13,686.3**	15,902.0	15,978.0	33.60	0.21	21,461.89	0.0000
U 3	12,138.03 **c**		12,070.0	12,186.0	51.76	0.43		

Note: Mean values with different letters within a column are statistically significantly different from each other at the 95% confidence level.

Accordingly, it is stated that the calorific value of coal depends on the sample, so U_2 has the highest calorific value and U_3 – the lowest. The former (U_2) represents a mixed sample from field B/C and field E, taken from location 2, while U_3 represents a coal sample taken from the western part of the Kolubara Basin, at the Drobilana-Kalenić loading point and represents mixed coal from Tamnava west field and field G. The lower calorific value of U_3 can be attributed to the larger amount of clay present in the sample itself, compared to other samples. The data in Table 2 also show that U_2 had the highest calorific value.

In addition to differences in calorific values, there are also differences in moisture and ash content; according to these, coal U_2 stood out, while the other two had identical values of these indicators (Table 3).

Table 3. Moisture and ash content of different coal samples

Sample	Moisture (%)	Ash content (%)
U1	44.51	25.4
U2	50.94	12.3
U3	44.56	25.5

Table 4 shows the statistical results of the calorific values (kJ/kg) of the mixture of all coal samples with different proportions of biomass.

The statistical results of calorific values (kJ/kg) of the mixture of coal with different proportions of biomass show that there are no statistically significant differences between the clones and the proportion of biomass when it comes to the mixture of all coal samples.

Mean calorific values for the mixture of coal and biomass ranged from 14,126.70 kJ/kg (clone 1 added to coal as 5% biomass) to 14,613.80 kJ/kg (clone 3 added to coal as 20% biomass), depending on the tested genotype as a proportion of biomass added to coal. Based on the arithmetic mean ($\bar{X}$) the calorific value of the mixtures increased with the increase in the proportion of added biomass, and for each clone, it had the smallest increase in calorific value when adding 5% of biomass, and the largest when adding 20%. If we look at the clones, the arithmetic mean ($\bar{\bar{X}}$) indicates that the greatest increase in thermal value is in clone 4, and the least in clone 1. Based on these data, it can be concluded that clone 4 is the clone with the highest energy potential. In second place in terms of energy potential is clone 3, followed by clone 2 and finally clone 1 with the lowest energy potential.

Table 4. Analysis of the variance of the calorific value (kJ/kg) of the mixture of coal and willow biomass

Clone	Share of biomass (%)	$\bar{X}$	$(\bar{\bar{X}})$	MIN	MAX	SD	CV	F	p
Clone 1	5	14,126.70 a	**14,356**	12,619.0	16,203.0	1,589.11	11.24	0.10	1.0000
	10	14,358.70 a		12,773.0	16,489.0	1,642.02	11.44		
	15	14,427.70 a		13,207.0	16,359.0	1,452.90	10.07		
	20	14,511.00 a		13,174.0	16,365.0	1,421.74	9.80		
Clone 2	5	14,140.70 a	**14,359**	12,521.0	16,417.0	1,742.65	12.32		
	10	14,271.10 a		13,002.0	15,987.0	1,298.72	9.10		
	15	14,526.30 a		13,121.0	16,579.0	1,559.22	10.73		
	20	14,498.70 a		13,259.0	16,279.0	1,350.96	9.32		
Clone 3	5	14,147.20 a	**14,372**	12,617.0	16,403.0	1,708.20	12.07		
	10	14,238.90 a		12,837.0	16,289.0	1,560.49	10.96		
	15	14,490.70 a		13,058.0	16,547.0	1,567.31	10.82		
	20	14,613.80 a		13,342.0	16,369.0	1,347.29	9.22		
Clone 4	5	14,327.30 a	**14,436**	12,801.0	16,506.0	1,663.39	11.61		
	10	14,298.00 a		12,796.0	16,476.0	1,635.92	11.44		
	15	14,551.30 a		13,192.0	16,368.0	1,398.78	9.61		
	20	14,570.60 a		13,203.0	16,476.0	1,437.15	9.86		

Note: Mean values with different letters within a column are statistically significantly different from each other at the 95% confidence level.

It should also be noted that, given that there are no significant differences between the mean values calculated for the calorific value of coal with the addition of biomass of genotypes in different proportions, it is economically justified to add 5%.

The minimum value was measured for coal with the addition of clone 1 biomass in the proportion of 10% and was 12,521.0 kJ/kg, while the maximum value was measured for coal with the addition of clone 2 biomass in the proportion of 15% (16,579.0 kJ/kg).

Low (<10%) to medium (10–20%) coefficients of variation were established for the calorific value of the mixture of coal and biomass, depending on the genotype and the proportion of biomass. Analysis of variance (ANOVA) determined that there is no statistically significant difference ($p = 1,0000$) between the mean values calculated for the calorific value of coal with the addition of biomass of genotypes in different proportions (Table 4). Based on this, it can be concluded that the calorific value of the mixture of coal and biomass does not depend on the genotype, as well as the proportion of biomass from 5% to 20%. Savolainen (2003) points out that with the concept of joint combustion of biomass and coal, it is possible to replace 5–30% of coal with renewable fuels – biomass.

Table 5. Analysis of variance for calorific and thermal values (kJ/kg) of mixtures of coal and willow biomass according to genotype (clone 1–4), coal sample (U_1–U_3) and biomass share (%)

Coal sample	Share of biomass (%)	$\bar{X}$	($\bar{\bar{X}}$)	Thermal difference (Δ)	Mean value of thermal differences $\bar{\bar{X}}$	SD	CV	F	*p*
Clone 1									
U_1	5	13,556.00 **g**	**13,769**	581	**794**	17.35	0.13	13,168.56	0.0000
	10	13,804.00 **e**		739		27.00	0.20		
	15	13,714.00 **f**		829		28.00	0.20		
	20	14,005.00 **d**		1030		20.00	0.14		
U_2	5	16,179.00 **c**	**16,334**	233	**388**	25.06	0.15		
	10	16,470.00 **a**		398		17.35	0.11		
	15	16,344.00 **b**		400		23.43	0.14		
	20	16,346.00 **b**		524		26.29	0.16		
U_3	5	12,645.00 **k**	**12,963**	507	**825**	23.58	0.19		
	10	12,802.00 **j**		664		31.80	0.25		
	15	13,225.00 **h**		1,087		15.87	0.12		
	20	13,182.00 **i**		1,044		7.55	0.06		
Clone 2									
U_1	5	13,476.00 **h**	13,759	551	**797**	33.00	0.24	4,354.20	0.0000
	10	13,733.30 **g**		758		24.17	0.18		
	15	13,859.00 **f**		884		23.00	0.17		
	20	13,970.00 **e**		995		36.00	0.26		
U_2	5	16,401.00 **b**	**16,296**	308	**539**	14.42	0.09		
	10	15,964.00 **d**		445		20.42	0.13		
	15	16,565.00 **a**		619		12.17	0.07		
	20	16,254.00 **c**		786		22.91	0.14		
U_3	5	12545.00 **k**	**13,022**	407	**884**	20.81	0.17		
	10	13116.00 **j**		978		108.0	0.82		
	15	13155.00 **j**		1,017		29.60	0.22		
	20	13272.00 **i**		1,134		20.81	0.16		
Clone 3									
U_1	5	13,419.70 **g**	**13,751**	445	**776**	14.05	0.10	27,345.67	0.0000
	10	13,600.70 **f**		626		11.06	0.08		
	15	13,857.00 **c**		882		7.55	0.05		
	20	14,129.30 **d**		1,154		17.16	0.12		
U_2	5	16,380.00 **b**	**16,384**	434	**438**	21.28	0.13		
	10	16,273.00 **c**		327		14.42	0.09		
	15	16,532.00 **a**		408		14.11	0.09		
	20	16,354.00 **b**		582		13.00	0.08		
U_3	5	12,642.00 **k**	**12,981**	504	**843**	26.06	0.20		
	10	12,843.00 **j**		705		8.72	0.07		
	15	13,083.00 **i**		945		21.70	0.17		
	20	13,358.00 **h**		1,220		14.00	0.10		

Coal sample	Share of biomass (%)	$\bar{X}$	($\bar{\bar{X}}$)	Thermal difference (Δ)	Mean value of thermal differences $\bar{\bar{X}}$	SD	CV	F	*p*
Clone 4									
U_1	5	13,667.00 **d**	**13,864**	692	**886**	17.00	0.12	3,405.03	0.0000
	10	13,661.00 **d**		669		98.09	0.72		
	15	14,084.00 **c**		1,109		65.87	0.47		
	20	14,047.70 **c**		1,073		40.50	0.29		
U_2	5	16,491.00 **a**	**16,423**	376	**452**	17.35	0.11		
	10	16,422.00 **ab**		402		47.15	0.29		
	15	16,348.00 **b**		483		25.71	0.16		
	20	16,428.00 **a**		545		41.68	0.25		
U_3	5	12,824.00 **f**	**13,023**	686	**885**	24.06	0.19		
	10	12,811.00 **f**		673		24.27	0.19		
	15	13,222.00 **e**		1,084		32.79	0.25		
	20	13,236.00 **e**		1,098		37.00	0.28		

Note: Mean values with different letters within a column are statistically significantly different from each other at the 95% confidence level.

The mean calorific values of the mixture of coal and willow biomass ranged from 12,545.00 kJ/kg (coal sample 3 with 5% biomass of clone 2) to 16,565.00 kJ/kg (coal sample 2 with 15% biomass of clone 2) depending on examined genotype (clone), coal sample and biomass share. For the calorific values of the examined mixtures of coal with biomass, low values of the coefficient of variation were established (CV=0.05–0.82%). According to the results of the analysis of variance (ANOVA), the mean values determined for the calorific value of the mixture of coal samples with the biomass of willow clones are statistically significantly different from each other (p = 0.0000) (Table 5).

It can be stated that the improvement of the calorific value of coal with willow biomass depends on the genotype, coal sample and biomass share, so the mixtures of coal sample 2 with 15% biomass of clones 2 or 3 have the highest calorific value, and the mixture of coal sample 3 with 5% of clone biomass 2 – the lowest calorific value. At the same time, the addition of only 5% of biomass of clone 4 to coal sample 2 gives a solid improvement in the calorific value, because the addition of three times less biomass of this clone to coal sample 2, compared to clones 2 and 3, only gives a minor 0.2–0.4% improvement in the calorific value of coal. With the addition of biomass, the calorific difference increased, with an increase in the amount of added biomass from 5–20%.

By analysing table 5, the greatest heterogeneity was obtained in clones 1,2,3, where there are 11 groups of variability, and the least in clone 4, with 7 groups of variability. When it comes to the type of coal and the share of biomass, the smallest dependence on the amount of biomass and type of coal is observed in clone 4, while coal U2 is the best and takes first place (a) regardless of the clone.

Thermal values of coal and willow clones clearly show that there are differences both between the types of coal and the willow clones (Table 6).

Table 6. Mean value of calorific differences ($\bar{\bar{X}}$) of coal types and willow clones regardless of the proportion of willow biomass (kJ/kg)

Type of coal	Clone 1	Clone 2	Clone 3	Clone 4	$\bar{X}$
U_1	794	797	776	886	**813**
U_2	388	539	438	452	**455**
U_3	825	883	843	885	**859**
$\bar{X}$	**669**	**740**	**685**	**741**	–

The type of coal contributes the least to the increase in calorific value when willow clones are added, which is understandable considering that it is the best coal in terms of calorific value. Among the willow clones, clone 4 and clone 2 stand out, the other two are similar. The addition of willow biomass to coal is most effective with U3, which is the worst thermally; then with U1 and finally with the best quality coal U2, the thermal difference is 455 (kJ/kg).

CONCLUSION

The application of biomass in co-combustion with lignite could successfully increase the calorific value of coal and thus save coal in the production of energy, but also reduce the carbon taxes that will be present in the future, thus properly following the path of decarbonization, with the aim of protecting and preserving the environment.

Based on the results obtained in this paper, the following can be concluded:

– The calorific value of the mixture of coal and biomass depends on the type of coal, as well as on the genotype and the proportion of the willow biomass. The basket willow, *Salix viminalis*, showed the lowest calorific potential compared to the examined white willow genotypes (*Salix alba*);

– Clones 347 and NS 73/6 (clones 3 and 4) of white willow showed the greatest energy potential compared to clones B-44 (clone 2) of white willow and basket willow (clone 1);

– The lignite sample taken at the Drobilana-Kalenić loading site, which represents mixed coal from the Tamnava west field and field G, is the coal sample with the lowest calorific value (U3), followed by sample U_1 which represents a mixed sample from field B/C and field E, while sample U_2 has the highest thermal value, which represents a mixed sample from field B/C and field E, but was taken from a different location compared to the sample U_1;

– With an increase in the proportion of willow biomass (5–20%) in coal, regardless of the genotype, the calorific difference (Δ) of coal increases and it is generally the largest with the highest proportion of willow biomass;

– The addition of willow biomass to coal is most effective with U3, which is the worst calorically, then with U1 and finally, with the best quality coal U2, the thermal difference is the smallest, so the most optimal is the co-combustion of biomass and low-calorie lignite;

– Although the thermal value of the mixture of biomass and coal increases with the increase in the proportion of willow biomass, it is economically justified to add 5–10% of biomass.

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ПРИМЕНА БИОМАСЕ РАЗЛИЧИТИХ КЛОНОВА ИЗ РОДА *Salix* L. У ПРОЦЕСУ ДЕКАРБОКСИЛАЦИЈЕ ПРИ ПРОИЗВОДЊИ ЕНЕРГИЈЕ

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РЕЗИМЕ: Биомаса се све више користи у разним видовима добијања или повећања енергетске ефикасности јер је карбонски неутрална, односно количина CO_2 која се ослободи приликом њеног сагоревања представља исту ону количину коју биомаса апсорбује током свог раста. Циљ овог рада је да се испита каква је енергетска ефикасност биомасе анализираних клонова брзорастућих врста врба у процесима косагоревања са лигнитом у различитим процентуалним односима, а све у циљу повећања калоријске вредности лигнита бољег сагоревања и смањења штетних ефеката сагоревања. Добијени резултати указују да је калоријска вредност врба виша од калоријских вредности угља. Калоријска вредност угља (лигнита) зависи од налазишта угља (поља), док калоријска вредност врба зависи од врста врба. Утврђено је да клонови 347 и NS 73/6 беле врбе (*Salix alba*), поседују највећи енергетски потенцијал у поређењу са клоновима B-44 беле врбе и кошарачке врбе (*S. viminalis*).

КЉУЧНЕ РЕЧИ: биомаса, врбе, декарбоксилација, енергетски потенцијал, косагоревање, угаљ

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EFFECT OF PEG-INDUCED DROUGHT STRESS ON SEED GERMINATION AND INITIAL GROWTH OF THREE CUCUMBER CULTIVARS

ABSTRACT: As the most common abiotic stress, drought seriously affects crop production. Since the impact of drought stress varies among species, this study aims to evaluate drought tolerance of domestic cucumber cultivars in the initial stages of growth under PEG-induced drought conditions. The germination assay was performed using three different cucumber varieties (Tajfun, Sunčani Potok and NS Kir) at four different drought levels (0, -0.15, -0.49, and -1.03 MPa). The results demonstrated that all the tested cucumber cultivars are sensitive to drought during germination and initial plant growth, while the osmotic potential of -0.49 MPa can be considered the tolerance threshold for cucumber.

KEYWORDS: *Cucumis sativus* L., initial seedling growth, PEG-induced drought, seed germination, seedling vigour index

INTRODUCTION

Drought is one of the most common abiotic stresses that adversely affect agriculture and food security in the era of climate change. Due to global warming, water loss occurs both in the soil and at the plant level (Seleiman et al., 2021). According to current estimates, one-fifth of the world's population will be affected by a serious water deficit as the air temperature increases by an estimated 2 °C above the current level (Ray et al., 2019; Seleiman et al., 2021). The severity of drought is determined by many factors, including the amount of rainfall and its distribution, soil moisture-holding capacity and evaporative requirements (Khan et al., 2018). According to Radić et al. (2018), up to 70% of the total arable land is affected by drought.

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The impact of drought on crop production depends on the plant species and the growth stage. Many crops are highly vulnerable to water deficit if it occurs at germination or seedling growth. Since seed germination is related to vegetative and generative plant growth in later stages, low moisture availability during seed germination impedes plant distribution and diversity (Lu et al., 2022). Previous studies demonstrated that drought may have detrimental effects on the seed germination and vegetative growth of rice (Evamoni et al., 2023), maize (Radić et al., 2018), wheat (Bayoumi et al., 2008), carrot and watermelon (Steiner and Zuffo, 2018) and other species, with significant differences between the tested varieties. Different taxa may exhibit distinct adaptation methods of survival under drought stress (Bhatt et al., 2020; 2022). Assessment of plant drought tolerance during seed germination can give insights into the patterns of population persistence and community assembly, as well as clarifying the potential effects of climate change on these attributes (Bhatt et al., 2022).

However, establishing and preserving pure water potential in soil, in order to assess drought tolerance, is almost impossible (Hellal et al., 2018). One of the most effective ways to examine the effects of drought stress on germination is to induce osmotic potential using various osmotic materials, such as polyethylene glycol (PEG), mannitol or salt treatments. Polyethylene glycol (PEG-6000), a harmless, non-ionic polymer with a defined molecular weight, causes drought stress without affecting cells or causing physiological injuries (Qi et al., 2023). PEG 6000 is often used in this type of research because it has excellent water-absorbing properties and may simulate drought stress by dehydrating plant cells (Murillo-Amador et al., 2002).

Cucumber (*Cucumis sativus* L.) is one of the most significant vegetable crops in the world. According to Campobenedetto et al. (2020) and Das et al. (2024), cucumber is the most cultivated vegetable globally and the fourth vegetable most frequently consumed in salads. As reported by Liu et al. (2016) and Kim et al. (2019), cucumbers have a high transpiration rate and sensitivity to drought. Plant growth and fruit yield of cucumbers are adversely affected by exposure to soil water deficits, which may also result in decreased fruit quality (Farag et al., 2019). Different osmotic potentials of PEG solution, which represent the tolerance threshold of cucumber, vary depending on the genotypes and range from 0.5% to 10% PEG solution (De Suoza Neta et al., 2024; Li et al., 2022). In Serbia, information about drought sensitivity and drought tolerance threshold for domestic cucumber cultivars is currently lacking. The study aims to identify the tolerance thresholds for domestic cucumber cultivars and investigate their susceptibility to drought.

MATERIALS AND METHODS

Experimental (Plant) materials

The seeds of three different cucumber cultivars (Tajfun, Sunčani Potok, and NS Kir) were obtained from the Department of Vegetable and Alternative

Crops, Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad. Seeds of all the examined cucumber cultivars were produced at the Rimski Šančevi experimental field of the Institute of Field and Vegetable Crops, Novi Sad, in 2023.

PEG-induced drought

Three different polyethylene glycol (PEG 6000) (Sigma Aldrich, St. Louis, MO, USA) treatments, -0.15, -0.49, and -1.03 MPa, were used to assess the response of cucumber cultivars to artificial drought. The respective solutions were prepared according to the procedures set by Money et al., (1989). Distilled water was used as a control.

Germination assay

Prior to the germination test, cucumber seeds were disinfected with 5% (v/v) sodium hypochlorite for 5 minutes and then rinsed thoroughly with distilled water thrice. Working samples consisted of 3 x 100 seeds. The cucumber seeds were placed in plastic boxes (240 × 150 mm) with filter paper moistened with the respective solutions. The samples were incubated for eight days in a germination chamber at 25 °C (ISTA, 2024). Germination energy, i.e. germination first count, was determined four days after seed placement in the germination chamber by counting the normal seedlings with well-developed essential structures. Final germination, abnormal seedlings, shoot and root length, and fresh seedling weight of the 10 normal seedlings were determined eight days after seed placement in the germination chamber (Conviron CMP 4030, Winnipeg, Canada). Dry weight of the cucumber seedlings was determined after drying the seedlings in an oven at 80 °C for 24 hours (Tamindžić et al., 2023).

The seedling vigour index (SVI) was determined according to the procedure of Abdul-Baki and Anderson (1973) and calculated using the following formula:

$$\text{SVI} = \text{Final Germination (\%)} \times \text{Seedling Length (cm)}$$

Following the procedure of Channaoui et al. (2019), the shoot elongation rate (SER) and root elongation rate (RER) were determined using the following formulas:

$$\text{SER} = \frac{\text{Shoot length on the } 8^{\text{th}} \text{ day} - \text{Shoot length on the } 4^{\text{th}} \text{ day}}{\text{Time duration between two measurements (days)}}$$

$$\text{RER} = \frac{\text{Root length on the } 8^{\text{th}} \text{ day} - \text{Root length on the } 4^{\text{th}} \text{ day}}{\text{Time duration between two measurements (days)}}$$

Statistical analysis

The statistical software Statistica 10 (StatSoft, Inc., 2007) was used to perform an analysis of variance. Duncan's multiple range test was employed to separate the mean values at the probability level of $p<0.05$.

RESULTS AND DISCUSSION

The results showed that cucumber cultivars differed in their response to drought stress (Table 1). As confirmed by the analysis of variance, drought level significantly altered all the tested parameters of the different cucumber cultivars. Moreover, all the tested parameters were significantly affected by the cultivar and cultivar × drought interaction.

Table 1. Two-way analysis of variance for the examined parameters of three cucumber cultivars under different artificial drought conditions

Trait	Cultivar	Drought level	Cultivar × drought level
Germination Energy	0.0000***	0.0000***	0.0000***
Final Germination	0.0000***	0.0000***	0.0000***
Abnormal Seedlings	0.0000***	0.0000***	0.0003***
Shoot Length	0.0000***	0.0000***	0.0000***
Root Length	0.0000***	0.0000***	0.0000***
Fresh Seedlings Weight	0.0000***	0.0000***	0.0000***
Dry Seedlings Weight	0.0000***	0.0000***	0.0000***
Seedling Vigour Index	0.0000***	0.0000***	0.0000***
Shoot Elongation Rate	0.0000***	0.0000***	0.0000***
Root Elongation Rate	0.0000***	0.0000***	0.0000***

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns – non-significant

In general, drought caused a decrease in all the examined parameters at a certain level (Table 2; Table 3). The control groups of the examined cucumber cultivars showed differences in germination energy, which ranged between 87.7% and 93.0%. The decrease in osmotic potential led to a decrease in germination energy compared to the control. A significant decrease was observed in cv. Tajfun and cv. Sunčani Potok at -0.49 MPa, while a significant decrease in germination energy occurred in cv. NS Kir at an osmotic potential of -1.03 MPa. Cucumber cultivars differed in their final germination as well; cv. Sunčani Potok had the lowest germination percentage of 88.0% in control, while the highest final germination was recorded in cv. NS Kir (96.0%). Final germination decreased in all the tested cultivars with the decrease in osmotic potential.

Compared to the control, a significant decrease in final germination was observed under drought at an osmotic potential of -0.49 MPa, while the greatest decrease was recorded at an osmotic potential of -1.03 MPa, which was significantly lower compared to control and at other tested drought levels. A similar pattern was also observed in the abnormal seedling occurrence. A significant increase in abnormal seedlings was noted under moderate drought (-0.49 MPa), while the highest was observed under severe drought (-1.03 MPa). Numerous studies confirmed the detrimental effects of drought on seed germination of different crops, such as carrot and eggplant (Steiner and Zuffo, 2018), pea (Petrović et al., 2021), maize (Radić et al., 2018), tomato (Esan et al., 2018) and spinach (Zargar et al., 2023), which is in agreement with the results obtained in the study. Germination is reduced under drought conditions due to reduced water infusibility via the seed coat and hampered water absorption (Channaoui et al., 2019; Hossain et al., 2024). Various factors, such as decreased water availability which impedes reserve mobilization, hormonal and enzymatic activities, respiration and protoplasm dilution required for effective embryonic growth, are considered responsible for the decrease in germination percentage (Li et al., 2021; Hossain et al., 2024).

Furthermore, initial growth was also affected by drought (Table 2). However, the response of cucumber cultivars to drought levels varied in terms of the shoot or root length. Shoot length of cv. Tajfun significantly decreased with the decrease of osmotic potential at -0.49 MPa and -1.03 MPa, while shoot length decreased gradually with the decrease of osmotic potential in cv. Sunčani Potok and cv. NS Kir. A similar pattern of root length decrease was observed in cv. Sunčani Potok and cv. NS Kir, where a significant reduction of root length was observed at -0.49 MPa and -1.03 MPa, compared to the control. In contrast, root length of cv. Tajfun decreased gradually with the decrease of osmotic potential. Shoot and root length of the tested cucumber cultivars, as crucial indicators of drought resistance, demonstrated that these cultivars are drought-sensitive, especially at a lower osmotic potential of PEG-induced drought. Cucumber cultivars also differed in their response to different levels of osmotic stress, indicating different levels of resistance. These findings corroborate the previous studies conducted on wheat (Peršić et al., 2022), maize (Mustamu et al., 2023) and tartary buckwheat (Hossain et al., 2024), indicating that seedling growth gradually declines as PEG concentration increases.

Table 2. Germination and initial plant growth parameters of cucumber cultivars under different artificial drought conditions

Cucumber cultivar	Drought Level	Germination Energy (%)	Final Germination (%)	Abnormal Seedlings (%)	Shoot Length (mm)	Root Length (mm)
Tajfun	Control	87.7 ± 1.5 a	93.3 ± 1.2 a	5.0 ± 1.5 c	47.7 ± 1.7 a	78.0 ± 2.0 a
	-0.15 MPa	86.7 ± 1.2 a	93.0 ± 1.0 a	6.3 ± 0.6 bc	46.8 ± 1.6 a	75.2 ± 1.0 b
	-0.49 MPa	73.7 ± 1.2 b	78.3 ± 0.6 b	8.0 ± 1.0 b	29.2 ± 0.3 b	50.3 ± 1.0 c
	-1.03 MPa	70.0 ± 1.0 c	72.3 ± 2.5 c	12.3 ± 0.6 a	27.8 ± 0.3 b	47.3 ± 0.8 d
	p	0.0000	0.0000	0.0000	0.0000	0.0000
Sunčani Potok	Control	82.3 ± 2.1 a	88.0 ± 1.0 a	5.7 ± 1.2 c	63.2 ± 1.7 a	57.7 ± 1.8 a
	-0.15 MPa	82.0 ± 1.5 a	87.3 ± 0.6 a	6.3 ± 0.6 c	58.7 ± 1.3 b	57.0 ± 1.7 a
	-0.49 MPa	70.7 ± 2.1 b	74.3 ± 0.6 b	11.0 ± 1.0 b	39.3 ± 0.6 c	52.0 ± 1.3 b
	-1.03 MPa	72.0 ± 1.0 b	72.7 ± 0.6 c	14.7 ± 1.2 a	25.5 ± 0.5 d	48.0 ± 1.3 c
	p	0.0000	0.0000	0.0000	0.0000	0.0000
NS Kir	Control	93.0 ± 2.0 a	96.0 ± 1.7 a	1.7 ± 0.6 b	74.2 ± 0.8 a	114.3 ± 0.8 a
	-0.15 MPa	93.0 ± 0.0 a	95.3 ± 1.2 a	2.0 ± 1.0 b	69.3 ± 0.8 b	113.0 ± 1.0 a
	-0.49 MPa	90.3 ± 1.2 a	91.7 ± 1.5 b	4.3 ± 0.6 a	46.3 ± 1.3 c	70.7 ± 2.6 b
	-1.03 MPa	86.3 ± 1.5 b	90.0 ± 1.0 c	5.3 ± 0.6 a	38.0 ± 0.5 d	58.3 ± 2.0 c
	p	0.0000	0.0000	0.0000	0.0000	0.0000

Data are presented as Means ± SD (n=3). Means within each trait followed by the same letters are not significantly different (Duncan's multiple range test, $p \leq 0.05$).

Regarding biomass accumulation, in cv. Tajfun and cv. NS Kir, an osmotic potential of -1.03 MPa, followed by -0.49 MPa significantly reduced fresh and dry seedling weight compared to the control (Table 3). In cv. Sunčani Potok, fresh seedling weight decreased gradually with the decreased osmotic potential, while dry seedling weight was reduced only at -1.03 MPa. Lower reduction in biomass accumulation during drought suggests that a genotype is more resistant to drought. Similar results were also obtained on tartary buckwheat (Hossain et al., 2024).

Seedling vigour index decreased gradually with the decrease in osmotic potential in all tested cultivars (Table 3). Hellal et al. (2018) also pointed out that seedling vigour index decreases with an increase in drought stress, which is in accordance with our results. Additionally, Magar et al. (2019) reported that PEG treatment up to -1.5 MPa reduced maize seedling vigour index, while Hellal et al. (2018) also observed the same reduction in barley.

The tested cultivars responded differently to osmotic stress in terms of the shoot elongation rate (SER) (Table 3). A significant decrease in SER was observed in cv. Sunčani Potok at -0.15 MPa and lower and in cv. Tajfun and cv. NS Kir at -0.49 MPa and -1.03 MPa, respectively. Contrary to this, all tested cultivars had a significantly decreased root elongation rate (RER) at an osmotic potential of -0.49 MPa and lower compared to the control, while no significant decrease was observed at mild stress levels. In this regard, PEG concentration proved to have a major impact on the histological size variations of root tissues, including the cortex, stele and epidermis (Mustamu et al., 2023). According to Zeid and Shedeed (2006), plant growth is a complex process of cell division and cell growth. It is considered one of the most drought-sensitive physiological processes due to the reduction of turgor pressure. The reduction in growth occurs due to impaired mitosis, cell elongation and expansion caused by drought (Wach and Skowron, 2022). Despite differences in cultivar response to certain drought stress levels, the results of shoot and root elongation rate confirmed drought sensitivity in all the tested cucumber cultivars.

Table 3. Biomass accumulation and growth-related parameters of cucumber cultivars under different artificial drought conditions

Cucumber Cultivar	Drought Level	Fresh Seedling Weight (g)	Dry Seedling Weight (g)	Seedling Vigour Index (SVI)	Shoot Elongation Rate (SER)	Root Elongation Rate (RER)
Tajfun	Control	2.84 ± 0.04 a	0.294 ± 0.001 a	1172.9 ± 22.2 a	8.58 ± 0.6 a	12.69 ± 0.6 a
	-0.15 MPa	2.82 ± 0.05 a	0.292 ± 0.003 a	1134.6 ± 23.2 b	8.51 ± 0.3 a	12.04 ± 0.4 a
	-0.49 MPa	1.87 ± 0.04 b	0.185 ± 0.001 b	622.8 ± 9.1 c	4.91 ± 0.1 b	7.48 ± 0.4 b
	-1.03 MPa	1.71 ± 0.03 c	0.176 ± 0.005 c	543.7 ± 20.7 d	4.58 ± 0.1 b	6.83 ± 0.2 b
	p	0.0000	0.0000	0.0000	0.0000	0.0000
Sunčani Potok	Control	2.66 ± 0.05 a	0.206 ± 0.003 a	1063.4 ± 24.3 a	12.5 ± 0.3 a	8.25 ± 0.5 a
	-0.15 MPa	2.54 ± 0.04 b	0.207 ± 0.006 a	1010.1 ± 24.0 b	11.4 ± 0.3 b	8.42 ± 0.3 a
	-0.49 MPa	2.28 ± 0.01 c	0.198 ± 0.006 a	678.9 ± 17.7 c	7.60 ± 0.1 c	5.92 ± 0.5 b
	-1.03 MPa	1.62 ± 0.03 d	0.174 ± 0.005 b	535.3 ± 11.2 d	4.56 ± 0.1 d	5.10 ± 0.5 b
	p	0.0000	0.0000	0.0000	0.0000	0.0000
NS Kir	Control	2.96 ± 0.03 a	0.286 ± 0.003 a	1809.5 ± 21.6 a	9.79 ± 0.4 b	11.68 ± 0.4 a
	-0.15 MPa	2.89 ± 0.02 a	0.284 ± 0.003 a	1738.3 ± 19.8 b	11.17 ± 0.6 a	11.42 ± 0.4 a
	-0.49 MPa	2.80 ± 0.07 b	0.264 ± 0.008 b	1072.7 ± 46.9 c	8.27 ± 0.2 c	4.83 ± 1.1 b
	-1.03 MPa	2.47 ± 0.01 c	0.224 ± 0.003 c	867.1 ± 22.7 d	7.07 ± 0.2 d	3.75 ± 0.3 b
	p	0.0000	0.0000	0.0000	0.0000	0.0000

Data are presented as Means ± SD (n=3). Means within each trait followed by the same letters are not significantly different (Duncan's multiple range test, $p \leq 0.05$).

CONCLUSION

Based on the obtained results, it can be concluded that domestic cucumber cultivars are susceptible to drought. All tested cultivars can withstand mild drought in the initial phase of plant growth, such as seed germination and initial seedling growth. However, all examined parameters of the tested cucumber cultivars declined at an osmotic potential of -0.49 MPa (moderate drought), which can be considered as the tolerance threshold for cucumber.

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ОРИГИНАЛНИ ЧЛАНАК
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УТИЦАЈ ПЕГ-ИНДУКОВАНЕ СУШЕ НА КЛИЈАВОСТ СЕМЕНА И ПОЧЕТНИ ПОРАСТ БИЉАКА ТРИ СОРТЕ КРАСТАВЦА

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САЖЕТАК: Као најчешћи абиотички стрес, суша озбиљно утиче на производњу усева. С обзиром да утицај стреса од суше варира међу врстама, ова студија је имала за циљ да процени толеранцију домаћих сорти краставца на сушу у почетним фазама раста у условима вештачке суше. Лабораторијско испитивање је изведено коришћењем три различите сорте краставца (Тајфун, Сунчани поток и НС Кир) и четири различита нивоа суше (0, -0,15, -0,49 и -1,03 MPa). Резултати су показали да су све испитиване сорте краставца осетљиве на сушу у фази клијања и почетног пораста биљака, као и да се осмотски потенцијал од -0,49 MPa може сматрати прагом толеранције за краставац.

КЉУЧНЕ РЕЧИ: *Cucumis sativus* L., индекс вигора семена, клијавост семена, почетни пораст изданака, PEG-индукована суша

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Anyone may inform the editors and/or Editorial Staff at any time of suspected unethical behavior or any type of misconduct by giving the necessary information/evidence to start an investigation.

Investigation

• Editor-in-Chief will consult with the Subject Editors on decisions regarding the initiation of an investigation.

• During an investigation, any evidence should be treated as strictly confidential and only made available to those strictly involved in investigating.

• The accused will always be given the chance to respond to any charges made against them.

• If it is judged at the end of the investigation that misconduct has occurred, then it will be classified as either minor or serious.

Minor misconduct

Minor misconduct will be dealt directly with those involved without involving any other parties, e.g.:

• Communicating to authors/reviewers whenever a minor issue involving misunderstanding or misapplication of academic standards has occurred.

• A warning letter to an author or reviewer regarding fairly minor misconduct.

Major misconduct

The Editor-in-Chief, in consultation with the Subject, and, when appropriate, further consultation with a small group of experts should make any decision regarding the course of action to be taken using the evidence available. The possible outcomes are as follows (these can be used separately or jointly):

• Publication of a formal announcement or editorial describing the misconduct.

• Informing the author's (or reviewer's) head of department or employer of any misconduct by means of a formal letter.

• The formal, announced retraction of publications from the journal in accordance with the Retraction Policy (see below).

• A ban on submissions from an individual for a defined period.

• Referring a case to a professional organization or legal authority for further investigation and action.

When dealing with unethical behavior, the Editorial Staff will rely on the guidelines and recommendations provided by the Committee on Publication Ethics (COPE).

Retraction policy

Legal limitations of the publisher, copyright holder or author(s), infringements of professional ethical codes, such as multiple submissions, bogus claims of authorship, plagiarism, fraudulent use of data or any major misconduct require retraction of an article. Occasionally a retraction can be used to correct errors in submission or publication. The main reason for withdrawal or retraction is to correct the mistake while preserving the integrity of science; it is not to punish the author.

Standards for dealing with retractions have been developed by a number of library and scholarly bodies, and this practice has been adopted for article retraction by MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES: in the electronic version of the retraction note, a link is made to the original article. In the electronic version of the original article, a link is made to the retraction note where it is clearly stated that the article has been retracted. The original article is retained unchanged; save for a watermark on the PDF indicating on each page that it is "retracted."

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INSTRUCTIONS FOR AUTHORS

1. General remarks

1.1. Matica Srpska Journal for Natural Sciences (short title: Matica Srpska J. Nat. Sci.) publishes manuscripts and review articles as well as brief communications from all scientific fields as referred to in the title of the journal. Review articles are published only when solicited by the editorial board of the journal. Manuscripts that have already been published in extenso or in parts or have been submitted for publication to other journal will not be accepted. The journal is issued twice a year.

1.2. The manuscripts should be written in correct English language regarding the grammar and style. The manuscripts should be submitted electronically as a separate file to npavlovic@maticasrpska.org.rs and enclosed with the author's written consent for the publishing of the manuscript.

1.3. Upon the reception of the manuscript, the author shall be assigned with a manuscript code, which has to be referred to in any further correspondence. The authors will be notified about the manuscript reception within seven days and about the reviewers' opinion within two months from submission. All submitted manuscripts are reviewed and proofread.

2. Planning and preparing of the manuscript

2.1. Type the manuscripts electronically on A4 (21 × 29.5 cm) format with 2.5 cm margins, first line indent, and 1.5 line spacing. When writing the text, the authors should use Times New Roman size 12 font and when writing the abstract, key words, summary, and footnotes use font size 10.

2.2. First name, middle initial and last name should be given for all authors of the manuscript and their institutional affiliations, institution name, and mailing address. In complex organizations, a full hierarchy should be mentioned (e.g. University of Novi Sad, Faculty of Sciences – Department of Biology and Ecology). The institution of employment of each author should be stated below the author's name. The position and academic degrees should not be cited. If there is more than one author, indicate separately institutional affiliation for each of the authors. Put the name and mailing address (postal or e-mail address) of the author responsible for correspondence at the bottom of the first page. If there is more than one author, write the address of only one author, usually the first one.

2.3. Structure the text of the original articles into Abstract, Key Words, Introduction, Material or Methods, or Material and Methods, Results or Results and Discussion, Discussion, Conclusion, References, Summary and Key Words in Serbian language, and Acknowledgement (if there is one). Original articles should not be longer than 10 pages, including the references, tables, legends, and figures.

2.4. Titles should be informative and not longer than 10 words. It is in the best interest of the authors and the journal to use words in titles suitable for indexing and electronic searching of the article.

2.5. The authors should submit the title of the article with last name and the initials of the first author.

(if the article has more than one author, et al. should be used for other authors) and running title of not more than five words.

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2.8. Write the information about financial support, advices, and other forms of assistance, if necessary, at the end of the article under the Acknowledgement. Financial support acknowledgement should contain the name and the number of the project, i.e. the name of the program from which the article originated, and the name of the institution that provided the financial support. In case of other forms of assistance the author should submit the first name, middle initial, last name, institutional affiliation, and the address of the person providing the assistance or the full name and the address of the assisting institution.

3. Structure the Review articles in Abstract, Key Words, Text of the manuscript, Conclusion, and References; submit Summary and Key Words in Serbian language.

4. Write brief communication according to the instructions for original articles but not be longer than five pages.

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(d) Dissertations: Last name VA (2009): *Title of the thesis*. Doctoral dissertation, City: University

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6.1. SI units of measurement (Système international d'unités) should be used but when necessary use other officially accepted units.

6.2. Write the names of living organisms using Italics font style.

6.3. Abbreviated form of a term should be put into parenthesis after the full name of the term first time it appears in the text.

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