



AGROSYM

# BOOK OF PROCEEDINGS



*XVI International Scientific Agriculture Symposium  
"Agrosym 2025"  
Jahorina, October 2-5, 2025*



AGRO 2025  
sym

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## FOREWORD

The world is facing a range of crises that require policymakers to base their decisions on scientific data and evidence to effectively address them. Issues such as climate change, loss of biodiversity, degradation of natural resources (e.g. land and water), ongoing conflicts, persistent inequalities, and economic disruptions are progressively weakening the ability of agri-food systems to ensure food security and nutrition for all. Research plays a vital role in providing evidence and technical expertise, acting as a facilitator and policy broker in the development of agri-food strategies, investment planning, innovation policies, and other important policy processes. Additionally, research is essential for generating the knowledge, evidence, and resources required to promote responsible and inclusive innovation. It also offers support to a range of stakeholders, including farmers' organizations, businesses, and civil society groups. Integrating research with long-term policy strategies can enhance the acceptance and adoption of innovations in the agri-food sector, particularly when these innovations are promoted through inclusive platforms. To achieve a sustainable impact at scale, it is essential to improve collaboration in agri-food research and innovation. Scientific events and gatherings can be crucial in reaching this objective.

The International Agriculture Symposium AGROSYM has served as an annual forum for sixteen years, facilitating global scientific discussions on agriculture, food, rural development, the environment, and forestry. This symposium offers an excellent opportunity to share ideas, strengthen existing networks, establish new academic connections, and stimulate dialogue among academia, public institutions, the private sector, and civil society organizations. Participants engage in discussions about recent global and regional trends in the agri-food sector. The multidisciplinary findings presented at AGROSYM contribute to the dissemination of knowledge and best practices among all stakeholders in the agri-food chain, including farmers, extension agents, researchers, and policymakers. Additionally, the symposium emphasizes the importance of agriculture and food science as vital components of many national research strategies, impacting the broader community as well.

This Book of Proceedings from the 16<sup>th</sup> International Scientific Agricultural Symposium “AGROSYM 2025” encompasses articles which illustrate a wide range of research and insights across the comprehensive spectrum of agricultural disciplines. These articles address the following themes: 1) Plant production, 2) Plant protection and food safety, 3) Organic agriculture and agroecology, 4) Environmental protection and natural resources management, 5) Animal husbandry, 6) Rural development and agro-economy, and 7) Forestry and agroforestry.

We wish to express our heartfelt appreciation to all authors, reviewers, and colleagues who assisted in preparing this Book of Proceedings. Our special thanks go to co-organizers, partners, and sponsors for their unwavering support and collaboration.

We hope this multidisciplinary and international collection of research findings serves as a valuable resource for all stakeholders and encourages continuous dialogue and exchange to facilitate the transition towards sustainable, resilient, and inclusive agri-food systems.

East Sarajevo, 1 November 2025

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## CONTENT

<b>PLANT PRODUCTION .....</b>	<b>27</b>
<b>SEED GERMINATION OF LENTIL GENOTYPES UNDER SALINITY STRESS AND NANO-SILICON APPLICATION</b>	
Naser SABAGHNIA, Mohsen JANMOHAMMADI, Fariborz SHEKARI.....	28
<b>USING GPS AND GIS IN PRECISION AGRICULTURE OPTIMIZATION IN A HUNGAIAN FARM</b>	
Ahmed EL SHAL , Péter RICZU, János TAMÁS .....	31
<b>ANALYSYS AND REVIEW OF RESPONSES AGRICULTURAL PLANTS TO HUMIC SUBSTANCES</b>	
Mirjana JOVOVIĆ, Zoranka MALEŠEVIĆ, Aleksandra GOVEDARICA – LUČIĆ.....	37
<b>RESISTANCE OF TOMATO GENOTYPES TO LATE BLIGHT (<i>Phytophthora infestans</i>) UNDER CONDITIONS OF NATURAL INFECTION</b>	
Milomirka MADIĆ, Nenad PAVLOVIĆ, Milan ŠEVIĆ, Vladeta STEVOVIĆ, Dalibor TOMIĆ, Branka GOVEDARICA, Dragan ĐUROVIĆ .....	46
<b>ADAPTABILITY AND STABILITY OF PROMOSING BREAD WHEAT LINES USING GGE BILOT ANALYSIS</b>	
Damla BALABAN GÖÇMEN, Oğuz BİLGİN, Alpay BALKAN, İsmet BAŞER.....	52
<b>RELATIONSHIPS BETWEEN YIELD AND YIELD TRAITS IN WHEAT UNDER DIFFERENT DROUGHT APPLICATIONS</b>	
İsmet BAŞER, Alpay BALKAN, Oğuz BİLGİN, Damla BALABAN GÖÇMEN, Kamil ÖZCAN.....	60
<b>THE INFLUENCE OF SOIL TYPE ON THE QUALITY TRAITS OF POTATO</b>	
Tanja JAKIŠIĆ, Milan JUGOVIĆ, Branka GOVEDARICA, Igor ĐURĐIĆ, Nevena RISTIČEVIĆ, Selena KOVAČEVIĆ .....	66
<b>COMPARATIVE ANALYSIS OF ESSENTIAL OIL OF <i>HELICHRYSUM ITALICUM</i> L. FROM PLANTATION AND RECLAIMED SOILS</b>	
Zoranka MALEŠEVIĆ, Jelena LAZAREVIĆ, Mirjana JOVOVIĆ, Selena KOVAČEVIĆ, Ivana BOŠKOVIĆ .....	72
<b>THE EFFECT OF ZEOLITE TYPE AND DOSAGE ON PEA SEED GERMINATION AND EARLY GROWTH TRAITS</b>	
Nevena RISTIČEVIĆ, Branka GOVEDARICA, Igor ĐURĐIĆ, Vesna MILIĆ, Tanja JAKIŠIĆ, Selena KOVAČEVIĆ, Aleksandar GAVRILOVIĆ .....	78
<b>NDVI-BASED ASSESSMENT OF PHYSIOLOGICAL STATUS IN PROKUPAC VINEYARDS UNDER DIFFERENT CROP PROTECTION STRATEGIES</b>	
Marija GAVRILOVIĆ, Vedran TOMIĆ, Biljana VELJKOVIĆ, Danijela ŽIVOJINOVIĆ, Anđelija OBRADOVIĆ .....	84
<b>EFFECTS OF ABIOTIC STRESS CONDITIONS ON GENE EXPRESSION IN <i>SOLANACEAE</i> SPECIES</b>	
Akife DALDA-SEKERCI, Hande Seda ÖZDAL .....	92

**RESPONSE OF WINTER WHEAT TO REDUCED TILLAGE AND FERTILIZATION**

Željko DOLIJANOVIĆ, Dušan KOVAČEVIĆ, Milena SIMIĆ, Snežana OLJAČA, Zoran JOVOVIĆ, Milena BILJIĆ ..... 101

**ANALYSIS OF THE FERTILITY POTENTIAL OF RED WINE VARIETIES GROWN IN THE TREBINJE AREA (BOSNIA AND HERZEGOVINA)**

Tijana BANJANIN, Milica GLIŠIĆ, Kristina MILIŠIĆ, Zorica RANKOVIĆ VASIĆ 108

**RESEARCH OF AGRICULTURE LAND FOR IRON (Fe) CONTENT IN THE AREA OF THE CITY OF SOMBOR IN SERBIA**

Vladimir SABADOŠ, Danijela ŽUNIĆ ..... 115

**IN VITRO PROPAGATION OF EUROPEAN VARIETIES OF *ARONIA MELANOCARPA* (MICHX.) ELLIOTT 'NERO' AND 'ALEXANDRINA'**

Ioana-Cătălina NICOLAE, Tatiana CALALB, Nina CHIORCHINĂ, Maria TABĂRA, Natalia ONICA, Iulia BOZBEI ..... 122

**BOTANICAL COMPOSITION OF PERMANENT GRASSLAND AND FORAGE QUALITY IN THE MUNICIPALITY OF LUČANI IN REPUBLIC OF SERBIA**

Vladimir ZORNIĆ, Nedeljko RACIĆ, Đorđe LAZAREVIĆ, Zoran LUGIĆ, Dalibor TOMIĆ, Nenad PAVLOVIĆ, Mirjana PETROVIĆ..... 129

**EFFECT OF NITROGEN FERTILIZERS WITH UREASE INHIBITORS ON THE QUALITY CHARACTERISTICS OF WINTER CEREALS**

Dimitrios BARTZIALIS, Stefania PASCHALI-PAPANASTASIOU, Dimitrios-Marios LEKKAS, Ippolitos GINTSIODIS, Kyriakos D. GIANNOULIS, Nikolaos G. DANALATOS ..... 135

**EFFECT OF BENZYLADENINE (BA) ON GROWTH AND FLOWER QUALITY OF PURPLE - PINK PICO (*Chrysanthemum* sp.)**

Pham THI PHUONG THAO, Le THANH TOAN, Le THI HOANG YEN, Le THI MONG CAM ..... 141

**INFLUENCE OF INTERCROPPING MAIZE WITH CLIMBING BEAN ON FORAGE YIELD AND QUALITY**

Darko UHER, Dubravko MACESIĆ, Goran KIŠ, Dario JAREŠ, Ivan HORVATIĆ.... 148

**CLASSIFICATION OF COMBINED STRESSES IN AUBERGINE (*SOLANUM MELONGENA* L.) USING MACHINE LEARNING AND HYPERSPECTRAL IMAGING**

Maria BEMPI, Aris KYPARISSIS ..... 154

**USE OF META-TOPOLIN AS AN ALTERNATIVE CYTOKININ FOR *IN VITRO* MULTIPLICATION OF *ACTINIDIA KOLOMIKTA***

Ioana-Cătălina NICOLAE, Maria TABĂRA, Melania GHEREG, Natalia ONICA, Oana VENAT, Liliana BĂDULESCU, Nina CHIORCHINĂ..... 161

**THE INFLUENCE OF DIFFERENT METHODS OF CALCIUM FERTILIZATION IN THE SOIL ON THE YIELD AND THE BLOSSOM-END ROT (BER) IN PEPPER FRUIT**

Aleksandra GOVEDARICA-LUČIĆ, Mirjana JOVOVIĆ, Alma MEMIĆ, Dragica GAGOVIĆ ..... 167

**SELECTED BREEDING LINES OF SUNFLOWER SEEDS IN REDUCING DROUGHT STRESS ON MORPHOLOGICAL CHARACTERISTICS DURING THE GERMINATION PHASE**

Tanja MIJATOVIĆ, Branka GOVEDARICA, Igor ĐURĐIĆ, Nevena RISTIČEVIĆ, Vesna MILIĆ ..... 172

**ORCHIDACEAE IN THE FLORA OF THE TREBEVIĆ NATURE PARK (BOSNIA AND HERZEGOVINA)**

Natasa MARIC, Sladjana PETRONIC ..... 180

**CONSERVATION STATUS OF THE MACROPHYTES OF THE TIŠINA PROTECTED HABITAT (BOSNIA AND HERZEGOVINA)**

Natasa MARIC, Sladjana PETRONIC, Zoran MATIC..... 187

**THE EFFECTS OF FERTILIZATION METHODS ON THE MECHANICAL COMPOSITION OF GRAPES OF THE VINE VARIETY VRANAC**

Nedim MARIĆ, Semira SEFO ..... 193

**ASSESSMENT OF DROUGHT IMPACT ON TRITICALE (X TRITICOSECALE WITTMAK) YIELD ACROSS VARIOUS ENVIRONMENTS**

Zakaria AL AJLOUNI..... 199

**BIOSTIMULANTS EFFECT ON LAVENDER (*LAVANDULA ANGUSTIFOLIA*) CULTIVATION IN THE SECOND GROWING YEAR**

Maria DALAKOURA, Kyriakos D. GIANNOULIS, Dimitrios BARTZIALIS, Ippolitos GINTSIODIS, Nikolaos G. DANALATOS ..... 204

**EFFECT ON THE YIELD OF THREE VARIETIES OF CHAMOMILE (*MATRICARIA CHAMOMILLA* L.) USING DIFFERENT BIOSTIMULANTS**

Anastasia-Charikleia KYRIAZAKI, Kyriakos D. GIANNOULIS, Dimitrios BARTZIALIS, Ippolitos GINTSIODIS, Nikolaos G. DANALATOS..... 210

**PROPAGATION OF *CORNUS ALBA* 'SIBIRICA' BY SOFTWOOD HEEL CUTTINGS**

Marija MARKOVIĆ, Mihailo GRBIĆ, Dragana SKOČAJIĆ, Danijela ĐUNISIJEVIĆ-BOJOVIĆ, Marijana MILUTINOVIĆ ..... 216

**MULTIVARIATE ANALYSIS OF BREAD WHEAT ROOT AND SHOOT TRAITS AT THE SEEDLING STAGE AND AT THE BOOTING STAGE FOR DROUGHT-TOLERANCE**

Milica BLAŽIĆ, Gordana BRANKOVIĆ, Dejan DODIG, Vesna KANDIĆ, Tomislav ŽIVANOVIĆ ..... 220

**COMPUTATIONAL INTELLIGENCE FOR INVESTIGATION ON GENETIC DIVERSITY AMONG GENOTYPES OF SAFFLOWER FOR YIELD PERFORMANCE**

Naser SABAGHNIA, Fariborz SHEKARI, Mohsen JANMOHAMMADI..... 228

**RESPONSE OF DRAGONHEAD TO SOWING DENSITY REGARDING ESSENTIAL OIL YIELD AND SOME BIOCHEMICAL TRAITS**

Naser SABAGHNIA, Mohsen JANMOHAMMADI, Fariborz SHEKARI..... 233

<b>FUNGAL COMMUNITY ASSOCIATED WITH SWEET POTATO ROOTS DURING STORAGE</b>	
Beatrice Michaela IACOMI, Elena DOBRIN, Lenuța CHIRA, Adrian CHIRA, Elena Maria DRAGHICI .....	237
<b>THE EFFECT OF ABIOTIC STRESS ON THE FLOWERING AND PRODUCTIVITY OF ZUCCHINI</b>	
Zdenka GIREK, Suzana PAVLOVIĆ, Milan UGRINOVIĆ, Jelena DAMNJANOVIĆ, Dejan CVIKIĆ, Nenad PAVLOVIĆ .....	243
<b>VARIATIONS OF MAIZE YIELD IN LONG-TERM CROP ROTATION</b>	
Milena SIMIĆ, Miodrag TOLIMIR, Vesna DRAGIČEVIĆ, Željko DOLIJANOVIĆ, Natalija PAVLOVIĆ, Milan BRANKOV .....	249
<b>EFFECTS OF THE DIFFERENT TERM GRAPE THINNING ON THE YIELD AND GRAPE QUALITY OF CABERNET SAUVIGNON CV. GRAPES</b>	
Milica GLIŠIĆ, Aleksandra MARKOVIĆ, Danijela ŽIVOJINOVIĆ, Andreja PREKOVIĆ, Zoran PRŽIĆ, Saša MATIJAŠEVIĆ .....	255
<b>PHYSICAL AND CHEMICAL CHARACTERIZATION OF PLUM CULTIVARS (<i>PRUNUS DOMESTICA</i> L.)</b>	
Mirjana RADOVIC, Mihajlo LIZDEK, Mirko KULINA, Slavisa MILOVANOVIC, Dejan ZEJAK .....	261
<b>QUALITY PROPERTIES OF WHEAT BREAD</b>	
Desimir KNEŽEVIĆ, Vesna DJUROVIĆ, Mirela MATKOVIĆ STOJŠIN, Dragan GRČAK, Milosav GRČAK, Dušan UROŠEVIĆ, Sarina REZAEI SHOJAEI, Simin HAGH-NAZARI .....	270
<b>VARIABILITY OF MASS OF SPIKE AMONG WHEAT VARIETIES</b>	
Dušan UROŠEVIĆ, Desimir KNEŽEVIĆ, Mirela MATKOVIĆ STOJŠIN, Artiona LAZE, Gordana BRANKOVIĆ, Jelena STOJILJKOVIĆ, Veselinka ZEČEVIĆ .....	277
<b>PHENOTYPIC VARIABILITY AND SIMILARITY OF NUMBER OF SPIKELETS IN PRIMARY SPIKE IN WHEAT VARIETIES (<i>Triticum aestivum</i> L.)</b>	
Dušan UROŠEVIĆ, Desimir KNEŽEVIĆ, Mirela MATKOVIĆ STOJŠIN, Jelica ŽIVIĆ, Danica MIĆANOVIĆ, Adriana RADOSAVAC, Vesna KANDIĆ .....	285
<b>EFFECT OF OAT INTERCROPPING ON <i>ERYSIPHE PISI</i> DISEASE INDEX IN WINTER PEA</b>	
Milosav GRČAK, Dragan GRČAK, Desimir KNEŽEVIĆ, Radivoje JEVTIĆ, Vesna ŽUPANSKI, Branka ORBOVIĆ, Vera RAJIČIĆ .....	293
<b>DETERMINATION OF YIELD AND YIELD COMPONENTS OF SOME SALSOLA AND SUAEDA SPECIES ACCORDING TO PHENOLOGICAL PERIODS</b>	
Bilal KESKİN, Süleyman TEMEL, Ali İhsan ATALAY, Faruk TOHUMCU, Seda AKBAY TOHUMCU .....	301
<b>MECHANICALLY-AIDED HARVESTING OF TABLE GRAPES WITH AN ELECTRICALLY PROPELLED PROTOTYPE</b>	
Roberto TOMASONE, Mauro PAGANO, Carla CEDROLA .....	308
<b>PLANT PROTECTION AND FOOD SAFETY .....</b>	<b>314</b>

**STUDY OF THE INFLUENCE OF STORAGE PERIOD ON THE ACCUMULATION OF HYDROXYMETHYLFURFURAL IN CANNED PRODUCTS BASED ON CARROT**

Zinaida YEGOROVA, Anastasia NAVROTSKAYA, Angelina BUTKO, Tatiana SHACHEK ..... 315

**THE HARMFULNESS OF LEAFMINER *NAPOMYZA GYMNOSTOMA* Loew (Diptera, Agromyzidae) IN BIJELJINA AREA (BOSNIA AND HERZEGOVINA)**

Dejana STANIĆ, Jovana OBRADOVIĆ ..... 324

**INFLUENCE OF GROWING LOCATION ON BIOACTIVE COMPOUNDS AND ANTIOXIDATIVE ACTIVITY OF 'CLERY' STRAWBERRY FRUITS IN HERZEGOVINA**

Maja KAZAZIC, Emina MEHIC, Amna OMANOVIC ..... 331

**ASSESSMENT OF NATURAL RADIOACTIVITY LEVELS IN AGRICULTURAL SOIL AND TRANSFER IN RICE IN THE KOCHANI REGION, NORTH MACEDONIA**

Aleksandra ANGELESKA, Radmila CRCEVA NIKOLOVSKA, Elizabeta DIMITRIESKA STOJKOVIKJ, Ljupco ANGELOVSKI, Igor ESMEROV, Risto UZUNOV ..... 337

**ASSESSMENT OF RADIOACTIVITY AND RADIOLOGICAL HAZARD FROM NATURAL RADIONUCLIDES CONTAINED IN RICE FROM NORTH MACEDONIA**

Aleksandra ANGELESKA, Radmila CRCEVA NIKOLOVSKA, Elizabeta DIMITRIESKA STOJKOVIKJ, Igor ESMEROV, Stefan JOVANOV, Ljupco ANGELOVSKI, Risto UZUNOV ..... 344

**SCIENTIFIC AND REGULATORY PERSPECTIVES ON IRRADIATED FOOD: DETECTION STANDARDS AND SAFETY EVALUATION**

Ljupco ANGELOVSKI, Elizabeta DIMITRIESKA STOJKOVIKJ, Radmila CRCEVA NIKOLOVSKA, Igor ESMEROV, Risto UZUNOV, Sandra MOJSOVA, Aleksandra ANGELESKA ..... 351

**THE ROLE OF RURAL TOURISM IN ADVANCING SUSTAINABLE DEVELOPMENT IN NORTH MACEDONIA**

Ljupco ANGELOVSKI, Aleksandra ANGELESKA, Radmila CRCEVA NIKOLOVSKA, Snezana DIMITROVSKA, Sandra MOJSOVA, Igor ESMEROV, Elizabeta DIMITRIESKA STOJKOVIKJ ..... 359

**LIST OF SOME INVASIVE INSECTS ESTABLISHED IN NORTH MACEDONIA**

Stanislava LAZAREVSKA, Sterja NACHESKI, Miroljub GOLUBOVSKI, Blagoj SURBEVSKI ..... 368

***MORINGA OLEIFERA* OVERDOSE: ALLELOPATHIC EFFECTS OF HIGH-CONCENTRATION EXTRACT ON BAMBARA GROUNDNUT**

Abdel Kader NAINO JIKA ..... 374

**VIALE BIOTECHNOLOGICAL WAYS FOR LEGUMICULTURE: INTEGRATION OF COMPOST AND BIOACTIVE SYSTEMS BASED ON BYOPOLIMERS**

Valentina-Elena GORGAN, Petronela NECHITA, Gabriela Elena BAHIRIM ..... 380

<b>BEE DIVERSITY IN SEMI-NATURAL HABITATS IN AGRICULTURAL LANDSCAPES IN VOJVODINA PROVINCE, SERBIA</b>	
Ivana KAVGIĆ, Zlata MARKOV RISTIĆ, Sonja MUDRI-STOJNIC	386
<b>EXPLORING THE POTENTIAL OF AUTOCHTHONOUS PROBIOTIC STARTER CULTURES STRAINS IN FRESH CHEESE PRODUCTION</b>	
Dušan BASTA, Ksenija ČOBANOVIĆ, Nataša GOLIĆ, Katarina VELJOVIĆ, Nikola POPOVIĆ, Snežana GLAMOČIĆ	392
<b>THE INFLUENCE OF SUBSTRATE ACIDITY ON PRODUCTION OF MUSHROOM <i>LENTINULA EDODES</i> (BERK.) PEGLER</b>	
Miroslava MARKOVIĆ, Renata GAGIĆ-SERDAR, Goran ČEŠLJAR	398
<b>EDIBLE MUSHROOMS AS NEW FOOD AND NEW ADDITIVES</b>	
Višnja SIKIMIĆ, Slavica ČABRILO, Nada JELIĆ	404
<b>EXTRACTION METHODS USED IN OBTAINING PHYTOMELATONIN-RICH EXTRACTS FROM CITRUS SPECIES</b>	
Özgül GERÇEKER, Sevcan ÜNLÜTÜRK, Tolga AKCAN, Şelale Öncü GALUE	413
<b>USE OF HALOCHROMIC SYSTEMS IN INTELLIGENT FOOD PACKAGING</b>	
Ozlem Kizilirmak ESMER, Sevgi CAY	422
<b>EFFECT OF RHIZOSPHERIC BACTERIA IN CONTROLLING <i>FUSARIUM OXYSPORUM</i></b>	
Redouan QESSAOUI, Safouane BENJAAA, Salahddine CHAFIKI, Abdelmalk MAHROUG, Soumaya EL ASSRI, Abdelhadi AJERRAR, Hasna ELHJOUJI, Mohamed ALOUANI, Rachid BOUHARROUD	427
<b>ORGANIC AGRICULTURE AND AGROECOLOGY</b>	<b>435</b>
<b>CHEMICAL COMPOSITION OF LEAVES FROM FIVE <i>Vitis vinifera</i> L. CULTIVARS</b>	
Ladislav VASILIŠIN, Goran VUČIĆ, Srđan LJUBOJEVIĆ, Nataša LAKIĆ-KARALIĆ, Staniša LATINOVIĆ	436
<b>INCREASING THE PRODUCTIVITY POTENTIAL OF THE CHERNOZEM IN THE REPUBLIC OF MOLDOVA IN CONDITIONS OF CLIMATE RESILIENCE</b>	
Tatiana DAVID, Leonid POPOV, Valerian CERBARI	444
<b>MULTIVARIATE VISUALIZATION UNLOCKS OPTIMAL SEED PRIMING STRATEGIES FOR SAHELIAN FARMERS: A MORINGA-BAMBARA GROUNDNUT CASE STUDY</b>	
Abdel Kader NAINO JIKA	450
<b>CHALLENGES, CONCEPTS AND INTERNATIONAL VIEWS ON ORGANIC WINE CERTIFICATION AND CONSUMPTION</b>	
Cosmina - Ionela VASILACHE, Adrian Gheorghe ZUGRAVU	456
<b>SUSTAINABLE MANAGEMENT OF THE PROTECTION OF VEGETABLE CROPS IN THE ORGANIC FARMING SYSTEM</b>	
Divna SIMIĆ, Snežana JANKOVIĆ, Željko DOLIJANOVIĆ, Vera POPOVIĆ, Vojin CVIJANOVIĆ, Jelena MARKOVIĆ, Vladan PEŠIĆ	463

**CITIZEN SCIENCE INSIGHTS INTO DECOMPOSITION ACROSS FARMS USING TEA BAGS AND COTTON FABRIC**

Dušanka VUJANOVIĆ, Andrijana ANDRIĆ, Jelena JOVIĆ, Kristina KALKAN, Nataša LJUBIČIĆ, Ljiljana ŠAŠIĆ ZORIĆ, Aleksandar IVEZIĆ, Tijana BAROŠEVIĆ ..... 469

**MORINGA PODS: AN ORGANIC WASTE SOLUTION FOR *TETRANYCHUS URTICAE* CONTROL**

Redouan QESSAOUI, Darbali Fatima ZAHRA, Salahddine CHAFIKI, Abdelmalk MAHROUG, Soumaya EL ASSRI, Bahoch SAID, Abdelhadi AJERRAR, Hasna ELHJOUJI, Ait Aabd NAIMA, Tahiri ABDEGHANI, Rachid BOUHARROUD ..... 477

**IMPACT OF SUCCESSIONAL AGROFORESTRY SYSTEMS ON SOIL BIOLOGY IN THE MEDITERRANEAN**

Pedro R. SOARES, Carla S. S. FERREIRA, Lyudmyla SYMOCHKO, Patrícia SANTOS, Coen RITSEMA, Luuk FLESKENS ..... 484

**ENVIRONMENT PROTECTION AND NATURAL RESOURCES**

**MANAGEMENT ..... 491**

**VALORIZATION OF ORGANIC WASTE, THE CASE OF DATE STONES, IN WATER TREATMENT**

Mimouna YAKOUBI, Nasser BELBOUKHARI, Khaled SEKKOUM ..... 492

**IMPERVIOUSNESS DENSITY OF LAND IN BOSNIA AND HERZEGOVINA**

Branislav DRAŠKOVIĆ, Ljiljana TANASIĆ, Milan GLIŠIĆ ..... 499

**A DAMAGE ASSESSMENT OF THE BEECH-FIR ECOSYSTEM AFTER EXPLOITATION**

Fatima MUHAMEDAGIĆ, Zemira DELALIĆ, Nihad GERZIĆ, Merjem HUSKIĆ .... 505

**INVASIVE ALIEN PLANT SPECIES OF THE MUNICIPALITY OF GRUDE (BOSNIA AND HERZEGOVINA)**

Mate BOBAN, Helena BREKALO, Safija BOŠKAILO, Toni GALIĆ, Aldin BOŠKAILO, Danijela PETROVIĆ ..... 511

**DIVERSITY OF ENDEMIC PLANTS AT SPRING OF BUNICA AND BUNA CHANNELS, BOSNIA AND HERZEGOVINA**

Lejla RIĐANOVIĆ, Robert KEPIĆ, Emina ADEMOVIĆ, Sanel RIĐANOVIĆ ..... 518

**ETHNOBOTANICAL ANALYSIS OF THE TRADITIONAL USE OF WILD PLANTS AND MUSHROOMS IN THE AREA OF BUSOVAČA (BOSNIA AND HERZEGOVINA)**

Iva RELOTA, Sandra MEDIĆ, Antonela MUSA, Safija BOŠKAILO, Aldin BOŠKAILO, Danijela PETROVIĆ ..... 524

**GERMPLASM COLLECTIONS OF NEGLECTED AND UNDERUTILIZED SPECIES AS RESEARCH AND CONSERVATION PLATFORMS FOR THEIR VALORIZATION**

Zakaria KIEBRE, Mariam KIEBRE, Romaric Kiswendsida NANEMA, Fanta Sheirita Reine TIETIAMBOU, Veli-Matti ROKKA, Hamid EL BILALI, Filippo ACASTO, Jacques NANEMA ..... 531

**WATER RETENTION CHARACTERISTICS OF TERRA ROSSA IN RELATION TO HUMUS CONTENT**

Aleksandra BENZA, Mirjam NIKOLIĆ, Danijela JUNGIC, Nikolina JURKOVIĆ BALOG..... 539

**SPATIAL AND TEMPORAL HISTORICAL LANDSCAPE CHANGE IN A MICRO BIODIVERSITY HOTSPOT OF GREECE**

Asimina SKOUTERI, Vassilios P. PAPANASTASIS..... 545

**INTEGRATED STATISTICAL APPROACHES TO SUSTAINABLE AQUACULTURE DEVELOPMENT: INSIGHTS FROM THE BALTIC SEA REGION FOR ENVIRONMENT PROTECTION AND NATURAL RESOURCE MANAGEMENT**

Inese SKAPSTE..... 551

**FLOWERING AND FRUITING BIOLOGY OF *PYRUS ELAEAGNIFOLIA* PALL. IN THE CONDITIONS OF THE REPUBLIC OF MOLDOVA**

Irina SFECLĂ, Elena TOFAN-DOROFEEV..... 557

***EPIPACTIS MUELLERI* GODFERY (ORCHIDACEAE) – A NEW SPECIES FOR THE FLORA OF THE REPUBLIC OF MOLDOVA**

Olga IONITA..... 568

**NITRATE NITROGEN AND NITRATE REDUCTASE ACTIVITY REGIME IN SOILS OF AGROCENOSSES OF THE DONETSK PEOPLE'S REPUBLIC**

Andrey BEREZOVSKIY, Dmitry SYSHCHYKOV, Irina AGUROVA..... 574

**ASSESSMENT OF ERODIBILITY INDICES AND THE DEGREE OF SOIL AGGREGATION DEPENDING ON THE TYPE OF LAND USE (EASTERN SERBIA)**

Boško GAJIĆ, Milan ĐORĐEVIĆ, Miloš MANIĆ, Mrđan ĐOKIĆ, Ranko DRAGOVIĆ, Aleksandar ČUPIĆ, Mihajlo JOVIĆ, Ivana SMIČIKLAS, Snežana DRAGOVIĆ..... 579

**LIGNOCELLULOSIC ARCHITECTURE AND BIOCONVERSION POTENTIAL OF *ARUNDO DONAX* AND *ZEA MAYS*: A FLUORESCENCE APPROACH**

Daniela ĐIKANOVIĆ, Dragana BARTOLIĆ, Mira STANKOVIĆ, Gabor STENBACH, Miloš PROKOPIJEVIĆ, Aleksandar KALAUZI, Ksenija RADOTIĆ..... 585

**INFLUENCE OF DRYING TREATMENT ON ANTIOXIDANT ACTIVITY IN FOUR DIFFERENTLY PIGMENTED MAIZE (*ZEA MAYS* L.) SEED CULTIVARS**

Daniela ĐIKANOVIĆ, Miloš PROKOPIJEVIĆ, Mira STANKOVIĆ, Olivera PRODANOVIĆ, Branka ŽIVANOVIĆ, Ksenija RADOTIĆ, Dragana BARTOLIĆ.... 591

**THE IMPACT OF HARVEST TIME ON THE CONTENT OF POLYPHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITY OF FRUITS OF *Chaenomeles japonica* (Thunb.) Lindl. ex Spach**

Mirjana OCOKOLJIĆ, Jelena ČUKANOVIĆ, Radenka KOLAROV, Djurdja PETROV, Nevenka GALEČIĆ, Dejan SKOČAJIĆ, Isidora SIMOVIĆ..... 595

**THE IMPACT OF LOCAL ADAPTATION ON THE PHENOLOGICAL PATTERNS OF FLOWERING IN *Buddleja davidii* 'PINK DELIGHT' IN KARADORDEV PARK, BELGRADE (SERBIA)**

Mirjana OCOKOLJIĆ, Nevenka GALEČIĆ, Dejan SKOČAJIĆ, Jelena ČUKANOVIĆ, Radenka KOLAROV, Dragan VUJIČIĆ, Djurdja PETROV .....	602
<b>A STUDY ON THE FLOWERING CHARACTERISTICS OF CHAENOMELES × SUPERBA 'PINK LADY'</b>	
Mirjana OCOKOLJIĆ, Djurdja PETROV, Nevenka GALEČIĆ, Dejan SKOČAJIĆ, Sara ĐORĐEVIĆ, Radenka KOLAROV, Jelena ČUKANOVIĆ .....	609
<b>SPATIAL USE AND THE NEEDS OF VISITORS OF THE BOJČIN FOREST NATURE MONUMENT (BELGRADE, SERBIA)</b>	
Jovana PETROVIĆ, Nenad STAVRETOVIĆ, Dušan JOKANOVIĆ, Vesna NIKOLIĆ JOKANOVIĆ .....	615
<b>INVASIVE AND ALLERGENIC PLANT SPECIES ON RUNNING TRAILS OF ADA CIGANLIJA (BELGRADE, SERBIA)</b>	
Jovana PETROVIĆ, Nenad STAVRETOVIĆ, Dušan JOKANOVIĆ, Vesna NIKOLIĆ JOKANOVIĆ .....	621
<b>STRENGTHENING ORGANIZATIONAL QUALITY THROUGH ISO 9001 AND ISO 10005 INTEGRATION</b>	
Marija PEROVIĆ, Marija TODOROVIĆ, Tatjana MITROVIĆ .....	628
<b>ENVIRONMENTAL BEHAVIOR AND TRACER POTENTIAL OF FLUORIDE IN GROUNDWATER</b>	
Marija PEROVIĆ, Tatjana MITROVIĆ .....	634
<b>BEE-ING IN THE NEWS: ANALYZING SERBIAN NEWSPAPER COVERAGE OF BEES</b>	
Snežana POPOV, Zlata MARKOV RISTIĆ, Dušanka VUJANOVIĆ .....	639
<b>TOXICOLOGICAL RISKS PEDICTION OF THE METOLACHLOR HERBICIDE AS ENVIRONMENTAL CONTAMINANT</b>	
Tatjana MITROVIĆ, Darija OBRADOVIĆ, Saša LAZOVIĆ, Marija PEROVIĆ .....	644
<b>ECOLOGICAL STATUS EVALUATION OF SERBIAN SURFACE WATERS BASED ON PHOSPHORUS CONCENTRATIONS</b>	
Tatjana MITROVIĆ, Marija PEROVIĆ, Tijana MILICEVIĆ .....	649
<b>APPLYING SWOT ANALYSIS TO THE VALUATION OF POLLINATION ECOSYSTEM SERVICES</b>	
Zlata MARKOV RISTIĆ, Sonja MUDRI-STOJNIĆ .....	655
<b>CULTIVATION OF HOVERFLIES ON FARMS: BIOLOGICAL, PRACTICAL AND ECONOMIC PERSPECTIVES</b>	
Zlata MARKOV RISTIĆ, Snežana POPOV .....	661
<b>IMPACTS OF AGRICULTURAL WATER USE AND SEASONAL DROUGHT ON WATER RESOURCES: A CASE STUDY OF KESKIN DAM, TURKIYE</b>	
Eray HARMAN .....	667
<b>HISTORICAL SHARK EXPLOITATION AND CONTEMPORARY CONSERVATION EFFORTS IN THE TURKISH SEAS</b>	
Ertan TASKAVAK, Halit FILIZ .....	674

<b>GEOGRAPHICALLY WEIGHTED REGRESSION BASED APPROACHES FOR ENHANCING PRECIPITATION ESTIMATION IN AGROECOSYSTEMS</b>	
Filiz DADASER-CELİK, Mete CELİK, Ali Ümran KÖMÜŞCÜ.....	680
<b>MODELLING HYDROLOGICAL PROCESSES AT BURDUR LAKE BASIN (TURKEY) WITH SWAT</b>	
Mehmet SOYLU, Meltem KACIKOC, Filiz DADASER-CELİK.....	685
<b>PRELIMINARY ASSESSMENT OF WATER QUALITY IN EĞİRDİR LAKE BASED ON FIELD MONITORING</b>	
Meltem KACIKOC, Eda BOYACIOGLU, Filiz DADASER-CELİK.....	691
<b>A SOLUTION TO CLIMATE CHANGE AND WATER SCARCITY: STUDIES ON RAINWATER HARVESTING IN TURKEY</b>	
Yoldaş EKTİREN.....	700
<b>IMPROVING WATER EFFICIENCY IN AGRICULTURE: A DEFICIT IRRIGATION APPROACH FOR TURKEY</b>	
Yoldaş EKTİREN.....	705
<b>CULTURAL LANDSCAPES OF THE BIOSPHERE RESERVE “OS ANCARES LUCENSES E MONTES DE CERVANTES, NAVIA E BECERREÁ”</b>	
Ignacio J. DIAZ-MAROTO.....	710
<b>HOW DO CULTURAL ECOSYSTEM SERVICES IN MOUNTAIN AREAS CONTRIBUTE TO THE WELL-BEING OF LOCAL COMMUNITIES?</b>	
Ignacio J. DIAZ-MAROTO.....	717
<b>THE ROLE OF SUSTAINABLE FOREST RESOURCE MANAGEMENT IN THE SOCIOECONOMIC GROWTH OF THE EASTERN MOUNTAINS OF GALICIA IN SPAIN</b>	
Ignacio J. DIAZ-MAROTO.....	723
<b>POTENTIAL ROLES OF MELATONIN AND ITS METABOLITES IN REDUCING HEAVY METAL TOXICITY IN VEGETABLES</b>	
Aygül KARACA .....	729
<b>AN ANALYSIS OF THE SOCIO-ECONOMIC CHARACTERISTICS OF PADDY FARMS IN TURKEY</b>	
Sema Ezgi YÜCEER, Sibel TAN.....	736
<b>THE USE OF SEAWEED EXTRACTS TO MITIGATE ABIOTIC STRESSES IN VEGETABLES</b>	
Aygül KARACA, Nusret ÖZBAY .....	743
<b>ANIMAL HUSBANDRY .....</b>	<b>749</b>
<b>RAW MILK QUALITY AS A BASIS FOR CHEESE PRODUCTION</b>	
Drazenko BUDIMIR, Milijana ŠKRBIĆ .....	750
<b>BACTERIA AND LOWER RESPIRATORY TRACT DISEASES IN HORSES</b>	
Mariyana NIKOLOVA, Sasho SABEV .....	755

**CLINICAL STUDIES OVER EQUINE GASTROULCERATIVE SYNDROME IN HORSES (EGUS)**

Sasho SABEV, Mariyana NIKOLOVA ..... 763

**PRODUCTIVITY AND EGG QUALITY OF LAYING HENS FED WITH BLACK SOLDIER FLY (*HERMETIA ILLUCENS*) LARVAE MEAL**

Aiga NOLBERGA-TRŪPA, Niklāvs KLEINS, Kārlis SAMS ..... 770

**THE INFLUENCE OF THE LEPTIN GENE ON MILK PRODUCTION IN THREE CONSECUTIVE LACTATIONS IN HOLSTEIN FRIESIAN CATTLE**

Igor ESMEROV, Radmila CRCEVA NIKOLOVSKA, Nikolay MARKOV, Risto UZUNOV, Ljupco ANGELOVSKI, Aleksandra ANGELESKA, Ljupco MICKOV ... 778

**PORTUGUESE CONSUMERS' PREFERENCE REGARDING THE PRESENTATION OF PROTECTED DESIGNATION OF ORIGIN TRANSMONTANO GOAT CHEESE**

António José FERNANDES, Fernando SOUSA, Dina AVEIRO, Maria Isabel RIBEIRO ..... 784

**PORK MEAT CONSUMPTION: A COMPARATIVE ANALYSIS BETWEEN BRAZILIAN AND PORTUGUESE CONSUMERS**

António José FERNANDES, Juan de Oliveira MORAIS, Maria Isabel RIBEIRO ..... 791

**PORTUGUESE CONSUMERS' PREFERENCE REGARDING THREE PROTECTED DESIGNATION OF ORIGIN TRANSMONTANO GOATLING BUTCHER PIECES**

António José FERNANDES, Fernando SOUSA, Dina AVEIRO, Maria Isabel RIBEIRO ..... 798

**PHARMACEUTICAL CONTAMINANTS IN AQUATIC ENVIRONMENTS: IMPACT ON EDIBLE FISH AND SUSTAINABLE MANAGEMENT PERSPECTIVES**

Daniela-Nicoleta ROPOTAN, Lorena DEDIU ..... 804

**EFFECT OF GLYCEROL SUPPLEMENTATION ON BLOOD CONCENTRATIONS OF INSULIN AND BIOCHEMICAL PARAMETERS IN PERIPARTUM DAIRY COWS**

Julijana TRIFKOVIĆ, Dušan BOŠNJAKOVIĆ, Slavica DRAŽIĆ, Ljubomir JOVANOVIĆ, Milica STOJKOVIĆ, Danijela KIROVSKI, Željko SLADOJEVIĆ ..... 810

**THE POSSIBILITY OF USING ESSENTIAL OILS OF *ORIGANUM VULGARE* L., *MENTHA X PIPERITA* L. AND *SATUREJA MONTANA* L. AGAINST GASTROINTESTINAL NEMATODES IN SHEEP**

Filip ŠTRBAC, Radomir RATAJAC, Nataša TOLIMIR, Divna SIMIĆ, Slađan STANKOVIĆ, Antonio BOSCO, Laura RINALDI, Nataša SIMIN, Dejan ORČIĆ, Slobodan KRNJAJIĆ, Dragica STOJANOVIĆ ..... 817

**THE INFLUENCE OF WEATHER ON THE QUALITY OF HONEY IN RASINA REGION FOR THE PERIOD OF 2019-2024**

Goran JEVIĆ, Snežana BABIĆ, Snežana ANDELKOVIĆ, Đorđe LAZAREVIĆ, Mirjana PETROVIĆ, Vladimir ZORNIĆ, Kazimir MATOVIĆ ..... 826

**PORCINE REPRODUCTIVE RESPIRATORY SYNDROME AS A HEALTH PROBLEM IN SWINE PRODUCTION**

Jovan BOJKOVSKI, Sreten NEDIĆ, Sveta ARSIĆ, Radiša PRODANOVIĆ, Aleksandra MITROVIĆ, Branko ANGELOVSKI, Milan NINKOVIĆ ..... 832

**CONSTRUCTIVE ALIGNMENT OF LEARNING OUTCOMES, TEACHING METHODS AND ASSESSMENT STRATEGIES IN THE FARM ANIMAL BIOSECURITY COURSE**

Slavca HRISTOV, Branislav STANKOVIC, Dimitar NAKOV, Milica RADJENOVIC ..... 837

**CHARACTERISTICS OF KAJMAK PRODUCED IN THE VALJEVO AREA IN SERBIA**

Tanja VUČIĆ, Snežana JOVANOVIĆ, Kristina SOFRONIĆ ..... 845

**EFFECT OF PROTEASE AND SEX ON CARCASS QUALITY AND ABDOMINAL FAT OF SLOW-GROWING HYBRID OF CHICKEN**

Vladimir DOSKOVIĆ, Snežana BOGOSAVLJEVIĆ-BOŠKOVIĆ, Zdenka ŠKRBIĆ, Miloš LUKIĆ, Bojan STOJANOVIĆ, Simeon RAKONJAC, Veselin PETRIČEVIĆ.. 851

**RISK ASSESSMENT OF ANIMAL WASTE FOR OCCUPATIONAL HEALTH AND SAFETY IN LIVESTOCK SECTOR**

Turgut AYGÜN ..... 857

**SOME ENVIRONMENTAL EFFECTS ON BODY WEIGHT AT WEANING AND SHEARING AND GREASY WOOL YIELD IN MORKARAMAN EWES**

Turgut AYGÜN ..... 863

**RAISING SYSTEMS IN SMALL RUMINANT HUSBANDRY IN EASTERN ANATOLIA OF TÜRKİYE**

Turgut AYGÜN ..... 869

**SUPPLEMENTATION WITH GARLIC POLYSULFIDES EFFECTIVELY REDUCES ENTERIC METHANE EMISSIONS IN DAIRY COWS**

Julijana TRIFKOVIĆ, Dušan BOŠNJAKOVIĆ, Sreten NEDIĆ, Ljubomir JOVANOVIĆ, Ivan VUJANAC, Željko SLADOJEVIĆ, Danijela KIROVSKI ..... 875

**FISH FEED: AN ANALYSIS OF OLD AND NEW TECHNOLOGIES**

Albana UKA ..... 883

**DEVELOPMENT OF COMPETENCES OF VETERINARIANS AND ANIMAL HUSBANDRY ENGINEERS FOR BIOSECURITY MEASURES IMPLEMENTATION ON ANIMAL FARMS**

Branislav STANKOVIC, Slavča HRISTOV, Dimitar NAKOV, Sonja OBRENOVIC, Milica RADJENOVIC, Ivana MILOSEVIC-STANKOVIC ..... 888

**EVALUATION OF EGGSHELL HYGIENE AND QUALITY OF TABLE EGGS FOLLOWING SURFACE DISINFECTION WITH LIQUID CHLORINE DIOXIDE**

Ajla ALIŠAH, Nedim BEGIĆ, Julijana TRIFKOVIĆ, Miroslav LALOVIĆ, Abdulah GAGIĆ ..... 895

**ANALYSIS OF THE USE AND PRESENCE RESIDUES OF ANTIBIOTICS IN TABLE EGGS IN MODERN LAYING HEN FARMS IN THE DAKAR REGION AND SURROUNDING AREA (SENEGAL)**

Khalifa Serigne Babacar SYLLA, Arame THIOUNE, Sadibou BA, Rianatou BADA-ALAMBEDJI..... 900

**RURAL DEVELOPMENT AND AGROECONOMY ..... 909**

**PUBLIC POLICIES, PRIVATE INITIATIVES AND RURAL DEVELOPMENT FOR CLIMATE-RESILIENT AGRICULTURE IN BOSNIA AND HERZEGOVINA**

Branka TOPIĆ-PAVKOVIĆ ..... 910

**BUYING BEHAVIOUR IN CONSUMERS OF FRESH VEGETABLES**

Elma SEFO, Zrinka KNEZOVIĆ, Anamarija ŠEVO..... 920

**FISH CONSUMPTION PREFERENCES AND CONSUMER CHARACTERISTICS IN HERZEGOVINA-NERETVA CANTON, BOSNIA AND HERZEGOVINA**

Predrag IVANKOVIĆ, Marija LASIĆ, Luka DRAGIĆEVIĆ ..... 927

**ANALYSIS OF THE ECONOMIC PROFITABILITY OF DIFFERENT BELL PEPPER PRODUCTION METHODS**

Radomir BODIROGA, Milica STOJANOVIĆ, Uroš GALINAC, Ljubiša ŠEVKUŠIĆ, Koštana VINČIĆ ..... 933

**SUPPLY CHAIN FINANCE AS A FUNDING OPPORTUNITY FOR FARMERS**

Zorica GOLIĆ, Saša LALIĆ..... 939

**INCLUSIVE GREEN FINANCE: IMPORTANCE FOR STRENGTHENING THE AGRICULTURAL RESILIENCE TO CLIMATE CHANGE**

Zorica GOLIĆ..... 948

**AGRICULTURAL BIOGAS PLANTS IN EUROPEAN RURAL DEVELOPMENT AND THE ENERGY TRANSITION**

Krzysztof PILARSKI, Agnieszka A. PILARSKA ..... 957

**HEALTH AND SAFETY OF FARMERS IN POLAND**

Władysław MIGDAŁ, Joanna MAKULSKA, Barbara TOMBARKIEWICZ, Michał CUPIAŁ..... 966

**DEMOGRAPHIC TRENDS AND THEIR IMPLICATIONS FOR SUSTAINABLE RURAL DEVELOPMENT: A CASE STUDY OF THE BUFTEA AREA, ILFOV COUNTY, ROMANIA**

Andreea Roxana FIRĂȚOIU, Elena SOARE, Aurelia-Ioana CHEREJI, Irina-Adriana CHIURCIU, Liviu MĂRCUȚĂ ..... 973

**RURAL LABOR: AN ANALYSIS OF THE PROSPECTS FOR EMPLOYMENT OF YOUTH IN THE AGRICULTURAL SECTOR**

Irina CHEKHOVSKIKH ..... 982

**THE ROLE OF KNOWLEDGE TRANSFER TRAININGS IN FIELD CROP AND VEGETABLE PRODUCTION: AN ANALYSIS OF A TEN-YEAR PERIOD**

Divna SIMIĆ, Snežana JANKOVIĆ, Vojin CVIJANOVIĆ, Vedran TOMIĆ, Marijana MASLOVARIĆ, Ivana STANIMIROVIĆ, Nataša TOLMIR ..... 989

<b>SMART AGRICULTURE AND CRYPTOECONOMICS: CHALLENGES AND OPPORTUNITIES FOR THE WESTERN BALKANS</b>	
Vedran TOMIĆ, Robert RADIŠIĆ, Marija GAVRILOVIĆ, Stevan ČANAK, Nikola LJILJANIĆ .....	998
<b>IMPORTANCE OF THE TEMPERATURE-HUMIDITY INDEX IN ENSURING OPTIMAL SHELTER CONDITIONS FOR ANIMAL PRODUCTION IN TURKEY</b>	
Merve Nur EKTIREN, Yoldaş EKTIREN .....	1004
<b>ASSESSING GENDER SENSITIVITY OF THE SMALL-SCALE MECHANIZED RAISED BED TECHNOLOGY (MRB) IN EGYPT</b>	
Gehan A.G. ELMENOFI, Aman ELGARHY, Bezaiet DESSALEGN, Biju GEORGE .....	1009
<b>AGRICULTURAL TRENDS AND CHALLENGES IN SLOVAKIA IN COMPARISON WITH BOSNIA AND HERZEGOVINA</b>	
Branislav DUDIC, Velibor SPALEVIC .....	1019
<b>EVALUATION OF THE ECONOMIC EFFICIENCY OF FRUIT ORCHARD PRODUCTION WITH RISK CONSIDERATION</b>	
Jozef REPISKÝ, Nándor NAGY .....	1025
<b>FORECASTING APRICOT PRODUCTION IN TÜRKİYE USING THE ARIMA (BOX-JENKINS) MODEL</b>	
Ashl DALGIC.....	1032
<b>FORESTRY AND AGRO-FORESTRY .....</b>	<b>1040</b>
<b>ANALYSIS OF CHAINSAW OPERATORS’ EXPERIENCE IN FORESTRY</b>	
Dane MARČETA, Miodrag ŠTRBAC, Vladimir PETKOVIĆ, Danijela PETROVIĆ, Milan SUKUR .....	1041
<b>BRANDING CHALLENGES AND PERSPECTIVES OF NON-TIMBER FOREST PRODUCTS IN SARAJEVO-ROMANIJA REGION IN BOSNIA AND HERZEGOVINA</b>	
Nedeljka ELEZ .....	1049
<b>CHEMICAL COMPOSITION OF <i>Morchella costata</i> (Vent.) Pers AND FOUR OTHER EDIBLE MUSHROOMS FROM BOSNIA AND HERZEGOVINA</b>	
Srđan LJUBOJEVIĆ, Ladislav VASILIŠIN, Goran VUČIĆ, Nataša LAKIĆ-KARALIĆ, Staniša LATINOVIĆ .....	1054
<b>GERMINATION OF <i>Fagus sylvatica</i> L. SEEDS DEPENDING ON X-RAY DOSE</b>	
Dina ELISOVETCAIA, Raisa IVANOVA, Jan BRINDZA .....	1064
<b>MORPHOLOGICAL DIVERGENCE AMONG SIX SAHELIAN TREE SPECIES: IMPLICATIONS FOR FUNCTIONAL AGROFORESTRY DESIGN</b>	
Abdel Kader NAINO JIKA .....	1072
<b>RESEARCH ON THE POSSIBILITY OF IMPLEMENTING FOREST CLIMATE PROJECTS IN THE FOREST FUND OF THE RUSSIAN FEDERATION</b>	
Svetlana MORKOVINA, Denis KUZNETSOV, Andrey TOPCHEEV .....	1078

<b>MONITORING LAND USE CHANGE IN VOJVODINA USING THE COLLECT EARTH TOOL</b>	
Dragan BOROTA, Damjan PANTIĆ, Brano VAMOVIĆ, Aleksandar GOLUBOVIĆ .....	1085
<b>HYDROLOGICAL REGIME OF HIGROPHILOUS FORESTS ON THE ISLANDS ALONG THE DANUBE RIVER</b>	
Dušan JOKANOVIĆ, Vesna NIKOLIĆ JOKANOVIĆ .....	1094
<b>MORPHOLOGICAL LEAF TRAITS VARIATION WITHIN AND AMONG FIVE PEDUNCULATE OAK (<i>Quercus robur</i> L.) POPULATIONS IN REPUBLIC OF SRPSKA (BOSNIA AND HERZEGOVINA)</b>	
Milena STANKOVIĆ NEĐIĆ, Srđan STOJNIĆ, Marko GUTALJ, Boban MILETIĆ, Branislav FILIPIĆ, Branislav KOVAČEVIĆ, Lazar KESIĆ, Miloš BAČIĆ Saša ORLOVIĆ.....	1101
<b>REHABILITATION OF DAMAGED FORESTS AS A RESULT OF CLIMATE CHANGE IN THE FUNCTION OF ENVIRONMENTAL PROTECTION</b>	
Zvonimir BAKOVIĆ, Vladimir VASIĆ, Bratislav KISIN .....	1108
<b>AUTHOR INDEX.....</b>	<b>1117</b>

## THE INFLUENCE OF SUBSTRATE ACIDITY ON PRODUCTION OF MUSHROOM *LENTINULA EDODES* (BERK.) PEGLER

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### Abstract

For the fungus to successfully colonize the substrate, counteract competitive species, and perform maximum fructification, certain basic environmental conditions must be met, depending on the type and strain of the fungus and the phase of wood decay. This paper examines the basic environmental conditions during the first phase of Shiitake development – the phase of substrate colonization. The aim of the research was to determine the optimal conditions for the development of the fungus *Lentinula edodes* (Berk.) Pegler. The results showed optimal acidity of the substrate stimulating the growth of this fungus, compared to the conditions under which the rival microorganisms developed. The highest weight of mycelial dry mass was formed at the substrate pH values between 3.00 and 3.63, meaning that this fungus could easily perform spontaneous infection. The changes in pH of the substrate where the examined strains of the fungus *L. edodes* grew shifted toward acidic reaction (5.15 to 3.52), suggesting that such acidity favors the development of the fungus investigated. Results show the stagnation of base pH value at the pH value of about 3.5, which indicates that these are the most favourable conditions for development of both strains of the fungus *L. edodes* in the substrate colonization phase (base pH of 3.6 being the most favourable for mycelial growth). The assumptions from the reference literature were thus confirmed by using the described methods. The fungus cannot thrive in an alkaline environment at pH 7.3.

**Key words:** *Substrate acidity, Mushroom Production, Shiitake.*

### Introduction

The Shiitake mushroom (*Lentinula edodes* (Berk.) Pegl., Syn. *Lentinus edodes* (Berk.) Sing.) grows wild in Japan on the dead wood of *Castanopsis cuspidata*, family Fagaceae (Chang and Buswell, 1996; Wasser, 2005). This mushroom is known for its medicinal properties and has been cultivated for centuries in Japan and China, while its production in Serbia is more recent. It is important to note that no chemical substances are used in its cultivation, unlike in the case of popular button mushrooms.

Shiitake significantly increases the strength and vitality of the organism and empowers the body to ward off a large number of organic disorders (Safaie *et al.*, 2024). Shiitake contains immunostimulants, components effective against tumours and viruses which lower the cholesterol level, prevent clogging of blood vessels, regulate the blood pressure, improve the circulation, balance the blood sugar level, regulate digestion, improve the respiratory system, have antirheumatic and anti-allergenic effect, stimulate or soothe the central nervous system in an innocuous way, boost physical strength and stamina and slow down the aging process (Camilleri *et al.*, 2025). Japanese and American researchers have demonstrated that this type of fungus contains a certain number of biologically active compounds that inhibit cell degeneration. The fungus contains medicinal polysaccharides - lentinan and LEM are compounds that have shown anti-fatigue, anti-virus, anti-bacterial and hepatoprotective effects (Hobbs, 2000; Maeda *et al.*, 1998; Mizuno, 1996; Yap & Ng, 2003; Ngai & Ng, 2003), its production does not entail use of chemicals and pollution of the environment and, finally, the

production itself requires minimal investment and pays off before long (Markovic, 2003; Stamets, 1993; Tsvileva *et al.*, 2008). The basic conditions that enable the fungus to perform infection are the substrate pH, the status of the nutrients within the substrate, the level of inoculum, virulence and the competition on the part of other microorganisms. For the fungus to carry out successful colonization of the substrate, to counteract the competitive species and perform maximum fructification, certain basic environmental conditions must be met, depending on the type and strain of the fungus and the phase of the wood decay. This paper examines the basic environmental conditions during the first phase of Shiitake development – the phase of substrate colonization. Concentration of hydrogen ions is one of the major factors that define the dynamics of the substrate-based fungal development (Grosser, 1985; Li, K. & Li, F., 1992, Lee *et al.*, 2008, Desisa *et al.*, 2023). Substrate acidity may affect the stimulation or inhibition of the growth of lignicolous fungi, while changes in pH value significantly influence the speed of nutrient consumption and substrate decomposition. Environmental acidity also affects the enzyme system of the fungi, which satisfies the vital organisms’ needs for food. Decomposing wood (by oxidation and hydrolysis of the wood constituents), epixylous fungi increase wood acidity by means of oxalic acid obtained as a product of these processes. Edible fungus *L. edodes* belongs to this group of fungi. In this way, the order and succession of nutritive substrate colonization by microorganisms are arranged under natural conditions. Succession is one of the key factors of the wood colonization process, in addition to the vitality of the host plant, degree of parasitism, and the manner fungi consume food (Schmidt, 1994), which in turn regulates connexion or succession of the species on the same substrate. In order to achieve more efficient mass production, this study examined the most important optimal conditions that stimulate its growth and development.

### **Material and Methods**

The influence of the substrate pH value on the mycelial growth and mass production investigation method

For the purpose of investigating the influence of the constant substrate pH values on the *L. edodes* mycelial growth and mass production, a buffered nutritive base was prepared according to the Wolpert method (Rehm & Reed, 2007). By mixing different volumes of 0.3 molar solutions of phosphates (H<sub>3</sub>PO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub> and K<sub>2</sub>HPO<sub>4</sub>), five series of phosphates with different pH values were obtained, yet they all had equal quantities of nutrients in certain parts of the buffer so that different quantities would not influence the results. A portion of double-concentrated malt and agar base (10 Bé sugar and 4% agar) was prepared separately and autoclaved independently of 0.3 M solutions of phosphates. Upon pH value check, the base was mixed with the phosphates under aseptic conditions. In this way, a base of standard concentration was obtained with the physiologically equal percentage of buffer (0.15 M). Thus prepared buffered base was poured into plastic Petri dishes. Five replications were used for each fungus strain and each pH value examined. The experiments were set in a poly-thermostat at the temperature of 21°C. Mycelial growth was measured every 24 hours along three axes: the dish radius and two additional lines angled at ±22.5° from the radius. To test the stability of the buffered system, a parallel culture was grown in a liquid medium. The dry mycelial mass from this culture was measured at the conclusion of the experiment.

The influence of the mycelium on the substrate pH change investigation method

For the study of the influence of the fungus on the change of nutritive substrate pH, the unbuffered (liquid) medium was prepared. A double-concentrated malt base was prepared with distilled water. According to the recipe, 1M-solutions of HCl or NaOH were added, thus providing five series of liquid base of standard sugar concentration (5 Bé). Base acidity for each series (five replications each) was measured before the sterilization. The base pH values were

also measured after the sterilization and treated as initial values. Upon inoculation of the substrate with strains of the fungus *L. edodes*, the changes in pH value of the substrate were measured every 7 days. Twenty-two days after the experiment began, the mycelium was extracted with a water vacuum pump, air-dried in a laminar flow chamber, and its dry mass was measured.

### Results and discussion

The influence of the substrate pH value on the *L. edodes* mycelial growth and mass production. The mycelia of both strains of the fungus *L. edodes* developed in all the series with acidic reaction (initial pH 3.0 to 4.9) as shown in Table 1. For the most part, the A strain had a more aggressive growth than the B strain while both strains had the fastest growth and formed the greatest mycelial mass in series 2 (at the initial pH 3.6). Both strains had the smallest mycelial mass and the lowest growth in series 3 (initial pH 4.9), whereas in series 5 (at the initial pH 7.3) there was no mycelial growth in either strain. Series 5 was not lethal for the fungus which was confirmed when fragments were transferred onto the substrate at optimal pH value 3.5. Results shows the most favourable initial pH value is 3.6 as the mycelial growth is approximately 4.25 mm/day in both strains of the fungus, while for all other initial pH values (3.0, 4.6 and 4.9), the growth amounts to 3.5 to 3 mm/day. At the initial pH value of 7.3 there was no mycelial growth at all.

Table 1. Average daily increment (mm/day) and weight of dry mycelial mass of the fungus *L. edodes* (A and B strain) (g) on buffered nutritive substrates

Series no.	Fungus strain	pH of base			Average daily increment of mycelium (mm/day)	Weight of dry mycelial mass (g)
		pH of buffer	Initial pH	pH at the end of experiment		
1	A	3.0	3.0	3.1	3.48	0.195
	B			3.2		
2	A	3.5	3.6	3.4	4.23	0.252
	B			3.3		
3	A	4.6	4.9	4.7	3.01	0.148
	B			4.5		
4	A	4.7	4.6	4.3	3.25	0.160
	B			4.2		
5	A	7.5	7.3	7.0	0.0	0.0
	B			7.1		

The influence of *L. edodes* mycelium on the change of pH of the nutritive substrate

Table 2 shows changes in the pH values of the malt substrate of the standard concentration under the influence of the *L. edodes* mycelium. After three weeks of the activity of the fungus, the pH of the base was reduced to the range of 3.52 to 5.15 for A strain and 3.63 to 5.05 for B strain in all the series investigated which means that those pH values were most favourable for the mycelia of both strains of the fungus *L. edodes* in this phase of growth.

Table 2. Changes of pH values of substrate provoked by fungus *L. edodes* (A and B strain) after 7, 14 and 21 days

Series no.	Initial pH	Fungus strain	Change in pH of the base				pH at the end of experiment	Weight of dry mycelial mass (g)
			After 7 days	After 14 days	After 21 days	Total change		
1	3.9	A	-0.15	-0.20	-0.03	-0.38	3.52	0.081
		B	-0.20	-0.05	-0.02	-0.27	3.63	0.085
2	5.4	A	-0.12	-0.04	-0.40	-0.54	4.86	0.062
		B	-0.07	-0.08	-0.25	-0.40	5.00	0.059
3	5.5	A	-0.10	-0.11	-0.14	-0.35	5.15	0.051
		B	0.02	-0.08	-0.45	-0.55	4.95	0.045

4	5.7	A	0.00	-0.14	-0.50	-0.64	5.06	0.040
		B	0.10	-0.15	-0.40	-0.65	5.05	0.042
5	6.1	A	-0.15	-0.20	-0.60	-0.95	5.15	0.033
		B	-0.12	-0.40	-1.05	-1.57	4.53	0.038

The greatest change of the substrate pH value through the effect of both strains of the fungi *L. edodes* was found in series 5 at the initial pH value 6.1 while the smallest one occurred in series 1 – initial pH 3.9. In three series (no. 3, 4 and 5), the B strain caused greater total change of the substrate pH than the A strain.

Results demonstrates that for the initial pH value of 3.9 (strain B) base pH slightly decreases until the seventh day when it reaches a constant value. This pH is optimal for *L. edodes*, meaning the substrate should be adjusted to this value during the colonization phase to ensure efficient production. The same occurs in the A strain at the initial substrate pH value of 3.9, the base pH decreases mildly until day 14 when it reaches its constant value. It is characteristic for the initial pH values of 5.4, 5.5 and 5.7 that both strains of the fungus (A and B strain) reach stagnation of the base pH value by the 14th day after which the base pH value suddenly drops. At the initial pH value of 6.1, the base pH value abruptly decreases since day 1 in both strains of the fungus.

Results shows that the percentage of decrease of the pH value ranges between 6 and 11% for the initial pH values of 3.9, 5.4, 5.5 and 5.7 in both strains of the fungus (A and B strain), whereas at the initial pH value of 6.1 the decrease of the acidity is far greater and amounts to approximately 16% for the A strain and 26% for the B strain.

Generally, during the experiment (22 days) it was determined that both strains of the fungus changed the initial pH values of the substrate shifting them towards the optimal values. As this shifting was within the range of tolerance, all buffered systems were considered stable. Both strains of the fungus *L. edodes* had the fastest growth and formed the greatest mycelial mass at the initial pH 3.6. According to the research results of other authors, the optimum pH for *L. edodes* mycelial growth was 3.0 to 3.5 (Hasegawa *et al.*, 2005). It is well known that all fungi need different pH values depending on the phase of development – germination of spores, mycelial growth, formation of fruiting bodies, sporulation etc. Hence the fungi may prefer more acidic substrate for germination of spores, whereas this changes in the phase of fruiting body formation, and quite often less acidic or almost neutral reaction in the substrate is more suitable. This phenomenon has particular importance in the production of the fruiting bodies of the fungi for commercial purposes as is the case with fungus *L. edodes*. Practice has shown that the formation of fruiting bodies for this species is best on substrates with an acidic reaction. This should certainly be taken into consideration for the pH value of the substrate is the key factor for the infection process, substrate colonization or rot progress and dynamics.

## Conclusions

The changes in pH of the substrate where the examined strains of the fungus *L. edodes* grew shifted toward acidic reaction (5.15 to 3.52), which suggests that such acidity favours the development of the fungus investigated. Results show the stagnation of base pH value at the pH value of about 3.5 which indicates that these are the most favourable conditions for development of both strains of the fungus *L. edodes* in the substrate colonization phase (base pH of 3.6 being the most favourable for mycelial growth). The assumptions from the reference literature were thus confirmed by using the described methods. On mildly acidic bases, the fungus *L. edodes* developed a colony of hyphae, which means that lower pH values resulting from the development of a rival species under natural conditions would not hinder the development of *L. edodes*. Alkaline conditions inhibit this fungus, with growth stopping at a pH of 7.3. Because development resumes when the pH is reduced, this suggests the fungus

enters a dormant state rather than losing its vitality. The competition of microorganisms on the same substrate, inhibition or growth, or the occurrence of antagonism, represents a phenomenon that may be the consequence of the metabolism of rival fungal species, excretion of mycotoxins or antibiotics from the growing front of the mycelia, and sensitivity, that is, the reaction of the rival species thereafter. This phenomenon has a direct impact on the speed, course, and consequences of the decomposition of wood as a substrate and food source.

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# AUTHOR INDEX

Abdel Kader NAINO JIKA.....	374, 450, 1072	Bezaiet DESSALEGN .....	1009
Abdelhadi AJERRAR .....	427, 477	Biju GEORGE .....	1009
Abdelmalk MAHROUG.....	427, 477	Bilal KESKİN .....	301
Abdulah GAGIĆ .....	895	Biljana VELJKOVIĆ .....	84
Adrian CHIRA .....	237	Blagoj SURBEVSKI.....	368
Adrian Gheorghe ZUGRAVU .....	456	Boban MILETIĆ .....	1101
Adriana RADOSAVAC.....	285	Bojan STOJANOVIĆ .....	851
Agnieszka A. PILARSKA .....	957	Boško GAJIĆ .....	579
Ahmed EL SHAL.....	31	Branislav DRAŠKOVIĆ .....	499
Aiga NOLBERGA-TRŪPA.....	770	Branislav DUDIĆ.....	1019
Ait Aabd NAIMA .....	477	Branislav FILIPIĆ.....	1101
Ajla ALIŠAH.....	895	Branislav KOVAČEVIĆ.....	1101
Akife DALDA-SEKERCI.....	92	Branislav STANKOVIĆ .....	837, 888
Albana UKA .....	883	Branka GOVEDARICA.....	46, 66, 78, 172
Aldin BOŠKAILO .....	511, 524	Branka ORBOVIĆ .....	293
Aleksandar ČUPIĆ.....	579	Branka TOPIĆ-PAVKOVIĆ.....	910
Aleksandar GAVRILOVIĆ.....	78	Branka ŽIVANOVIĆ.....	591
Aleksandar GOLUBOVIĆ.....	1085	Branko ANGJELOVSKI .....	832
Aleksandar IVEZIĆ .....	469	Brano VAMOVIĆ.....	1085
Aleksandar KALAUZI.....	585	Bratislav KISIN .....	1108
Aleksandra ANGELESKA..337, 344, 351, 359, 778		Carla CEDROLA .....	308
Aleksandra BENSA .....	539	Carla S. S. FERREIRA .....	484
Aleksandra GOVEDARICA – LUČIĆ .....	37	Coen RITSEMA .....	484
Aleksandra GOVEDARICA-LUČIĆ .....	167	Cosmina - Ionela VASILACHE .....	456
Aleksandra MARKOVIĆ.....	255	Dalibor TOMIĆ .....	46, 129
Aleksandra MITROVIĆ.....	832	Damjan PANTIĆ .....	1085
Ali İhsan ATALAY .....	301	Damla BALABAN GÖÇMEN .....	52, 60
Ali Ümran KÖMÜŞÇÜ .....	680	Dane MARČETA.....	1041
Alma MEMIĆ .....	167	Danica MIĆANOVIĆ .....	285
Alpay BALKAN .....	52, 60	Daniela ĐIKANOVIĆ .....	585, 591
Aman ELGARHY.....	1009	Daniela-Nicoleta ROPOTAN .....	804
Amna OMANOVIC .....	331	Danijela ĐUNISIJEVIĆ-BOJOVIĆ.....	216
Anamarija ŠEVO .....	920	Danijela JUNGIĆ.....	539
Anastasia NAVROTSKAYA.....	315	Danijela KIROVSKI.....	810, 875
Anastasia-Charikleia KYRIAZAKI .....	210	Danijela PETROVIĆ .....	511, 524, 1041
Anđelija OBRADOVIĆ .....	84	Danijela ŽIVOJINOVIĆ .....	84, 255
Andreea Roxana FIRĂȚOIU .....	973	Danijela ŽUNIĆ.....	115
Andreja PREKOVIĆ.....	255	Darbali Fatima ZAHRA.....	477
Andrey BEREZOVSKIY .....	574	Darija OBRADOVIĆ.....	644
Andrey TOPCHEEV .....	1078	Dario JAREŠ.....	148
Andrijana ANDRIĆ .....	469	Darko UHER.....	148
Angelina BUTKO .....	315	Dejan CVIKIĆ .....	243
Antonela MUSA .....	524	Dejan DODIG .....	220
Antonio BOSCO .....	817	Dejan ORČIĆ.....	817
António José FERNANDES .....	784, 791, 798	Dejan SKOČAJIĆ .....	595, 602, 609
Arame THIOUNE .....	900	Dejan ZEJAK.....	261
Aris KYPARISSIS .....	154	Dejana STANIĆ.....	324
Artiona LAZE .....	277	Denis KUZNETSOV .....	1078
Asimina SKOUTERI .....	545	Desimir KNEŽEVIĆ.....	270, 277, 285, 293
Aslı DALGIC.....	1032	Dimitar NAKOV.....	837, 888
Aurelia-Ioana CHEREJI .....	973	Dimitrios BARTZIALIS .....	135, 204, 210
Aygül KARACA.....	729, 743	Dimitrios-Marios LEKKAS .....	135
Bahoch SAID .....	477	Dina AVEIRO.....	784, 798
Barbara TOMBARKIEWICZ .....	966	Dina ELISOVETCAIA.....	1064
Beatrice Michaela IACOMI.....	237	Divna SIMIĆ.....	463, 817, 989

Djurđja PETROV .....	595, 602, 609	Irina SFECLĂ .....	557
Dmitry SYSHCHYKOV .....	574	Irina-Adriana CHIURCIU .....	973
Đorđe LAZAREVIĆ .....	129, 826	Isidora SIMOVIĆ .....	595
Dragan BOROTA .....	1085	İsmet BAŞER .....	52, 60
Dragan ĐUROVIĆ .....	46	Iulia BOZBEI .....	122
Dragan GRČAK .....	270, 293	Iva RELOTA .....	524
Dragan VUJIĆ .....	602	Ivan HORVATIĆ .....	148
Dragana BARTOLIĆ .....	585, 591	Ivan VUJANAC .....	875
Dragana SKOČAJIĆ .....	216	Ivana BOŠKOVIĆ .....	72
Dragica GAGOVIĆ .....	167	Ivana KAVGIĆ .....	386
Dragica STOJANOVIĆ .....	817	Ivana MILOSEVIC-STANKOVIC .....	888
Drazenko BUDIMIR .....	750	Ivana SMIČIKLAS .....	579
Dubravko MACESIĆ .....	148	Ivana STANIMIROVIĆ .....	989
Dušan BASTA .....	392	Jacques NANEMA .....	531
Dušan BOŠNJAKOVIĆ .....	810, 875	Jan BRINDZA .....	1064
Dušan JOKANOVIĆ .....	615, 621, 1094	János TAMÁS .....	31
Dušan KOVAČEVIĆ .....	101	Jelena ČUKANOVIĆ .....	595, 602, 609
Dušan UROŠEVIĆ .....	270, 277, 285	Jelena DAMNJANOVIĆ .....	243
Dušanka VUJANOVIĆ .....	469, 639	Jelena JOVIĆ .....	469
Eda BOYACIOGLU .....	691	Jelena LAZAREVIĆ .....	72
Elena DOBRIN .....	237	Jelena MARKOVIĆ .....	463
Elena Maria DRAGHICI .....	237	Jelena STOJILJKOVIĆ .....	277
Elena SOARE .....	973	Jelica ŽIVIĆ .....	285
Elena TOFAN-DOROFEEV .....	557	Joanna MAKULSKA .....	966
Elizabeta DIMITRIESKA STOJKOVIKJ .	337, 359	Jovan BOJKOVSKI .....	832
Elizabeta DIMITRIESKA STOJKOVIKJ .....	344	Jovana OBRADOVIĆ .....	324
Elma SEFO .....	920	Jovana PETROVIĆ .....	615, 621
Emina ADEMOVIĆ .....	518	Jozef REPISKÝ .....	1025
Emina MEHIC .....	331	Juan de Oliveira MORAIS .....	791
Eray HARMAN .....	667	Julijana TRIFKOVIĆ .....	810, 875, 895
Ertan TASKAVAK .....	674	Kamil ÖZCAN .....	60
Fanta Sheirita Reine TIETIAMBOU .....	531	Kārlis SAMS .....	770
Fariborz SHEKARI .....	28, 228, 233	Katarina VELJOVIĆ .....	392
Faruk TOHUMCU .....	301	Kazimir MATOVIĆ .....	826
Fatima MUHAMEDAGIĆ .....	505	Khaled SEKKOUM .....	492
Fernando SOUSA .....	784, 798	Khalifa Serigne Babacar SYLLA .....	900
Filip ŠTRBAC .....	817	Koštana VINČIĆ .....	933
Filippo ACASTO .....	531	Kristina KALKAN .....	469
Filiz DADASER-CELİK .....	680, 685, 691	Kristina MILIŠIĆ .....	108
Gabor STENBACH .....	585	Kristina SOFRONIĆ .....	845
Gabriela Elena BAHRIM .....	380	Krzysztof PILARSKI .....	957
Gehan A.G. ELMENOFI .....	1009	Ksenija ČOBANOVIĆ .....	392
Goran ČEŠLJAR .....	398	Ksenija RADOTIĆ .....	585, 591
Goran JEVTIĆ .....	826	Kyriakos D. GIANNOULIS .....	135, 204, 210
Goran KIŠ .....	148	Ladislav VASILIŠIN .....	436, 1054
Goran VUČIĆ .....	436, 1054	Laura RINALDI .....	817
Gordana BRANKOVIĆ .....	220, 277	Lazar KESIĆ .....	1101
Halit FILIZ .....	674	Le THANH TOAN .....	141
Hamid EL BILALI .....	531	Le THI HOANG YEN .....	141
Hande Seda ÖZDAL .....	92	Le THI MONG CAM .....	141
Hasna ELHJOUJI .....	427, 477	Lejla RIDANOVIĆ .....	518
Helena BREKALO .....	511	Lenuța CHIRA .....	237
Ignacio J. DIAZ-MAROTO .....	710, 717, 723	Leonid POPOV .....	444
Igor ĐURĐIĆ .....	66, 78, 172	Liliana BĂDULESCU .....	161
Igor ESMEROV .....	337, 344, 351, 359, 778	Liviu MĂRCUȚĂ .....	973
Inese SKAPSTE .....	551	Izabeta DIMITRIESKA STOJKOVIKJ .....	351
Ioana-Cătălina NICOLAE .....	122, 161	Ljiljana ŠAŠIĆ ZORIĆ .....	469
Ippolitos GINTSIODIS .....	135, 204, 210	Ljiljana TANASIĆ .....	499
Irina AGUROVA .....	574	Ljubiša ŠEVKUŠIĆ .....	933
Irina CHEKHOVSKIKH .....	982	Ljubomir JOVANOVIĆ .....	810, 875

Ljupco ANGELOVSKI.....	337, 344, 351, 359, 778	Mirela MATKOVIĆ STOJŠIN.....	270, 277, 285
Ljupco MICKOV .....	778	Mirjam NIKOLIĆ .....	539
Lorena DEDIU.....	804	Mirjana JOVOVIĆ.....	37, 72, 167
Luka DRAGIĆEVIĆ .....	927	Mirjana OCOKOLJIĆ.....	595, 602, 609
Luuk FLESKENS .....	484	Mirjana PETROVIĆ .....	129, 826
Lyudmyla SYMOCHKO .....	484	Mirjana RADOVIĆ.....	261
Maja KAZAZIC.....	331	Mirko KULINA .....	261
Maria BEMPI.....	154	Miroļjub GOLUBOVSKI .....	368
Maria DALAKOURA.....	204	Miroslav LALOVIĆ.....	895
Maria Isabel RIBEIRO.....	784, 791, 798	Miroslava MARKOVIĆ.....	398
Maria TABĀRA.....	122, 161	Mohamed ALOUANI.....	427
Mariam KIEBRE .....	531	Mohsen JANMOHAMMADI.....	28, 228, 233
Marija GAVRILOVIĆ .....	84, 998	Mrđan ĐOKIĆ .....	579
Marija LASIĆ .....	927	Nada JELIĆ.....	404
Marija MARKOVIĆ .....	216	Nándor NAGY.....	1025
Marija PEROVIĆ.....	628, 634, 644, 649	Naser SABAGHNIA.....	28, 228, 233
Marija TODOROVIĆ.....	628	Nasser BELBOUKHARI .....	492
Marijana MASLOVARIĆ.....	989	Natalia ONICA .....	122, 161
Marijana MILUTINOVIĆ.....	216	Natalija PAVLOVIĆ.....	249
Mariyana NIKOLOVA .....	755, 763	Nataša GOLIĆ .....	392
Marko GUTALJ .....	1101	Nataša LAKIĆ-KARALIĆ .....	436, 1054
Mate BOBAN .....	511	Nataša LJUBIČIĆ .....	469
Mauro PAGANO .....	308	Natasa MARIC .....	180, 187
Mehmet SOYLU .....	685	Nataša SIMIN .....	817
Melania GHEREG .....	161	Nataša TOLIMIR .....	817, 989
Meltem KACIKOC .....	685, 691	Nedeljka ELEZ .....	1049
Merjem HUSKIĆ .....	505	Nedeljko RACIĆ.....	129
Merve Nur EKTIREN.....	1004	Nedim BEGIĆ.....	895
Mete CELİK.....	680	Nedim MARIĆ .....	193
Michał CUPIAŁ .....	966	Nenad PAVLOVIĆ.....	46, 129, 243
Mihailo GRBIĆ.....	216	Nenad STAVRETOVIĆ .....	615, 621
Mihajlo JOVIĆ.....	579	Nevena RISTIĆEVIĆ .....	66, 78, 172
Mihajlo LIZDEK .....	261	Nevenka GALEČIĆ .....	595, 602, 609
Milan BRANKOV .....	249	Nihad GERZIĆ .....	505
Milan ĐORĐEVIĆ .....	579	Niklāvs KLEINS.....	770
Milan GLIŠIĆ .....	499	Nikola LJILJANIĆ.....	998
Milan JUGOVIĆ .....	66	Nikola POPOVIĆ.....	392
Milan NINKOVIĆ .....	832	Nikolaos G. DANALATOS.....	135, 204, 210
Milan ŠEVIĆ .....	46	Nikolay MARKOV .....	778
Milan SUKUR .....	1041	Nikolina JURKOVIĆ BALOG .....	539
Milan UGRINOVIĆ.....	243	Nina CHIORCHINĂ.....	122, 161
Milena BILJIĆ .....	101	Nusret ÖZBAY .....	743
Milena SIMIĆ .....	101, 249	Oana VENAT .....	161
Milena STANKOVIĆ NEĐIĆ .....	1101	Oğuz BİLGİN .....	52, 60
Milica BLAŽIĆ.....	220	Olga IONITA .....	568
Milica GLIŠIĆ .....	108, 255	Olivera PRODANOVIĆ .....	591
Milica RADJENOVIC .....	837, 888	Özgül GERÇEKER.....	413
Milica STOJANOVIĆ.....	933	Ozlem Kizilirmak ESMER .....	422
Milica STOJKOVIĆ .....	810	Patrícia SANTOS.....	484
Milijana ŠKRBIĆ.....	750	Pedro R. SOARES .....	484
Milomirka MADIĆ .....	46	Péter RICZU .....	31
Miloš BAČIĆ .....	1101	Petronela NECHITA .....	380
Miloš LUKIĆ .....	851	Pham THI PHUONG THAO .....	141
Miloš MANIĆ.....	579	Predrag IVANKOVIĆ .....	927
Miloš PROKOPIJEVIĆ .....	585, 591	Rachid BOUHARROUD .....	427, 477
Milosav GRČAK .....	270, 293	Radenka KOLAROV .....	595, 602, 609
Mimouna YAKOUBI.....	492	Radiša PRODANOVIĆ .....	832
Miodrag ŠTRBAC .....	1041	Radivoje JEVTIĆ.....	293
Miodrag TOLIMIR .....	249	Radmila CRCEVA NIKOLOVSKA.....	337, 344, 351, 359, 778
Mira STANKOVIĆ.....	585, 591		

Radomir BODIROGA.....	933	Staniša LATINOVIĆ .....	436, 1054
Radomir RATAJAC.....	817	Stanislava LAZAREVSKA.....	368
Raisa IVANOVA.....	1064	Stefan JOVANOVIĆ .....	344
Ranko DRAGOVIĆ .....	579	Stefania PASCHALI-PAPANASTASIOU .....	135
Redouan QESSAOUI.....	427, 477	Sterja NACHESKI.....	368
Renata GAGIĆ-SERDAR.....	398	Stevan ČANAK .....	998
Rianatou BADA-ALAMBEDJI.....	900	Süleyman TEMEL .....	301
Risto UZUNOV .....	337, 344, 351, 778	Suzana PAVLOVIĆ.....	243
Robert KEPIĆ.....	518	Sveta ARSIĆ.....	832
Robert RADIŠIĆ.....	998	Svetlana MORKOVINA .....	1078
Roberto TOMASONE.....	308	Tahiri ABDEGHANI.....	477
Romarić Kiswendsida NANEMA.....	531	Tanja JAKIŠIĆ .....	66, 78
Sadibou BA.....	900	Tanja MIJATOVIĆ.....	172
Safija BOŠKAILO .....	511, 524	Tanja VUČIĆ.....	845
Safouane BENJAAA.....	427	Tatiana CALALB.....	122
Salahddine CHAFIKI.....	427, 477	Tatiana DAVID.....	444
Sandra MEDIĆ.....	524	Tatiana SHACHEK.....	315
Sandra MOJSOVA.....	351, 359	Tatjana MITROVIĆ.....	628, 634, 644, 649
Sanel RIĐANOVIĆ .....	518	Tijana BANJANIN .....	108
Sara ĐORĐEVIĆ.....	609	Tijana BAROŠEVIĆ.....	469
Sarina REZAEI SHOJAEI.....	270	Tijana MILICEVIĆ.....	649
Saša LALIĆ .....	939	Tolga AKCAN.....	413
Saša LAZOVIĆ.....	644	Tomislav ŽIVANOVIĆ .....	220
Saša MATIJAŠEVIĆ .....	255	Toni GALIĆ .....	511
Saša ORLOVIĆ .....	1101	Turgut AYGÜN .....	857, 863, 869
Sasho SABEV .....	755, 763	Uroš GALINAC.....	933
Seda AKBAY TOHUMCU.....	301	Valentina-Elena GORGAN.....	380
Şelale Öncü GALUE.....	413	Valerian CERBARI .....	444
Selena KOVAČEVIĆ .....	66, 72, 78	Vassilios P. PAPANASTASIS .....	545
Sema Ezgi YÜCEER .....	736	Vedran TOMIĆ.....	84, 989, 998
Semira SEFO .....	193	Velibor SPALEVIC .....	1019
Sevcan ÜNLÜTÜRK .....	413	Veli-Matti ROKKA .....	531
Sevgi CAY .....	422	Vera POPOVIĆ.....	463
Sibel TAN .....	736	Vera RAJIČIĆ.....	293
Simeon RAKONJAC .....	851	Veselin PETRIČEVIĆ .....	851
Simin HAGH-NAZARI .....	270	Veselinka ZEČEVIĆ.....	277
Slađan STANKOVIĆ.....	817	Vesna DJUROVIĆ.....	270
Sladjana PETRONIC .....	180, 187	Vesna DRAGIČEVIĆ.....	249
Slavca HRISTOV.....	837	Vesna KANDIĆ .....	220, 285
Slavča HRISTOV.....	888	Vesna MILIĆ .....	78, 172
Slavica ČABRILO .....	404	Vesna NIKOLIĆ JOKANOVIĆ .....	615, 621, 1094
Slavica DRAŽIĆ.....	810	Vesna ŽUPANSKI.....	293
Slavisa MILOVANOVIĆ .....	261	Višnja SIKIMIĆ.....	404
Slobodan KRNJAJIĆ .....	817	Vladan PEŠIĆ .....	463
Snežana ANĐELKOVIĆ .....	826	Vladeta STEVOVIĆ .....	46
Snežana BABIĆ .....	826	Vladimir DOSKOVIĆ .....	851
Snežana BOGOSAVLJEVIĆ-BOŠKOVIĆ .....	851	Vladimir PETKOVIĆ .....	1041
Snezana DIMITROVSKA .....	359	Vladimir SABADOŠ .....	115
Snežana DRAGOVIĆ .....	579	Vladimir VASIĆ .....	1108
Snežana GLAMOČIĆ.....	392	Vladimir ZORNIĆ .....	129, 826
Snežana JANKOVIĆ .....	463, 989	Vojin CVIJANOVIĆ .....	463, 989
Snežana JOVANOVIĆ .....	845	Władysław MIGDAŁ.....	966
Snežana OLJAČA.....	101	Yoldaş EKTİREN .....	1004
Snežana POPOV .....	639, 661	Yoldaş EKTİREN .....	700, 705
Sonja MUDRI-STOJNIĆ.....	386, 655	Zakaria AL AJLOUNI .....	199
Sonja OBRENOVIC .....	888	Zakaria KIEBRE.....	531
Soumaya EL ASSRI.....	427, 477	Zdenka GIREK .....	243
Srdan LJUBOJEVIĆ .....	436, 1054	Zdenka ŠKRBIĆ.....	851
Srdan STOJNIĆ .....	1101	Željko DOLJANOVIĆ .....	101, 249, 463
Sreten NEDIĆ .....	832, 875	Željko SLADOJEVIĆ.....	810, 875

Zemira DELALIĆ .....	505	Zoran PRŽIĆ.....	255
Zinaida YEGOROVA.....	315	Zoranka MALEŠEVIĆ .....	37, 72
Zlata MARKOV RISTIĆ .....	386, 639, 655, 661	Zorica GOLIĆ.....	939, 948
Zoran JOVOVIĆ .....	101	Zorica RANKOVIĆ VASIĆ .....	108
Zoran LUGIĆ .....	129	Zrinka KNEZOVIĆ .....	920
Zoran MATIĆ .....	187	Zvonimir BAKOVIĆ .....	1108