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April 10 - 12, 2026

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APRIL 10 – 12, 2026
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İlgi : 27.03.2024 tarihli ve E--903.07-474236 sayılı yazı

Fakültemiz Tıbbi Biyokimya Anabilim Dalı'nda görevli öğretim üyesi Prof. Dr. Hülya ÇİÇEK'in Yükseköğretim Genel Kurulunun 15.06.2023 tarihli, 10 sayılı oturumunda alınan 2023.10.183 sayılı kararı gereğince Doçentlik Başvuru Şartlarında bulunan ve doçent olacak adaylardan istenen "Diğer uluslararası/ ulusal bilimsel toplantının düzenleme komitesinde resmi olarak görevlendirilmiş üniversite akademisyen temsilcisi bulunması zorunludur." maddesi gereğince, Academy Global Conference & Journals tarafından yapılan kongrelerin düzenleme kurullarında yolluksuz ve yevmiyesiz olarak görevlendirilme talebi ile ilgili dilekçesi ekte gönderilmiştir

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Salon	Moderator		Bildiri No ve Başlığı / Paper ID and Title	Authors
HALL / SALON 1	**Doç. Dr. LATİFE CEYDA İRKİN**	1	GENETIC ENGINEERING AND SYNTHETIC BIOLOGY	Doç. Dr. LATİFE CEYDA İRKİN
		2	BIOMATERIALS AND NEXT GENERATION APPLICATIONS	Doç. Dr. LATİFE CEYDA İRKİN
		3	HIERARCHICAL DYNAMICS IN HUMAN FRATAXIN: BRIDGING NANOSECOND LOCAL FLUCTUATIONS TO MICROSECOND-MILLISECOND CONFORMATIONAL EXCHANGE THROUGH TRANSFER ENTROPY ANALYSIS OF MOLECULAR DYNAMICS SIMULATIONS	Res. Asst. Kevser Kübra Kırboğa Prof. Dr., Ecir Uğur Küçüksille
		4	THE HIDDEN DANGER IN HONEYCOMBS: THE IMPACT OF CHEMICAL RESIDUE RISKS ON BEE AND HUMAN HEALTH	Dr., Ekin VAROL Prof. Dr., Banu YÜCEL
		5	THE PARADIGM EFFECT OF HONEY BEES IN PLANT POLLINATION	Dr., Ekin VAROL Prof. Dr., Banu YÜCEL
		6	DISULFIDPTOSIS AND THE 2-HG AXIS IN NEURO-ONCOLOGY: CSF-BASED MULTIMODAL THERANOSTIC APPROACHES	Assist. Prof. Dr. Halil İbrahim AKBAY,
		7	THE ONCOMETABOLOMIC VISION OF CLINICAL BIOCHEMISTRY: METABOLIC REPROGRAMMING IN CANCER AND SPECIFIC ONCOMETABOLITES	Assist. Prof. Dr. Halil İbrahim AKBAY,

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Salon	Moderator		Bildiri No ve Başlığı / Paper ID and Title	Authors
HALL / SALON 2	**Assistant Professor İbrahim GECİLİ**	1	IMPACT OF BORON FERTİLİZATİON ON YİELD AND OİL QUALİTY OF SUNFLOWER (Helianthus annuus L.)	Dr., Hakan YILDIZ
		2	STRATEGIC RATIONAL REDESIGN AND MULTI-PARAMETRIC IN SILICO PROFILING OF A NOVEL PARABUTOPORIN MUTANT (PARP-MTN) FOR ENHANCED ANTIMICROBIAL EFFICACY	Master's Student Derya ÇAĞATAY KAYIŞ Dr.Öğr.Üyesi Handan Açelya KAPKAÇ Doç.Dr. Hülya KARACA ATSOROS
		3	IRISIN'S ASSOCIATION WITH GENE AND MIRNA EXPRESSION ALTERATIONS IN AN MPP^+-INDUCED IN VITRO PARKINSON'S DISEASE MODEL	Tutku KARABAŞ Arş. Gör Hilal SANCAR Öğr. Gör. Dr. Deniz ŞUMNULU Prof. Dr. Lokman AYAZ
		4	PROTECTIVE EFFECTS OF GALLIC ACID AGAINST ARSENIC-INDUCED CYTOTOXICITY IN Caco-2 CELLS	Assistant Professor İbrahim GECİLİ Associate Professor Adem GÜNER
		5	PROTECTIVE ROLE OF SINAPIC ACID AGAINST HYDROGEN PEROXIDE-INDUCED OXIDATIVE NEURONAL INJURY IN SH-SY5Y CELLS	Assist. Prof. Dr. İbrahim Gecili Assoc. Prof. Dr. Adem Güner
		6	ITIR BİTKİSİNİN ENDOFİTİK FUNGAL ÇEŞİTLİLİĞİ	Sanem UZGUR Sevinç KARAGÖZ Doç. Dr. Gülsüm Ebru ÖZER UYAR
		7	KULLANILMIŞ KAHVE ATIĞI EKSTRAKTININ FİTOPATOJENİK KÜFLERE KARŞI ANTİFUNGAL ETKİSİNİN DEĞERLENDİRİLMESİ	Ceren KAPLAN Doç. Dr. Gülsüm Ebru ÖZER UYAR

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HALL / SALON 3	**Dr. Öğr. Üyesi Ebru BOĞA BARAN**	1	YAPAY ZEKÂ DESTEKLİ EĞİTİM ARAÇLARININ ERKEN ÇOCUKLUK EĞİTİMİNDE KULLANIM POTANSİYELİ	Dr. Öğr. Üyesi Ebru BOĞA BARAN
		2	DENEYSEL YÖNTEMLERİN SANATTA KULLANIMI: ISI İLE FORM DÖNÜŞÜMÜ ÜZERİNE BİR UYGULAMA	Dr. Öğretim Üyesi SİBEL ARMAĞAN BENEK
		3	FROM TEXT TO CRITICAL TRANSFORMATION: EXPLORING CRITICAL READING AND WRITING PRACTICES IN ELT TEACHER EDUCATION	Asst. Prof. Dr. MANOLYA SAĞLAM
		4	REFRAMING LISTENING AND PRONUNCIATION PEDAGOGY THROUGH IELTS-ORIENTED TASKS: A CASE STUDY IN ELT TEACHER EDUCATION	Asst. Prof. Dr. MANOLYA SAĞLAM
		5	EXAMINING PRIMARY SCHOOL TEACHERS' NEEDS IN SCIENCE TEACHING: A MIXED METHODS STUDY	Dr., ZAFER HANEDAR Dr. Öğrt. Üyesi, ALİ RIZA ERDEM
		6	ENGLISH CURRICULUM CHANGE AND TEACHER AGENCY UNDER TÜRKİYE'S NEW EDUCATION MODEL	Lecturer PhD, ÖZLEM UTKU BİLİCİ
		7	ARTIRILMIŞ GERÇEKLİK DESTEKLİ FEN ÖĞRETİMİ SÜRECİNDE ÖĞRETMEN ADAYLARININ EĞİTİM TEKNOLOJİLERİ ÖZYETERLİLİĞİ VE BİLGİ İŞLEMSEL DÜŞÜNME BECERİLERİ ARASINDAKİ İLİŞKİ	Prof. Dr. Ali Günay BALIM Prof. Dr. Yasin ÖZARSLAN Doktora Öğrencisi, Betül ÖZTAŞ Doktora Öğrencisi, Ece ALTAY
		8	SANAL SOSYAL DESTEK VE SOSYAL ONAY İHTİYACININ SOSYAL VE DUYGUSAL YALNIZLIK ÜZERİNDEKİ YORDAYICI ROLÜ: GENÇ YETİŞKİNLER ÖRNEĞİ	Yüksek Lisans Öğrencisi, Beril YALAMA Dr. Öğr. Üyesi Muhammet Fatih YILMAZ
		9	TÜBİTAK 2209-A PROJESİ KAPSAMINDA TEZHİP SANATI İLE TOPLUMSAL KATKI: "KADIN ELİYLE ALTIN RENKLER"	Ayşenur ASKER Doçent, Betül COŞKUN ÇELİK

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HALL / SALON 4	**Assistant Professor ARZU USLU**	1	ASSESSMENT OF NUMBER SENSE SKILLS IN PRIMARY SCHOOL STUDENTS WITH HEARING LOSS	Audiologist, NAZLI ÖZSÖZ Prof. Dr., PELİN PİŞTAV AKMEŞE
		2	EFFECTS OF PRENATAL YOGA ON OXIDATIVE STRESS AND ITS CONTRIBUTIONS TO MATERNAL–FETAL HEALTH	Dr. Öğr. Üyesi Suzan ONUR Uzm. Ebe Hatice TOPÇU
		3	THE RELATIONSHIP BETWEEN SLEEP DISORDERS, MATERNAL FATIGUE, AND OXIDATIVE STRESS DURING PREGNANCY	Dr. Öğr. Üyesi Suzan ONUR Zaytuna HASHİMİ
		4	INTERGENERATIONAL FAMILY TRAUMA AND PSYCHOLOGICAL RESILIENCE: THE MEDIATING ROLE OF PSYCHOLOGICAL FLEXIBILITY	Asst. Prof. Dr. NURTEN ARSLAN IŞIK Res. Asst. İREM NUR SANDIKÇI
		5	CYBERTHERAPY-SUPPORTED ONCOLOGICAL CARE IN OLDER ADULTS WITH CANCER	Assistant Professor ARZU USLU
		6	İNVAZİV OLMAYAN DOĞUM ÖNCESİ TESTLER VE CİNSİYET SEÇİMİ: ETİK BOYUT VE GÜNCEL UYGULAMALAR	Dr. Ebe, TUĞBA KAVAS Prof. Dr., ÖZLEM KARABULUTLU Dr. Öğr. Üyesi, CANSU MİNE AYDIN Uzman Ebe, YAĞMUR KOTANCI Öğr. Gör. Dr., GÜLHAN AKDEMİR
		7	POLİKİSTİK OVER SENDROMU VE TEDAVİ YÖNTEMLERİ	Dr. Ebe, TUĞBA KAVAS Prof. Dr., ÖZLEM KARABULUTLU Uzman Ebe, YAĞMUR KOTANCI Dr. Öğr. Üyesi, CANSU MİNE AYDIN Öğr. Gör. Dr., GÜLHAN AKDEMİR
		8	EVALUATION OF OPERATING ROOM NURSES' EXPERIENCE, KNOWLEDGE LEVEL, CHALLENGES, AND INTRAOPERATIVE COMMUNICATION IN MICROSURGERY	Dr. Yavuz ÖNEL

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HALL / SALON 5	**Prof. Dr. Meryem YAVUZ Van GİERSBERGEN**	1	PREOPERATIVE NURSING CARE COMPETENCE AMONG NURSING STUDENTS: FINDINGS FROM NURSING EDUCATION RESEARCH	Prof. Dr. Meryem YAVUZ Van GİERSBERGEN Nurse Beyza SÜZEN
		2	EVIDENCE-BASED PRACTICE COMPETENCE İN NURSING EDUCATION	Prof. Dr. Meryem YAVUZ Van GİERSBERGEN Nurse Beyza SÜZEN
		3	GENERAL SELF-EFFICACY AMONG TURKISH NURSING STUDENTS: A LITERATURE REVIEW	Semiha TİMOÇİN Prof. Dr. Meryem YAVUZ Van GİERSBERGEN
		4	AN EXAMINATION OF ACADEMIC SELF-EFFICACY AMONG STUDENTS IN TÜRKIYE	Semiha TİMOÇİN Prof. Dr. Meryem YAVUZ Van GİERSBERGEN
		5	GENERATIONS OF PATIENT-CENTERED PERSPECTIVES IN CANCER SURGERY: ALIGNING TREATMENT PATHWAYS WITH PATIENT VALUES	Öğr. Gör. Tülin KARAKOÇ Prof. Dr. Meryem YAVUZ Van GİERSBERGEN
		6	PREPARATION FOR COLORECTAL CANCER SURGERY: STUDIES USING THE PCSQ-PRE 24	Öğr. Gör. Tülin KARAKOÇ Prof. Dr. Meryem YAVUZ Van GİERSBERGEN
		7	MAPPING RESEARCH ON DIABETIC FOOT CARE İN NURSING: AN ANALYSIS OF GRADUATE THESES İN TÜRKIYE	Arş. Gör. Züleyha SÖNMEZ Prof. Dr. Meryem YAVUZ Van GİERSBERGEN

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HALL / SALON 6	**Prof. Dr. NALAN DEMİRCİOĞLU**	1	SİNEMA UYARLAMALARI ÜZERİNDEN KONUT İÇ MEKÂNININ KÜLTÜREL KODLAR BAĞLAMINDA ANALİZİ: BENİM DÜNYAM FİLMİ	Yük. Lisans Öğrencisi, MERVE KARAKURT Yük. Lisans Öğrencisi, ESİN ARSLAN Doç. Dr., HARE KILIÇASLAN
		2	MİMARLIK EĞİTİMİNDE UZAMSAL YETENEĞİN ÖNEMİNE BİR BAKIŞ	Doktora Öğrencisi, ÖZLEM NUR SAMANCI Doç. Dr., HARE KILIÇASLAN
		3	AİLE SAĞLIĞI MERKEZLERİNDE HAVALANDIRMA ve BULAŞ RİSKİ: CFD ANALİZİ	KÜBRA YILDIZ ULAŞ Dr. Öğr. Üyesi MELTEM EZEL ÇIRPI
		4	AKILLI ŞEHİRLER VE YAPAY ZEKA BAĞLAMINDA ÇEVRESEL SÜRDÜRÜLEBİLİRLİK ARAŞTIRMALARIN BİBLİYOMETRİK ANALİZİ	Yük. Lis. Öğr. Mevhibe DEMİRCAN Yük. Lis. Öğr. Gülbanu ÇUBUKÇU Prof. Dr. Nalan DEMİRCİOĞLU
		5	YEŞİL ÇATI SİSTEMLERİNİN SÜRDÜRÜLEBİLİRLİK VE İKLİM DEĞİŞİKLİĞİ ÜZERİNDEKİ ETKİLERİ: BİR BİBLİYOMETRİK ANALİZ	Yük. Lis. Öğr., GÜLBANU SÜMEYYE ÇUBUKÇU Yük. Lis. Öğr., MEVHİBE DEMİRCAN Prof. Dr. NALAN DEMİRCİOĞLU
		6	EVALUATION OF CULTURAL BUILDINGS DESIGNED WITH A PARAMETRIC DESIGN APPROACH WITHIN THE SCOPE OF ICONIC STRUCTURES	M.Arch., Edanur BİRİNCİ Prof. Dr. Çiğdem Belgin DİKMEN
		7	EVALUATION OF ICONIC STRUCTURES IN THE CONTEXT OF SUSTAINABLE TOURISM THROUGH EXAMPLES	M.Arch., Edanur BİRİNCİ Prof. Dr. Çiğdem Belgin DİKMEN

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HALL / SALON 7	**Asst. Prof. Dr. DÜNYA KARAPINAR**	1	BIFURCATION ANALYSIS AND CHAOS CONTROL IN A NONSTANDARD DISCRETIZED POPULATION MODEL	Cahit KÖME
		2	DYNAMICAL ANALYSIS OF A MEMORY-DEPENDENT 3D POPULATION MODEL	Merve KARTAL Cahit KÖME
		3	DOUBLE DİZİLER İÇİN RİESZ LACUNARY DÜZGÜN İNTEGRALLENEBİLİRLİK, RİESZ İSTATİSTİKSEL YAKINSAKLIK VE RİESZ KUVVETLİ YAKINSAKLIK	Beyzanur COŞAR Prof.Dr.Mustafa YILDIRIM
		4	MEAN SQUARED ERROR MATRIX COMPARISONS BETWEEN WEIGHTED MIXED REGRESSION ESTIMATION	Asst. Prof. Dr. DÜNYA KARAPINAR MURAT POLAT Assoc. Prof. Dr. NİMET ÖZBAY Prof. Dr. SELAHATTİN KAÇIRANLAR
		5	SOME INTEGRAL TYPE BEST PROXIMITY CIRCLE RESULTS ON G-METRIC SPACES	Hatice Merve PEKER Assoc. Prof. Dr. Nihal TAŞ
		6	λ-STATISTICAL CONVERGENCE OF ORDER α IN CONE METRIC SPACES	Arş. Gör. SÜLEYMAN SARIKAYA Prof. Dr. YAVUZ ALTIN

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HALL / SALON 8	**Associate Prof. Makbulenur ONUR**	1	ARTIFICIAL INTELLIGENCE IN LANDSCAPE ARCHITECTURE EDUCATION	Prof. Dr. Elif Merve ALPAK Prof. Dr. Tuğba DÜZENLİ
		2	SUSTAINABLE URBAN OPEN SPACE DESIGN	Prof. Dr. Elif Merve ALPAK Prof. Dr. Tuğba DÜZENLİ
		3	EFFECTS OF WINDOW-TO-WALL RATIO AND SOLAR TRANSMITTANCE ON ENERGY PERFORMANCE IN HOT CLIMATES	Res. Assist. Dr. OKAN ŞİMŞEK
		4	RECONSTRUCTING TOPOGRAPHY: FORM, PERFORMANCE, AND SPATIAL EXPERIENCE IN LANDSCAPE ARCHITECTURE	Prof. Dr. SERAP YILMAZ Res.Asst. Nida KURAK SEZGİN
		5	AN ANALYSIS OF SPATIAL AFFORDANCE IN URBAN GREEN SPACES THROUGH THE LENS OF WOMEN'S EXPERIENCES: THE CASE OF TRABZON BOTANICAL GARDEN	Res.Asst., Nida KURAK SEZGİN Prof.Dr., Serap YILMAZ
		6	IMITATING NATURE: BIOMIMICRY IN LANDSCAPE DESIGN	Associate Prof. Makbulenur ONUR Asist.Prof. Dr., Demet Ulku GULPINAR SEKBAN
		7	LANDSCAPE VALUE OF ORCHID GARDENS: THE CASE OF SINGAPORE	Associate Prof. Makbulenur ONUR Asist.Prof. Dr., Demet Ulku GULPINAR SEKBAN

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HALL / SALON 1	**Assoc. Prof. Dr. Dimitri Stavros**	1	NONLINEAR DYNAMICAL SYSTEMS ANALYSIS FOR PREDICTING CHAOTIC BEHAVIOR IN ALGERIAN DESERT ECOSYSTEMS	Prof. Dr. Karim Belkacem Dr. Nadia Zerhouni Yacine Amrane
		2	OPTIMAL CONTROL THEORY APPLICATIONS IN ALGERIAN WATER RESOURCE MANAGEMENT MODELS	Assoc. Prof. Dr. Samir Lounis Amina Bouzid Karim Mansouri Zahra Haddad
		3	FRACTIONAL CALCULUS METHODS FOR SOLVING HEAT TRANSFER EQUATIONS IN INDIAN INDUSTRIAL PROCESSES	Dr. Rajesh Kumar Priya Sharma
		4	GRAPH THEORY ALGORITHMS FOR NETWORK OPTIMIZATION IN INDIAN TRANSPORTATION SYSTEMS	Prof. Dr. Sanjay Patel Anjali Desai Rohan Mehra
		5	STOCHASTIC PROCESSES MODELING FOR KAZAKHSTANI FINANCIAL MARKET VOLATILITY PREDICTION	Assoc. Prof. Dr. Gulnara Abayeva Yerzhan Tolegenov
		6	NUMERICAL SOLUTIONS TO PARTIAL DIFFERENTIAL EQUATIONS IN KAZAKH PETROLEUM RESERVOIR SIMULATIONS	Dr. Aizhan Kassenova Nurlan Bekbolatov Dina Sarsenbayeva
		7	DIFFERENTIAL GEOMETRY APPLICATIONS IN ANALYZING GREEK ANCIENT ARCHITECTURAL STRUCTURES	Prof. Dr. Eleni Papadopoulos Dr. Nikos Karamanlis
		8	FUNCTIONAL ANALYSIS TECHNIQUES FOR QUANTUM MECHANICS PROBLEMS IN GREEK RESEARCH	Assoc. Prof. Dr. Dimitri Stavros Maria Kostas

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HALL / SALON 2	**Assoc. Prof. Dr. Mira Gashi**	1	NONLINEAR DYNAMICAL SYSTEMS ANALYSIS FOR CHAOTIC BEHAVIOR PREDICTION IN IRANIAN HYDROLOGICAL MODELS	Dr. Soraya Karimi Ali Jafari
		2	FRACTIONAL CALCULUS APPLICATIONS IN OPTIMIZING IRANIAN RENEWABLE ENERGY GRIDS	Assoc. Prof. Dr. Hassan Tavakoli Fatemeh Hosseini
		3	GRAPH THEORY APPROACHES TO ALBANIAN URBAN TRANSPORTATION NETWORK OPTIMIZATION	Dr. Arben Hoxha Elsa Deda Prof. Dr. Luan Kuci
		4	NUMBER THEORY INVESTIGATIONS INTO ALBANIAN CRYPTOGRAPHIC SECURITY PROTOCOLS	Prof. Dr. Ivan Petrov Dr. Natalia Sokolova Mikhail Ivanov
		5	STOCHASTIC PROCESSES MODELING FOR RUSSIAN FINANCIAL MARKET VOLATILITY FORECASTING	Assoc. Prof. Dr. Mira Gashi Krenar Lleshi
		6	TOPOLOGICAL DATA ANALYSIS FOR RUSSIAN BIOMEDICAL IMAGE PROCESSING ALGORITHMS	Assoc. Prof. Dr. Elena Kuznetsova
		7	DIFFERENTIAL EQUATIONS SOLUTIONS FOR EGYPTIAN POPULATION GROWTH PROJECTIONS	Prof. Dr. Ahmed El-Sayed Dr. Fatima Mahmoud Karim Abdel Nadia Salem

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HALL / SALON 3	**Assoc. Prof. Dr. Firew Bekele**	1	MICROBIAL DIVERSITY AND FUNCTIONAL ANALYSIS IN TAIWAN HIGH-MOUNTAIN HOT SPRINGS	Prof. Dr. Li-Wei Chen Dr. Mei-Hui Lin Hao-Ting Wu Yi-Chen Huang
		2	PHYTOREMEDIATION POTENTIAL OF TAIWANESE NATIVE PLANTS FOR INDUSTRIAL WASTEWATER TREATMENT	Assoc. Prof. Dr. Chun-Yen Wang Shu-Fen Tsai
		3	GENETIC ADAPTATION MECHANISMS OF ETHIOPIAN COFFEE ARABICA VARIETIES TO DROUGHT STRESS	Dr. Alemayehu Tadesse Meron Getachew Prof. Dr. Solomon Abate
		4	BIODIVERSITY CONSERVATION STRATEGIES FOR ETHIOPIAN HIGHLAND FROG SPECIES	Assoc. Prof. Dr. Firew Bekele Tigist Demissie
		5	MOLECULAR IDENTIFICATION OF IRANIAN PISTACHIO PATHOGENS AND RESISTANCE GENES	Dr. Reza Jalali Fatemeh Karimi Prof. Dr. Mohammad Hosseini
		6	PHYTOCHEMICAL SCREENING OF MEDICINAL HERBS FROM IRANIAN ZAGROS MOUNTAINS	Assoc. Prof. Dr. Sara Mahmoudi Ali Rezaei
		7	ENDANGERED BIRD POPULATION DYNAMICS IN GEORGIAN CAUCASUS MOUNTAIN ECOSYSTEMS	Dr. Giorgi Tsivtsivadze Nino Chikhradze Lasha Kapanadze
		8	FUNGAL DIVERSITY SURVEY IN GEORGIAN BLACK SEA COASTAL FORESTS	Prof. Dr. Mariam Lomtadze Irakli Beridze
		9	GENETIC DIVERSITY ASSESSMENT OF GEORGIAN WINE GRAPE CULTIVARS	Assoc. Prof. Dr. Tamar Gelashvili Koba Kervalishvili

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HALL / SALON 4	**Assoc. Prof. Dr. Elnur Hasanov Dr. Aysel Mammadova**	1	ENZYMATIC SYNTHESIS OF NOVEL GLYCOLIPIDS FOR BIOMEDICAL APPLICATIONS USING ENGINEERED LIPASES	Prof. Dr. Lim Wei Ming Dr. Tan Soo Ling Chen Yi Xuan
		2	MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS BIOMARKERS IN NEURODEGENERATIVE DISEASE MODELS	Assoc. Prof. Dr. Rajesh Kumar Ng Hui Fen Dr. Amirul Al-Ashraf
		3	BIOCHEMICAL CHARACTERIZATION OF DATE PALM PHOSPHATASES UNDER DROUGHT STRESS CONDITIONS	Dr. Ahmed El-Sayed Fatima Mahmoud
		4	METABOLIC PATHWAY ANALYSIS OF ANTIBIOTIC-PRODUCING ENDOPHYTES FROM EGYPTIAN DESERT PLANTS	Prof. Dr. Nadia Karim Omar Hassan Layla Abdel
		5	INVESTIGATION OF LIPID PEROXIDATION PRODUCTS IN CARDIOVASCULAR DISEASE PATIENTS	Assoc. Prof. Dr. Elnur Hasanov Dr. Aysel Mammadova
		6	PROTEOMIC PROFILING OF STRESS-INDUCED CHANGES IN AZERBAIJANI GRAPE CULTIVARS	Leyla Ibrahimova Kamal Gurbanov Dr. Nigar Aliyeva
		7	GLYCOGENOLYSIS REGULATION IN HIGH-ALTITUDE ADAPTED LIVESTOCK FROM KAZAKHSTAN STEPPES	Prof. Dr. Aigerim Zhumaliyeva Nursultan Bektasov
		8	MICROBIAL ENZYME DISCOVERY FOR BIOFUEL PRODUCTION FROM KAZAKH WHEAT RESIDUES	Dr. Dana Tulegenova Assoc. Prof. Dr. Yerzhan Mukhammedov Aidana Kassenova

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HALL / SALON 5	**Assoc. Prof. Dr. Leyla Hasanova**	1	CRISPR-CAS9 MEDIATED GENE EDITING FOR DROUGHT RESISTANCE IN ROMANIAN WHEAT VARIETIES	Prof. Dr. Andrei Popescu Dr. Maria Ionescu Ioana Vasilescu
		2	EPIGENETIC MODIFICATIONS ASSOCIATED WITH CANCER PROGRESSION IN ROMANIAN POPULATIONS	Assoc. Prof. Dr. Radu Stanescu Elena Dragomir Mihai Popa
		3	NANOPARTICLE DELIVERY SYSTEMS FOR SIRNA THERAPEUTICS IN EGYPTIAN LIVER CANCER MODELS	Dr. Ahmed Khalil Fatima Salem Omar Hassan Prof. Dr. Nadia El-Gendy
		4	MITOCHONDRIAL DNA MUTATIONS LINKED TO METABOLIC DISORDERS IN EGYPTIAN COHORTS	Assoc. Prof. Dr. Karim Abdel Layla Mahmoud
		5	TELOMERE LENGTH VARIATIONS AND AGING PROCESSES IN AZERBAIJANI ELDERLY POPULATIONS	Dr. Elnur Mammadov Assoc. Prof. Dr. Leyla Hasanova Nigar Aliyeva
		6	NON-CODING RNA REGULATION OF GENE EXPRESSION IN AZERBAIJANI CARDIOVASCULAR PATIENTS	Prof. Dr. Kamal Huseynov Aysel Ibrahimova
		7	GENOME-WIDE ASSOCIATION STUDIES FOR DIABETES SUSCEPTIBILITY IN PAKISTANI POPULATIONS	Dr. Arif Khan Sana Malik Assoc. Prof. Dr. Bilal Ahmed Hina Gul
		8	MICRORNA BIOMARKERS FOR BREAST CANCER EARLY DETECTION IN PAKISTANI WOMEN	Prof. Dr. Faisal Rehman Zainab Noor
		9	PROTEIN-PROTEIN INTERACTION NETWORKS IN PAKISTANI LUNG CANCER PATHOGENESIS	Dr. Omar Siddiqui Rabia Saleem Muhammad Arif

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HALL / SALON 6	**D Assis. Prof. Dr. Nyoman Artawa**	1	PHYTOREMEDIATION POTENTIAL OF ROMANIAN DANUBE DELTA PLANTS FOR HEAVY METAL POLLUTION	Prof. Dr. Andrei Popescu Dr. Maria Ionescu Ioana Vasilescu
		2	MICROBIAL DIVERSITY IN CARPATHIAN CAVE ECOSYSTEMS AND THEIR BIOTECHNOLOGICAL APPLICATIONS	Assoc. Prof. Dr. Radu Stanescu Elena Dragomir Victor Popa
		3	CORAL BLEACHING RESISTANCE MECHANISMS IN INDONESIAN RAJA AMPAT MARINE PROTECTED AREAS	Dr. Sari Wijaya Budi Santoso Lina Pratiwi Dewi Kusuma I Made Setiawan
		4	GENETIC DIVERSITY ASSESSMENT OF SUMATRAN ORANGUTAN POPULATIONS USING NON-INVASIVE SAMPLING	Assis. Prof. Dr. Nyoman Artawa
		5	PHYTOCHEMICAL ANALYSIS OF PERSIAN GULF MANGROVE SPECIES FOR ANTICANCER ACTIVITY	Assoc. Prof. Dr. Reza Jalali Fatemeh Karim Mohammad Rezaei
		6	MICROPLASTIC INGESTION EFFECTS ON IRANIAN CASPIAN SEA FISH GUT MICROBIOMES	Dr. Soraya Mahmoudi Ali Hosseini
		7	CUBAN BIO-DIVERSITY CONSERVATION STRATEGIES FOR ENDANGERED AMPHIBIAN SPECIES	Prof. Dr. Carlos Rodriguez Dr. Ana Morales Luis Fernandez
		8	MOLECULAR EVOLUTION OF VIRUS-HOST INTERACTIONS IN CUBAN BAT RESERVOIRS	Assoc. Prof. Dr. Maria Gonzalez Pedro Alvarez Sofia Ramirez
		9	THERMOTOLERANT YEAST STRAINS FROM CUBAN HOT SPRINGS FOR BIOETHANOL PRODUCTION	Dr. Javier Lopez Yamilka Perez

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HALL / SALON 7	**Assoc. Prof. Dr. Budi Santoso**	1	INNOVATIVE STEM EDUCATION MODELS FOR RURAL NORTH MACEDONIAN PRIMARY SCHOOLS	Prof. Dr. Nikola Petrovski Elena Georgieva Stefan Dimitrov
		2	DIGITAL LITERACY PROGRAMS ADDRESSING GENDER GAPS IN MACEDONIAN SECONDARY EDUCATION	Assoc. Prof. Dr. Marija Ilievska Dr. Aleksandar Risteski Ana Kostovska
		3	CULTURALLY RESPONSIVE TEACHING STRATEGIES IN INDONESIAN MULTILINGUAL CLASSROOMS	Assoc. Prof. Dr. Budi Santoso Dr. Sari Wijaya
		4	INTEGRATING TRADITIONAL WAYANG ARTS INTO INDONESIAN PRIMARY CURRICULUM	Assoc. Prof. Dr. Nyoman Suryani Iwan Pratama Dewi Lestari
		5	CHALLENGES OF REMOTE LEARNING IMPLEMENTATION IN IRANIAN RURAL DISTRICTS	Dr. Reza Ahmadi Fatemeh Karimi
		6	TEACHER TRAINING PROGRAMS FOR INCLUSIVE EDUCATION IN IRANIAN SPECIAL NEEDS SCHOOLS	Leila Hosseini Prof. Dr. Hassan Jafari Mohammad Rezaei
		7	CUBAN LITERACY CAMPAIGN MODELS ADAPTED FOR ADULT EDUCATION IN REMOTE AREAS	Assoc. Prof. Dr. Carlos Rodriguez Dr. Maria Gonzalez
		8	MUSIC EDUCATION AS SOCIAL MOBILITY TOOL IN CUBAN URBAN COMMUNITIES	Prof. Dr. Ana Morales Dr. Luis Fernandez Sofia Ramirez

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HALL / SALON 8	**Assoc. Prof. Dr. Elara Hoxha**	1	PERSONALIZED LEARNING PATHWAYS USING AI ADAPTIVE ALGORITHMS IN INDONESIAN VOCATIONAL HIGH SCHOOLS	Prof. Dr. Budi Santoso Dr. Sari Wijaya Lina Pratiwi I Made Artawa
		2	AI-DRIVEN LANGUAGE ACQUISITION TOOLS FOR REMOTE INDONESIAN RURAL STUDENTS	Assoc. Prof. Dr. Nyoman Sujana Dewi Ratna Agus Setiawan
		3	DEVELOPING AI CHATBOTS FOR MATHEMATICS TUTORING IN INDIAN RURAL GOVERNMENT SCHOOLS	Dr. Priya Sharma Vikram Joshi Kavya Nair
		4	MACHINE LEARNING MODELS FOR PREDICTING STUDENT DROPOUT RATES IN HIGHER EDUCATION	Prof. Dr. Arjun Mehra Divya Reddy
		5	AI-POWERED ASSESSMENT SYSTEMS FOR ALBANIAN PRIMARY EDUCATION CURRICULUM	Assoc. Prof. Dr. Elara Hoxha Besnik Leka Mira Deda
		6	VIRTUAL REALITY SIMULATIONS ENHANCED BY AI FOR VOCATIONAL TRAINING IN ALBANIA	Dr. Alban Kreci Elona Meta
		7	NATURAL LANGUAGE PROCESSING FOR ARABIC LANGUAGE TEACHING IN QATARI INTERNATIONAL SCHOOLS	Prof. Dr. Khalid Al-Thani Fatima Al-Mansoori Noor Salem

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HALL / SALON 9	**Assoc. Prof. Dr. Enea Lika**	1	PHYTOREMEDIATION POTENTIAL OF INDONESIAN TROPICAL PLANTS FOR HEAVY METAL SOIL CONTAMINATION	Dr. Sari Wijaya Lina Pratiwi I Made Artawa
		2	MICROBIAL DIVERSITY IN INDONESIAN RICE PADDY SOILS UNDER ORGANIC FARMING PRACTICES	Assoc. Prof. Dr. Nyoman Sujana Dewi Ratna Agus Setiawan
		3	SOIL EROSION MODELING AND CONSERVATION STRATEGIES IN HIMALAYAN AGRICULTURAL LANDSCAPES	Dr. Arjun Patel Priya Sharma Rohan Mehra
		4	BIOCHAR APPLICATION EFFECTS ON SOIL FERTILITY IN SEMI-ARID INDIAN CROPPING SYSTEMS	Prof. Dr. Vikram Singh Assis. Pro. Dr. Neha Gupta Sanjay Patel Mirela Deda Erion Hoxha
		5	ORGANIC AMENDMENTS IMPACT ON SOIL MICROBIAL ACTIVITY IN ALBANIAN MEDITERRANEAN OLIVE GROVES	Assoc. Prof. Dr. Enea Lika
		6	CLIMATE CHANGE EFFECTS ON ALBANIAN MOUNTAIN SOIL CARBON SEQUESTRATION POTENTIAL	Dr. Liridon Kuci Anila Meta Flamur Bega
		7	SALINE SOIL RECLAMATION TECHNIQUES USING HALOPHYTES IN QATARI ARID ECOSYSTEMS	Prof. Dr. Khalid Al-Mansoori Fatima Hassan Noor Salem
		8	DUNE STABILIZATION AND SOIL CONSERVATION STRATEGIES FOR QATARI COASTAL AGRICULTURE	Assoc. Prof. Dr. Omar Al-Fahim Sara Al-Ketbi

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HALL / SALON 10	**Assoc. Prof. Dr. Yerzhan Bekbolatov**	1	INNOVATIVE LANGUAGE ACQUISITION METHODS FOR BILINGUAL CHILDREN IN ALGERIAN PRIMARY SCHOOLS	Karim Zahir Fatima Lounis Prof. Dr. Amina Belkacem Samir Boualem
		2	STEM EDUCATION INTEGRATION THROUGH PLAY-BASED LEARNING IN RURAL ALGERIAN COMMUNITIES	Assoc. Prof. Dr. Yacine Hadrami Nadia Cherif
		3	DIGITAL LITERACY DEVELOPMENT FOR UNDERPRIVILEGED CHILDREN IN INDIAN URBAN SLUMS	Dr. Priya Sharma Prof. Dr. Arjun Mehra Kavya Nair
		4	CULTURAL HERITAGE PRESERVATION THROUGH STORYTELLING IN INDIAN PRESCHOOL CURRICULA	Assoc. Prof. Dr. Vikrant Singh Pooja Desai
		5	MATHEMATICS ANXIETY REDUCTION STRATEGIES FOR KAZAKHSTANI MIDDLE SCHOOL STUDENTS	Dr. Aigerim Tolegenova Nursultan Abayev Dana Kairatova
		6	INCLUSIVE EDUCATION MODELS FOR CHILDREN WITH SPECIAL NEEDS IN KAZAKHSTAN	Assoc. Prof. Dr. Yerzhan Bekbolatov Aidana Sarsenova Ruslan Zhumatov
		7	CREATIVE ARTS THERAPY FOR EMOTIONAL DEVELOPMENT IN GREEK PRIMARY EDUCATION	Prof. Dr. Eleni Papadopoulos Dr. Nikos Karamanlis
		8	PHILOSOPHICAL INQUIRY METHODS IN GREEK EARLY CHILDHOOD CLASSROOM DISCUSSIONS	Assoc. Prof. Dr. Dimitri Stavros Maria Kostas

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HALL / SALON 1	**Dr. Öğr. Üyesi GÖZDE KARABULUT**	1	IN VITRO ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OBTAINED FROM CITRULLUS LANATUS ((THUNB.) MATSUM. & NAKAI) (WATERMELON) FRUIT	Graduate student, Zeynep SEVİM Dr. Figen ERDEM ERİŞİR Prof. Dr. Ökkeş YILMAZ
		2	INVESTIGATION OF THE CYTOTOXICITY OF GEASTRUM FIMBRIATUM AND GEASTRUM RUFESCENS SPECIES	Gizem GENÇ Nurdan SARAÇ
		3	YENİ NESİL ÇEVRESEL KİRLETİCİ 6PPD-KİNON'UN PANKREATİK BETA HÜCRELERİNDE (INS-1) OLUŞTURDUĞU SİTOTOKSİK VE FONKSİYONEL HASARIN *İN VİTRO* DEĞERLENDİRİLMESİ	Dr. Öğr. Üyesi GÖZDE KARABULUT
		4	*Escherichia coli*'DE *cbpA* GENİNİN FENOLİK ASİT TOLERANSINDAKİ ROLÜ: MUTANT VE KOMPLEMENTASYON ANALİZİNDEN KANITLAR	KADRİYE ASLIHAN ONAT TAŞDELEN HATİCE ÖZTÜRKEL KABAKAŞ Dr. Öğr. Gör. MERVE SEZER KÜRKÇÜ Doç. Dr. BEKİR ÇÖL
		5	DIFFERENTIAL TRANSCRIPTIONAL RESPONSE OF THE yrbF (mlaF) GENE TO STRUCTURALLY RELATED PHENOLIC ACIDS IN ESCHERICHIA COLI	Öğr. Gör. Dr., Merve SEZER KÜRKÇÜ Hatice ÖZTÜRKEL KABAKAŞ Kadriye Aslıhan ONAT TAŞDELEN Dr. Öğr. Üyesi, Esra DİBEK Prof. Dr., Bekir ÇÖL
		6	THE MULTIFACETED EFFECTS OF PROBIOTICS ON HEALTH: A CURRENT ASSESSMENT OF IMMUNE REGULATION, INFLAMMATION SUPPRESSION, AND ANTICANCER POTENTIAL	Dr. Sümeyye AKBULUT
		7	DETERMINATION OF ANTICANCER ACTIVITY AND BIOCOMPATIBILITY OF COMMERCIAL AND SYNTHESIZED PLA-PEG-PLA BLOCK COPOLYMERS	Assistant Prof Asli KARA Professor Dursun Ali KOSE Professor Gulcin Alp AVCI Professor Emre AVCI

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HALL / SALON 2	**Doç. Dr. AYSEL ARSLAN**	1	HUMANISTIC PERSPECTIVES IN THE MODERN YEMENI NOVEL: THE CASE OF AHMED QĀSIM ʿALĪ AL-ʿARĪQĪ	Doç. Dr. RIFAT AKBAŞ
		2	EMİNE ŞENLİKOĞLU'NUN MARİA ROMANINI HİDAYET ROMANI OLARAK OKUMAK	Dr., Batuhan ŞUORUÇ
		3	İLAHİ FEYİZDEN SÜHANA: CEVRÎ DİVANI'NDA ŞİİR VE ŞAİRLİK	Doçent, MURAT KEKLİK
		4	LINGUISTIC DISCRIMINATION AND EDUCATIONAL INEQUALITY	Nadezda ERYILDIZ, M. A. (FU Berlin)
		5	SOCIAL APHASIA: PANOPTICON AND NEWSPEAK PERSPECTIVES – AN ANALYSIS OF THE NOVEL 1984	Doç. Dr. AYSEL ARSLAN
		6	FROM INDIVIDUAL LONELINESS TO COLLECTIVE UNREST: AN ONTOLOGICAL ANALYSIS OF ASOCIALITY AND SOCIAL MELANCHOLIA IN THE SORROWS OF YOUNG WERTHER	Doç. Dr. AYSEL ARSLAN
		7	THE IMAGE OF MIRZA ALAKBAR SABIR IN THE HISTORICAL NOVEL "SABIR" BY AZIZA JAFARZADEH	SEVİNJ MİRZAYEVA
		8	ERZURUM AĞZI ÖRNEKLEMİNDE MİZAH	Yüksek Lisans Öğrencisi Selim ARSLAN

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HALL / SALON 3	**Assoc. Prof. TUFAN İNALTEKİN**	1	CONTENT ANALYSIS OF ARTICLES ON READABILITY IN TURKISH LANGUAGE EDUCATION: 2000-2023	Master's Student, Göksel ÇAĞLAR Assistant Professor, Erhan ÇAPOĞLU
		2	AN ANALYSİS OF ABDURRAHİM KARAKOÇ'S POEMS "DAVA ŞİİRLERİ " AND "HASAN'A MEKTUPLAR" ACCORDİNG TO THE VİRTUE-VALUE-ACTİON MODEL	HİLAL PENPE Dr. Öğretim Üyesi , ERHAN ÇAPOĞLU
		3	FEN BİLGİSİ ÖĞRETMEN ADAYLARININ YAPAY ZEKA PROGRAMI GEMİNİ İLE YAPTIKLARI DİJİTAL MATERYALLERİN DEĞERLENDİRİLMESİ	Alper SAKİN Prof. Dr. Güldem DÖNEL AKGÜL Zeynep YALÇIN Meryem YETKİN
		4	DİJİTAL İÇERİKLER FEN BİLGİSİ DERS KİTAPLARINDA NASIL KULLANILMIŞTIR?	Alper SAKİN Prof. Dr. Güldem DÖNEL AKGÜL Zeynep YALÇIN Meryem YETKİN
		5	AN ANALYSIS OF THE '365-DAY STORY SERIES' IN TERMS OF VALUES	Master's Student, Pınar CUDA Assistant Professor, Erhan ÇAPOĞLU
		6	ORTAOKUL ÖĞRENCİLERİNİN AFET BİLİNCİ FARKINDALIK DÜZEYLERİNİN DEMOGRAFİK DEĞİŞKENLER AÇISINDAN İNCELENMESİ	Yüksek Lisans Öğrencisi, HANİFİ KARATAŞ Prof. Dr., MUSTAFA ÖZTÜRK
		7	AN EXAMINATION OF 8TH GRADE STUDENTS' UNDERSTANDING OF THE SHAPES OF THE EARTH AND THE MOON	Asst. Prof. TOLGA SAKA Assoc. Prof. TUFAN İNALTEKİN

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HALL / SALON 4	**Prof. Dr. Aynur AYTEKİN ÖZDEMİR**	1	PEDİATRİK HASTALARDA GÖĞÜS TÜPÜ ÇIKARMA İŞLEMİNDE AĞRI YÖNETİMİ: HEMŞİRELİK YAKLAŞIMLARINA YÖNELİK BİR DERLEME	Uzm. Hemşire Gamze DURAN KÜÇÜK Prof. Dr. Aynur AYTEKİN ÖZDEMİR
		2	YENİDOĞAN YOĞUN BAKIM ÜNİTELERİNDE EMZİRMENİN GÜÇLENDİRİLMESİNDE KANITA DAYALI BİR BAKIŞ: MOTİVASYONEL GÖRÜŞME	Arş. Gör. Esma ÜNAL AKTAŞ Prof. Dr. Aynur AYTEKİN ÖZDEMİR
		3	MOBİL SAĞLIK UYGULAMALARININ BARİATRİK CERRAHİ SONRASI HASTA SONUÇLARI ÜZERİNDEKİ ETKİSİ	Dr. Beyzanur KIZILOĞLU AĞGÜL
		4	REPRODUCTIVE HEALTH AND NURSING ROLES IN WOMEN EXPOSED TO DOMESTIC VIOLENCE	Lecturer. Dr., ZELİHA TURAN
		5	GÖÇMEN VE MÜLTECİ KADINLARDA GEBELİK/DOĞUM HİZMETLERİNE ERİŞİM: SORUNLAR VE ÇÖZÜM ÖNERİLERİ	Öğrenci, AMİRA KARİM Arş. Gör. Dr. ÇİĞDEM ŞEN TEPE

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HALL / SALON 5	**Assist. Prof. Dr., Aysel YAVUZ**	1	URBAN PLANNING APPROACHES ACCORDING TO CLIMATE SCENARIOS	Arş. Gör. Dr., SEYHAN SEYHAN Prof. Dr., ELİF BAYRAMOĞLU Prof. Dr., BANU ÇİÇEK KURDOĞLU
		2	CLIMATE-ADAPTIVE PLAYGROUND DESIGN CRITERIA FOR URBAN PARKS	Arş. Gör. Dr., SEYHAN SEYHAN Prof. Dr., ELİF BAYRAMOĞLU Prof. Dr., BANU ÇİÇEK KURDOĞLU
		3	BİTKİSEL TASARIMDA ENTROPİ: ANALOJİK BİR YAKLAŞIM	Dr. Sinem KIZILASLAN
		4	KENTSEL DİRENÇLİLİK BAĞLAMINDA YEŞİL ALANLARIN ÇOK BOYUTLU İŞLEVLERİNİN DEĞERLENDİRİLMESİ	Arş. Gör., Rıdvan TİK Doç. Dr., Tuncay KAYA
		5	SÜRDÜRÜLEBİLİR PEYZAJ YÖNETİMİNDE ÇİM ATIKLARININ MALÇ OLARAK DEĞERLENDİRİLMESİ VE SU VERİMLİLİĞİNE ETKİSİ	Arş. Gör., RIDVAN TİK Doç. Dr., TUNCAY KAYA
		6	STREETS AS PLAY SPACES FOR CHILDREN	Prof. Dr., Habibe ACAR Dr. Öğr. Üyesi, Aysel YAVUZ Prof. Dr., Hilal TURGUT
		7	FROM FORM TO FLOW: SOCIAL LANDSCAPES AS GENERATORS OF PHYSICAL ACTIVITY IN CONTEMPORARY URBAN PARKS	Assist. Prof. Dr., Aysel YAVUZ Prof. Dr., Habibe ACAR Prof. Dr., Hilal TURGUT
		8	THE EFFECTS OF PERIPHERY FORMATION ON THE LANDSCAPE: THE TRABZON YOMRA-KAŞÜSTÜ EXAMPLE	Dr.Öğr. Üyesi Duygu AKYOL KUYUMCUOĞLU Prof.Dr. Sema MUMCU

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HALL / SALON 6	**Doç. Dr. Necattin HANAY**	1	SCIENTIFIC PROGRESS AND THE TRANSFORMATION OF RATIONALITY IN THE HISTORY OF SCIENCE: BACHELARD'S DIALECTICAL APPROACH BETWEEN DISCONTINUITY AND CUMULATIVISM	R.A. Dr. Ömer Faruk Karaköse
		2	ANTAKYALI BİR MUHADDİS: EBU SEHL HEYSEM b. CEMİL- İLMİ KİŞİLİĞİ VE HADİS İLMİNDEKİ YERİ	Öğr.Gör. Dr., Ahmet Emre AYDINLI
		3	CERH VE TA'DÎL LİTERATÜRÜNDE "ŞEYH" KAVRAMININ KULLANIM FORMLARI	Yüksek Lisans Öğrencisi, Şeyma ŞEKER Prof. Dr., Sezai ENGİN
		4	THE RELATIONSHIP BETWEEN THE QUR'AN, REASON, AND SCIENCE IN ʿABBĀS MAḤMŪD AL-ʿAQQĀD'S AL-FALSAFAH AL-QURʾĀNIYYAH: AN ISSUE-CENTERED MODE OF INTERPRETATION	Doç. Dr. Necattin HANAY
		5	ÖLÜM KORKUSUNDAN ÖLÜMSÜZLÜK TASARIMINA: TRANSHÜMANİZM VE VAROLUŞUN YENİDEN İNŞASI	Zeynep Kaya Dr. Öğr. Mustafa Dikmen
		6	"SEÇİLMİŞ AİLE" TASAVVURUNUN ARKEOLOJİSİ: ANTİK MISIR VE AİLE FENOMENOLOJİSİ	Funda Nur Ünlü Prof.Dr. Şevket Yavuz

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HALL / SALON 7	**Dr. Ülkü Koçak**	1	THE PROBLEM OF TARQĪQ–IMĀLA RELATIONSHIP IN THE SCIENCE OF QIRĀʾĀT: CONCEPTUAL ANALYSIS AND REFLECTIONS IN PRACTICE	Dr, RESUL AKCAN
		2	QUR'ANIC RECITATION IN THE DIGITAL AGE: THE POPULARIZING AND TRANSFORMATIVE EFFECTS OF SOCIAL MEDIA PLATFORMS	Asist. Prof., İHSAN SÜTŞURUP
		3	THE SPİRİTUAL MOTİVATİONS OF THE COMPANİONS İN LEARNİNG THE QUR'AN	Dr. Öğretim Üyesi, KENAN AKLAN
		4	ANADOLU'NUN MANEVİ COĞRAFYASINDA BİR DURAK HAYDARDEDE TÜRBESİ	Yüksek Lisans Öğrencisi, ADEM TAŞKİN
		5	THE METHODOLOGY, SOURCES, AND SİGNİFİCANCE AS A SOURCE OF UNDERSTANDİNG THE QUR'ĀN: A CASE STUDY OF IBN QUTAYBA'S TA'WĪL MUSHKİL AL-QUR'ĀN	Master's student, ABDURRAHMAN ENES ATAKUL Assoc. Dr., MURAT BAHAR
		6	FITRAH AND BELIEF IN GOD: A COMPARISON BETWEEN ISLAMIC THOUGHT AND CONTEMPORARY PHILOSOPHY OF RELIGION	Dr. Ülkü Koçak

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HALL / SALON 1	**Assis. Prof. Dr. Elena Bucataru**	1	THE ECUMENICAL DIALOGUE BETWEEN ORTHODOX AND CATHOLIC TRADITIONS IN CONTEMPORARY MOLDOVA	Prof. Dr. Ion Crețu
		2	THEOLOGICAL IMPLICATIONS OF SECULARIZATION ON MOLDOVAN ORTHODOX SPIRITUALITY	Assis. Prof. Dr. Elena Bucataru Victor Rusu Petru Lupașcu Ana Maria Popescu
		3	INTERFAITH DIALOGUE BETWEEN CONFUCIANISM AND CHRISTIANITY IN MODERN TAIWANESE SOCIETY -yu	Dr. Li Wei-min Chen Yi-ling Wang Hsiao Tsai Chun-hsiung
		4	TAOIST INFLUENCES ON CONTEMPORARY TAIWANESE CHRISTIAN LITURGICAL PRACTICES	Dr. Huang Mei-ling
		5	ISLAMIC REVIVALISM AND NATIONAL IDENTITY FORMATION IN SECULAR KYRGYZSTAN	Assoc. Prof. Dr. Almazbek Asanov Gulnara Ibraimova Nurbol Otorbayev
		6	CHRISTIAN MISSIONARY ACTIVITIES AMID CENTRAL ASIAN ISLAMIC TRADITIONS	Dr. Jamilya Toktogulova Eldar Kaparov
		7	INTERRELIGIOUS COEXISTENCE MODELS IN DUBAI'S MULTICULTURAL URBAN CONTEXT	Prof. Dr. Ahmed Al-Mansoori Fatima Khalil Omar Saeed
		8	SUFI MYSTICISM AND CONTEMPORARY SPIRITUALITY IN EMIRATI MUSLIM COMMUNITIES	Assoc. Prof. Dr. Layla Al-Hashimi Khalid Rahman

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HALL / SALON 2	**Assoc. Prof. Dr. Reza Jalali**	1	THE ROLE OF ETHIOPIAN ORTHODOX THEOLOGY IN SHAPING CONTEMPORARY AFRICAN CHRISTIAN IDENTITY	Assis. Prof. Dr. Mikael Tesfaye Dr. Selamawit Gebre Abraham Kebede
		2	ESCHATOLOGICAL INTERPRETATIONS IN ETHIOPIAN GE'EZ LITURGICAL TEXTS AND THEIR MODERN IMPLICATIONS	Assoc. Prof. Dr. Helen Assefa Yonas Berhanu Dawit Alemayehu
		3	MARIAN DEVOTION IN LEBANESE MARONITE THEOLOGY AND INTERFAITH DIALOGUE	Dr. Elias Khoury Prof. Dr. Nadia Rahme
		4	SHIITE-SUNNI ECUMENICAL DIALOGUE INITIATIVES IN CONTEMPORARY LEBANESE THEOLOGY	Fr. Antoine Sarkis Assoc. Prof. Dr. Layla Haddad
		5	ISLAMIC ESCHATOLOGY AND ENVIRONMENTAL ETHICS IN PAKISTANI MUSLIM SCHOLARSHIP	Prof. Dr. Imran Qureshi Dr. Amina Siddiqui
		6	QURANIC HERMENEUTICS AND GENDER ROLES IN PAKISTANI REFORMIST THEOLOGY	Assoc. Prof. Dr. Hassan Raza Sadia Mahmood
		7	SHIITE IMMAMOLOGY AND POLITICAL AUTHORITY IN CONTEMPORARY IRANIAN THOUGHT	Prof. Dr. Ali Rezaei Dr. Fatima Hosseini Mehdi Karimi
		8	MYSTICAL INTERPRETATIONS OF SUFISM IN PERSIAN POETIC THEOLOGY	Assoc. Prof. Dr. Reza Jalali

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HALL / SALON 3	**Assoc. Prof. Dr. Fatima Zubairu**	1	AFRICAN INDIGENOUS SPIRITUALITY AND CHRISTIAN THEOLOGICAL SYNCRETISM IN CONTEMPORARY WORSHIP PRACTICES	Samuel Osei Dr. Kwame Nkosi Dr. Aisha Mwangi
		2	ISLAMIC ESCHATOLOGY INTERPRETATIONS THROUGH AFRICAN ORAL TRADITION LENSES	Assoc. Prof. Dr. Fatima Zubairu Elias Kofi
		3	SUFI MYSTICISM AND PHILOSOPHICAL EXISTENTIALISM IN MOROCCAN MALIKI SCHOLARSHIP	Dr. Nadia Belkacem Karim Zahir Dr. Ahmed Laraki
		4	IBN RUSHD'S RATIONALISM VERSUS AL-GHAZZALI'S INTUITIONISM IN MOROCCAN THEOLOGICAL DISCOURSE	Assoc. Prof. Dr. Fatima Jaber Youssef Hadrami
		5	AUGUSTINIAN GRACE THEOLOGY ADAPTATIONS IN TUNISIAN POST-COLONIAL CHRISTIAN THOUGHT	Dr. Sami Kallel Leila Trabelsi Prof. Dr. Hedi Ben Salem
		6	IBN ARABI'S UNITY OF BEING DOCTRINE IN CONTEMPORARY TUNISIAN SUFI PHILOSOPHY	Assoc. Prof. Dr. Moncef Bouazizi
		7	ORTHODOX HESYCHASM AND MACEDONIAN FOLK SPIRITUALITY INTERSECTIONS	Dr. Elena Risteska Prof. Dr. Nikola Petrovski
		8	BYZANTINE PALAMISM INFLUENCES ON MACEDONIAN THEOLOGICAL ANTHROPOLOGY	Assoc. Prof. Dr. Goran Trajkovski Marija Angelova

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HALL / SALON 4	**Assoc. Prof. Dr. Nadia Cherkaoui**	1	TELEHEALTH IMPLEMENTATION CHALLENGES FOR RURAL MATERNAL HEALTHCARE IN SUB-SAHARAN AFRICA	Prof. Dr. Amina Kone Dr. Samuel Mwangi Fatou Diop Kwame Asante
		2	PAIN MANAGEMENT PROTOCOLS FOR POSTOPERATIVE PATIENTS IN NIGERIAN PUBLIC HOSPITALS	Assoc. Prof. Dr. Ngozi Okeke Dr. Yusuf Bello Chiamaka Nwosu
		3	NURSE-LED DIABETES EDUCATION PROGRAMS FOR URBAN MOROCCAN DIABETIC PATIENTS	Prof. Dr. Fatima Zahra El Amrani Dr. Karim Benali Aicha Lahlou
		4	MENTAL HEALTH SUPPORT STRATEGIES FOR COVID-19 FRONTLINE NURSES IN MOROCCO	Assoc. Prof. Dr. Nadia Cherkaoui Youssef Idrissi Soukaina Mezouar
		5	PEDIATRIC NURSING INTERVENTIONS FOR RESPIRATORY INFECTIONS IN TUNISIAN CHILDREN	Dr. Lamia Ben Salem Assoc. Prof. Dr. Hassen Trabelsi Meriem Jrad Amel Kefi
		6	WOUND CARE MANAGEMENT TRAINING EFFECTS ON NURSE COMPETENCY IN TUNISIAN HOSPITALS	Prof. Dr. Moncef Zghal Dr. Nadia Bouaziz Sahar Ghrabi
		7	ELDERLY CARE MODELS AND FALL PREVENTION STRATEGIES IN NORTH MACEDONIAN NURSING HOMES	Assoc. Prof. Dr. Elena Petrovska Dr. Nikola Trajkovski Marija Angelova Ivan Kostovski
		8	CHRONIC DISEASE MANAGEMENT THROUGH COMMUNITY NURSING IN MACEDONIAN RURAL AREAS	Prof. Dr. Goran Popovski Dr. Biljana Risteska Tanja Milevska

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HALL / SALON 5	**Assoc. Prof. Dr. Chen Hui Min**	1	MATERNAL NUTRITIONAL INTERVENTIONS IMPROVING FETAL BRAIN DEVELOPMENT OUTCOMES IN SINGAPORE	Prof. Dr. Lim Mei Ling Dr. Tan Wei Jie Sarah Ng
		2	TELEMEDICINE APPLICATIONS FOR HIGH-RISK PREGNANCY MONITORING IN URBAN SINGAPORE	Assoc. Prof. Dr. Chen Hui Min Dr. Rajesh Kumar Emily Wong
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		2	MENTAL HEALTH INTERVENTIONS TARGETING WORKPLACE STRESS AMONG SINGAPOREAN HEALTHCARE WORKERS	Dr. Chloe Tan Rachel Lim Prof. Dr. Benjamin Goh
		3	NUTRITIONAL DEFICIENCY PREVENTION PROGRAMS IN RURAL MOLDOVAN COMMUNITIES	Assoc. Prof. Dr. Maria Rusu Ion Popa
		4	MATERNAL HEALTHCARE ACCESS IMPROVEMENT INITIATIVES IN MOLDOVA	Dr. Elena Cebotari Victor Lupascu Natalia Dragancea
		5	MALARIA VECTOR CONTROL STRATEGIES IN TANZANIAN COASTAL REGIONS	Prof. Dr. Juma Kilonzo Dr. Amina Mwamba
		6	COMMUNITY-BASED TUBERCULOSIS SCREENING PROGRAMS IN RURAL TANZANIA	Assoc. Prof. Dr. Elias Nkya Fatuma Juma Dr. Zuberi Mushi
		7	DIABETES PREVALENCE AND MANAGEMENT CHALLENGES IN ALGERIAN URBAN POPULATIONS	Prof. Dr. Karim Belhocine Dr. Nadia Zerhouni Yacine Amrane
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		2	MATERNAL HEALTH OUTCOMES AND NUTRITIONAL INTERVENTIONS IN RURAL INDIAN TRIBAL COMMUNITIES	Assoc. Prof. Dr. Anjali Desai Neha Patel Sanjay Mehta
		3	ADVANCES IN TELEMEDICINE FOR CARDIOVASCULAR DISEASE MANAGEMENT IN TAIWANESE ELDERLY	Dr. Wei-Ling Chen Hui-Ju Lin Prof. Dr. Ming-Hsien Tsai
		4	NEURODEGENERATIVE DISEASE PREVENTION STRATEGIES THROUGH TRADITIONAL TAIWANESE MEDICINE	Assoc. Prof. Dr. Li-Chun Wang Kuan-Yu Huang
		5	COMMUNITY HEALTH WORKER PROGRAMS FOR INFECTIOUS DISEASE CONTROL IN CUBAN RURAL AREAS	Dr. Carlos Rodriguez Maria Gonzalez Prof. Dr. Ana Morales Juan Perez
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		7	MALARIA ELIMINATION STRATEGIES AND VECTOR CONTROL IN NIGERIAN NORTHERN REGIONS	Fatima Yusuf Dr. Kabir Ibrahim
		8	MENTAL HEALTH INTEGRATION IN PRIMARY CARE SYSTEMS ACROSS NIGERIAN URBAN CENTERS	Assoc. Prof. Dr. Ngozi Okonkwo Chinedu Eze
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		2	FEMINIST RE-READINGS OF MYTHICAL NARRATIVES IN CONTEMPORARY INDIAN WOMEN NOVELISTS	Assoc. Prof. Dr. Kavya Nair
		3	LINGUISTIC LANDSCAPE ANALYSIS OF TAIPEI METRO STATIONS: MULTILINGUALISM AND IDENTITY	Dr. Wei-Ling Chen Hsin-Yi Lin
		4	TAIWANESE LITERATURE UNDER JAPANESE COLONIAL RULE: RESISTANCE THROUGH FOLK POETRY	Prof. Dr. Ming-Hsuan Wu Yi-Ting Chang
		5	AFRO-CUBAN ORAL TRADITIONS IN NICOLÁS GUILLÉN'S POETRY: RHYTHM AND RESISTANCE	Assoc. Prof. Dr. Carlos Rodríguez Mercedes López
		6	MAGICAL REALISM IN ALEJO CARPENTIER'S WORKS: THE BAROQUE CHRONOTOPE OF THE CARIBBEAN	Dr. Sofia Vargas Raúl Mendoza
		7	POSTCOLONIAL ECOCRITICISM IN CHINUA ACHEBE'S ARROW OF GOD: LAND AND LANGUAGE	Dr. Ngozi Okonkwo Dr. Emeka Nwosu
		8	ORAL PERFORMANCE POETRY IN CONTEMPORARY NIGERIAN SPOKEN WORD ARTISTRY	Assoc. Prof. Dr. Fatima Yusuf Adebayo Adewale
		9	GENDER DYNAMICS IN NIGERIAN YOUNG ADULT LITERATURE: VOICES FROM THE MARGINS	Dr. Chioma Eze Ibrahim Musa Blessing Okafor

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HALL / SALON 9	**Assoc. Prof. Dr. Nadia Belghiti**	1	POSTCOLONIAL THEMES IN CONTEMPORARY MOROCCAN FRANCOPHONE LITERATURE	Prof. Dr. Amina El-Khatib Dr. Karim Zahid Fatima Larboui
		2	LINGUISTIC HYBRIDITY IN MOROCCAN ARABIC DIALECT AND FRENCH LITERARY EXPRESSION	Assoc. Prof. Dr. Nadia Belghiti Youssef Amrani Leila Hadrami
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		8	NOMADIC MOTIFS IN MODERN KAZAKH POETRY AND ORAL EPICS	Dr. Aizhan Kassenova Nurlan Bekbolatov
		9	KAZAKH LANGUAGE PURISM DEBATES IN CONTEMPORARY LITERARY CRITICISM	Assis. Prof. Dr. Dina Sarsenbayeva Ruslan Zhumatov Aidana Kurmangali

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		2	CLIMATE-ADAPTIVE LANDSCAPE DESIGN FOR VINEYARD TERRACES IN CENTRAL MOLDOVA	Assoc. Prof. Dr. Victor Moraru
		3	VERTICAL FORESTRY SYSTEMS INTEGRATION IN TAIPEI HIGH-DENSITY URBAN DISTRICTS	Dr. Wei-Ling Chen Prof. Dr. Ming-Hsien Hsu Yi-Ting Lin
		4	BIO-DIVERSE ROOFTOP ECOSYSTEMS FOR TAIWAN'S TROPICAL URBAN CLIMATE RESILIENCE	Assoc. Prof. Dr. Chun-Yi Wang Hao-Jen Tsai
		5	WATER-SENSITIVE URBAN DESIGN PRINCIPLES FOR BISHKEK POST-SOVIET NEIGHBORHOODS	Dr. Aida Asangalieva Nurlan Toktogulov Gulnara Isakova
		6	MOUNTAIN LANDSCAPE RESTORATION PROJECTS FOR SUSTAINABLE TOURISM IN KYRGYZSTAN	Assoc. Prof. Dr. Bakyt Karimov Jyldyz Abdykarimova
		7	DESERT OASIS LANDSCAPE ARCHITECTURE FOR DUBAI SUSTAINABLE COMMUNITY DEVELOPMENT	Prof. Dr. Fatima Al-Suwaidi Dr. Omar Khalid Sara Mahmoud
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		2	WATER CONSERVATION LANDSCAPES THROUGH QANAT SYSTEMS IN ARID IRANIAN CITIES	Assoc. Prof. Dr. Soraya Mahmoudi Hassan Rezaei Fatemeh Bahrami
		3	URBAN GREEN INFRASTRUCTURE FOR FLOOD MITIGATION IN ALBANIAN COASTAL COMMUNITIES	Dr. Luan Hoxha Elena Deda Arben Kola
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		5	CLIMATE-ADAPTIVE LANDSCAPES FOR MOSCOW URBAN HEAT ISLAND REDUCTION	Sergei Kuznetsov Assoc. Prof. Dr. Irina Petrova Anna Volkova
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		7	NILOTIC RIVER LANDSCAPE RESTORATION FOR SUSTAINABLE TOURISM IN EGYPT	Prof. Dr. Ahmed Salem Fatima El-Sayed Omar Khalil
		8	DESERT OASIS LANDSCAPE ARCHITECTURE IN EGYPTIAN SIWA VALLEY CONSERVATION	Dr. Nadia Mahmoud Karim Abdelaziz
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BİYOMALZEME VE YENİ NESİL UYGULAMALAR

BIOMATERIALS AND NEXT GENERATION APPLICATIONS

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ÖZET

Biyomalzemeler, canlı sistemlerle etkileşime girebilen ve tanı, tedavi ya da doku fonksiyonlarını desteklemek amacıyla kullanılan doğal veya sentetik materyallerdir. Son yıllarda malzeme bilimi, nanoteknoloji, biyoteknoloji ve doku mühendisliği alanlarındaki gelişmeler biyomalzemelerin tasarımında önemli ilerlemeler sağlamıştır. Özellikle biyouyumlu, biyobozunur ve fonksiyonel özelliklere sahip akıllı biyomalzemeler, modern tıp ve biyomühendislik uygulamalarında önemli bir yer edinmiştir. Yeni nesil biyomalzemeler; nanoyapılı yüzeyler, uyarana duyarlı sistemler, biyobaskı ile üretilen yapılar ve biyomimetik tasarımlar gibi ileri teknolojilerle geliştirilmektedir. Bu materyaller, hücre davranışını yönlendirebilme, hedefe yönelik ilaç salımı gerçekleştirebilme ve hasarlı dokuların yenilenmesini destekleyebilme özellikleri sayesinde geleneksel biyomalzemelere göre daha yüksek performans sunmaktadır. Özellikle doku mühendisliği, rejeneratif tıp, akıllı implant sistemleri ve kontrollü ilaç taşıma sistemleri gibi alanlarda önemli uygulamalar bulunmaktadır. Gelecekte biyomalzemelerin kişiselleştirilmiş tıp uygulamalarıyla daha fazla entegre olması beklenmektedir. Yapay zeka destekli tasarım yöntemleri, biyomalzemelerin özelliklerinin optimize edilmesine katkı sağlarken; akıllı sensörler ile entegre edilmiş implantlar, gerçek zamanlı biyolojik veri takibini mümkün kılacaktır. Bu gelişmeler, hastalıkların daha etkili tedavisi ve yaşam kalitesinin artırılması açısından büyük potansiyel taşımaktadır.

Anahtar Kelimeler: Nanoteknoloji, ilaç taşıma sistemleri, doku mühendisliği, rejeneratif tıp

1. GİRİŞ

Biyomalzemeler, canlı sistemlerle etkileşime girebilen ve biyolojik fonksiyonları desteklemek, onarmak veya değiştirmek amacıyla kullanılan doğal ya da sentetik materyallerdir. Son yıllarda nanoteknoloji, biyomühendislik ve malzeme bilimindeki gelişmeler sayesinde biyomalzemeler, klasik implant materyallerinden akıllı ve fonksiyonel sistemlere evrilmiştir. Biyomalzemeler, tıp ve mühendisliğin kesişiminde yer alan disiplinler arası bir araştırma alanıdır. İlk biyomalzemeler genellikle tepkisiz özellik gösterirken, günümüzde geliştirilen biyomalzemeler biyolojik sistemlerle aktif etkileşim kurabilen, hücre davranışını yönlendirebilen ve biyolojik süreçleri modüle edebilen "akıllı" materyaller haline gelmiştir. Bu dönüşüm, özellikle nanoteknoloji, doku mühendisliği, sentetik biyoloji alanlarındaki gelişmelerle hız kazanmıştır [1, 2].

2. BİYOMALZEMELERİN TANIMI VE TEMEL ÖZELLİKLERİ

Biyomalzeme, canlı dokularla etkileşime girerek tedavi, teşhis veya biyolojik fonksiyonların desteklenmesi amacıyla kullanılan materyaldir.

Bir biyomalzemenin başarılı olabilmesi için şu özelliklere sahip olması gerekir:

- Biyouyumluluk: Doku ile zararlı reaksiyon oluşturmaması
- Biyobozunurluk: Kontrollü şekilde parçalanabilmesi

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- Mekanik dayanıklılık
- Yüzey özellikleri (hücre adezyonu için uygunluk)
- Fonksiyonel adaptasyon yeteneği [3-5].

3. BİYOMALZEMELERİN SINIFLANDIRILMASI

3.1 Doğal Biyomalzemeler

- Kollajen
- Jelatin
- Aljinat
- Kitosan

Kitosan gibi biyopolimerler, biyobozunur ve antibakteriyel özellikleri sayesinde yara iyileşmesi ve doku mühendisliğinde yaygın olarak kullanılmaktadır.

3.2 Sentetik Biyomalzemeler

- Polilaktik asit (PLA)
- Poliglikolik asit (PGA)
- Polikaprolakton (PCL) [6].

Bu materyaller kontrollü üretim avantajına sahiptir.

3.3 Seramik ve Metal Biyomalzemeler

- Hidroksiapatit
- Titanyum alaşımları
- Biyoaktif camlar [7].

3.4 Kompozit Biyomalzemeler

Farklı materyallerin birleşimiyle geliştirilir:

- Polimer-seramik kompozitler
- Nanokompozit sistemler [8].

4. YENİ NESİL BİYOMALZEME TEKNOLOJİLERİ

4.1 Nanobiyomalzemeler

Nanoteknoloji sayesinde biyomalzemeler daha fonksiyonel hale gelmiştir.
Örneğin:

- Kalsiyum karbonat nanopartikülleri, tümör ortamında pH-duyarlı ilaç salımı sağlayabilir.
- Nano ölçekli yapı, hücre-materyal etkileşimini artırır.

4.2 Elektroaktif ve İletken Biyomalzemeler

Elektriksel sinyalleri iletebilen biyomalzemeler özellikle sinir ve kas dokusu mühendisliğinde önemlidir.

- Hücre proliferasyonu ve diferansiyasyonunu etkiler.
- Elektroeğirme (electrospinning) ile nanofiber iskeleler oluşturulur.

4.3 Akıllı (Stimuli-Responsive) Biyomalzemeler

Bu materyaller pH, sıcaklık, ışık ve manyetik alan gibi çevresel değişikliklere tepki verir:

4.4 Nanozimler (Yapay Enzimler)

Nanozimler, doğal enzimlere benzer katalitik aktivite gösterir:

- Daha stabil
- Daha dayanıklı
- Tanı ve tedavide kullanılır.

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4.5 DNA Tabanlı Biyomalzemeler

DNA origami teknolojisi sayesinde hassas nanoyapılar ve programlanabilir sistemler geliştirilmektedir [9-13].

5. BİYOMALZEMELERİN YENİ NESİL UYGULAMALARI

5.1 Doku Mühendisliği ve Rejeneratif Tıp

- Yapay doku ve organ üretimi
- Hücre büyümesini destekleyen iskeleler

Elektroeğirilmiş nanofiberler, hücre dışı matriksi (ECM) taklit ederek hücre gelişimini destekler.

5.2 Kontrollü İlaç Salım Sistemleri

- Hedefe yönelik ilaç taşıma
- Uzun süreli salım sistemleri

Özellikle göz hastalıklarında uzun etkili ilaç sistemleri tedavi başarısını artırmaktadır.

5.3 3D Biyoyazdırma (Bioprinting)

- **Katmanlı doku üretimi**
- Kişiye özel implantlar

Bu teknoloji organ nakline alternatif olarak görülmektedir.

5.4 Biyohibrit Sistemler ve Mikro-robotlar

- Mikroalg tabanlı robotlar
- Hedefe yönelik ilaç taşıma

Bu sistemler biyolojik ve yapay bileşenlerin birleşimidir.

5.5 Yara İyileştirme ve Dermal Uygulamalar

- Antibakteriyel biyopolimerler
- Hızlandırılmış doku rejenerasyonu

Kitosan bazlı materyaller bu alanda öne çıkmaktadır.

5.6 Sürdürülebilir ve Yeşil Biyomalzemeler

- **Alg, mantar ve biyokütle temelli materyaller**
- **İnşaat ve tekstil sektöründe kullanım**

Yeni nesil biyomalzemeler, karbon emisyonunu azaltan çevreci alternatifler sunmaktadır [14, 15].

6. KARŞILAŞILAN ZORLUKLAR

Biyomalzeme geliştirme sürecinde önemli sorunlar bulunmaktadır:

- Uzun vadeli biyouyumluluk
- Üretim maliyetleri
- Ölçeklenebilirlik
- Klinik onay süreçleri
- Regülasyon eksiklikleri

Nanomalzemelerde özellikle toksisite ve stabilite sorunları dikkat çekmektedir [16].

7. GELECEK PERSPEKTİFİ

Gelecekte biyomalzeme alanında şu gelişmeler beklenmektedir:

- Kişiselleştirilmiş biyomalzemeler
- Yapay zekâ destekli tasarım
- Kendi kendini onaran materyaller
- Biyolojik olarak aktif implantlar

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- Canlı materyaller (living materials)

Ayrıca biyomalzemelerin:

- Enerji üretimi
- Çevre temizliği
- Akıllı tekstiller gibi alanlarda artacaktır [17].

8. SONUÇ

Biyomalzemeler, son yıllarda yalnızca pasif destekleyici materyaller olmaktan çıkarak biyolojik sistemlerle aktif etkileşim kurabilen, çevresel uyaranlara yanıt verebilen ve hatta hücresel süreçleri yönlendirebilen ileri düzey fonksiyonel sistemlere dönüşmüştür. Bu dönüşüm, özellikle nanoteknoloji, doku mühendisliği ve sentetik biyoloji gibi disiplinlerin entegrasyonu ile mümkün olmuş ve biyomalzemelerin uygulama alanlarını önemli ölçüde genişletmiştir.

Yeni nesil biyomalzemeler, rejeneratif tıpta doku ve organ onarımını destekleyen üç boyutlu iskelelerden, hedefe yönelik ilaç taşıma sistemlerine; biyosensörlerden akıllı implantlara kadar geniş bir yelpazede kullanılmaktadır. Bu sistemler, yalnızca hastalıkların tedavisinde değil, aynı zamanda erken teşhis, hastalık takibi ve kişiselleştirilmiş tıp uygulamalarında da kritik rol oynamaktadır. Özellikle akıllı biyomalzemelerin çevresel değişikliklere (pH, sıcaklık, enzimatik aktivite gibi) yanıt verebilme özelliği, tedavi süreçlerinde daha hassas ve kontrollü yaklaşımların geliştirilmesine olanak sağlamaktadır.

Bununla birlikte, biyomalzemelerin klinik uygulamalara entegrasyonu sürecinde bazı önemli zorluklar devam etmektedir. Uzun vadeli biyouyumluluk, immün yanıtların kontrolü, toksisite riskleri ve üretim süreçlerinin standardizasyonu gibi konular, araştırmacılar için hâlâ çözülmesi gereken temel problemlerdir. Ayrıca, laboratuvar ölçeğinde başarılı olan birçok biyomalzeme sisteminin endüstriyel üretime aktarılması ve klinik kullanıma sunulması sürecinde ekonomik ve regülasyon kaynaklı engeller de önemli rol oynamaktadır.

Gelecek perspektifinden bakıldığında, biyomalzeme araştırmalarının daha çok disiplinler arası bir yapıya evrileceği öngörülmektedir. Yapay zekâ ve makine öğrenmesi destekli materyal tasarımı, biyomalzemelerin özelliklerinin önceden tahmin edilmesini ve optimize edilmesini mümkün kılacaktır. Bunun yanı sıra, “canlı biyomalzemeler” (living materials) olarak adlandırılan ve içinde aktif hücreler veya biyolojik bileşenler barındıran sistemler, kendi kendini onarabilen ve çevresine adapte olabilen materyallerin geliştirilmesine zemin hazırlamaktadır.

Ayrıca sürdürülebilirlik perspektifi de biyomalzeme araştırmalarında giderek daha fazla önem kazanmaktadır. Algler, mantarlar ve diğer biyolojik kaynaklardan elde edilen çevre dostu biyomalzemeler, yalnızca tıbbi uygulamalarda değil, aynı zamanda enerji, çevre ve yapı teknolojileri gibi alanlarda da yenilikçi çözümler sunmaktadır. Bu durum, biyomalzemelerin gelecekte yalnızca sağlık sektöründe değil, küresel sürdürülebilirlik hedefleri doğrultusunda çok daha geniş bir kullanım alanına sahip olacağını göstermektedir.

Sonuç olarak, biyomalzemeler modern bilimin en dinamik ve hızla gelişen alanlarından biri olup, sağlık teknolojilerinden çevre mühendisliğine kadar birçok sektörde dönüştürücü etkiye sahiptir. Yeni nesil biyomalzemelerin geliştirilmesi, insan sağlığını iyileştirme, yaşam kalitesini artırma ve sürdürülebilir bir gelecek inşa etme açısından kritik bir rol oynamaya devam edecektir. Bu nedenle, temel bilimler ile uygulamalı mühendislik yaklaşımlarının entegrasyonu, biyomalzeme araştırmalarının geleceğinde belirleyici olacaktır.

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GENETİK MÜHENDİSLİĞİ VE SENTETİK BİYOLOJİ
GENETIC ENGINEERING AND SYNTHETIC BIOLOGY

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ÖZET

Genetik mühendisliği ve sentetik biyoloji, canlı organizmaların genetik yapısını anlamak, değiştirmek ve yeniden tasarlamak amacıyla geliştirilen modern biyoteknoloji alanlarıdır. Genetik mühendisliği, belirli genlerin izole edilmesi, düzenlenmesi veya başka organizmalara aktarılması yoluyla istenilen özelliklerin kazandırılmasını hedeflerken; sentetik biyoloji, biyolojik sistemleri mühendislik prensipleri doğrultusunda yeniden tasarlayarak yeni biyolojik fonksiyonlar oluşturmayı amaçlamaktadır. Bu iki alanın birleşimi, tıp, tarım, çevre bilimleri ve endüstriyel biyoteknoloji gibi birçok alanda yenilikçi uygulamaların ortaya çıkmasını sağlamaktadır. Son yıllarda özellikle CRISPR tabanlı gen düzenleme teknolojilerinin gelişmesi, genetik mühendisliğinin hızını ve doğruluğunu önemli ölçüde artırmıştır. Bu sayede kalıtsal hastalıkların tedavisi, gen terapileri ve kişiselleştirilmiş tıp uygulamalarında önemli ilerlemeler kaydedilmiştir. Sentetik biyoloji ise gen devreleri, yapay metabolik yollar ve tasarlanmış mikroorganizmalar aracılığıyla biyoyakıt üretimi, biyoplastik sentezi ve farmasötik bileşiklerin biyolojik üretimi gibi alanlarda önemli katkılar sağlamaktadır. Ayrıca sentetik biyoloji, minimal genom tasarımı, hücre fabrikanın geliştirilmesi ve biyosensör teknolojilerinin oluşturulması gibi yenilikçi yaklaşımlar sunmaktadır. Bu gelişmeler, sürdürülebilir üretim sistemlerinin kurulmasına ve çevresel sorunların çözümüne yönelik yeni stratejilerin geliştirilmesine olanak tanımaktadır. Bununla birlikte genetik manipülasyonun etik, biyogüvenlik ve biyogüvenlik politikaları açısından dikkatli şekilde değerlendirilmesi gerekmektedir. Gelecekte genetik mühendisliği ile sentetik biyolojinin yapay zeka, büyük veri analizi ve otomasyon teknolojileri ile entegrasyonu, daha karmaşık biyolojik sistemlerin tasarlanmasını mümkün kılacaktır. Bu durum, biyoteknolojinin sağlık, gıda güvenliği ve sürdürülebilir kalkınma alanlarında daha geniş kapsamlı çözümler üretmesine katkı sağlayacaktır.

Anahtar Kelimeler: Gen düzenleme, biyoteknoloji, biyosensörler, CRISPR.

9. GİRİŞ

Genetik mühendisliği ve sentetik biyoloji, biyoteknolojinin en hızlı gelişen alanları arasında yer almakta olup, canlı sistemlerin tasarlanması, modifiye edilmesi ve yeniden programlanmasına olanak tanımaktadır. Genetik mühendisliği, belirli genlerin hedeflenerek değiştirilmesine odaklanırken; sentetik biyoloji, biyolojik sistemleri mühendislik prensipleriyle yeniden tasarlamayı amaçlayan daha geniş kapsamlı bir yaklaşımdır. Biyoteknolojideki ilerlemeler, DNA'nın yapısının anlaşılmasıyla hız kazanmış ve genetik mühendisliği tekniklerinin

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geliştirilmesiyle yeni bir boyut kazanmıştır. Genetik mühendisliği, organizmaların genetik materyalinin doğrudan değiştirilmesini içerirken, sentetik biyoloji bu yaklaşımı genişleterek yeni biyolojik sistemlerin tasarımını mümkün kılar. Sentetik biyoloji, mühendislik ilkelerini biyolojiye uygulayarak "tasarla–inşa et–test et–öğren" (design-build-test-learn) döngüsünü temel alır ve biyolojik sistemlerin modüler şekilde oluşturulmasını hedefler [1].

10. GENETİK MÜHENDİSLİĞİ

2.1 Tanım ve Temel İlkeler

Genetik mühendisliği, bir organizmanın DNA'sının doğrudan manipülasyonu yoluyla yeni özellikler kazandırılmasıdır. Bu süreçte genler:

- Eklenebilir (gen transferi)
- Silinebilir (knock-out)
- Değiştirilebilir (mutasyon)

Bu teknikler sayesinde insülin üretimi, gen tedavisi ve transgenik organizmalar geliştirilmiştir.

2.2 Kullanılan Teknolojiler

Genetik mühendisliğinde kullanılan başlıca teknikler:

- **CRISPR-Cas9 sistemi:** Hedef DNA dizilerini keserek düzenleme yapar
- **Rekombinant DNA teknolojisi:** Farklı organizmalardan gen transferi sağlar
- **PCR ve DNA klonlama:** DNA çoğaltma ve analiz
- **MAGE (Multiplex Automated Genome Engineering):** Çoklu gen düzenleme

Bu araçlar, genom düzeyinde hassas düzenlemeler yapılmasını mümkün kılmaktadır [2, 3].

2.3 Uygulama Alanları

- **Tıp:** Gen tedavisi, rekombinant protein üretimi
- **Tarım:** Hastalığa dayanıklı GDO bitkiler
- **Endüstri:** Enzim ve biyoyakıt üretimi [4].

3. SENTETİK BİYOLOJİ

3.1 Tanım ve Kapsam

Sentetik biyoloji, genetik mühendisliğinin ötesine geçerek tamamen yeni biyolojik sistemlerin tasarlanmasını hedefler. Bu alan:

- Biyolojik devreler oluşturur
- Sentetik genomlar üretir
- Hücresel sistemleri yeniden programlar

Sentetik genom mühendisliği, tüm genomların tasarlanmasını ve inşa edilmesini kapsar.

3.2 Temel Yaklaşımlar

Sentetik biyoloji üç ana yaklaşımı içerir:

1. **Standart biyolojik parçalar (BioBricks):** Modüler genetik bileşenler
2. **Metabolik mühendislik:** Hücre içi yolların yeniden düzenlenmesi
3. **Minimal genom tasarımı:** Gereksiz genlerin çıkarılması [5].

3.3 DNA Tasarım ve Montaj Teknikleri

Modern sentetik biyoloji, gelişmiş DNA montaj tekniklerine dayanır:

- Gibson Assembly
- Golden Gate Cloning
- In vivo rekombinasyon

Bu teknikler, karmaşık genetik yapıların hızlı ve hassas şekilde oluşturulmasını sağlar.

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3.4 Sentetik Biyolojinin Uygulamaları

3.4.1 Tıp ve Sağlık

- CAR-T hücre tedavileri
- Gen düzenleme temelli hastalık tedavileri
- Biyosensörler

Sentetik biyoloji, hücrelerin hastalıklara özgü sinyallere yanıt vermesini sağlayacak şekilde programlanmasına olanak tanır. [7].

3.4.2 Tarım

- Kuraklığa dayanıklı bitkiler
- Verimi artırılmış mahsuller
- Bitkilerde yeni metabolik yollar

Bitki sentetik biyolojisi, tarımsal üretimi ve sürdürülebilirliği artırma potansiyeline sahiptir.

3.4.3 Endüstriyel Biyoteknoloji

- Biyoyakıt üretimi
- Biyoplastikler
- Mikroorganizmalarla kimyasal üretimi

3.4.4 Çevresel Uygulamalar

- Atık biyodönüşümü
- Karbon yakalama sistemleri
- Biyoremediasyon [8].

4. GENETİK MÜHENDİSLİĞİ VE SENTETİK BİYOLOJİ ARASINDAKİ FARKLAR

Özellik	Genetik Mühendisliği	Sentetik Biyoloji
Kapsam	Tek gen veya sınırlı değişiklik	Komple sistem tasarımı
Yaklaşım	Modifikasyon	Tasarım ve inşa
Amaç	Var olanı değiştirmek	Yeni sistem oluşturmak
Karmaşıklık	Daha düşük	Daha yüksek

Çizelge 1. Genetik mühendisliği ve sentetik biyoloji arasındaki farklar [9].

5. ETİK, GÜVENLİK VE BİYOGÜVENLİK

Bu teknolojiler önemli etik ve güvenlik tartışmalarını beraberinde getirir:
Genetik manipülasyonun sınırları
Biyolojik silah riski
Ekosistem üzerindeki etkiler
İnsan genomu düzenlemesi
Sentetik biyoloji ve yapay zekâ birleşimi, biyogüvenlik açısından yeni riskler doğurmakta ve düzenleyici çerçevelerin geliştirilmesini gerektirmektedir.- [9].

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6. GELECEK PERSPEKTİFLERİ

Yapay hücre üretimi, tam sentetik organizmalar, kişiselleştirilmiş tıp, otomatik biyolojik tasarım sistemleri olarak karşımıza çıkmaktadır. DNA sentezi ve dizileme teknolojilerindeki gelişmeler, sentetik biyolojinin hızla ilerlemesini sağlamaktadır.

7. SONUÇ

Genetik mühendisliği ve sentetik biyoloji, modern biyoteknolojinin temel taşlarıdır. Genetik mühendisliği mevcut organizmaları geliştirirken, sentetik biyoloji tamamen yeni biyolojik sistemlerin tasarlanmasına olanak tanımaktadır. Bu iki alanın birleşimi, tıp, tarım ve endüstride devrim niteliğinde yenilikler sunmakta olup, aynı zamanda etik ve güvenlik açısından dikkatli yönetilmesi gereken güçlü araçlardır.

Genetik mühendisliği ve sentetik biyoloji, 21. yüzyıl biyoteknolojisinin en dönüştürücü disiplinleri arasında yer almakta olup, canlı sistemlerin anlaşılması, yeniden programlanması ve yeniden tasarlanması açısından çığır açıcı olanaklar sunmaktadır. Genetik mühendisliği, mevcut organizmaların genetik yapısını hedefli ve kontrollü biçimde değiştirerek daha verimli, dirençli ve fonksiyonel hale getirilmesini sağlarken; sentetik biyoloji, biyolojik parçaların modüler bir yaklaşımla bir araya getirilmesi yoluyla doğada bulunmayan yeni biyolojik sistemlerin ve organizmaların tasarlanmasına imkan tanımaktadır.

Bu iki alanın entegrasyonu, özellikle kişiselleştirilmiş tıp, gen tedavileri, biyoyakıt üretimi, sürdürülebilir tarım uygulamaları ve çevresel biyoteknoloji gibi kritik alanlarda devrim niteliğinde gelişmelere zemin hazırlamaktadır. Örneğin, hastalığa özgü genetik müdahaleler, hedefe yönelik ilaç tasarımı ve sentetik mikroorganizmalar aracılığıyla gerçekleştirilen biyosentez süreçleri, geleceğin sağlık ve üretim paradigmalarını yeniden şekillendirmektedir.

Bununla birlikte, bu teknolojilerin sunduğu güçlü imkanlar, beraberinde önemli etik, biyogüvenlik ve biyogüvenlik (biosecurity) risklerini de gündeme getirmektedir. Genetik müdahalelerin uzun vadeli etkileri, ekosistem üzerindeki potansiyel sonuçlar, biyolojik silahların kötüye kullanım riski ve genetik veri gizliliği gibi konular, disiplinler arası bir yaklaşımla ele alınması gereken kritik başlıklar arasında yer almaktadır. Bu nedenle, bilimsel ilerlemenin sürdürülebilir ve güvenli bir şekilde devam edebilmesi için uluslararası düzenlemeler, etik kurallar ve denetim mekanizmalarının geliştirilmesi büyük önem taşımaktadır.

Sonuç olarak, genetik mühendisliği ve sentetik biyoloji, insanlığın karşı karşıya olduğu sağlık, gıda ve çevre sorunlarına yenilikçi çözümler sunma potansiyeline sahip olmakla birlikte, bu potansiyelin sorumlu, etik ve kontrollü bir şekilde yönetilmesi gerekmektedir. Gelecekte bu alanlardaki ilerlemelerin, multidisipliner iş birlikleri ve küresel yönetişim çerçevesinde şekilleneceği öngörülmektedir.

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HIERARCHICAL DYNAMICS IN HUMAN FRATAXIN: BRIDGING NANOSECOND LOCAL FLUCTUATIONS TO MICROSECOND-MILLISECOND CONFORMATIONAL EXCHANGE THROUGH TRANSFER ENTROPY ANALYSIS OF MOLECULAR DYNAMICS SIMULATIONS

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ABSTRACT

Protein dynamics span multiple timescales, yet the mechanistic relationship between fast local fluctuations and slow collective motions remains incompletely understood. Here, we investigated hierarchical dynamics in human frataxin, a mitochondrial iron chaperone whose deficiency causes Friedreich's ataxia, using molecular dynamics simulations (1.5 μs total sampling) combined with transfer entropy (TE) and dynamic cross-correlation matrix (DCCM) analyses. Residues exhibiting elevated nanosecond-scale flexibility, quantified by RMSF profiles, correspond precisely to primary allosteric signaling hubs identified by TE. LEU47 and LEU51 displayed intermediate RMSF values (0.8–1.2 Å) distinct from the rigid β-sheet core (<0.8 Å) and flexible termini (>1.5 Å), while functioning as dominant information sources with net TE values of 0.415 and 0.249, respectively. DCCM identified a mechanical hinge between GLY48 and GLU100 ($r = -0.431$), resolved by TE as a directional signal from LEU47 to GLU100, establishing causal rather than correlational relationships. Experimental validation through ^{15}N NMR relaxation at two field strengths (600 and 800 MHz) confirmed that hub residues exhibit microsecond-to-millisecond conformational exchange (R_{ex} = 3–5 s^{-1} at 600 MHz; 5–8 s^{-1} at 800 MHz with B_0^2-dependent scaling), with intermediate order parameters (S^2 = 0.68–0.84). The correlation between RMSF-derived flexibility rankings and NMR-detected exchange (R^2 = 0.67) demonstrates that nanosecond dynamics encode the energy landscape

topology giving rise to slower functional motions. These findings establish fast local fluctuations as reliable predictors of slower conformational transitions in frataxin, providing a computational strategy for identifying dynamic hotspots in disease-relevant proteins.

Keywords: hierarchical dynamics, frataxin, Friedreich's ataxia, molecular dynamics simulation, transfer entropy, conformational exchange, NMR relaxation, allosteric communication

1. INTRODUCTION

Proteins are not static structures; they exhibit a rich spectrum of motions spanning from sub-picosecond bond vibrations to second-timescale domain rearrangements. This multi-timescale dynamic behavior is hierarchically organized: fast local fluctuations of individual atoms and side chains occur on the picosecond-to-nanosecond (ps-ns) timescale, while slower collective motions involving loops, secondary structure elements, and domains emerge on the microsecond-to-millisecond (µs-ms) timescale [1,2]. A fundamental question in protein biophysics is whether these fast and slow motions are mechanistically coupled—that is, whether knowledge of fast dynamics can predict the location and magnitude of slower conformational transitions relevant to protein function.

Buchenberg et al. [1] provided direct computational evidence for such hierarchical coupling in Aib peptide, demonstrating that picosecond hydrogen bond dynamics serve as a prerequisite for nanosecond local conformational transitions, which in turn enable microsecond global rearrangements. Ernst et al. [2] extended this framework to T4 lysozyme, revealing a four-state model where fast nanosecond opening-closing motions are hierarchically coupled to slow microsecond locking transitions mediated by a single residue (Phe4). These studies establish a conceptual framework in which the topology of the fast-timescale energy landscape encodes information about slower functional motions.

Molecular dynamics (MD) simulations, which typically sample dynamics on the nanosecond-to-microsecond timescale, can in principle capture the fast tier of this hierarchy. If hierarchical coupling holds generally, then analyses of ns-timescale MD trajectories should predict which residues participate in slower conformational exchange processes detectable by NMR spectroscopy. Transfer entropy (TE), a measure of directed information flow between residue pairs originally introduced by Schreiber [3] and adapted to protein systems by Kamberaj and van der Vaart [4], provides a powerful framework for identifying causal relationships within MD-derived dynamics. Hacısüleyman and Erman [5] demonstrated that TE can map allosteric communication pathways in ubiquitin, identifying entropy sources and sinks that correlate with experimentally validated functional residues. Recent applications have extended TE analysis to mitochondrial phenylalanyl-tRNA synthetase [6] and β_2-adrenergic receptor [7], confirming its broad utility for allosteric network characterization.

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Frataxin, a mitochondrial iron chaperone essential for iron-sulfur cluster assembly, represents an ideal model system for investigating hierarchical dynamics. Its compact size (~14 kDa, 119 residues in the structured core) enables both extensive MD sampling and detailed NMR characterization, while the approximately 15 Å separation between the hydrophobic core and the iron-binding acidic ridge provides suitable geometry for long-range dynamic communication. Previous studies have characterized frataxin's conformational flexibility through various approaches: Noguera et al. [8] demonstrated that Loop-1 modifications profoundly affect protein dynamics and stability, Roman et al. [9] showed that C-terminal region truncation causes extreme alterations in dynamics spanning multiple timescales, and Correia et al. [10] established connections between clinical mutations and altered dynamic properties. Furthermore, our recent work on aggregation-resistant frataxin design confirmed strong correlations between MD-derived flexibility parameters and NMR relaxation measurements [11]. However, no study has systematically examined whether frataxin's nanosecond dynamics predict its microsecond-millisecond conformational exchange within a hierarchical dynamics framework.

In this study, we employ 1.5 µs of MD simulations combined with DCCM and transfer entropy analyses to identify residues with enhanced nanosecond flexibility and directional information flow in human frataxin. We then validate these computational predictions against independent ^{15}N NMR relaxation measurements to test the hypothesis that fast dynamics encode the locations of slower conformational exchange, thereby demonstrating hierarchical coupling in a disease-relevant protein.

2. COMPUTATIONAL AND EXPERIMENTAL METHODS

2.1. Molecular Dynamics Simulations

Three independent 500 ns all-atom MD simulations of human frataxin (PDB: 1EKG) were performed using GROMACS 2022.2 with the AMBER99SB-ILDN force field and TIP3P water model. The protein was solvated in a dodecahedral box with 150 mM NaCl. Following energy minimization (steepest descent, 50,000 steps) and two-phase equilibration (100 ps NVT at 310 K, 100 ps NPT at 310 K and 1 bar), production runs were conducted under NPT conditions using the Parrinello-Rahman barostat and V-rescale thermostat. Three replicates with distinct initial velocity seeds generated a total sampling of 1.5 µs. Coordinates were saved every 10 ps, yielding 150,003 frames for analysis. Internal residue numbering (1–119) corresponds to UniProt Q16595 residues 90–208.

2.2. Dynamic Cross-Correlation and Transfer Entropy Analysis

DCCM was calculated from Cα displacement vectors across the concatenated trajectory to identify correlated and anticorrelated residue motions. Transfer entropy was computed using a regression-based approach analogous to Granger causality. For each residue pair (i, j), the future fluctuation of residue j was regressed on: (i) its own past alone (residual variance σ_1^2) and (ii) both its own past and the past of residue i (residual variance σ_2^2). Transfer entropy was calculated as $TE(i \rightarrow j) = 0.5 \times \ln(\sigma_1^2/\sigma_2^2)$ with a lag time of 50 ps. Net information flow per residue was computed as total outgoing minus total incoming TE, distinguishing signal sources

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(positive net TE) from sinks (negative net TE). The complete 119 × 119 TE matrix was validated against a subsampled 60 × 60 analysis (Spearman $\rho = 0.94$).

2.3. NMR Relaxation Measurements

Backbone ^{15}N relaxation parameters (R_1, R_2, heteronuclear NOE) were measured for uniformly ^{15}N-labeled frataxin at 298 K on Bruker Avance III 600 MHz and Avance NEO 800 MHz spectrometers equipped with TCI cryoprobes. CPMG relaxation dispersion experiments were performed at both field strengths (νCPMG = 50–1000 Hz, constant relaxation period = 40 ms). Model-free analysis extracted order parameters (S^2), internal correlation times (τe), and exchange contributions (Rex). Two-field global fitting of dispersion data used the Carver-Richards equation for two-site exchange.

3. RESULTS AND EVALUATION

3.1. MD Simulations Reveal Hierarchically Organized Dynamics

The simulations achieved stable equilibrium (backbone RMSD = 1.19 ± 0.24 Å; Rg = 12.81 ± 0.07 Å) with consistent behavior across all three replicates. Per-residue RMSF analysis revealed a tri-modal flexibility distribution: the rigid β-sheet core (<0.8 Å), the flexible N- and C-terminal regions (>1.5 Å), and—critically—an intermediate flexibility class (0.8–1.2 Å) corresponding to residues at the interface between secondary structure elements. This intermediate flexibility tier proved to be the most functionally significant, as it encompasses residues subsequently identified as allosteric signaling hubs by transfer entropy analysis.

3.2. Transfer Entropy Identifies Directional Information Flow Hierarchy

Transfer entropy analysis revealed a clear hierarchy of information flow. LEU47 dominated as the primary signal source (net TE = 0.415; outgoing-to-incoming ratio = 8.7), transmitting nearly nine times more information than it receives. LEU51 served as the secondary source (net TE = 0.249; ratio = 9.3). These two residues form a hydrophobic signaling hub at the core of the protein, positioned approximately 7.3 Å from the iron-binding acidic ridge. Critically, the DCCM-identified mechanical hinge between GLY48 and GLU100 ($r = -0.431$) was resolved by TE as a directional signal: LEU47 drives GLU100, not the reverse. This demonstrates how TE decomposes symmetric correlations into causal, directional relationships—revealing the hierarchical organization invisible to standard correlation analysis (Figure 1).

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Figure 1. Hierarchical allosteric communication network in human frataxin (PDB: 1EKG). **A)** Schematic topology diagram showing secondary structure elements colored by RMSF-derived flexibility classification: rigid β-sheet core (blue, RMSF < 0.8 Å), intermediate flexibility hub region (gold, RMSF 0.8–1.2 Å), and flexible termini (red, RMSF > 1.5 Å). Signal source hubs LEU47 and LEU51 (red circles; net TE = 0.415 and 0.249, respectively) transmit directional information to signal sinks at the iron-binding acidic ridge (ASP33, ASP35, GLU100; orange circles) via relay stations LEU101 and LEU105 (blue circles). Red dashed arrow: TE-directed allosteric signal path (7.3 Å). Blue dashed line: DCCM anticorrelation hinge between GLY48 and GLU100 (r = −0.431). **B)** Three-dimensional structure rendered in ChimeraX with corresponding color scheme. Sphere sizes reflect relative TE magnitude. NMR validation summary: shared kex = 1720 ± 180 s^{-1}, R^2 = 0.67 (TE vs R_2/R_1, $p < 0.001$), Rex B_0^2 ratio = 1.73–1.76.

3.3. NMR Relaxation Validates Hierarchical Coupling

NMR relaxation measurements independently confirmed that the computationally identified hierarchy of fast dynamics predicts the locations of slower conformational exchange **(Figure 1B)**. Hub residues LEU47 and LEU51 exhibited the highest R_2/R_1 ratios (9.90 and 10.00, respectively) among all structured residues, significantly above the mean of 8.6 ± 0.4 ($p < 0.001$). These residues displayed substantial Rex contributions (4.02 ± 0.38 and 4.28 ± 0.22 s^{-1} at 600 MHz), confirming µs-ms conformational exchange. The Rex values scaled with B_0^2 at 800 MHz (ratio = 1.73–1.76; expected = 1.78), definitively confirming genuine exchange rather than anisotropic tumbling artifacts. Model-free analysis revealed intermediate S^2 values

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(0.68–0.84) for hub residues—precisely the flexibility regime predicted by intermediate RMSF values in MD simulations.

In contrast, signal sink residues (ASP33, ASP35, VAL55) exhibited low R_2/R_1 ratios (8.22–8.29) with no detectable Rex at either field strength, confirming they receive but do not generate conformational signals. Global CPMG fitting yielded a shared exchange rate kex = 1720 ± 180 s^{-1} for all hub residues, with field-independent kex values (1718 vs. 1724 s^{-1} at 600 vs. 800 MHz), supporting coordinated conformational dynamics across the hub network.

3.4. Quantitative Correlation Between Timescales

The correlation between computational predictions (ns-timescale) and experimental validation (µs-ms timescale) was quantitatively robust. Transfer entropy magnitudes correlated significantly with NMR R_2/R_1 ratios ($R^2 = 0.67$, $p < 0.001$), demonstrating that residues with enhanced nanosecond information transfer capacity also undergo slower conformational exchange. The RMSF-based flexibility classification correctly predicted the R_2/R_1 ranking for all analyzed residues: intermediate RMSF → elevated R_2/R_1 and Rex; low RMSF → baseline R_2/R_1 without Rex. This cross-timescale correspondence supports the hierarchical dynamics model in which the nanosecond energy landscape topology—captured by MD simulations—encodes the propensity for slower functional motions.

4. GENERAL EVALUATION AND CONCLUSIONS

This study demonstrates hierarchical coupling between nanosecond and microsecond-millisecond dynamics in human frataxin, a protein of direct relevance to Friedreich's ataxia. The key findings are:

First, MD-derived RMSF profiles and transfer entropy analysis identify the same subset of residues (LEU47, LEU51) as dynamically distinct, establishing that nanosecond flexibility metrics predict allosteric signaling capacity. Second, transfer entropy resolves the directionality invisible to DCCM, revealing that the hydrophobic core drives signal propagation toward the iron-binding acidic ridge rather than vice versa. Third, NMR relaxation measurements at two independent field strengths confirm that these computationally identified hub residues undergo genuine µs-ms conformational exchange, with quantitative agreement between predicted and measured parameters ($R^2 = 0.67$ for TE vs. R_2/R_1).

These results are consistent with the hierarchical dynamics framework established by Buchenberg et al. [1] for Aib peptide and Ernst et al. [2] for T4 lysozyme, extending this paradigm to a disease-relevant human protein. The intermediate flexibility of signaling hub residues—neither too rigid nor too flexible—represents an optimal dynamic regime for conformational signal transduction, analogous to the intermediate protection factors observed in hydrogen-deuterium exchange experiments at these same positions.

From a methodological perspective, this work validates that standard MD simulations (500 ns timescale) combined with transfer entropy analysis can reliably predict the locations of functionally important slower dynamics, without requiring enhanced sampling methods. This has practical implications for computational screening of allosteric hotspots in therapeutic

protein targets. Future studies should extend this hierarchical analysis to iron-bound frataxin states and apply Markov state modeling to directly bridge the nanosecond-to-millisecond timescale gap. The identified hub–relay–sink architecture provides specific structural targets for future experimental interrogation of frataxin's allosteric mechanism and potential therapeutic intervention in Friedreich's ataxia.

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THE HIDDEN DANGER IN HONEYCOMBS: THE IMPACT OF CHEMICAL RESIDUE RISKS ON BEE AND HUMAN HEALTH

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ABSTRACT

Beeswax is a natural product with a complex chemical structure, synthesized by honeybees and used in beekeeping to produce basic honeycomb. However, because beeswax is lipophilic and has a high absorption capacity, environmental pollutants and chemical residues resulting from beekeeping practices accumulate in this matrix. Synthetic acaricides (fluvalinate, coumaphos, bromopropylate) used in the control of the Varroa destructor mite, pesticides, and substances such as naphthalene used in the honeycomb storage process can persist in beeswax for extended periods and, over time, migrate into honey, potentially reaching humans through consumption. The long-term use of old combs within the hive leads to a cumulative increase in chemical residues and creates a suitable environment for pathogenic microorganisms. This negatively impacts bee health, causing issues such as delayed queen development, reduced egg-laying capacity, behavioral abnormalities, and decreased reproductive performance. These residues accumulating in beeswax not only affect colony health but also pose significant risks to human health by transferring into honey and other bee products. It has been reported that chronic exposure to lipophilic pesticides and acaricides can cause toxic effects on the nervous system, endocrine system, immune system, and metabolic processes. Additionally, the bioaccumulation potential of certain residues may lead to cumulative health risks for humans over the long term. The mixing of waxes from different sources in wax foundation production and inadequate sterilization practices contribute to the spread of disease-causing spores as well as the transport of chemical residues over wide areas. Furthermore, bees' use of the same nectar sources facilitates the indirect transfer of residues between colonies. In recent years, the use of organic compounds such as organic acids and essential oils—as alternatives to synthetic chemicals—has gained importance, and it is reported that these substances carry a lower risk of residues. However, these applications must be used at appropriate doses and durations. In conclusion, chemical residues accumulating in beeswax pose significant risks to both bee and human health; therefore, sustainable beekeeping requires regular comb renewal, residue-minimizing control methods, standardized production processes, and effective monitoring systems.

Key words: Beeswax, chemical residues, contamination, sustainable beekeeping

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1. INTRODUCTION

Beeswax, one of the unique products produced by honeybees, is a biological substance secreted by the wax glands located in the last four abdominal segments of worker bees aged 12–18 days. Upon secretion, it emerges between the abdominal segments in a liquid and transparent form, rapidly solidifying into thin flakes when exposed to air. Honeybees produce beeswax by metabolizing carbohydrates derived from honey and combining them with specific enzymatic secretions from their bodies. Chemically, beeswax is a highly complex and heterogeneous material characterized by a honey-like scent and aromatic flavor, consisting of more than 300 identified compounds, including esters, hydrocarbons, free fatty acids, fatty alcohols, and minor bioactive constituents. Approximately 50 distinct aromatic compounds have been reported to contribute to its characteristic odor profile. Beeswax is primarily synthesized from the sugars present in honey—namely glucose, fructose, and sucrose—through biochemical conversion pathways involving lipid metabolism.

The primary biological function of beeswax is to enable the construction and expansion of honeycomb cells, which serve as essential structures for brood rearing, food storage (honey and pollen), and colony organization [1,2,3]. Although beeswax is insoluble in water, it exhibits high solubility in organic solvents such as ether, benzene, acetone, gasoline, and chloroform due to its lipophilic nature. This physicochemical property also underlies its ability to accumulate and retain lipophilic compounds, including pesticides and veterinary drug residues, making it a potential reservoir for contaminants within the hive environment.

The production of beeswax is an energetically demanding process; it is estimated that bees must consume approximately 6–10 kg of honey to synthesize 1 kg of beeswax. At an optimal hive temperature of approximately 35°C, worker bees form chain-like clusters (festooning behavior), during which wax secretion is stimulated. Each worker bee can produce up to eight wax plates from its abdominal glands. These wax scales are subsequently transferred from the abdominal segments to the mandibles via the legs, where they are mechanically processed, mixed with saliva, and molded into hexagonal comb structures. The remarkable geometric precision of the hexagonal comb architecture provides both structural strength and efficient space utilization, representing an optimal solution in terms of material economy and mechanical stability.

Seasonal dynamics and colony physiology strongly influence beeswax production within the colony. It is known to be most intensive during the spring season, when brood rearing activity is at its peak and the demand for new comb construction increases. The quantity of beeswax produced varies depending on several factors, including the queen’s oviposition rate, colony strength and developmental stage, nectar and pollen availability, environmental temperature, and overall colony management practices. According to recent statistical data from Türkiye, approximately 4,095 tons of beeswax are produced annually from 8,984,676 colonies, highlighting its economic and apicultural significance [4].

Beyond its biological role in the hive, pure beeswax produced by honeybees has a wide range of industrial, pharmaceutical, and technological applications due to its unique physicochemical properties, biocompatibility, and stability. In beekeeping, it is primarily used for the production

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of wax foundation sheets, which facilitate comb building and improve colony productivity. In the pharmaceutical industry, beeswax is utilized as a coating agent for tablets and capsules, as well as a stabilizing and emulsifying component in ointments and drug delivery systems. In the cosmetics industry, it is extensively employed in the formulation of creams, lotions, lip balms, masks, and soaps due to its moisturizing, protective, and film-forming properties. Additionally, beeswax is used in dental applications (e.g., impression materials), candle production, metal casting (lost-wax technique), textile finishing, paint and varnish formulations, furniture and floor polishes, optical lens processing, and waterproofing technologies. In the food industry, it serves as a glazing agent and additive in products such as confectionery and chewing gum. Its versatility and natural origin make beeswax an indispensable raw material across multiple sectors, further emphasizing the importance of sustainable and residue-free beeswax production systems [3,5].

1. TRANSPORTING COMBS TO THE FACTORY AND WAX FOUNDATION PRODUCTION

In our country, beekeepers take their old, expanded combs to the comb factory by cutting them from the frames and stacking them, or by melting and straining the combs through a straining process, and receive them as processed wax foundation sheets. The melting process can be carried out by directly melting the combs using solar heat or by boiling a mixture of wax and water in equal parts. The melted beeswax is then poured into a large bag made of plant fibers (telis), and pressure is applied to allow the clean wax to flow through the bag's pores while the impurities remain inside. As the water-wax mixture flowing out cools, the clean wax solidifies into a layer on the surface of the water. There are also press machines used for this process [3]. The 'wax foundation' is formed by melting the beeswax under heat and passing it through cylinders with hexagonal prism cells to create plates. Two methods are used in production: hot casting and cold casting. In both methods, the process uses sterilized, pure, and natural beeswax. While hot-rolled combs are more brittle, cold-rolled combs offer advantages in terms of ease of use and a higher number of combs per kilogram. The beeswax used in wax foundation production must be pure and free of additives, containing no foreign substances such as paraffin, ceresin, internal oil, resin, oxalic acid, or bleaching agents. Honeybees accept and build upon pure, additive-free foundation, but do not build upon foundation containing synthetic chemical additives. Wax foundation factories collect combs from a large number of beekeepers; these are mixed, melted in the same boiler, and used to produce wax foundation sheets. If the collected combs are obtained from colonies carrying disease-causing spores, these spores adhere to the walls of the boiler during the melting process, making it inevitable that the wax foundation sheets produced by pouring the mixture into molds will contain disease spores. For this reason, the sterilization of foundation sheets at the frame factory is of great importance. The beeswax used in the production of foundation sheets must be sterilized by holding it in special boilers at 120°C and 1 atm pressure for 15 minutes or by subjecting it to an equivalent sterilization process. In our country, the use of inadequately sterilized foundation in hives leads to the uncontrolled spread of bee diseases, particularly mandatory-reporting diseases such as American Foulbrood. By ensuring that the necessary sterilization conditions are met, the risk of the spread of disease-causing microorganisms is reduced, and the production of healthy wax foundation sheets becomes possible [6,7,8].

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2. THE USE OF OLD COMBS IN COLONIES

The continued use of old combs in hives is a factor that contributes to the spread of disease-causing microorganisms among honey bees. Due to the bees' brood-rearing activity, old combs containing remnants of body parts create a suitable environment for the reproduction of disease-causing pathogens. Additionally, since worker bees emerging from these combs tend to be smaller in size, this negatively impacts colony development and performance. The high concentration of pathogenic microorganisms in old combs not only threatens bee health within the hive but also poses a risk to human health when considering the consumption of bee products. The continuous use of old combs within the hive leads to an increased accumulation of pesticides applied each season in the beeswax, resulting in higher toxic levels. In particular, beekeepers risk easily transmitting disease-causing microorganisms if they transfer frames between hives without proper control or use contaminated materials in the apiary. Therefore, care must be taken to clean and disinfect tools and equipment used in the apiary and hives, and every effort should be made to use new frames in the hives whenever possible. Even if no medication is applied to the hive, drug residues may still be found in the foundation frames provided to the hive, including those provided for the first time. While this situation might suggest that proper sterilization is not being carried out in the wax produced by comb factories, the presence of certain pesticide residues in comb samples taken from the hives of beekeepers who do not use synthetic chemical pesticides in their hives may stem from the fact that their bees share the same nectar source with the bees of beekeepers in the same region who do use pesticides in their hives. Bees from colonies where synthetic chemical pesticides are used for disease and pest control in the apiary transfer pesticide residues to flowers during flight, and bees from colonies that do not use pesticides, when landing on these flowers, become contaminated with the pesticides and carry these substances back to their hives [9,10,11].

3. STORAGE CONDITIONS FOR HONEYCOMBS

The preservation of honeycombs begins once the honey harvest is complete. The most significant problem our producers face during storage is the honeycomb moth. This is a particularly serious issue in warm regions. Another problem is mold and souring, which can occur during the storage of honeycombs containing honey and pollen. Humidity and temperature accelerate the emergence of these undesirable damages in the combs. Storing combs in cold conditions is the most effective method against both the wax moth and spoilage and mold. Storing combs below 10 °C—for example, in cold storage facilities—prevents the hatching of wax moth eggs and inhibits larval development. Exposing the combs to −12°C for 3 hours or −15°C for 2 hours kills all living organisms, including eggs, at every stage of development. In regions where winters are cold and air temperatures remain below the specified levels, no additional measures are necessary. Another method used for storing combs is the application of sulfur smoke. For this, the combs are arranged on a rack system in a room, and 20–50 grams of sulfur are burned per 1 m^3 during each application. The smoke emitted from this burning is effective against the wax moth but does not affect wax moth eggs. Therefore, the application should be performed 1–2 weeks after the combs are removed from the hive and repeated after 2 weeks. Organic acids such as formic acid and acetic acid can be used in

honeycomb storage rooms. Since organic acids are heavier than air, they are placed on top of the honeycombs and a few drops are distributed. For a room volume of 10 liters, 20 ml of 60–80% acetic acid or 8 ml of 85% formic acid should be used. These applications help protect the combs from the wax moth pest and spoilage/mold without causing any residue issues and are recommended for beekeepers [3,12,13].

4. THE PROBLEM OF RESIDUES IN BEESWAX

Bee diseases are generally management-related conditions caused by inadequate or improper care and feeding conditions. These diseases can be prevented through proper care and feeding, regular selection, and the development of disease-resistant strains, leading to healthier and more productive beekeeping. However, beekeepers often tend to use medications even when no disease is present in the hive and the bees are healthy. This practice negatively impacts the bees' natural defense systems against diseases and pests, leading to inadequate defense mechanisms when they actually encounter a disease or pest. Currently, none of the synthetic chemicals used against bee diseases and pests are medications specifically designed to target "bee diseases and pests." Therefore, the healthiest, most cost-effective, and easiest method of combating bee diseases and pests is for beekeepers to maintain their colonies in a strong population balance and provide appropriate care and management for their bees.

The synthetic chemicals used in hives against bee diseases and pests have a negative impact on bee health in the long term and on the quality of honey produced by the hive in the short term. Even the use of licensed medications for Varroa control, when used for extended periods or at high doses, leads to Varroa developing resistance to the medication within the hive. In this process, both the health of the larvae and adult bees deteriorates, and the hive population weakens. It is known that varroacides pose a risk of residues in honey. The pesticides used to combat bee diseases, however, represent another aspect of the issue. In some studies [14,15,16,17], it was found that medications used to combat Varroa in colonies—such as Apistan (active ingredient: fluvalinate), Perizin (active ingredient: coumaphos), and Folbex-VA (active ingredient: bromopropylate)—can cause residue issues in the comb within the hive depending on the dose and duration of use. Additionally, these studies reveal that these treatments hinder queen development, reduce egg-laying, decrease sperm count in drones, cause behavioral abnormalities in bees, and lead to difficulties in accepting a new queen. These active ingredients, present at high levels in beeswax, can migrate into honey and potentially cause disorders in the nervous, circulatory, excretory, and immune systems of consumers.

Natural compounds such as formic acid, lactic acid, and oxalic acid, which have become increasingly preferred in Varroa control in recent years, do not leave residues in honey or beeswax like other chemical pesticides, and yield positive results in Varroa control due to their rapid half-life. In today's world, where the consumption of organic food has gained importance and consumer awareness has grown, honey production compliant with European Union standards has become a necessity. Therefore, beekeepers must take the necessary precautions due to the sensitivity of the issue and manage their colonies in accordance with the guidelines for Varroa control. Additionally, care must be taken to use organic-based chemicals at the appropriate dose and for the appropriate duration [3]. One of the highly harmful chemical substances that has been used for many years in the storage of honeycombs is naphthalene.

Naphthalene is an antiseptic hydrocarbon obtained by distilling coal tar; it is white, has a distinctive odor, is insoluble in water, and dissolves easily in alcohol, benzene, and ether. It rapidly vaporizes, transitioning from a solid to a gaseous state. The use of naphthalene to protect beeswax combs against wax moths results in increased naphthalene concentrations in the combs. Naphthalene reaching high levels can be toxic to bees and lead to outcomes such as colony losses. Because of its absorbent nature, beeswax traps naphthalene within its structure, and since a significant portion of it transfers into the honey, the residue problem persists and worsens. Pesticide and drug residues accumulating in honey can harm humans when the honey is harvested and consumed; however, naphthalene and other pesticides accumulating in the beeswax are more dangerous because the combs are used for several seasons. This is because, in such cases, pesticides previously accumulated in the combs gradually mix with the honey along with the pesticides introduced into the hive during the year, making old combs a potential source of pesticides within the hive [9,18].

5. CONCLUSION

In conclusion, ensuring the sustainability, safety, and quality of beewax requires a comprehensive and coordinated approach. Beekeepers must take care to use healthy combs, maintain and manage hives, and properly harvest and store bee products; they must also be trained by qualified professionals in the control of bee diseases and pests, and the public must be made aware of these issues. In addition to these fundamental practices, the implementation of good beekeeping practices (GBP), routine monitoring systems, and traceability mechanisms is essential to ensure both product quality and colony health. Regular comb renewal, residue monitoring, and the adoption of preventive management strategies should be prioritized to minimize contamination risks and maintain sustainable production systems. Furthermore, the lack of an official scientific laboratory authorized by the state to address bee diseases and pests, as well as the insufficient number and effectiveness of experts in this field, contribute to the persistence of these issues in Turkey. Strengthening institutional infrastructure, increasing the number of accredited laboratories, and improving collaboration between universities, research institutions, and governmental bodies are critical steps for effective disease surveillance, diagnosis, and control. In addition, developing standardized protocols and national monitoring programs would enhance the early detection and management of emerging threats. Although the extremely negative effects of chemical substances used against bee diseases and pests on both bee and human health have been demonstrated, it is evident that the use of chemical substances will continue, and the issue will remain a subject of debate for many years to come unless alternative control methods are utilized. In this context, there is a growing need to promote sustainable and eco-friendly approaches such as biotechnical methods, organic acids, essential oils, breeding for disease-resistant bee lines, and integrated pest management (IPM) strategies. These alternatives not only reduce chemical residues in bee products but also support long-term colony resilience and environmental sustainability. To prevent all these negative consequences, significant responsibilities fall upon our beekeepers, beekeeping associations, universities and research institutions, our ministry, and consumers. In addition, policymakers and regulatory authorities must play an active role in establishing effective legislation, ensuring compliance with international quality standards, and supporting educational and extension

programs. Consumers also play a critical role in shaping production practices by prioritizing safe, traceable, and high-quality bee products, thereby indirectly promoting compliance with quality standards and responsible beekeeping practices. The alignment of stakeholder awareness and accountability across the apicultural value chain is therefore essential to mitigating current challenges in the production, quality, and marketing of bee products. As conclusion, a multidisciplinary and coordinated approach—integrating scientific research, field practices, policy development, and public awareness—will be essential for achieving sustainable, safe, and high-quality apicultural production systems in the future.

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THE PARADIGM EFFECT OF HONEY BEES IN PLANT POLLINATION

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ABSTRACT

The increased importance of the global food supply following the recent pandemic has once again brought to the forefront the vital role of agricultural production and the need to ensure its continuity. In crop production, yield and quality depend largely on effective pollination. Honey bees (*Apis mellifera* L.) play a critical role as the primary pollinators, particularly in many crop plants that require cross-pollination. Honeybees are a decisive factor in the sustainability of agricultural production not only through the production of high-value-added products, but also through the pollination function they provide as part of ecosystem services. The role of honeybees in pollination should not be viewed only as a mechanical transfer of pollen; this process should be considered a multifaceted effect that regulates gene flow between plants, supports biodiversity, and shapes both the quantitative and qualitative dimensions of agricultural production. In this context, honeybees serve as fundamental biological actors that bridge natural ecosystems and agricultural production systems, functioning as ecosystem engineers. However, in recent years, the expansion of agricultural lands, the proliferation of monoculture production systems, increased pesticide use, and anthropogenic factors disrupting ecosystem balance have negatively impacted natural pollination processes. This situation creates pressure on honey bee populations, leading to a decline in pollination efficiency and, therefore, resulting in yield and quality losses in crop production. In this context, to maximize the benefits of honey bees' pollination efficiency, it is necessary to understand bee-plant interactions, develop appropriate colony management strategies, and promote the widespread adoption of bee-friendly agricultural practices. In conclusion, honeybees should not be considered merely a supporting element in crop production; rather, they should be recognized as a central biological actor that reshapes agricultural production paradigms through their genetic, ecological, and economic dimensions.

Key words: Honey bee, pollination, agriculture, plant production

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2. INTRODUCTION

Many flowering plants cannot produce fruit or seeds on their own and rely on pollinators to transfer pollen between flowers. Honeybees are particularly important because they visit a large number of flowers, carry large amounts of pollen on their hairy bodies, and show a strong preference for certain plant species while foraging for food. All cultivated crops in agricultural production are flowering plants. Seed production is of vital importance for the continuity of agricultural production. In other words, cultivated plants must produce seeds. For this, plants require pollination. High-quality and high-volume plant production depends on the successful occurrence of cross-pollination. The honeybee is nature's best pollinator capable of performing this function. While collecting nectar and pollen from nature to sustain the systematic life within the colony, the honeybee also plays a crucial role as a pollinator of vegetables and fruits, which are of great importance to human life. In plants where healthy pollination does not occur, seed formation may fail, or even if it does occur, the resulting deformities due to insufficient pollination negatively affect product quality. This situation not only makes marketing the harvested product difficult but also leads to the inadequate development of functions such as germination and seedling vigor in the plant [1]. In recent years, the conversion of open areas to agricultural use, increased and irregular pesticide use, and numerous other factors disrupting the biological balance, along with the practice of monoculture farming over vast areas, have contributed to the escalation of pollination problems [2]. Pollination is of vital importance in plant production. In particular, the production constraints resulting from insufficient pollination in large agricultural areas constitute a significant problem. In recent years, extensive and effective studies have been conducted to ensure optimal pollination in plants that rely on honeybee pollination. Furthermore, if the necessary importance is given to pollination, the resulting increase in crop yields will contribute to the global expansion of crop production. Many major crops (fruits, nuts, oilseeds, vegetables) depend heavily on bee pollination; honey bees provide a large share of this service—often around two thirds of insect pollination in farm [3,4,5,6,7]. In some fruit orchards, over 80% of pollination is done by bees, and yields rise sharply when hives are added [4,8,9]. It is reported that increasing pollination efficiency results in yield increases of 45–50% in sunflowers, 56–60% in apples and pears, 75–90% in cucumbers, 95–100% in melons and watermelons, 25–30% in tomatoes and grapes, and 35–40% in alfalfa, clover, and vetch. These increases cannot be achieved through fertilization, irrigation, or other agronomic practices; honeybee pollination is absolutely essential. It is noteworthy that in areas of intensive agriculture, the value of the crop yield is 10–12 times greater than the value of the products produced by bees, such as honey, pollen, and royal jelly, and the immense contribution they make to plant production is striking [2,10].

3. DEFINITION OF POLLINATION AND BEE POLLINATION

Pollination is the transfer of pollen grains from the anther (male part of the flower) to the stigma (female part) [11,12]. This process can occur within the same flower or among flowers of the same species. After the pollen grain reaches the stigma, it absorbs water, germinates, and extends a pollen tube along the style toward the ovule, carrying two sperm cells for double fertilization (embryo + endosperm) [12,13]. Pollination can occur through both abiotic and biotic agents. Abiotic pollination involves the transfer of pollen by non-living factors such as

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wind and water, whereas biotic pollination is mediated by living organisms including insects, birds, bats, and even ant [12,14,15]. In agricultural systems, however, the primary pollination agents are wind and insects, with bees playing a particularly significant role due to their efficiency and specialization in pollen transfer. Cross pollination (cross fertilization) is the transfer of pollen from one plant to another plant of the same species or a compatible variety [8,11,12,16]. Cross-pollination plays a critical role in both natural ecosystems and agricultural production. It increases genetic diversity within plant populations, which is generally favored by natural selection as it enhances adaptability and resilience [11,17]. In many crop species, cross-pollination leads to improved seed set as well as higher fruit quantity and quality, even in plants that are capable of self-pollination [16,18,19]. Furthermore, some plant species exhibit self-incompatibility mechanisms, whereby their own pollen is recognized and rejected, making pollen transfer from another individual essential for successful fertilization and seed production [17]. Plants have evolved various strategies to promote cross-pollination. Floral traits such as color, scent, nectar production, and morphological adaptations are specifically designed to attract particular pollinators and facilitate efficient pollen transfer between different plants [11,20]. In addition to these physical adaptations, genetic mechanisms such as self-incompatibility systems actively prevent self-fertilization and enforce outcrossing, thereby maintaining genetic diversity and reproductive success [21,22,23].

3.1 Why Many Flowers Need Honey Bee Pollination

Many flowering plants cannot set fruit or seeds well on their own; they need animals to transfer pollen between flowers. Honey bees are especially important because they visit many flowers, carry lots of pollen on their hairy bodies, and show strong loyalty to particular plant species while foraging. When a honey bee visits a flower for nectar or pollen, grains stick to its body and are rubbed onto the next flower's stigma, allowing fertilization so fruits and seeds can develop [5,24]. Cross pollination by honey bees increases fruit and seed set, improves seed quality, and boosts yield in many crops and wild plants. For some crops and wildflowers, open pollination with bees produces many times more seeds than self pollination or no pollination [4,6,8,9]. Plants have evolved a range of adaptive traits to attract bees and enhance pollination efficiency. In this mutualistic relationship, plants effectively "invest" in bees by offering visual, olfactory, and nutritional cues. Bright flower colors and distinctive scents are aligned with bee vision and olfactory sensitivity, making flowers more detectable and attractive. In addition, nectar and nutrient-rich pollen serve as essential food rewards, encouraging repeated visits by bees. Structural adaptations, such as specialized petal surfaces or flower movements, further facilitate landing and efficient foraging behavior. Together, these traits increase the frequency and effectiveness of bee visitation, ultimately improving pollination success and plant reproductive outcomes [3,24,25].

4. HONEYBEE BEHAVIORS ON FLOWERS

Not every visit by a honeybee to a flower results in pollination. During the visit, the bee may not come into contact with the flower's stigma, or the body parts that do touch the stigma may not be carrying pollen. Whether pollination occurs depends on whether the bee is collecting nectar or pollen. Pollen-collecting bees move around the anthers of the plant. In this way, the bee's entire body becomes covered in pollen. The bee then acts as a "carrier" in pollination by

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touching the stigma. To determine whether a nectar-collecting bee is pollinating, one must observe which part of the flower the bee is on. Bees collecting nectar from fruit flowers may be found on the anthers, on the petals, or on both. Nectar-collecting bees on the anthers generally also touch the stigma to facilitate pollination. However, pollen-carrying bees on the petals may sometimes collect nectar without touching the stigma. In this case, pollination does not occur. Bees visiting flowers with prominent stamens (plum, pear, peach, apricot) perform pollination while collecting both pollen and nectar [26]. A honeybee that finds a rich nectar source maintains its relationship with that source until it finds a richer one. Thanks to this "flower fidelity" behavior, the honeybee helps plants pollinate more reliably. When plants produce sufficient levels of nectar and pollen, bees do not tend to travel long distances; instead, they maintain an economic flight range and tend to utilize nearby nectar and pollen sources [1].

5. FACTORS AFFECTING POLLINATION EFFICIENCY

The timing of bringing bees to the plant source is of great importance for pollination efficiency. As a general rule, bringing honeybee colonies to the plant source one week before the start of flowering is considered appropriate for good pollination. The most productive period for pollination efficiency is assessed as when flowering begins at a level of 10–25%. However, for certain plant species where pollination is relatively more challenging (such as apple, almond, and strawberry), it is more appropriate to bring the hives to the pollination area before flowering begins. Additionally, to ensure effective pollination, the number of colonies per unit area must also be increased [26]. It is seen that specialized pollinator plant varieties play a significant role in the establishment of orchards. The placement of these varieties within the orchard should also be planned during the orchard's establishment. Since honeybees placed in an orchard for pollination will primarily prefer the flowers on trees near their hives as a source of nectar and pollen, care must be taken to ensure that the beehives are distributed evenly throughout the orchard. Otherwise, the bees will fly to a limited number of trees, resulting in better pollination, fruit set, and fruit quality on those trees. This situation is particularly evident in fruit trees that are less attractive to bees, such as apple, cherry, and almond trees. Fruit set and fruit quality are lower in trees that bees do not visit or visit insufficiently. Due to this risk, positioning honeybee colonies near pollinator plant varieties is crucial for effective pollination and the production of high-quality fruit [27,28]. In honeybee colonies, the colony's strength—and particularly the level of the forager bee population—directly determines pollination effectiveness. Beekeepers aim to have strong colonies to produce better yields, while vineyard and orchard owners seek them to ensure effective pollination. One of the most important criteria determining colony strength is the distribution and size of the brood area. Colonies containing 5–6 brood frames are generally considered sufficient for effective pollination; however, the effectiveness of the colony count may vary depending on the nature of the crop to be pollinated and weather conditions. The recommended number of hives per hectare for crop plants varies: 5–8 for clover, 5 for apples and cherries, 5–12 for almonds, 8–10 for strawberries and kiwis, and 5–7 for onions. Generally, effective pollination is achieved by calculating 1–4 colonies per 4 decares. In the area where pollination will take place, care should be taken to maintain a minimum distance of 150 m between bee colonies in winter and 300 m in summer, and to position the flight entrances so they can receive morning sunlight. Orienting the hive's entrance

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so that it faces the plants in the pollination area and is exposed to a light breeze allows honeybees to quickly detect the scent from the flowers and respond more efficiently [27]. The distribution pattern and density of colonies across the landscape vary depending on the plant species, flowering period, flower density and attractiveness, colony strength, flower competition, land area, and weather conditions. The timing of pollination is another important factor. Generally, the flowering period in plants is short. When weather conditions are unfavorable, insect activity and pollination slow down and become insufficient. In such cases, growers reduce the risk of insufficient pollination by placing bee colonies in their orchards. Bee pollination improves both the quality and quantity of the crop. The most effective pollination temperature begins at 21°C, when bees start working actively. Pollination is negatively affected by cold, rainy, and windy weather. Crops obtained through adequate pollination have normal shape and form, which increases their marketability and value. Additionally, seed quality improves. Flowers exposed to bee visits are also less affected by spring frosts [1]. To ensure effective pollination, care must be taken to ensure that no other plant species within a 3-kilometer radius of the target plant species is in bloom to a degree that creates competition. Therefore, by carefully calculating the flowering periods of plants to be planted for pollination, the ability of surrounding plants to attract honeybees—which could create competition—is significantly reduced. Crop plants with intensive production, such as sunflowers and cotton, have a significant need for bee pollination. High fiber quality in cotton plants is only possible with adequate pollination. With bee pollination, germination rate and 1,000-seed weight in cotton increase by 20–30%, and the maturation and vegetative growth period of cotton bolls is shortened by 5–8 days. Sunflower is a high-quality plant that provides abundant nectar. It has two types of flowers, and because these flowers mature at different times, the female and male reproductive organs cannot self-pollinate. At this point, bee visits are required. Bees account for 95–98% of pollination. Approximately 100–150 bee colonies can pollinate 200 hectares of sunflowers. The amount and quality of oil in the seeds produced through bee pollination increase. Forage plants such as alfalfa, clover, white clover, red clover, rock clover, and sainfoin are important sources of nectar and pollen for bees. There is a linear relationship between nectar and pollen production in these plants and bee visits. In other words, the greater the amount of nectar and pollen in the plants, the higher the likelihood of them being visited by bees. Additionally, the composition of sugars in the nectar is one of the factors influencing bee visits. The higher the sugar concentration in the nectar, the more likely bees are to prioritize visiting those plants [10,29,30].

6. GUIDING BEES TO SPECIFIC PLANTS

Bees recognize flowers by their color, shape, and scent. Bees also have a highly developed sense of smell. In this way, bees can be guided to flowers with specific scents. Nectar-gathering bees also prefer flowers that contain the pollen found in the hive. Furthermore, feeding honeybee colonies with a sugar syrup prepared using the essence of flowers from the plant species intended for pollination helps direct the bees directly to a specific plant. Another method involves spraying sugar syrup onto the plants intended for pollination to attract the bees to them. However, this method is neither practical nor does it allow the bee to spend sufficient time working on the flower.

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5.1 Making Plants More Attractive to Bees

Making plants attractive to honeybees for pollination purposes—ensuring that nectar and pollen can be easily collected by the bees—increases pollination. There is a direct correlation between the amount of nectar in a plant and the number of bee visits.

When plants do not receive sufficient pollination from bees, two methods are applied:

1. Pollen from the plant variety to be pollinated is collected and placed in front of the hive entrance; as a result, bees become coated with pollen as they exit the hive and pollinate other varieties they visit.

2. Flowers from the plant to be pollinated are cut and placed in plastic containers filled with water on the branches of plants of a different variety; pollination between the two different varieties is achieved through bee visits [26,30].

5.2 Breeding Bee Colonies That Visit Specific Plants

Pollen selection in honeybees is not random; genetic makeup also influences this selection. Research is being conducted to develop bee lines that visit specific plants. For example, in fruit orchards, the best pollinators have been identified as Caucasian-Italian hybrids and pure Caucasian breeds. Additionally, the Caucasian breed is considered the best pollinator for alfalfa and other legumes. Pure Italian and Italian-Caucasian hybrids are highly effective in sunflower pollination.

5.3 Making Pollination Profitable for Both Beekeepers and Growers

Transforming the use of bee colonies and vineyard-orchard lands for pollination into a mutually beneficial arrangement for both beekeepers and growers is a widespread practice in many countries. In countries such as the United States and Israel, a significant portion of honeybee colonies are leased to landowners for specific periods solely for pollination purposes. In the colony rental method, which serves as an important source of income for beekeepers, a fee schedule varies depending on the plant species and the pollination period. For example, in the U.S., a landowner pays $150 per hive placed on their land for melon pollination during the summer, while receiving $20 per hive for almond pollination during the winter months. The beekeeper and the landowner-grower enter into a mutual agreement before the flowering period. In this agreement, the beekeeper guarantees the landowner that they will bring a sufficient number and strength of bee colonies to the land before the plants bloom to ensure adequate pollination, and will remove them after the flowering period ends. The landowner, in turn, guarantees payment to the beekeeper based on the strength of the colonies, the type of plant, and the season. Additionally, the landowner guarantees that no pesticide applications will be made while the colonies are on the land. Through this mutual agreement, the beekeeper ensures stronger hives by providing a source of nectar and pollen for the bees, while the landowner will earn money from the high-quality and high-yield crop.

7. CONCLUSION

In our country, since the high pollination efficiency of honeybees is either unknown to or not utilized by crop growers, the desired level of crop production is not being achieved. In fact, in many places, colonies are kept away from crop production areas due to unfounded beliefs that honeybees harm crops. This situation causes significant economic losses both in terms of honey production and crop production. Institutions involved in both beekeeping and crop production must implement necessary regulations, educate producers, and prevent the loss of national wealth. On the other hand, there is a need for more scientific studies with broad impact, particularly regarding seed production, pollination, and the utilization of bees under greenhouse conditions.

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IMPACT OF BORON FERTILIZATION ON YIELD AND OIL QUALITY OF SUNFLOWER (*Helianthus annuus* L.)

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ABSTRACT

Sunflower is one of the primary crops that plays a significant role in oilseed production worldwide and in our country. However, the effective realization of this potential depends on the implementation of appropriate and balanced plant nutrition strategies. Sunflower, one of the crop species highly susceptible to boron (B) deficiency, is considered not only a micronutrient but also a key nutrient that influences the plant's physiological processes and reproductive capacity. Boron plays a structural role in cell wall synthesis and the cross-linking of pectin, thereby ensuring tissue integrity. Furthermore, by regulating auxin (IAA) metabolism, it contributes to cell division and the development of meristematic tissues. Boron also has a direct effect on fertilization biology, particularly during the flowering period, where it supports pollen viability and pollen tube development, thereby increasing seed set. It also plays a regulatory role in the transport of photosynthetic products from leaves to reproductive organs and the root system. Furthermore, it actively participates in fundamental physiological processes such as lignification, carbohydrate metabolism, RNA synthesis, and cellular respiration. In plants with boron deficiency, increased sensitivity to high light intensity and the occurrence of photooxidative damage have been reported. This condition leads to the disruption of cell membrane integrity and impairs the plant's physiological functions. Consequently, light -which is essential for plant growth- can become a stress factor when proper nutritional conditions are not met. When evaluated in terms of yield components, boron applications have been shown to yield significant increases in sunflowers. Studies have reported that boron application resulted in an approximate 20% increase in grain yield per decare. Regarding thousand-seed weight, a key indicator of seed quality, an increase of approximately 45% was observed with boron application. Boron not only significantly affects grain yield but also substantially impacts oil yield per unit area. Indeed, it has been reported that applying boron at a dose of 600 ppm resulted in an approximate 65% increase in oil yield. Additionally, it has been determined that the oleic acid content -a critical factor for oil quality- increased to 38.22% with boron applications, and in some studies, even reached levels as high as 52.71%. Furthermore, it is stated that boron application resulted in an approximate 8.72% increase in the synthesis of linoleic acid, an important unsaturated fatty acid. To address boron deficiency in sunflower cultivation, it is recommended to apply 700-1,000 g of boron per decare before or during planting. However, under conditions where boron uptake from the soil is limited or in cases of drought stress, a foliar application can be made by preparing a solution of 150 g of boron per 100 liters of water. The most critical period for boron fertilization is the onset of head formation; applications made approximately 10-15 days before flowering are reported to be more effective. In conclusion, it is evident that boron has significant effects on seed yield, oil yield, and unsaturated fatty acid content in sunflowers. Furthermore, considering its positive contributions

to plant growth and the root system, incorporating boron into production programs whether applied to the soil before planting or via foliar application during critical growth stages is regarded as an important agronomic approach for enhancing yield and quality.

Key words: *Helianthus annuus*, Boron fertilization, Yield, Oil Quality

1. INTRODUCTION

Today, there is a wide variety of oilseed crops grown worldwide, and species such as jojoba, palm, and coconut -which are not dependent on specific ecological conditions -are able to adapt successfully to Turkiye's diverse agroecological regions. This highlights our country's potential for oilseed production and contributes to agricultural diversity [1]. However, while annual per capita consumption of vegetable oil in Turkiye is estimated to be approximately 18 kg, this figure averages around 24 kg in European Union countries. Although per capita vegetable oil consumption in Turkiye remains at lower levels compared to European Union countries, significant gaps in meeting domestic demand arise due to insufficient production of oilseed crops. In this context, it is reported that as a result of this supply-demand imbalance, Turkiye is forced to import over 300,000 tons of vegetable oil annually [2].

Sunflower (*Helianthus annuus* L.) ranks third worldwide among oilseed crops, and is widely cultivated in various agroecological regions, particularly due to its high ecological adaptability. The oil derived from it offers a rich content of polyunsaturated fatty acids, including linoleic and oleic acids, providing high nutritional value in human diets while also contributing to the regulation of blood cholesterol levels and positive effects on cardiovascular health. However, sunflower is considered a crop highly sensitive to boron deficiency, and today, boron deficiency has become a widespread problem due to inadequate soil management and improper fertilization practices in agricultural soils; as the boron element plays a critical role in vital plant metabolic functions such as sugar transport, pollen yield, stigma receptivity, and nectar production, thereby directly contributing to an increase in the number of filled seeds. In this context, symptoms of boron deficiency are typically observed in leaves, stems, reproductive organs, and flower heads; such deficiency can lead to morphological and physiological abnormalities and may cause a significant reduction in potential yield [3].

Findings in the literature indicate that boron applications have positive effects on the growth parameters, yield characteristics, and nutrient uptake of sunflower plants, thereby increasing their productivity; In this context, studies conducted by various researchers have systematically and comprehensively examined the effects of boron applications—whether applied to the soil or foliage—on sunflower production, and have thoroughly demonstrated the effects of these applications on the plant's morphological and physiological development [4].

Sunflower (*Helianthus annuus* L.) is one of the strategically important oilseed crops worldwide and can be cultivated in many agroecological regions, particularly due to its high oil content and broad adaptability. In Turkiye, sunflower cultivation is concentrated in specific regions, primarily the Thrace Region. The crop holds significant economic importance due to its role as a staple in crop rotation systems, its high suitability for mechanization, and its ability to adapt to diverse environmental conditions. Indeed, while sunflower production areas in our country vary by year, they generally range around 550–600 thousand hectares, and approximately 73% of total production is carried out in the Thrace Region. This clearly highlights the region's central role in sunflower production [2].

Among soil nutrients, micronutrients are of critical importance for plant growth and product quality. Boron (B) stands out as a key element among these micronutrients, exerting a direct influence on plants' metabolic activities and morphological development. The total boron content of soils generally ranges from 2 to 200 ppm, but the amount of available boron accessible to plants constitutes only a small fraction of these total values. Research indicates that plants can directly utilize less than approximately 5% of the total boron content. For example, in a study conducted in Canada, while the total boron content of soils ranged from 45-124 ppm, the amount of boron soluble in hot water -and thus available to plants- was found to be only 0.38-4.67 ppm. These findings demonstrate a significant difference between the total boron present in the soil and the amount of available boron accessible to plants, highlighting the critical importance of this distinction for plant nutrition strategies [5].

Turkiye possesses significant global potential in terms of boron reserves. According to 1995 data from the State Planning Organization, approximately 63% of the world's boron reserves are located within Turkiye's borders, placing the country in a strategic position both economically and agriculturally [6]. However, boron uptake by plants varies significantly among species. Even among plants grown under the same soil and environmental conditions, boron uptake capacities vary; generally, it has been reported that monocotyledonous plants have lower boron uptake capacities compared to dicotyledonous plants and are therefore more sensitive to boron nutrition [7].

With the realization that boron is an essential nutrient for plants, it has been established that many physiological disorders previously attributed to various factors are actually caused by boron deficiency. In this context, conditions such as tip dieback in tobacco, core rot in sugar beets, fungal core rot in apples, brown rot in cauliflower, cracked stems in celery, brown core in radishes, internal browning in potatoes, and yellowing of young leaves in clover are reported to develop due to boron deficiency [8]. This clearly highlights the critical role of boron in plant metabolism.

The forms of boron available to plants in the soil are typically H_3BO_3, $B(OH)_3$, or the ionic form $B(OH)_4^-$, which are generally found in a dissolved state and can be directly taken up by plant roots [9]. Boron uptake by plants primarily occurs through a passive absorption mechanism, and this process is significantly influenced by soil pH, soil moisture, temperature, and other environmental factors. In particular, soil reaction and water status are among the key factors that directly determine boron solubility and, consequently, its uptake by plants [10].

Studies conducted in the Thrace Region indicate that approximately 16% of sunflower-planted fields suffer from severe boron deficiency and that yield increases of 10–20% can be achieved in these areas through appropriate boron fertilization practices. Therefore, soil analysis are mandatory to accurately determine boron status in areas where sunflowers are to be grown, and the rate, application method (soil or foliar), and timing of boron fertilization must be determined based on analysis results and in accordance with expert recommendations. Such informed fertilization practices are of critical importance for both yield increases and improvements in product quality [11].

2. EXPERIMENTAL STUDIES AND APPLICATIONS

In the studies conducted, researchers comprehensively investigated the effects of the boron element on the growth and development parameters of the Sambro sunflower (*Helianthus annuus* L.) variety. The findings reveal that while boron deficiency has a stimulating effect on root growth, it leads to significant decreases in total pigment content and stem length in the plant. On the other hand, it has been reported that boron excess exerts an inhibitory effect on

root length, while simultaneously increasing the plant's pigment content and, particularly, stem length, thereby exerting divergent effects on morphological development [4].

In another study, boron in the form of H_3BO_3 was applied to sunflower plants via foliar application at concentrations of 25, 50, and 75 mg L^{-1}. In this study, conducted under monoculture growing conditions, the effects of these boron doses on the plant's physiological characteristics were evaluated; the findings revealed that, compared to the control group, the total chlorophyll and carotenoid contents -determined on a dry matter (DM) basis- generally showed a decreasing trend in the leaves. This result also suggests that increasing boron concentrations may have adverse effects on the synthesis and accumulation of photosynthetic pigments [12].

In another study conducted on the Shumos variety of sunflower, boron applications of 0, 50, 100, 150, 200, and 250 mg L^{-1} were applied to spring- and fall-planted plots, and the treatments were designed using a randomized block experimental design. While a linear relationship was observed between boron and the leaf area index (LAI) in spring planting, a linear relationship was found between boron and dry matter yield in fall planting. Applications above 100 mg L^{-1} resulted in significant increases in dry matter yield compared to the control group. Additionally, in the fall planting, applications of 100, 200, and 250 mg L^{-1} resulted in significant increases in seed number, while only the 150 mg L^{-1} application increased seed weight. The empty seed rate decreased linearly with applications above 150 mg L^{-1}, particularly in spring planting, while in fall planting, applications of 200 and 250 mg L^{-1} significantly increased seed yield [13].

3. ANALYSIS

Given the critical role of boron in plant nutrition, it is evident that significant yield and quality losses occur in plants under both deficiency and excess conditions. Therefore, it is of great importance that plants receive the boron they require in appropriate amounts and at the right times throughout their growth stages. Within the framework of a balanced and sustainable fertilization program, the adequate, balanced, and regular application of boron alongside other nutrient elements is considered a fundamental requirement for both improving quality parameters and minimizing yield losses [14]

When the findings of numerous studies conducted in the Thrace Region are evaluated using a holistic approach, it is evident that fertilization practices implemented in agricultural areas where boron deficiency has been identified—whether applied to the soil or via foliar application—have a significant and statistically notable impact on crop production; indeed, the fact that these applications result in an approximate 10–20% increase in yield provides strong evidence that boron is a critical micronutrient for plant development. This clearly demonstrates that boron fertilization does not merely support vegetative growth and development processes but also plays a decisive role in generative development, product composition, and quality parameters.

However, in light of the data obtained, it has become clear that scientific data based on soil analyses and expert opinions must be taken into account to enhance the effectiveness of boron applications; for it is through such comprehensive and informed approaches that both soil and foliar applications can be optimized, thereby ensuring that the amount of boron available to the plant is brought to the most appropriate level. In this context, adopting an integrated fertilization strategy that combines the right dosage, appropriate timing, and proper application methods enables the more effective and sustainable use of available resources, while also making significant contributions to minimizing yield and quality losses.

4. CONCLUSION

When the findings of the studies are evaluated together, it is clearly demonstrated that boron, just as it is for many agricultural crops, is an indispensable micronutrient for the proper functioning of physiological and biochemical processes, particularly in sunflower cultivation; indeed, insufficient or excessive levels of boron within the plant can lead to significant and often irreparable losses not only in yield parameters but also in quality attributes. In this context, taking into account the varying boron requirements of plants during different growth and development stages, and supplying this element to the plant in appropriate doses at the right time, is of critical importance for ensuring optimal plant development.

Furthermore, planning boron applications while taking into account their interactions with other macro- and micronutrients, and evaluating them within the framework of a balanced fertilization program, makes a significant contribution to both improving crop quality parameters and increasing the economic yield per unit area. Furthermore, given boron's role in plant metabolism and its effects on cell wall formation, membrane integrity, and various enzymatic activities, the proper and informed use of this element within the context of sustainable agricultural practices is considered a strategic necessity for long-term plant health and the sustainability of agricultural production systems.

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GELİŞMİŞ ANTİMİKROBİYAL ETKİNLİK İÇİN YENİ BİR PARABUTOPORİN MUTANTININ (PARP-MTN) STRATEJİK RASYONEL YENİDEN TASARIMI VE ÇOK PARAMETRELİ *IN SILICO* PROFİLLEMESİ

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ÖZET

Antibiyotik direncinin küresel çapta tırmanışı, yeni antimikrobiyal stratejilere duyulan acil ihtiyacı vurgulamaktadır. Bu çalışmada; örümcek zehrinden türetilen katyonik bir peptit olan Parabutoporin'in, hedeflenmiş dizi kısaltması ve elektrostatik optimizasyon yoluyla fizikokimyasal ve yapısal özelliklerini iyileştirmeyi amaçlayan rasyonel yeniden tasarımı sunulmaktadır.

PARP-MTN olarak adlandırılan, yeniden tasarlanmış 31 kalıntılı mutant peptit; artırılmış bir teorik net yük (+14) ve optimize edilmiş bir alfa-sarmal topolojisi sergilemektedir. Rasyonel mutasyon bölgeleri; hücre içine nüfuz etme potansiyelini artırmak için CellPPD, antimikrobiyal eğilimi tahmin etmek için CAMPR3 algoritmaları ve potansiyel immünojenik epitopları en aza indirmek için Predicted Antigenic Peptides aracını kullanan bütünleşik bir hesaplamalı strateji ile belirlenmiştir.

Yeniden tasarlanan peptitin amfipatik profili HeliQuest kullanılarak değerlendirilirken; öngörülen biyouyumluluğu, alerjenite ve hemolitik potansiyeli tahmin etmek amacıyla AllerTOP ve HemoPI üzerinden analiz edilmiştir. Ayrıca, DBAASP çerçevesinde ön moleküler kenetlenme simülasyonları gerçekleştirilmiştir. Genel olarak, in-silico yeniden tasarım; PARP-MTN peptitinin, yeni nesil bir antimikrobiyal peptit olarak ileri deneysel değerlendirmeler için gelecek vadeden bir aday olabileceğini göstermektedir.

Anahtar Kelimeler: Parabutoporin, *In Silico* Rasyonel Tasarım, Antimikrobiyal Peptitler, Moleküler Kenetlenme

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1. GİRİŞ

Enfeksiyon hastalıklarının tedavisinde antibiyotiklerin yaygın ve uygunsuz kullanımı, dirençli mikroorganizmaların ortaya çıkmasına ve yayılmasına yol açmıştır. Antibiyotik direnci, ciddi bir küresel halk sağlığı sorunu haline gelmiştir [1]. Geleneksel antibiyotiklerin giderek etkinliğini kaybetmesi nedeniyle, antimikrobiyal dirençle mücadele için yeni terapötik stratejilere duyulan ihtiyaç artmaktadır [2]. Doğada çok çeşitli organizmalar tarafından üretilen antimikrobiyal peptitler (AMP'ler), çeşitli mikroorganizmalara karşı geniş spektrumlu aktiviteleri nedeniyle bu bağlamda öne çıkan biyomoleküller haline gelmiştir [3]. Bu peptitlerin mikroorganizmalar üzerindeki etki mekanizmaları, geleneksel antibiyotiklerden farklıdır [4]. Bakteriyel hücre zarlarıyla elektrostatik olarak etkileşime girerek zar bütünlüğünü bozabilirler ve ayrıca DNA, RNA ve protein sentezi gibi hücre içi hedefleri inhibe edebilirler [5].

Antimikrobiyal dirençle mücadelede doğal peptitlerin modifikasyonu stratejik bir öneme sahiptir. Bu çalışmada, Güney Afrika akrebi *Parabuthus schlechteri*'den izole edilen Parabutoporin peptiti temel alınarak, biyoinformatik araçlar yardımıyla antimikrobiyal aktiviteyi korurken toksisiteyi en aza indirmek amacıyla rasyonel tasarımı yapılmış yeni bir PARP-MTN isimli mutant form geliştirilmiştir.

2. *IN SILIKO* ANALİZLER

2.1. Rasyonel Peptit Tasarımı

Parabutoporin'in doğal sekansı Antimicrobial Peptide Database (APD3) temel alınarak, biyolojik etkinliği artırmak amacıyla rasyonel tasarım ilkeleri uygulanmıştır [6]. Rasyonel tasarımda peptit dizisindeki potansiyel antijenik bölgeler, Universidad Complutense de Madrid tarafından geliştirilen Predicting Antigenic Peptides aracı [7] ile belirlenmiş ve bu bölgeleri ortadan kaldırmak için hedefe yönelik mutasyonlar gerçekleştirilmiştir. Peptitlerin antimikrobiyal aktivite tahminleri CAMPR3 veri tabanındaki SVM ve RF gibi sınıflandırma algoritmaları kullanılarak yapılmıştır. Oluşturulan varyant dizilerinin antimikrobiyal etkinlikleri, CAMPR3 arayüzü kullanılarak skorlanmış ve sınıflandırılmıştır. Bu skorlama yöntemi, dizilerde yapılan modifikasyonların etkinliğini kıyaslamaya olanak tanıyarak, çalışma kapsamındaki ön eleme süreci için belirleyici bir kriter teşkil etmiştir [8].

CAMPR3 analizlerinden elde edilen veriler, farklı biyoinformatik platformlardan gelen çıktılarla entegre edilerek kapsamlı bir değerlendirmeye tabi tutulmuştur. Bu bütünleşik

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yaklaşım; antimikrobiyal performansını muhafaza eden veya geliştiren peptit adaylarının tespit edilmesinde temel bir referans noktası olarak kullanılmıştır.

2.2. Fizikokimyasal Profilleme ve 3D Modelleme

Tasarlanan PARP-MTN sekansının moleküler ağırlık, teorik pI, net yük ve hidrofobiklik gibi parametreleri ProtParam/ExPasy sunucusu ile hesaplanmıştır [9]. Peptitin üç boyutlu konformasyonu, yüksek doğruluklu yapı tahmini sunan AlphaFold2 algoritması kullanılarak modellenmiştir [10]. Elde edilen PDB çıktıları, moleküler docking ve dinamik simülasyon çalışmalarında kullanılmak üzere en uygun yapısal oryantasyonlar baz alınarak seçilmiştir."

2.3. Biyolojik Aktivite ve Toksisite Tahminleri

Hücreye nüfuz eden peptit potansiyeli (CPP), CellPPD aracı [11] ile alerjenite potansiyeli ise AllerTOP v.2.0 üzerinden analiz edilmiştir [12]. Amfipatik heliks yapısı ve hidrofobik moment değerleri HeliQuest sunucusu kullanılarak haritalandırılmıştır [13]. Ayrıca, peptitler arasındaki dizi benzerliklerini belirlemek için Clustal Omega programı ile çoklu dizi hizalaması gerçekleştirildi [14]. Toksisite ve hemolitik potansiyel ise sırasıyla ToxinPred [15] ve HemoPI araçları kullanılarak değerlendirildi [16].

2.4. Moleküler Docking Analizi

Parabutoporin ve PARP-MTN peptiti antimikrobiyal potansiyeli, antibiyotik direnci veya virülansla ilişkili 4 farklı hedef proteine (8CPL, 2FRH, 2V50, 3W9I) karşı manuel moleküler kenetleme yöntemiyle incelendi. Moleküler docking çalışmaları; Parabutoporin ve PARP-MTN peptitlerinin, belirlenen dört farklı hedef proteinle olan etkileşim mekanizmalarını ve bağlanma enerjilerini belirlemek amacıyla gerçekleştirilmiştir. Hedef proteinlerin üç boyutlu kristal yapıları RCSB Protein Data Bank (PDB) veri tabanından [17] temin edilmiş; UCSF Chimera yazılımı kullanılarak yapıdaki su molekülleri, mevcut ligandlar ve diğer hetero atomlar uzaklaştırılarak temizlenmiştir [18].

Temizlenen protein yapılarının ve modellenen peptitlerin yapısal kararlılığını optimize etmek amacıyla UCSF Chimera üzerinde enerji minimizasyonu uygulanmıştır. Ardından, AutoDock Tools (ADT) yazılımı [19] aracılığıyla proteinlere hidrojenler eklenmiş ve Kollman birleşik atom yükleri atanmıştır. Ligand olarak kullanılan Parabutoporin ve PARP-MTN peptiti için ADT kullanılarak peptitlerin esnek yapısını temsil eden aktif rotasyonel bağlar (Torsion Tree) tanımlanmıştır.

Kenetleme bölgesi olarak proteinlerin aktif bölgelerini veya literatürde tanımlanmış etkileşim yüzeylerini kapsayacak şekilde uygun boyutlarda Grid Box parametreleri belirlenmiştir. Moleküler kenetleme simülasyonları AutoDock Vina (1.2.7.) aracı kullanılarak

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gerçekleştirilmiş, her bir etkileşim için en düşük bağlanma enerjisine sahip pozisyonlar seçilmiştir. Elde edilen docking sonuçları, amino asit düzeyindeki etkileşimlerin (hidrojen bağları, hidrofobik etkileşimler ve elektrostatik kuvvetler) tespiti için PyMOL [20] ve Discovery Studio Visualizer yazılımları ile analiz edilerek görselleştirilmiştir.

3. SONUÇLAR VE DEĞERLENDİRME

3.1. Rasyonel Tasarım ve Sekans Analizi Bulguları

Antijenik bölgelerin eliminasyonu ve yük optimizasyonu amacıyla yapılan stratejik trunkasyon sonucunda, 45 amino asitlik doğal Parabutoporin sekansı 31 amino asitlik PARP-MTN formuna dönüştürülmüştür.

```
EMBOSS_001
FKLGSFLKKAWKSKLAKKLRAKGKEMLKDYAKGLLEGGSEEVPGQ 45
EMBOSS_002      KLIFRFLKKAWKSKA-KKLRAKGKKRLLPIPK---------- 31
                : ********* ********: * *
```

Görsel 1. Wild Parabutoporin ve Mutant Peptitin Cluster Omega Algoritması ile Dizi Hizalaması

3.2. Fizikokimyasal Özelliklerin Değerlendirilmesi

ProtParam verilerine göre, PARP-MTN'nin net yükü +14 değerine yükseltilerek bakteriyel membranlara olan elektrostatik afinitesi maksimize edilmiştir. Mutasyonlar sonucu toplam 45 amino asitlik yapı, sentez verimliliği ve stabilite odaklı olarak 31 amino aside optimize edilmiştir. Moleküler ağırlıktaki azalma, peptitin doku penetrasyon kabiliyetini artırırken sentez maliyetini düşürmüştür. Yapılan analizler sonucunda PARP-MTN'nin net yükü +14'e yükseltilmiştir; bu değer orijinal yapının (+7) iki katıdır ve membran afinitesi açısından kritiktir.

Çizelge 1. Wild Parabutoporin ve Mutant Peptitin Karşılaştırmalı Fizikokimyasal Parametreleri

Parametre	**Wild Parabutoporin**	**Mutant Peptit**
Amino asit sayısı	45	31
Moleküler ağırlık (Da)	4994.96	3720.77
Teorik pI	10.00	12.33
Negatif yüklü kalıntılar (Asp+Glu)	5	0
Pozitif yüklü kalıntılar (Arg+Lys)	12	14

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Parametre	Wild Parabutoporin	Mutant Peptit
Net Yük	+7	+14
Kararsızlık indeksi	19.43	18.00
Stabilite sınıfı	Stabil	Stabil
Alifatik indeks	76.00	97.74
GRAVY	-0.700	-0.732
Yarı ömür (memeli retikülosit)	1.1 saat	1.3 saat
Toplam atom sayısı	731	576
Hidrofobisite	-0.23	-0.36
Hidrofobik Moment (µM)	0.211	0.279

3.3. Biyolojik Aktivite ve Güvenlik Tahminleri

CAMPR3 analizleri, PARP-MTN'nin AMP olasılık değerinin 0.9925 gibi en üst seviyeye ulaştığını ortaya koymuştur (Çizelge 2.). AllerTOP sonuçlarında ise peptitin "Alerjen" etiketinden kurtularak biyouyumlu bir profil kazanabileceği öngörülmektedir.

Çizelge 2. Wild Parabutoporin ve Mutant Peptitin Karşılaştırmalı Biyolojik Aktivite ve Güvenlik Tahminleri

	Wild Parabutoporin	Mutant Peptit
SVM Skoru	-0.55	0.73
AMP Olasılığı	0.8395	0.9925
CPP Tahmini	CPP Değil	CPP
Alerjenite	Muhtemel Alerjen	Muhtemel Alerjen Değil

Görsel 2. Wild Parabutoporin ve Mutant Peptit Varyantların Hemolitik İndeks Karşılaştırması

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Hesaplamalı analiz sonuçları, peptitin katyonik yükündeki belirgin artışın membran etkileşim dinamiklerini değiştirerek teorik hemolitik eşiği (32 µM)) etkileyebileceğine işaret etmektedir. Mutant peptitin HC50µM değerindeki değişim, artırılmış katyonik yükün (+14) ve güçlenen amfipatik karakterin membran selektivitesi üzerindeki beklenen bir yansıması olarak değerlendirilmektedir

3.4. Yapısal Modelleme ve Amfipatik Karakterizasyon

AlphaFold2 modelleri, doğal formda gözlenen merkezi esnek bölgenin PARP-MTN'den yerini stabil bir alfa-heliks yapısına bıraktığını doğrulamıştır. PyMOL üzerinden alınan Elektrostatik Yüzey Potansiyeli (ESP) haritaları, mutant peptitin yüzeyinde homojen bir pozitif yük dağılımı olduğunu göstermektedir. HeliQuest analizlerinde elde edilen PARP-MTN hidrofobik moment değerinin doğal varyanta oranla 0.211'den 0.279'a yükseldiği tespit edilmiştir. Bu artış amfipatik karakterin güçlendiğini göstermektedir (Görsel 3.).

Görsel 3. Wild Parabutoporin ve Mutant Peptitin Karşılaştırmalı Üç Boyutlu Modelleri, Elektrostatik Yüzey Potansiyeli (ESP) Haritaları ve Heliks Tekerleği Projeksiyonları (Sol: Parabutoporin, Sağ: PARP-MTN). (A) ve (B): Sırasıyla Parabutoporin ve PARP-MTN'nin PyMOL kullanılarak oluşturulan Elektrostatik Yüzey Potansiyeli (ESP) haritaları. Mavi bölgeler pozitif yük yoğunluğunu, kırmızı bölgeler negatif yükü, beyaz bölgeler ise hidrofobik/nötr yüzeyleri temsil etmektedir. (C) ve (D): Sırasıyla peptitlerin amfipatik heliks

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yapısını ve amino asit dağılımını gösteren HeliQuest helis tekerleği (helical wheel) projeksiyonları. Oklar hidrofobik moment vektörünü simgelemektedir. Sarı kalıntılar hidrofobik, mavi kalıntılar ise bazik/pozitif yüklü amino asitleri ifade eder.

3.5. Moleküler Kenetlenme (Docking) Analizleri

AutoDock Vina simülasyonları, PARP-MTN'nin 8CPL, 2FRH, 2V50 ve 3W9I hedef proteinlerine karşı doğal peptitten daha düşük bağlanma enerjileri sergilediğini göstermiştir. Discovery Studio analizleri, bu güçlü etkileşimin mutant peptitteki artırılmış katyonik kalıntılar (Lys, Arg) ile hedef proteinlerin aktif bölgeleri arasındaki hidrojen bağları ve elektrostatik kuvvetlerden kaynaklandığını düşündürmektedir (Görsel 4.).

	3W9İ-3D	3W9İ
Wild Parabutoporin		
Mutant Peptit		
	2FRH-3D	2FRH-2D
Wild Parabutoporin		
Mutant Peptit		
	2V50-3D	2V50-2D

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Wild Parabutoporin		
Mutant Peptit		
	8CPL-3D	8CPL2D
Wild Parabutoporin		
Mutant Peptit		

Görsel 4. Wild Parabutoporin ve Mutant Peptitin Karşılaştırmalı Moleküler Kenetlenme Simülasyonları ve Genel Bağlanma Oryantasyonları

Gerçekleştirilen *in siliko* analizlerle, stratejik ve elektrostatik optimizasyon işlemlerinin peptitin fonksiyonel özelliklerini iyileştirdiği görülmüştür. PARP-MTN'nin doğal forma kıyasla daha kararlı bir alfa-heliks yapısı sergilediği, net yük artışıyla birlikte hedef bakteriyel proteinlere olan bağlanma afinitesinin yükseldiği tespit edilmiştir. Rasyonel tasarımla peptitin biyolojik aktivitesi korunurken sitotoksisite potansiyelini minimize ettiği görülmüştür Tasarlanan PARP-MTN ve doğal Parabutoporin peptitlerinin bakteriyel virülans ve direnç mekanizmalarında rol oynayan kritik proteinlere karşı bağlanma afiniteleri, AutoDock Vina algoritması kullanılarak

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moleküler kenetlenme simülasyonları ile analiz edilmiştir. Elde edilen docking verileri (Çizelge 3), rasyonel tasarım sürecinde gerçekleştirilen modifikasyonların, peptitin hedef proteinlerle olan etkileşim gücünü çalışılan protein modellerinde artırdığını ortaya koymaktadır. Bu durum, PARP-MTN'nin biyouyumlu bir aday olabileceğini düşündürmektedir.

Çizelge 3. Wild Parabutoporin ve Mutant Peptitin Hedef Proteinlere Karşı Bağlanma Serbest Enerjileri

Gen	PDB ID	Wild Parabutoporin (kcal/mol)	Mutant Peptit (kcal/mol)
SarA	2FRH	-4.790	-5.964
Spa	8CPL	-6.601	-7.946
mexB	2V50	-4.184	-7.950
mexB	3W9İ	-5.956	-6.342

Sonuç olarak, bu araştırma; hesaplamalı biyoloji araçlarının, dirençli mikroorganizmalara karşı yeni nesil terapötik ajanların tasarımında maliyet ve zaman açısından sunduğu etkinliği ortaya koymaktadır. PARP-MTN, sahip olduğu yüksek antimikrobiyal olasılık skoru ve güvenli profil ile ileri aşama *in vitro* ve *in vivo* çalışmalar için gelecek vadeden bir aday olarak görülmektedir. Gelecek çalışmalarda, tasarlanan bu peptitin spesifik dirençli suşlar üzerindeki deneysel etkinliğinin doğrulanması hedeflenmektedir.

4. GENEL DEĞERLENDİRME VE SONUÇLAR

Bu çalışmada, doğal bir antimikrobiyal peptit olan Parabutoporin, rasyonel tasarım ilkeleri ve bütünleşik biyoinformatik stratejiler kullanılarak yeniden yapılandırılmış; biyolojik etkinliği maksimize edilmiş yeni bir türev olan PARP-MTN geliştirilmiştir. Gerçekleştirilen *in siliko* analizler, stratejik dizi kısaltması ve elektrostatik optimizasyon işlemlerinin peptitin fonksiyonel özelliklerini iyileştirdiğini doğrulamıştır. 45 amino asitlik doğal sekansın 31 kalıntıya düşürülmesiyle sentez verimliliği artırılırken, AlphaFold2 ve HeliQuest analizleri mutant formun doğal yapıdaki düzensizlikleri gidererek çok daha kararlı ve amfipatik bir alfa-heliks yapısı kazandığı görülmüştür.

Peptitin net yükünün +7'den +14 değerine yükseltilmesi, negatif yüklü bakteriyel membranlara olan elektrostatik afiniteyi üst düzeye çıkarmıştır. Bu durum, CAMPR3 algoritmalarından elde edilen 0.9925'lik AMP olasılık skoruyla da desteklenerek peptitin antimikrobiyal potansiyelinin rasyonel mutasyonlar aracılığıyla artırıldığını göstermiştir. Doğal AMP bileşenlerinde sıklıkla karşılaşılan sitotoksisite risklerinin azaltıldığı görülmüştür. AllerTOP

verileri, PARP-MTN'nin memeli hücreleri için güvenli, non-alerjen bir profil sergilediğini ortaya koyarak biyouyumlu bir ilaç adayı profili çizmiştir.

Moleküler kenetlenme analizleri ve etkileşim haritaları, PARP-MTN'nin dirençli patojenlerle ilişkili hedef proteinlere karşı doğal peptitten daha yüksek bir bağlanma afinitesine sahip olduğunu doğrulamıştır. Özellikle mutantın kazandığı hücreye nüfuz eden peptit (CPP) karakteri, bu yeni molekülün sadece membran düzeyinde değil, hücre içi moleküler hedeflerde de etkinlik gösterebileceğine dair işaretler sunmaktadır. Dışa akım pompası (efflux pump) sisteminin önemli bir bileşeni olan mexB (2V50) proteini ile yapılan kenetlenme simülasyonunda, PARP-MTN'nin bağlanma enerjisinin-7.950 kcal/mol değerine ulaştığı gözlenmiştir. Doğal formun-4.184 kcal/mol olan enerjisine kıyasla sergilenen bu belirgin düşüş, mutant peptitin direnç mekanizmalarını inhibe etme potansiyelinin yüksek olduğuna işaret etmektedir.Bu enerji skorlarındaki artışın, mutant peptitin artırılmış pozitif yükü ve stabilize edilen alfa-heliks yapısı sayesinde hedef proteinlerin negatif yüklü aktif bölgeleriyle daha stabil hidrojen bağları ve elektrostatik etkileşimler kurmasından kaynaklandığı değerlendirilmektedir. Sonuç olarak bu araştırma, doğal kaynaklı peptitlerin biyoinformatik mutasyonlar ve hesaplamalı yöntemlerin sağladığı avantajlar kullanılarak rasyonel tasarımına olanak tanımış; PARP-MTN gibi varyantların potansiyel birer aday molekül olabileceğine dair öncül bulgular sunmuştur. Gelecek çalışmalarda, tasarlanan bu peptitin spesifik dirençli bakteri suşları üzerindeki deneysel etkinliğinin doğrulanması ve etki mekanizmasının moleküler düzeyde aydınlatılması hedeflenmektedir. Bu bulgular ışığında geliştirilen PARP-MTN, optimize edilmiş fizikokimyasal parametreleri ve biyouyumluluğu ile ileri aşama *in vitro* ve *in vivo* çalışmalar için bir adaydır.

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IRISIN'S ASSOCIATION WITH GENE AND MIRNA EXPRESSION ALTERATIONS IN AN MPP$^+$-INDUCED IN VITRO PARKINSON'S DISEASE MODEL

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ABSTRACT

Parkinson's disease is characterized by progressive neuronal degeneration and mitochondrial dysfunction. Irisin, an exercise-induced myokine, has emerged as a potential neuroprotective molecule due to its modulatory effects on cellular survival pathways. This study aimed to investigate the neuroprotective effects of irisin in an MPP$^+$-based SH-SY5Y in vitro model of Parkinson's disease by focusing on miRNA-associated molecular regulatory responses involving the PGC-1α/FNDC5/BDNF signaling axis. Neurotoxicity was induced by exposing SH-SY5Y cells to 1500 µM MPP$^+$ for 24 hours. A non-cytotoxic concentration of irisin (20 nM) was determined using the MTT assay. Cells were divided into control, MPP$^+$, irisin pre-treatment, and post-treatment groups. Expression profiles of PGC-1α, FNDC5, and BDNF, along with their associated miRNAs, were analyzed using RT-qPCR. Compared with the control group, MPP$^+$-induced Parkinsonian conditions were associated with decreased expression levels of BDNF and FNDC5. In contrast, irisin administration led to statistically significant increases in PGC-1α expression under both pre-treatment and post-treatment conditions. Relative to the MPP$^+$ group, PGC-1α levels remained significantly elevated in both treatment groups, whereas FNDC5 expression showed a statistically significant increase particularly in the irisin pre-treatment group. Similarly, miRNA expression analysis revealed significant reductions in miR-129-1-3p and miR-143-3p levels in the MPP$^+$-induced Parkinsonian model compared with control cells. Irisin pre-treatment significantly increased miR-143-3p and miR-182-5p expression, while post-treatment resulted in marked elevations in miR-129-1-3p, miR-138-5p, and miR-182-5p levels. When compared with the MPP$^+$ group, both treatment approaches were associated with upregulation of miR-129-1-3p and miR-182-5p. Moreover, the increase in miR-143-3p was more pronounced in the pre-treatment condition,

whereas miR-138-5p exhibited a statistically significant elevation exclusively in the post-treatment group. Taken together, these findings indicate that irisin exerts condition-dependent modulatory effects on the PGC-1α/FNDC5/BDNF signaling network and related miRNAs, highlighting its potential role as a neuroprotective candidate in Parkinson's disease.

Keywords: Parkinson disease, MPP^+, exercise, irisin, miRNA

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1. INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by severe motor dysfunction. Although current therapeutic strategies primarily provide symptomatic relief, their long-term efficacy remains limited, highlighting the need for novel mechanistic targets [1]. Emerging evidence identifies physical exercise as a potent modulator of neurodegeneration, with effects extending beyond symptom control to influence disease progression [2]. The neuroprotective effects of exercise are closely associated with activation of the PGC-1α/FNDC5/BDNF axis. Irisin, a cleavage product of FNDC5 induced by PGC-1α, has been shown to promote neuronal survival and synaptic plasticity through the upregulation of brain-derived neurotrophic factor (BDNF), positioning this pathway as a critical regulator of neural resilience.[3]

MicroRNAs (miRNAs) have recently emerged as key post-transcriptional regulators that orchestrate gene networks involved in neuronal homeostasis in PD [4]. Altered miRNA expression profiles across multiple biological compartments further support their roles in disease pathogenesis and underscore their potential as robust biomarkers[5].

In this study, we investigated the neuroprotective and therapeutic potential of irisin in an MPP^+-induced Parkinson's disease model in SH-SY5Y cells, focusing on miRNA-mediated regulation of the PGC-1α/FNDC5/BDNF axis. Candidate miRNAs (miR-129-1-3p, miR-138-5p, miR-143-3p, and miR-182-5p), identified through TargetScan and miRWalk analyses, were selected to elucidate their contributions to PD-related molecular mechanisms and their potential utility as biomarkers.

2. MATERIAL AND METHOD

2.1 Cell Culture

In this study, human SH-SY5Y neuroblastoma cells were utilized as an in vitro model. Cells were maintained in DMEM/F12 culture medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Cell morphology and confluency were monitored daily using an inverted microscope. Upon reaching approximately 90% confluence, cells were detached using trypsin and subcultured for further experiments.

Effect of MPP^+ on Cell Viability

SH-SY5Y cells at approximately 90% confluence were seeded into 96-well microplates. Cells were treated with different concentrations of MPP^+ (250, 500, 1000, and 1500 µM) and incubated for 24 hours. Cell viability was assessed using the MTT assay.

Following incubation, 10 µL of MTT solution was added to each well, and plates were further incubated for 2 hours at 37°C under 5% CO_2. Subsequently, the medium was removed, and the resulting formazan crystals were dissolved in 200 µL of dimethyl sulfoxide (DMSO). Absorbance was measured spectrophotometrically at 570 nm using a microplate reader. Cell viability was expressed as a percentage relative to the untreated control group.

2.2 Effect of Irisin on Cell Viability

The effects of irisin on SH-SY5Y cell viability were evaluated under the same experimental conditions described above. Cells were treated with irisin at concentrations of 5, 10, 20, and 40 nM for 48 hours. Cell viability was subsequently determined using the MTT assay as described

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for MPP^+ treatment.

2.3 Experimental Groups

The study was designed with four experimental groups: (i) control group, (ii) MPP^+-treated group representing the acute PD model (MPP^+, 24 h), (iii) irisin pre-treatment group, in which cells were treated with 20 nM irisin prior to MPP^+ exposure (irisin + MPP^+), and (iv) irisin post-treatment group, in which irisin (20 nM) was applied following MPP^+-induced toxicity (MPP^+ + irisin).

The MPP^+ concentration was selected as the highest dose that did not reduce cell viability below 50%, while the irisin concentration was determined as a non-cytotoxic dose that neither reduced viability nor induced excessive proliferation.

2.4 Sample Collection

Following treatment, culture media were removed, and cells were collected into microcentrifuge tubes. Samples were centrifuged to pellet the cells, and the supernatant was carefully discarded to eliminate residual medium and trypsin. Cell pellets were lysed using 1 mL of miRNA extraction reagent and incubated at room temperature for 5–10 minutes. Lysed samples were stored at −80°C until further processing.

2.5 microRNA Isolation

Total microRNA was isolated from SH-SY5Y cells using the SanPrep Column MicroRNA Mini-Prep Kit (Cat. No. SK8811, Bio Basic), following the manufacturer's instructions. Briefly, frozen samples were thawed at room temperature, followed by chloroform addition and centrifugation to achieve phase separation. The aqueous phase was collected and mixed with absolute ethanol before being transferred onto spin columns for RNA binding.

After sequential washing steps, miRNAs were eluted in RNase-free water. The concentration and purity of isolated miRNAs were determined using a NanoDrop spectrophotometer. Purified samples were stored at −80°C until further analysis.

2.6 cDNA Synthesis from microRNAs

Isolated microRNAs were reverse-transcribed into complementary DNA (cDNA) using the miRNA All-In-One cDNA Synthesis Kit (Cat. No. G899, abmGood), in accordance with the manufacturer's protocol. Briefly, defined amounts of miRNA were combined with the provided reverse transcription reagents, including cDNA synthesis super mix and enzyme mix, and brought to a final reaction volume of 20 µL with RNase-free water.

Reverse transcription was carried out using a thermal cycler under recommended cycling conditions. Synthesized cDNA samples were stored at −20°C until use in quantitative PCR analysis.

2.7 Quantification of microRNA Expression by RT-qPCR

The expression levels of selected microRNAs (miR-129-1-3p, miR-138-5p, miR-143-3p, and miR-182-5p) were determined using real-time quantitative PCR (RT-qPCR). Amplification reactions were prepared using BlasTaq 2X qPCR Master Mix (Cat. No. MasterMix-MS, abmGood), miRNA-specific primer mixes, and cDNA templates, according to the manufacturer's recommendations. RT-qPCR was performed under standard cycling conditions using a real-time PCR system. RNU6B was used as the endogenous reference gene for normalization. All reactions were conducted in technical replicates to ensure reproducibility. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method

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2.8 Statistical Analysis

miRNA expression data were normalized to the housekeeping gene RNU6B and analyzed using the ΔΔCt method. Statistical significance between control and treatment groups was evaluated using Student's t-test based on 2^{-}ΔΔCt values. A p-value of <0.05 was considered statistically significant.

3. RESULT AND DISCUSSION

3.1. Cell Viability

MPP^+ treatment resulted in a dose-dependent decrease in SH-SY5Y cell viability after 24 hours. Among the tested concentrations, 1500 µM MPP^+ significantly reduced cell viability compared to the control group and was therefore selected to establish the in vitro Parkinson's disease model (Fig. 1).Irisin treatment for 48 hours did not induce cytotoxicity at lower concentrations, and 20 nM was identified as the optimal dose, as it preserved cell viability comparable to the control group (Fig. 2)

Fig.1. Dose-Dependent Cell Viability Graph in SHSY-5Y Cells Treated with MPP+ for 24 Hours

Fig.2 Dose-Dependent Cell Viability Graph in SH-SY5Y Cells Treated with Irisin for 48 Hours

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3.2 Gene Expression Levels

In the MPP^+-treated group, BDNF and FNDC5 expression levels were reduced compared to the control group, although these changes were not statistically significant. In contrast, both pre-treatment and post-treatment with irisin resulted in increased expression of these genes. Notably, PGC-1α expression was significantly elevated in both irisin pre-treatment and post-treatment groups compared to the control group ($p < 0.05$). Furthermore, when compared to the MPP^+-induced PD model, PGC-1α expression was significantly increased in both treatment groups. FNDC5 expression showed a significant increase particularly in the pre-treatment group ($p < 0.05$).

Table1. Gene Expression Levels Compared to Control and MPP+

Gene	Reference	Group	Mean_FC	SD
BDNF	Control	Control	1,03	0,09
	Control	MPP+	0,70	0,35
	Control	İrisin+ MPP+	1,51	0,16
	Control	MPP+ irisin	1,33	0,25
	MPP+	MPP+	1,06	0,40
	MPP+	İrisin+ MPP+	1,72	0,06
	MPP+	MPP+ irisin	1,26	0,13
PGC1 α	Control	Control	1,05	0,25
	Control	MPP+	1,28	0,38
	Control	İrisin+ MPP+	4,65	1,26
	Control	MPP+ irisin	3,73	0,05
	MPP+	MPP+	1,03	0,05
	MPP+	İrisin+ MPP+	1,69	0,46
	MPP+	MPP+ irisin	1,45	0,23
FNDC5	Control	Control	1,03	0,05
	Control	MPP+	0,69	0,28
	Control	İrisin+ MPP+	1,36	0,20
	Control	MPP+ irisin	0,99	0,33
	MPP+	MPP+	1,05	0,06
	MPP+	İrisin+ MPP+	1,47	0,20
	MPP+	MPP+ irisin	1,21	0,08

3.3 miRNA Expression Levels

In the MPP^+-induced PD model, miR-129-1-3p and miR-143-3p expression levels were significantly decreased compared to the control group ($p < 0.05$), whereas changes in miR-138-5p and miR-182-5p were not statistically significant. Irisin pre-treatment significantly increased the expression levels of miR-143-3p and miR-182-5p ($p < 0.05$), while changes in miR-129-1-3p and miR-138-5p remained non-significant. In the post-treatment group, miR-129-1-3p, miR-138-5p, and miR-182-5p expression levels were significantly increased compared to the control group ($p < 0.05$), whereas miR-143-3p expression was significantly decreased. When compared to the MPP^+ group, miR-129-1-3p expression was significantly upregulated in both pre-

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treatment and post-treatment groups. miR-143-3p exhibited a marked increase specifically in the pre-treatment group (11.31-fold, $p < 0.05$). Additionally, miR-138-5p expression was significantly increased only in the post-treatment group (1.62-fold, $p < 0.05$), while miR-182-5p expression was significantly elevated in both treatment groups ($p < 0.05$).

Table 2. miRNA Expression Levels Compared to Control and MPP+

miRNA	Reference	Group	Mean_FC	SD
miR-138-5p	Control	Control	1,04	0,1
	Control	MPP+	1,21	0,05
	Control	İrisin+ MPP+	1,34	0,07
	Control	MPP+ irisin	1,97	0,01
	PD	MPP+	1	0,05
	PD	İrisin+ MPP+	1,06	0,03
	PD	MPP+ irisin	1,55	0,08
miR-129-1-3p	Control	Control	1	0,1
	Control	MPP+	0,67	0,07
	Control	İrisin+ MPP+	0,9	0,11
	Control	MPP+ irisin	2,35	0,01
	PD	MPP+	1,01	0,11
	PD	İrisin+ MPP+	1,36	0,17
	PD	MPP+ irisin	3,56	0,01
miR-182-5p	Control	Control	1	0,1
	Control	MPP+	0,93	0,06
	Control	İrisin+ MPP+	1,39	0,13
	Control	MPP+ irisin	1,43	0,07
	PD	MPP+	1	0,07
	PD	İrisin+ MPP+	1,51	0,17
	PD	MPP+ irisin	1,52	0,1
miR-143-3p	Control	Control	1,07	0,1
	Control	MPP+	0,2	0,01
	Control	İrisin+ MPP+	2,27	0,01
	Control	MPP+ irisin	0,43	0,08
	PD	MPP+	1,02	0,18
	PD	İrisin+ MPP+	11,31	0,04
	PD	MPP+ irisin	2,36	0,15

3.4 Discussion of Result

This study evaluated the neuroprotective effects of irisin, an exercise-induced myokine, in a Parkinson's disease model through the PGC-1α/FNDC5/BDNF axis and its associated miRNAs. While the neuroprotective effects of exercise are well-defined in the literature [6], how irisin regulates these effects at the molecular level is a significant area of research. The 20 nM irisin

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dose used in this study was selected to be consistent with previous in vitro studies and aimed at producing a biologically significant effect [7,8]. The findings show that both gene expression and miRNA levels are significantly altered in the MPP^+-induced Parkinson's model. In particular, miR-129-1-3p, associated with FNDC5, was decreased in the MPP^+ group and increased again with irisin administration. This suggests that miR-129-1-3p, which has been reported to be decreased in Parkinson's disease [9], may be re-regulated via irisin and support dopaminergic neuron survival. Furthermore, this miRNA's association with mitochondrial function and apoptotic processes is consistent with previous studies [10,11,12]. Regarding PGC-1α-associated miR-138-5p, this miRNA is known to be associated with cellular stress, inflammation, and apoptosis [13]. The significant increase, particularly in the post-treatment group, suggests that irisin regulates this miRNA in a conditional manner. The literature reports that miR-138-5p has suppressive effects on the SIRT1/PGC-1α/FNDC5/BDNF axis and can modulate processes associated with neuroinflammation [14,15]. Another miRNA associated with FNDC5, miR-143-3p, is reported in the literature to exhibit both pro-apoptotic and neuroprotective effects [16,17]. In this study, it was observed to be decreased in the MPP^+ model and increased, especially in the pre-treatment group. Its parallel change with FNDC5 gene expression suggests that this miRNA may play a role in the regulation of the FNDC5 axis [18].Regarding BDNF-associated miR-182-5p, regulatory and neuroprotective effects have been reported in the literature in both depression and Parkinson's disease contexts [19,20]. In this study, BDNF levels decreased in the MPP^+ group, while they showed an increasing trend with irisin application. The significant increase in miR-182-5p levels, particularly in the pre-treatment group, suggests that irisin may activate a protective miRNA response in the early stages. In contrast, the lack of a further increase in the post-treatment group indicates that this effect occurs primarily in the preventive phase

4. CONCLUSION

Overall, this study demonstrates that irisin can support neuronal survival in an MPP^+-induced Parkinson's cell model by modulating the PGC-1α/FNDC5/BDNF axis and the miRNAs associated with this axis. The observation of more pronounced effects, especially under pre-treatment conditions, suggests that irisin may be more effective as a protective agent. However, further functional studies are needed to establish direct causal relationships between these molecular interactions.

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SİNAPİK ASİDİN SH-SY5Y HÜCRELERİNDE HİDROJEN PEROKSİT KAYNAKLI OKSİDATİF NÖRONAL HASARA KARŞI KORUYUCU ROLÜ

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ÖZET

Hidrojen peroksit (H_2O_2), oksidatif nöronal hasarın yaygın kullanılan bir indükleyicisidir ve reaktif oksijen türleri (ROT) aracılığıyla gerçekleşen nörotoksisite için güvenilir bir in vitro model teşkil etmektedir. H_2O_2'ye aşırı maruz kalınması, hücresel redoks dengesini bozmakta, antioksidan savunma sistemlerini zayıflatmakta, membran bütünlüğünü tehlikeye atmakta ve nöronal hücre hasarına katkıda bulunmaktadır.

Bu çalışmada, SH-SY5Y nöroblastoma hücrelerinde H_2O_2 ile uyarılan sitotoksisite üzerine sinapik asitin (SAc) olası koruyucu etkilerinin değerlendirilmesi amaçlanmıştır. Hücreler, SAc (10, 25 ve 50 µM) varlığında veya yokluğunda 24 saat boyunca H_2O_2'ye (250 µM) maruz bırakılmıştır. Hücre canlılığı MTT testi kullanılarak belirlenmiş; membran bütünlüğü ise laktat dehidrojenaz (LDH) salınımı ölçülerek değerlendirilmiştir. Bulgularımız, sinapik asitin H_2O_2'ye bağlı nöronal sitotoksisiteyi hafifletebileceğine işaret etmektedir.

Anahtar Kelimeler: Sinapik asit, H_2O_2, oksidatif stres, SH-SY5Y, nöroproteksiyon, MTT, LDH

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1. GİRİŞ

Oksidatif stres, nörolojik hastalıkların patogenezinde kritik bir rol oynayan ve reaktif oksijen türlerinin (ROT) aşırı üretimiyle endojen antioksidan savunma sistemlerinin kapasitesini aşmasıyla karakterize edilen bir süreçtir. Bu redoks dengesizliği; lipid peroksidasyon, protein oksidasyonu ve DNA hasarı gibi hücresel hasar mekanizmalarını tetikleyerek nöronal fonksiyon bozukluğuna ve hücre ölümüne yol açmaktadır (Chen, 2016).

Hidrojen peroksit (H_2O_2), deneysel modellerde güvenilir bir oksidatif stres indükleyicisi olarak yaygın biçimde kullanılan bir ROT türüdür. H_2O_2'ye maruz kalınması; antioksidan savunma enzimlerinin tükenmesi, mitokondriyal işlev bozukluğu ve membran geçirgenliğinde artışla ilişkilendirilmiştir (Feng ve ark., 2016). SH-SY5Y insan nöroblastoma hücreleri, nöronal karakteristikleri ve yüksek metabolik aktiviteleri nedeniyle oksidatif stres aracılıklı nöronal hasarın araştırılmasında sıklıkla tercih edilen bir in vitro model olarak kabul görmektedir (Kovalevich ve Langford, 2013).

Sinapik asit (3,5-dimetoksi-4-hidroksisinnamik asit; SAc), çeşitli bitkisel gıdalarda doğal olarak bulunan bir hidroksisinnamik asit türevidir. Antioksidan, antiinflamatuvar ve nöroprotektif özellikleri nedeniyle giderek artan bir ilgi görmektedir (Tungalag ve Yang, 2021; Zare ve ark., 2015). Bununla birlikte, H_2O_2 ile uyarılmış nöronal toksisiteye karşı koruyucu etkisinin altında yatan sitoprotektif mekanizmalar henüz tam olarak aydınlatılmamıştır.

Bu çalışmada, sinapik asitin SH-SY5Y hücrelerinde H_2O_2'ye bağlı oksidatif hasara karşı koruyucu etkilerinin hücre canlılığı ve membran bütünlüğü temel parametreleri aracılığıyla değerlendirilmesi amaçlanmıştır.

2. MATERYAL VE METOT

2.1. Hücre Kültürü

SH-SY5Y insan nöroblastoma hücreleri, %10 fetal sığır serumu (FBS), 100 U/mL penisilin ve 100 µg/mL streptomisin içeren DMEM/F12 besiyerinde, %5 CO_2'li nemlendirici bir ortamda 37°C'de kültüre edilmiştir.

2.2. Deneysel Uygulama

Hücreler, SAc'nin (10, 25 ve 50 µM) varlığında veya yokluğunda H_2O_2 (250 µM) ile 24 saat boyunca muamele edilmiştir. Sinapik asit, deneyden 1 saat önce uygulanmış; tüm bileşikler DMSO içinde çözülmüş ve nihai DMSO konsantrasyonu %0.1'i geçmeyecek şekilde hazırlanmıştır.

2.3. Hücre Canlılığı (MTT Testi)

Hücre canlılığı, MTT (3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolyum bromid) yöntemiyle değerlendirilmiştir. MTT çözeltisi (0.5 mg/mL) hücrelere eklenmiş ve 4 saat inkübasyon sonrasında DMSO ile formazan kristalleri çözündürülmüş; 570 nm dalga boyunda spektrofotometrik olarak ölçüm yapılmıştır.

2.4. LDH Salınım Analizi

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Membran bütünlüğü, hücre kültürü süpernatantına salınan LDH miktarı ticari bir kolorimetrik kit (Sigma-Aldrich) kullanılarak ölçülmüştür. LDH salınımı, tamamen lizatı alınmış hücrelerden elde edilen toplam LDH'nin yüzdesi olarak ifade edilmiştir.

2.5. İstatistiksel Analiz

Veriler, üç bağımsız deneyin ortalama ± standart sapması (SS) olarak sunulmuştur. İstatistiksel analizler, tek yönlü varyans analizi (ANOVA) ve ardından Tukey çoklu karşılaştırma testi kullanılarak gerçekleştirilmiştir. $p < 0.05$ değeri istatistiksel olarak anlamlı kabul edilmiştir.

3. BULGULAR

H_2O_2 uygulaması, kontrol grubuna kıyasla (97.6 ± 3.7%) hücre canlılığını istatistiksel olarak anlamlı şekilde 58.7 ± 4.5%'e düşürmüştür ($p < 0.001$). SAc ile ko-muamele ise canlılığı sırasıyla 10, 25 ve 50 µM için 71.3 ± 4.1%, 86.8 ± 4.4% ve 95.2 ± 4.6%'ye yükseltmiştir. Bu artış 10 µM'de anlamlı ($p < 0.01$), 25 ve 50 µM'de ise daha belirgin düzeyde anlamlı ($p < 0.001$) bulunmuştur.

LDH salınımı, kontrol grubuna kıyasla (11.6 ± 1.4%) H_2O_2 uygulamasıyla belirgin biçimde artmış (73.1 ± 5.0%, $p < 0.001$), SAc ise bu artışı konsantrasyona bağlı bir şekilde sırasıyla 52.7 ± 4.1%, 33.8 ± 3.2% ve 20.9 ± 2.5% değerlerine indirmiştir. Azalma 10 µM'de anlamlı ($p < 0.01$), 25 ve 50 µM'de ise yüksek düzeyde anlamlı ($p < 0.001$) olarak saptanmıştır.

Çizelge 1. Sinapik asitin SH-SY5Y hücrelerinde H_2O_2 ile uyarılmış sitotoksisite üzerine etkileri

Parametre	Kontrol	H_2O_2	SAc 10 µM + H_2O_2	SAc 25 µM + H_2O_2	SAc 50 µM + H_2O_2
Canlılık (%)	97.6 ± 3.7	58.7 ± 4.5***	71.3 ± 4.1##	86.8 ± 4.4###	95.2 ± 4.6###
LDH (% total)	11.6 ± 1.4	73.1 ± 5.0***	52.7 ± 4.1##	33.8 ± 3.2###	20.9 ± 2.5###

Değerler üç bağımsız deneyin ortalama ± SS olarak verilmiştir (n=3). ***$p < 0.001$ kontrol grubuna göre anlamlı farkı göstermektedir. ##$p < 0.01$, ###$p < 0.001$ H_2O_2 grubuna göre anlamlı farkı göstermektedir.

TARTIŞMA

Bu çalışmada, sinapik asitin SH-SY5Y nöroblastoma hücrelerinde H_2O_2'ye bağlı oksidatif sitotoksisiteye karşı koruyucu etkiler sergilediği gösterilmiştir. SAc uygulaması hücre canlılığını konsantrasyona bağlı biçimde geri kazandırmış ve LDH salınımını belirgin şekilde azaltmış; bu bulgular hem metabolik hem de membran bütünlüğü düzeyinde sitoprotektif bir aktiviteye işaret etmektedir.

SAc'nin nöroprotektif etkisi büyük olasılıkla hidroksil ve süperoksit radikallerini süpürme kapasitesinden kaynaklanmaktadır. Buna ek olarak, SAc'nin Nrf2/HO-1 sinyal yolunu aktive ederek endojen antioksidan savunmayı güçlendirdiği bildirilmiştir (Tungalag ve Yang, 2021).

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Bu mekanizmalar, oksidatif hasar tarafından tetiklenen mitokondriyal disfonksiyon ve apoptotik kaskadın baskılanmasına da katkıda bulunabilir. Bu bulgularla tutarlı biçimde, SAc'nin glutamat kaynaklı eksitotoksisiteye karşı da nöroprotektif özellikler sergilediği gösterilmiş olup bu etki, bir C6 glioma hücre modelinde endoplazmik retikulum stresi ve proinflamatuvar sitokin sinyalleşmesinin baskılanmasıyla ilişkilendirilmiştir (Ortasoz ve ark., 2025).

Çalışmamızda gözlemlenen belirgin LDH salınım azalması, SAc'nin plazma membran bütünlüğünü koruduğuna işaret etmekte olup bu etki, lipid peroksidasyon inhibisyonuyla bağlantılı olabilir (Zare ve ark., 2015; Tungalag ve Yang, 2021). H_2O_2'ye bağlı nöronal hasara karşı benzer koruyucu etkiler; likopen ve hespertin gibi diğer fenolik bileşikler için de raporlanmıştır (Feng ve ark., 2016; Moon ve ark., 2023). Ayrıca SH-SY5Y hücrelerinde MAPK ve NF-κB sinyal yollarının baskılanması aracılığıyla oksidatif hasarın azaltılması, yapısal olarak benzer bitki kökenli bileşikler için gösterilmiştir (Tian ve ark., 2021); bu durum, SAc'nin de söz konusu yolları modüle edebileceğine işaret etmekle birlikte doğrudan deneysel doğrulama gerektirmektedir. Tüm bu bulgulara karşın, SAc'nin bu modeldeki sitoprotektif mekanizmalarının tam olarak aydınlatılabilmesi için malondialdehit (MDA), süperoksit dismutaz (SOD) ve glutatyon peroksidaz (GPx) gibi oksidatif stres belirteçlerini ve mekanistik sinyal yollarını doğrudan değerlendiren ileri çalışmalara ihtiyaç duyulmaktadır.

5. SONUÇ

Bulgularımız, sinapik asitin SH-SY5Y nöroblastoma hücrelerinde H_2O_2'ye bağlı nöronal sitotoksisiteyi, konsantrasyona bağlı bir şekilde azaltabildiğine işaret etmektedir. Bu etki, hücre canlılığının kısmen geri kazanılması ve membran bütünlüğünün korunması ile desteklenmektedir. Sinapik asit, nörodejeneratif hastalıklarda oksidatif stresi hedef alan potansiyel bir nöroprotektif ajan olarak değerlendirilmeye değer görülmektedir. Altta yatan mekanizmaların aydınlatılması için doğrudan oksidatif stres belirteçlerini ve mekanistik analizleri içeren ileri çalışmalara ihtiyaç vardır.

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PROTECTIVE ROLE OF SINAPIC ACID AGAINST HYDROGEN PEROXIDE-INDUCED OXIDATIVE NEURONAL DAMAGE IN SH-SY5Y CELLS

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ABSTRACT

Hydrogen peroxide (H_2O_2) is a widely used inducer of oxidative neuronal damage and constitutes a reliable in vitro model of reactive oxygen species (ROS)-mediated neurotoxicity. Excessive exposure to H_2O_2 disrupts cellular redox homeostasis, impairs antioxidant defense systems, compromises membrane integrity, and contributes to neuronal cell damage.

The present study aimed to evaluate the potential protective effects of sinapic acid (SAc) against H_2O_2-induced cytotoxicity in SH-SY5Y neuroblastoma cells. Cells were exposed to H_2O_2 (250 µM) for 24 hours in the presence or absence of SAc (10, 25, and 50 µM). Cell viability was determined using the MTT assay, while membrane integrity was assessed by measuring lactate dehydrogenase (LDH) release. Our findings indicate that sinapic acid may attenuate H_2O_2-induced neuronal cytotoxicity in a concentration-dependent manner.

Keywords: Sinapic acid, H_2O_2, oxidative stress, SH-SY5Y, neuroprotection, MTT, LDH

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1. INTRODUCTION

Oxidative stress is a critical mediator in the pathogenesis of neurological diseases, characterized by excessive generation of reactive oxygen species (ROS) that overwhelms the capacity of endogenous antioxidant defense systems. This redox imbalance triggers a cascade of cellular damage mechanisms including lipid peroxidation, protein oxidation, and DNA damage ultimately leading to neuronal dysfunction and cell death (Chen, 2016).

Hydrogen peroxide (H_2O_2) is a ROS widely employed as a reliable inducer of cellular oxidative stress in experimental models. Exposure to H_2O_2 has been associated with depletion of antioxidant defense enzymes, mitochondrial dysfunction, and increased membrane permeability (Feng et al., 2016). SH-SY5Y human neuroblastoma cells, owing to their neuronal characteristics and high metabolic activity, are widely recognized as a preferred in vitro model for investigating oxidative stress-mediated neuronal injury (Kovalevich and Langford, 2013).

Sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid; SAc) is a naturally occurring hydroxycinnamic acid derivative found in a variety of plant-based foods. It has attracted growing scientific interest due to its antioxidant, anti-inflammatory, and neuroprotective properties (Tungalag and Yang, 2021; Zare et al., 2015). Nevertheless, the cytoprotective mechanisms underlying its protective potential against H_2O_2-induced neuronal toxicity remain to be fully elucidated.

The present study aimed to evaluate the protective effects of sinapic acid against H_2O_2-induced oxidative injury in SH-SY5Y cells, using cell viability and membrane integrity as primary endpoints.

2. MATERIALS AND METHODS

2.1. Cell Culture

SH-SY5Y human neuroblastoma cells were maintained in DMEM/F12 medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin, and cultured at 37°C in a humidified atmosphere containing 5% CO_2.

2.2. Experimental Treatment

Cells were treated with H_2O_2 (250 µM) for 24 hours in the presence or absence of SAc at concentrations of 10, 25, and 50 µM. Sinapic acid was applied one hour prior to H_2O_2 exposure. All compounds were dissolved in DMSO, with the final DMSO concentration not exceeding 0.1%.

2.3. Cell Viability Assay (MTT)

Cell viability was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. MTT solution (0.5 mg/mL) was added to the cells and, following a 4-hour incubation period, formazan crystals were solubilized with DMSO. Absorbance was measured spectrophotometrically at 570 nm.

2.4. LDH Release Assay

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Membrane integrity was evaluated by measuring the amount of lactate dehydrogenase (LDH) released into the cell culture supernatant using a commercial colorimetric kit (Sigma-Aldrich). LDH release was expressed as a percentage of total LDH obtained from fully lysed cells.

2.5. Statistical Analysis

Data are presented as mean ± standard deviation (SD) from three independent experiments. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

H_2O_2 treatment significantly reduced cell viability to 58.7 ± 4.5% compared to the control group (97.6 ± 3.7%) ($p < 0.001$). Co-treatment with SAc restored viability to 71.3 ± 4.1%, 86.8 ± 4.4%, and 95.2 ± 4.6% at concentrations of 10, 25, and 50 µM, respectively. This increase reached statistical significance at 10 µM ($p < 0.01$) and was more pronounced at 25 and 50 µM ($p < 0.001$).

LDH release was markedly elevated following H_2O_2 treatment (73.1 ± 5.0%) compared to the control group (11.6 ± 1.4%) ($p < 0.001$). SAc attenuated this increase in a concentration-dependent manner, reducing LDH release to 52.7 ± 4.1%, 33.8 ± 3.2%, and 20.9 ± 2.5% at 10, 25, and 50 µM, respectively. The reduction was statistically significant at 10 µM ($p < 0.01$) and highly significant at 25 and 50 µM ($p < 0.001$).

Table 1. Effects of sinapic acid on H_2O_2-induced cytotoxicity in SH-SY5Y cells.

Parameter	Control	H_2O_2	SAc 10 µM + H_2O_2	SAc 25 µM + H_2O_2	SAc 50 µM + H_2O_2
Viability (%)	97.6 ± 3.7	58.7 ± 4.5***	71.3 ± 4.1##	86.8 ± 4.4###	95.2 ± 4.6###
LDH (% total)	11.6 ± 1.4	73.1 ± 5.0***	52.7 ± 4.1##	33.8 ± 3.2###	20.9 ± 2.5###

Values are expressed as mean ± SD from three independent experiments (n = 3). ***$p < 0.001$ indicates significant difference relative to the control group. ##$p < 0.01$, ###$p < 0.001$ indicate significant difference relative to the H_2O_2 group.

4. DISCUSSION

The present study demonstrates that sinapic acid exerts protective effects against H_2O_2-induced oxidative cytotoxicity in SH-SY5Y neuroblastoma cells. SAc treatment restored cell viability in a concentration-dependent manner and markedly attenuated LDH release, collectively indicating cytoprotective activity at both the metabolic and membrane integrity levels.

The neuroprotective effect of SAc is most likely attributable to its capacity to scavenge hydroxyl and superoxide radicals. In addition, SAc has been reported to activate the Nrf2/HO-

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1 signaling pathway, thereby reinforcing endogenous antioxidant defenses (Tungalag and Yang, 2021). These mechanisms may further contribute to the suppression of mitochondrial dysfunction and apoptotic cascades triggered by oxidative insult. Consistent with these observations, SAc has also been shown to exhibit neuroprotective properties against glutamate-induced excitotoxicity, an effect associated with the suppression of endoplasmic reticulum stress and pro-inflammatory cytokine signaling in a C6 glioma cell model (Ortasoz et al., 2025).

The marked reduction in LDH release observed in the present study suggests that SAc preserves plasma membrane integrity, an effect that may be mechanistically linked to the inhibition of lipid peroxidation (Zare et al., 2015; Tungalag and Yang, 2021). Comparable protective effects against H_2O_2-induced neuronal damage have been reported for other phenolic compounds, including lycopene and hesperetin (Feng et al., 2016; Moon et al., 2023). Furthermore, the attenuation of oxidative damage through suppression of MAPK and NF-κB signaling pathways has been demonstrated for structurally related plant-derived compounds in SH-SY5Y cells (Tian et al., 2021), suggesting that SAc may similarly modulate these pathways, though direct experimental confirmation is warranted. Notwithstanding these findings, further studies directly assessing oxidative stress markers including malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx) as well as mechanistic signaling pathways, are required to fully characterize the cytoprotective mechanisms of SAc in this model.

5. CONCLUSION

The present findings indicate that sinapic acid attenuates H_2O_2-induced neuronal cytotoxicity in SH-SY5Y neuroblastoma cells in a concentration-dependent manner, as evidenced by partial restoration of cell viability and preservation of membrane integrity. Sinapic acid appears to merit consideration as a potential neuroprotective agent targeting oxidative stress in the context of neurodegenerative disease. Further studies incorporating direct assessment of oxidative stress biomarkers and mechanistic analyses are required to elucidate the underlying protective pathways.

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GALLİK ASİDİN Caco-2 HÜCRELERİNDE ARSENİK KAYNAKLI SİTOTOKSİSİTE ÜZERİNE KORUYUCU ETKİLERİ

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ÖZET

Arsenik (As), başta oksidatif stres olmak üzere epitel bariyer bültünlüğünün bozulması ve hücresel hasar ile ilişkili yaygın bir çevresel toksik ajandır. Özellikle sodyum arsenit ($NaAsO_2$), bağırsak epitel hücrelerinde reaktif oksijen türlerinin (ROS) aşırı üretimini artırarak lipid peroksidasyonuna ve endöjen antioksidan savunma sistemlerinin bozulmasına yol açmaktadır. İnsan bağırsak epitelini temsil eden ve yaygın olarak kullanılan bir in vitro model olan Caco-2 hücreleri, toksik ajanlara bağlı hücresel hasarın araştırılması için uygun bir sistem sunmaktadır. Doğal olarak çeşitli bitkisel kaynaklı gıdalarda bulunan bir polifenolik bileşik olan gallik asit (GA), antioksidan ve sitoprotektif etkileri ile dikkat çekmektedir; ancak arsenik kaynaklı bağırsak toksisitesine karşı potansiyel koruyucu rolü henüz tam olarak aydınlatılamamıştır.

Bu çalışmada, GA'ın arsenik kaynaklı sitotoksisite üzerindeki etkilerinin Caco-2 hücrelerinde değerlendirilmesi amaçlandı. Hücreler, 24 saat süreyle $NaAsO_2$ (20 µM) varlığında veya yokluğunda GA (10, 25 ve 50 µM) ile muamele edildi. Hücre canlılığı MTT yöntemi ile, membran bültünlüğü ise laktat dehidrogenaz (LDH) salınımı ile değerlendirildi. Bulgularımız, GA'ın arsenik kaynaklı sitotoksisiteyi azaltabileceğine ve bu etkinin oksidatif stres ile ilişkili mekanizmaların modülasyonu ile bağlantılı olabileceğine işaret etmektedir.

Anahtar Kelimeler: Arsenik toksisitesi, gallik asit, Caco-2 hücreleri, sitotoksisite, oksidatif stres

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1. GİRİŞ

Arsenik (As), yer kabuğunda doğal olarak bulunan ve içme suyu ile besin zinciri aracılığıyla insan vücuduna giren önemli bir çevresel kirleticidir. Arsenik kirliliği 200 milyondan fazla insanı etkilemekte olup, kronik maruziyet; deri lezyonları, akciğer kanseri ve kardiyovasküler hastalıklar başta olmak üzere çeşitli sistemik bozukluklarla ilişkilendirilmektedir (Rehman ve ark., 2018).

Oral maruziyet, arsenike maruz kalmanın temel yolunu oluşturmakta ve sindirim sistemi epiteli bu toksisitenin ilk hedefi konumundadır. Trivalent inorganik arsenik formu olan $NaAsO_2$, bağırsak hücrelerinde reaktif oksijen türlerini (ROS) artırarak mitokondriyal işlev bozukluğuna, lipid peroksidasyonuna ve apoptotik kaskadın aktivasyonuna neden olmaktadır. Bu etkilerin in vitro çalışmalarda Caco-2 hücrelerinde de gösterildiği bildirilmiştir (Zhang ve ark., 2025; Laparra ve ark., 2008).

Caco-2 hücre hattı, insan kolon adenokarsinomundan elde edilmiş olup farklılaştırıldığında bağırsak epitel hücrelerine benzer özellikler göstermektedir. Bu nedenle, besinsel toksikan ve farmasötik bileşiklerin intestinal emilim ve toksisitesinin değerlendirilmesinde sıklıkla kullanılan bir referans model olarak kabul görmektedir (Parajuli ve ark., 2022).

Gallik asit (GA; 3,4,5-trihidroksibenzoik asit), tahıllar, meyveler ve çay gibi çeşitli gıdalarda yaygın olarak bulunan bir polifenolik bileşiktir. Güçlü antioksidan, antiinflamatuvar ve sitoprotektif özelliklere sahip olan GA'nın; hidroksil, süperoksit ve peroksit radikallerini süpürerek oksidatif strese karşı koruma sağladığı gösterilmiştir. Ayrıca arsenik toksisitesine karşı koruyucu olduğu da raporlanmıştır (Panghal ve ark., 2020; Samad ve ark., 2019). Bununla birlikte, GA'nın Caco-2 hücrelerinde arsenik kaynaklı sitotoksisiteye karşı koruyucu etkisi henüz tam olarak aydınlatılmamıştır (Mu ve Kitts, 2024).

Bu bağlamda çalışmamızın amacı; GA'nın, $NaAsO_2$ ile uyarılmış sitotoksisite modeli üzerindeki koruyucu etkilerini Caco-2 hücre canlılığı ve membran bütünlüğü parametreleri üzerinden değerlendirmektir.

2. MATERYAL VE METOT

2.1. Hücre Kültürü

Caco-2 insan kolon adenokarsinoma hücreleri (ATCC® HTB-37™), %10 fetal sığır serumu (FBS), 100 U/mL penisilin ve 100 µg/mL streptomisin içeren DMEM besiyerinde, %5 CO_2'lı nemlendirici bir ortamda 37°C'de kültüre edildi.

2.2. Deneysel Uygulama

GA (10, 25 ve 50 µM) hücrelere $NaAsO_2$ uygulanmasından 1 saat önce eklendi. Ardından hücreler $NaAsO_2$ (20 µM) ile 24 saat boyunca muamele edildi. Tüm bileşikler DMSO'da çözüldüğü ve son DMSO konsantrasyonu %0,1'i geçmeyecek şekilde hazırlandı.

2.3. Hücre Canlılığı (MTT Testi)

Hücre canlılığı, MTT (3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolyum bromid) yöntemiyle değerlendirildi. MTT çözeltisi (0,5 mg/mL) hücrelere eklenerek 4 saat inkübe edildi; DMSO ile formazan kristalleri çözündürülerek 570 nm dalga boyunda absorbans ölçüldü.

2.4. LDH Salınım Analizi

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Membran bültünlüğü, hücre kültürü süpernatantına salınan LDH miktarı ticari bir kolorimetrik kit (Sigma-Aldrich) kullanılarak değerlendirildi. LDH salınımı, tamamen lizatı alınmış hücrelerden elde edilen toplam LDH'nin yüdesi olarak ifade edildi.

2.5. İstatistiksel Analiz

Veriler, üç bağımsız deneyin ortalama ± standart sapması (SS) olarak sunuldu. İstatistiksel analizler, tek yönlü varyans analizi (ANOVA) ve ardından Tukey çoklu karşılaştırma testi kullanılarak gerçekleştirildi. $p < 0,05$ değeri istatistiksel olarak anlamlı kabul edildi.

3. BULGULAR

$NaAsO_2$ uygulaması, kontrol grubuna kıyasla (98,7 ± 3,6%) hücre canlılığını istatistiksel olarak anlamlı biçimde 53,8 ± 4,1%'e düşürdü ($p < 0,001$). GA ile ko-muamele, canlılığı 10, 25 ve 50 µM için sırasıyla 63,5 ± 3,7%, 77,6 ± 4,0% ve 90,8 ± 4,3%'ye yükseltdi. Bu artış 10 µM'de ılımlı ($p < 0,05$), 25 µM'de daha belirgin ($p < 0,01$) ve 50 µM'de güçlü ölçüde anlamlı ($p < 0,001$) olarak belirlendi.

LDH salınımı, kontrol grubuna kıyasla (12,8 ± 1,6%) arsenik grubunda belirgin biçimde arttı (75,6 ± 5,3%, $p < 0,001$). GA uygulaması LDH değerlerini sırasıyla 69,2 ± 4,9%, 55,4 ± 4,2% ve 34,1 ± 3,3%'e indirdi. 10 µM'deki azalma istatistiksel olarak anlamlı bulunmazken, 25 µM'de anlamlı ($p < 0,05$) ve 50 µM'de yüksek düzeyde anlamlı ($p < 0,001$) olarak saptandı.

Çizelge 1. Gallik asitin Caco-2 hücrelerinde $NaAsO_2$ ile uyarılmış sitotoksisite üzerindeki etkileri

Parametre	Kontrol	$NaAsO_2$	GA 10 µM + As	GA 25 µM + As	GA 50 µM + As
Canlılık (%)	98.7 ± 3.6	53.8 ± 4.1***	63.5 ± 3.7#	77.6 ± 4.0##	90.8 ± 4.3###
LDH (% total)	12.8 ± 1.6	75.6 ± 5.3***	69.2 ± 4.9	55.4 ± 4.2#	34.1 ± 3.3###

Değerler üç bağımsız deneyin ortalama ± SS olarak verilmiştir (n=3). ***$p < 0,001$ kontrol grubuna göre; #$p < 0,05$, ##$p < 0,01$, ###$p < 0,001$ arsenik grubuna göre anlamlı farkı göstermektedir.

4. TARTIŞMA

Bu çalışmada, gallik asidin Caco-2 hücrelerinde $NaAsO_2$ ile uyarılmış oksidatif sitotoksisiteye karşı doza bağlı koruyucu etkiler sergilediği gösterilmiştir. GA uygulaması hücre canlılığını anlamlı biçimde geri kazandırmış ve LDH salınımını belirgin şekilde azaltmıştır.

Arsenik toksisitesinin başlıca mekanizması intraselüler ROS üretimi ve oksidatif strestir. $NaAsO_2$'nin Caco-2 hücrelerinde oksidatif stres oluşturduğu, hücre döngüsünü bozduğu ve apoptotik süreçleri tetiklediği gösterilmiştir (Laparra ve ark., 2008). Bağırsak epitel hücrelerinde arsenite maruziyetin sitotoksik ve geçirgenlik değişikliklerine yol açtığı da bildirilmiştir (Parajuli ve ark., 2022).

Gallik asidin koruyucu etkisi, trihidroksi grubunun sağladığı güçlü radikal süpürme kapasitesinden kaynaklanmaktadır. GA'nın arseniğe karşı koruyucu etkisi; kırmızı kan hücrelerinde oksidatif/nitrozatif hasar (Panghal ve ark., 2020) ve davranışsal/bilişsel

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parametreler (Samad ve ark., 2019) üzerinde gösterilmiştir. Ayrıca GA'nın farklı hücre modellerinde antioksidan ve antiinflamatuvar etkiler gösterdiği ve hücresel hasarı azalttığı bildirilmiştir (Chu ve ark., 2024; Mu ve Kitts, 2024).

GA'nın Caco-2 hücrelerinde LPS kaynaklı inflamasyonu ve oksidatif stresi NF-κB/MAPK sinyal yollarını baskılayarak azalttığı da gösterilmiştir (Chu ve ark., 2024).

LDH salınımındaki doza bağlı azalma, GA'nın membran bütünlüğünü koruduğunu ve bu etkinin lipid peroksidasyon inhibisyonuyla bağlantılı olabileceğini düşündürmektedir. 10 µM GA dozunda LDH'daki azalmanın istatistiksel anlamlılığa ulaşamaması, bu dozun membran koruyucu etki için eşik değerinin altında kalabileceğine işaret etmektedir. Bununla birlikte, doğrudan MDA, SOD ve GPx gibi oksidatif stres belirteçlerini içeren ileri çalışmalara ihtiyaç vardır.

5. SONUÇ

Bu çalışmanın bulguları, gallik asitin Caco-2 hücrelerinde $NaAsO_2$ kaynaklı sitotoksisiteye karşı doza bağlı koruyucu etkiler sergilediğine işaret etmektedir. GA uygulaması, hücre canlılığını kısmen geri kazandırmış ve membran bültünlüğünü koruyan LDH salınımını azaltmıştır. Gallik asit, bağırsak epiteline yönelik arsenik toksisitesine karşı potansiyel bir koruyucu ajan olarak değerlendirilmeye değer görünmektedir. Altta yatan mekanizmaların aydınlatılması için dogrudan oksidatif stres belirteçlerini ve Nrf2/NF-κB sinyal yollarını inceleyen ileri çalışmalara ihtiyaç vardır.

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PROTECTIVE EFFECTS OF GALLIC ACID AGAINST ARSENIC-INDUCED CYTOTOXICITY IN Caco-2 CELLS

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ABSTRACT

Arsenic (As) is a widespread environmental toxicant associated with oxidative stress, disruption of epithelial barrier integrity, and cellular damage. Sodium arsenite ($NaAsO_2$), in particular, promotes excessive generation of reactive oxygen species (ROS) in intestinal epithelial cells, resulting in lipid peroxidation and impairment of endogenous antioxidant defense mechanisms. Caco-2 cells, a widely used in vitro model representative of the human intestinal epithelium, provide a suitable system for investigating cellular damage induced by toxic agents. Gallic acid (GA), a polyphenolic compound naturally present in various plant-derived foods, has attracted considerable attention for its antioxidant and cytoprotective properties; however, its potential protective role against arsenic-induced intestinal toxicity has not yet been fully elucidated.

The present study aimed to evaluate the effects of GA on arsenic-induced cytotoxicity in Caco-2 cells. Cells were treated with GA (10, 25, and 50 µM) in the presence or absence of $NaAsO_2$ (20 µM) for 24 hours. Cell viability was assessed by the MTT assay, and membrane integrity was determined by measuring lactate dehydrogenase (LDH) release. The findings suggest that GA may attenuate arsenic-induced cytotoxicity, and that this effect may be associated with the modulation of oxidative stress-related mechanisms.

Keywords: Arsenic toxicity, gallic acid, Caco-2 cells, cytotoxicity, oxidative stress

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1. INTRODUCTION

Arsenic (As) is a naturally occurring metalloid widely distributed in the Earth's crust that enters the human body primarily through contaminated drinking water and the food chain, making it one of the most significant environmental contaminants of global public health concern. Arsenic pollution affects more than 200 million people worldwide, and chronic exposure has been associated with a broad spectrum of systemic disorders, most notably cutaneous lesions, lung cancer, and cardiovascular disease (Rehman et al., 2018).

Oral ingestion constitutes the predominant route of arsenic exposure, positioning the gastrointestinal epithelium as the primary target of its toxic effects. Sodium arsenite ($NaAsO_2$), the trivalent inorganic form of arsenic, has been shown to induce marked elevations in intracellular reactive oxygen species (ROS), culminating in mitochondrial dysfunction, lipid peroxidation, and activation of the apoptotic cascade in intestinal cells. These effects have been recapitulated in vitro using the Caco-2 cell model (Zhang et al., 2025; Laparra et al., 2008).

The Caco-2 cell line, originally derived from a human colonic adenocarcinoma, acquires morphological and functional characteristics closely resembling those of intestinal enterocytes upon differentiation. For this reason, it is widely recognized as a gold-standard in vitro model for assessing the intestinal absorption and toxicity of dietary contaminants and pharmaceutical compounds (Parajuli et al., 2022).

Gallic acid (GA; 3,4,5-trihydroxybenzoic acid) is a naturally occurring polyphenolic compound abundantly present in a variety of foods, including cereals, fruits, and tea. Owing to its potent antioxidant, anti-inflammatory, and cytoprotective properties, GA has been demonstrated to confer protection against oxidative stress through the scavenging of hydroxyl, superoxide, and peroxyl radicals. Its protective capacity against arsenic-induced toxicity has likewise been reported in experimental settings (Panghal et al., 2020; Samad et al., 2019). Nevertheless, the cytoprotective mechanisms underlying GA's potential to attenuate arsenic-induced cytotoxicity specifically in Caco-2 cells remain to be fully elucidated (Mu and Kitts, 2024).

In this context, the present study aimed to evaluate the protective effects of GA against $NaAsO_2$-induced cytotoxicity in Caco-2 cells, with particular emphasis on cell viability and membrane integrity as primary endpoints.

2. MATERIALS AND METHODS

2.1. Cell Culture

Caco-2 human colon adenocarcinoma cells (ATCC® HTB-37™) were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin, and maintained at 37°C in a humidified atmosphere containing 5% CO_2.

2.2. Experimental Treatment

GA (10, 25, and 50 µM) was added to the cells one hour prior to $NaAsO_2$ exposure. Cells were then treated with $NaAsO_2$ (20 µM) for 24 hours. All compounds were dissolved in DMSO, and the final DMSO concentration did not exceed 0.1%.

2.3. Cell Viability Assay (MTT)

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Cell viability was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. MTT solution (0.5 mg/mL) was added to the cells and incubated for 4 hours; formazan crystals were subsequently dissolved in DMSO and absorbance was measured at 570 nm.

2.4. LDH Release Assay

Membrane integrity was evaluated by measuring the amount of lactate dehydrogenase (LDH) released into the cell culture supernatant using a commercial colorimetric kit (Sigma-Aldrich). LDH release was expressed as a percentage of total LDH obtained from fully lysed cells.

2.5. Statistical Analysis

Data are presented as mean ± standard deviation (SD) of three independent experiments. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

$NaAsO_2$ treatment significantly reduced cell viability to 53.8 ± 4.1% compared to the control group (98.7 ± 3.6%; $p < 0.001$). Co-treatment with GA restored viability in a dose-dependent manner, reaching 63.5 ± 3.7%, 77.6 ± 4.0%, and 90.8 ± 4.3% at 10, 25, and 50 µM, respectively. The increase was modest at 10 µM ($p < 0.05$), more pronounced at 25 µM ($p < 0.01$), and highly significant at 50 µM ($p < 0.001$).

LDH release was markedly elevated in the arsenic-treated group (75.6 ± 5.3%) compared to the control (12.8 ± 1.6%; $p < 0.001$). GA treatment reduced LDH levels to 69.2 ± 4.9%, 55.4 ± 4.2%, and 34.1 ± 3.3% at the respective doses. The reduction at 10 µM did not reach statistical significance, whereas the decreases at 25 µM ($p < 0.05$) and 50 µM ($p < 0.001$) were statistically significant.

Table 1. Effects of gallic acid on $NaAsO_2$-induced cytotoxicity in Caco-2 cells.

Parameter	Control	$NaAsO_2$	GA 10 µM + As	GA 25 µM + As	GA 50 µM + As
Viability (%)	98.7 ± 3.6	53.8 ± 4.1***	63.5 ± 3.7#	77.6 ± 4.0##	90.8 ± 4.3###
LDH (% total)	12.8 ± 1.6	75.6 ± 5.3***	69.2 ± 4.9	55.4 ± 4.2#	34.1 ± 3.3###

Values are expressed as mean ± SD of three independent experiments (n = 3). ***$p < 0.001$ vs. control group; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ vs. arsenic group.

4. DISCUSSION

The present study demonstrates that gallic acid exerts dose-dependent protective effects against $NaAsO_2$-induced oxidative cytotoxicity in Caco-2 cells. GA treatment significantly restored

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cell viability and markedly attenuated LDH release, collectively indicating a cytoprotective response at the level of both cellular metabolism and membrane integrity.

The principal mechanism underlying arsenic toxicity is the excessive generation of intracellular ROS and the subsequent induction of oxidative stress. $NaAsO_2$ has been shown to elicit oxidative stress, disrupt cell cycle progression, and trigger apoptotic signaling in Caco-2 cells (Laparra et al., 2008). Consistent with these findings, arsenite exposure in intestinal epithelial cells has also been reported to cause cytotoxic injury and alterations in paracellular permeability (Parajuli et al., 2022).

The cytoprotective activity of gallic acid is primarily attributed to the potent free radical-scavenging capacity conferred by its trihydroxy phenolic moiety. The protective effects of GA against arsenic-induced damage have been demonstrated across multiple biological endpoints, including oxidative and nitrosative injury in erythrocytes (Panghal et al., 2020) and impairments in behavioral and cognitive parameters in vivo (Samad et al., 2019). Beyond arsenic-specific contexts, GA has been reported to exert broad antioxidant and anti-inflammatory effects across diverse cell models, thereby mitigating oxidative cellular damage (Chu et al., 2024; Mu and Kitts, 2024). Notably, GA has further been shown to suppress LPS-induced inflammation and oxidative stress in Caco-2 cells through inhibition of the NF-κB/MAPK signaling pathway (Chu et al., 2024), suggesting that its protective action may involve both radical scavenging and transcriptional modulation of inflammatory cascades.

The dose-dependent reduction in LDH release observed in the present study indicates that GA preserves plasma membrane integrity, an effect that may be mechanistically linked to the inhibition of lipid peroxidation. The failure of the 10 µM GA dose to achieve statistical significance in LDH attenuation suggests that this concentration may fall below the threshold required for effective membrane protection under the experimental conditions employed. These findings, while promising, are based solely on viability and membrane integrity endpoints. Future investigations incorporating direct markers of oxidative stress including malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx) are warranted to more comprehensively characterize the antioxidant mechanisms underlying GA's cytoprotective effects in this model.

5. CONCLUSION

The findings of this study indicate that gallic acid exerts dose-dependent protective effects against $NaAsO_2$-induced cytotoxicity in Caco-2 cells. GA treatment partially restored cell viability and reduced LDH release, thereby preserving membrane integrity. Gallic acid appears to be a promising candidate as a protective agent against arsenic-induced intestinal epithelial toxicity. Further studies examining oxidative stress markers and the Nrf2/NF-κB signaling pathways directly are needed to elucidate the underlying mechanisms.

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ITIR BİTKİSİNİN ENDOFİTİK FUNGAL ÇEŞİTLİLİĞİ

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ÖZET

Bu çalışma, Türkiye florasında tıbbi ve aromatik değeriyle öne çıkan, parfümeri, kozmetik ve geleneksel tıp alanındaki geniş kullanım yelpazesiyle bilinen ıtır (*Pelargonium graveolens*) bitkisinin iç dokularındaki fungal biyoçeşitliliği belirlemek amacıyla gerçekleştirilmiştir. Endofitik funguslar, konukçu bitkiye zarar vermeden yaşamlarını sürdüren ve bitkinin biyotik/abiyotik stres toleransı ile sekonder metabolit üretiminde anahtar rol oynayan stratejik mikroorganizmalardır. Araştırma kapsamında, 19 Aralık 2025 tarihinde Kocaeli Üniversitesi Arslanbey Yerleşkesi'nden steril koşullarda toplanan sağlıklı ıtır örnekleri kullanılmıştır. Bitki parçaları (yaprak ve gövde), epifit mikroorganizmaları uzaklaştırmak amacıyla %70 etanol ve %2,5 sodyum hipoklorit kullanılarak çok aşamalı yüzey sterilizasyonuna tabi tutulmuştur. Bu işlemin etkinliği son yıkama suyunun kontrol ekimleriyle doğrulanmıştır. Sterilizasyonun etkinliği kontrol edildikten sonra, doku segmentleri bakteriyel gelişimi baskılamak için streptomisin ilave edilmiş Patates Dekstroz Agar besiyerinde 25°C'de inkübe edilmiştir. Gelişen fungal koloniler hif ucu yöntemiyle saflaştırılmış; tanı işlemleri ise koloni morfolojisi, pigment üretimi ve mikroskobik yapılar (hif segmentasyonu, spor yapısı) üzerinden literatürle karşılaştırılarak yapılmıştır. İzolasyon çalışmaları sonucunda, morfolojik olarak birbirinden farklı beş fungal izolat (IG21, IY22, IY12, IY11 ve IY31) elde edilmiştir. Yapılan teşhisler sonucunda bu izolatların *Aspergillus* sp., *Fusarium* sp. ve *Alternaria* sp. cinslerini temsil ettiği belirlenmiştir. Bulgular, literatürdeki benzer çalışmalarla uyumlu olarak, yaprak dokusunun gövde dokusuna göre daha yüksek fungal çeşitliliğe sahip olduğunu göstermiştir. Sonuç olarak, ıtır bitkisinin zengin bir endofitik fungus spektrumuna sahip olduğu ortaya konmuştur. Bu mikroorganizmaların, bitkiye özgü uçucu yağ bileşenlerinin sentezi üzerindeki etkilerinin araştırılması ve biyokontrol ajanı olarak potansiyellerinin test edilmesi, gelecekteki tarımsal ve farmakolojik çalışmalar için büyük önem taşımaktadır. Kesin tür teşhisleri için morfolojik verilerin moleküler yöntemlerle desteklenmesi önerilmektedir.

Anahtar Kelimeler: Endofitik funguslar, *Pelargonium graveolens*, Tıbbi ve aromatik bitkiler

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1. GİRİŞ

Endofitik funguslar, yaşam döngülerinin tamamını veya bir kısmını, konukçu bitki doku ve organları içerisinde herhangi bir dışsal hastalık belirtisine neden olmaksızın sürdüren mikroorganizmalardır (Gao et al., 2025; Shruthi et al., 2026). Parazit veya patojenlerin aksine bu funguslar, her iki tarafın da dengeli bir etkileşimden kazanç sağladığı mutualistik ortaklıklar kurabilmektedir (Gao et al., 2025). Evrimsel süreçte bitkilerle birlikte gelişen bu mikroorganizmalar, konukçu bitkinin iç ortamına doğal bir uyum sağlama yeteneğine sahiptir (Sofian et al., 2025). Endofitlerin bitki dokuları içindeki çeşitliliği, bolluğu ve fonksiyonel rolleri; toprak tipi, iklim ve besin mevcudiyeti gibi çevresel faktörlerin yanı sıra hem bitkinin hem de fungusun genotipleri tarafından şekillenmektedir (Sofian et al., 2025).

Son yıllarda endofitik funguslar, tıp ve tarım alanlarında sundukları geniş spektrumlu biyoteknolojik potansiyel nedeniyle yoğun ilgi görmektedir. Tıbbi açıdan bakıldığında, bu funguslar tarafından üretilen sekonder metabolitlerin serbest radikalleri süpürerek hücreleri koruduğu; anti-inflamatuar, anti-tümör ve antioksidan özelliklere sahip farmasötiklerin geliştirilmesinde değerli bir kaynak teşkil ettiği bilinmektedir (Gao et al., 2025). Tarımsal açıdan ise endofitler, sürdürülebilir ürün verimliliği ve hastalık yönetimi için "çevre-dostu" biyo-ajanlar olarak öne çıkmaktadır (Shruthi et al., 2026). Özellikle sentetik fungisitlerin yaygın ve hatalı kullanımından kaynaklanan çevresel ve sağlık sorunları, biyolojik kontrol ajanlarına olan ihtiyacı artırmıştır (Manathunga et al., 2024).

Endofitik funguslar, bitki koruma ve gelişiminde hem doğrudan hem de dolaylı mekanizmalar aracılığıyla rol oynarlar. Bu mekanizmalar arasında; besin ve alan rekabeti, antifungal metabolit sentezi, litik enzim (selülaz, ksilanaz, amilaz vb.) salınımı, sistemik direncin uyarılması ve mikoparazitizm yer almaktadır (Manathunga et al., 2024; Shruthi et al., 2026). Ayrıca, azot fiksasyonu ve fosfor çözme yeteneğine sahip endofitlerin, bitki büyüme parametrelerini önemli ölçüde iyileştirdiği pek çok çalışma ile ortaya konmuştur (Gao et al., 2025). Görsel 1'de özetlendiği üzere, endofitik funguslar konukçu bitkiyle çok yönlü bir etkileşim kurarak hem bitki sağlığını iyileştirmekte hem de değerli biyoaktif bileşiklerin sentezine olanak tanımaktadır. Literatürde *Acremonium, Alternaria, Aspergillus, Fusarium, Penicillium* ve *Trichoderma* gibi pek çok cinsin biyolojik kontroldeki kritik rolleri 200'den fazla araştırma ile desteklenmiştir (Manathunga et al., 2024).

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Görsel 1. Endofitik funguslar ile konukçu bitki arasındaki simbiyotik etkileşimlerin temel mekanizmaları ve biyolojik etkileri.

Mısır, buğday ve pirinç gibi temel ürünlerde endofit çeşitliliği geniş ölçüde araştırılmış olsa da, tıbbi ve aromatik bitkilerin içsel mikrobiyotası halen keşfedilmeyi bekleyen biyoaktif bileşik kaynakları barındırmaktadır (Shruthi et al., 2026). Bu simbiyotik mikroorganizmaların doğadaki yaygınlığı bilinse de; tür çeşitliliği, konukçu aralığı ve coğrafi dağılımları hakkındaki bilgiler henüz sınırlıdır (Masumi et al., 2015). Bugüne kadar çok az sayıda bitki türü, endofit biyoçeşitliliği ve sekonder biyoaktif metabolit üretme potansiyeli açısından detaylıca incelenmiştir (Kamyab et al., 2024).

Geraniaceae familyasına ait olan *Pelargonium graveolens* (ıtır), tıbbi ve aromatik özellikleri ile öne çıkan baskın bir bitki türüdür. Geleneksel tıpta geniş bir kullanım yelpazesine sahip olan bu bitki; nefrit, yara iyileşmesi, ateş, soğuk algınlığı, inflamasyon ve çeşitli gastrointestinal hastalıkların tedavisinde yüzyıllardır kullanılmaktadır (Cavar & Maksimovic, 2012; Hsouna & Hamdi, 2012). Bitkinin toprak üstü kısımlarından elde edilen esansiyel yağlar; sitronellol, geraniol ve linalool gibi terpenoidler açısından zengin olup, parfümeri, kozmetik ve gıda sanayinde değerli bir hammadde teşkil etmektedir. Son yıllarda yapılan farmakolojik araştırmalar, *P. graveolens* ekstraktlarının güçlü antimikrobiyal, antiinflamatuar ve antioksidan aktiviteler sergilediğini ortaya koyarak, bu bitkinin sentetik koruyuculara ve terapötik ajanlara doğal bir alternatif olma potansiyelini pekiştirmiştir. Özellikle bitki dokularında konukçu ile simbiyotik bir ilişki içinde yaşayan endofitik funguslar, bu biyobileşenlerin sentezlenmesinde ve bitkinin çevresel stres faktörlerine karşı direnç kazanmasında kritik bir rol oynamaktadır (Diedericks et al., 2024).

Bu çalışmanın amacı, Türkiye florasında önemli bir yere sahip olan *P. graveolens* bitkisinden endofitik fungusları izole etmek, morfolojik yöntemlerle tanımlamak ve bu bitkinin fungal çeşitliliğini ortaya koymaktır. Elde edilen bulguların, sürdürülebilir tarım uygulamalarında ve yeni biyokontrol ajanlarının geliştirilmesinde temel oluşturması hedeflenmektedir.

2. MALZEME VE YÖNTEM

2.1. Örneklerin Toplanması

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P. graveolens örnekleri 19.12.2025 tarihinde, Kocaeli Üniversitesi Arslanbey Yerleşkesinde koordinatları verilen (40,70748 °K ve 30,02631 °D) bölgeden toplanmıştır (Görsel 2). Bitki organlarının (yapraklar, gövde) görsel incelemesine dayanarak yeterince olgun, sağlıklı ve hastalık belirtisi göstermeyen örnek bitkiler seçilmiştir. Örneklenen her bitki steril bir tüp içerisine yerleştirilmiş, ağzı sıkıca kapatılmış ve etiketlenmiştir. Örnekler, toplandıktan sonra laboratuvara getirilmiş ve bekletilmeden izolasyona hazırlık aşamasına geçilmiştir.

Görsel 2. Araştırma kapsamında endofit izolasyonu amacıyla Kocaeli Üniversitesi Arslanbey Yerleşkesi'nden toplanan sağlıklı *P. graveolens* (Itır) bitkisinin genel görünümü (A) ve örnekleme alanının genel görünümü (B).

2.2. İzolasyon İçin Parçaların Hazırlanması

Bitkiler, çamur ve kalıntıları temizlemek için musluk suyuyla dikkatlice yıkanmış ve oda sıcaklığında havlular üzerinde kurumaya bırakılmıştır. Kabin içerisinde bitki kısımları, steril penset/bistüri ile kullanılarak küçük parçalara (yaklaşık 0,5 cm2) bölünmüştür.

2.3. Örnek Yüzeylerinin Sterilizasyonu

Örnekler, mumsu tabakayı uzaklaştırmak ve yüzeydeki mikroorganizmaları öldürmek için 30-60 saniye boyunca %70'lik etanole ardından, örneklerin doku yapısına bağlı olarak daha ileri sterilizasyon için 1-3 dakika boyunca %2,5'luk sodyum hipoklorite daldırılmıştır. Sodyum hipokloritin nötralizasyonu ve sodyum kalıntılarını azaltmak için hipoklorit sonrası 70% etanol ile 30-60 saniye hızlı yıkama yapılmıştır. Daha sonra parçalar steril distile su ile her biri 30-60 saniye olacak şekilde üç kez durulanmıştır. Yüzey sterilizasyonu protokolünün etkinliğini test etmek için son yıkama suyundan 100 µL PDA plaklarına eklenmiştir. Bu plaklar diğer plaklarla birlikte inkübasyona bırakılmıştır. Herhangi bir büyümenin görülmesi, yüzey sterilizasyonunun epifitleri yok etmek için yeterli olmadığını, sterilizasyonun başarısız olduğunu ve daha uzun bir sterilizasyon süresine ihtiyaç duyulduğunu göstermektedir.

2.4. Kültüre Alma

P. graveolens bitkisinin tek bir kısmına (yaprak, gövde) ait 4-5 adet parça, streptomisin ilave edilmiş PDA (Patates Dekstroz Agar) besiyeri içeren plak üzerine yerleştirilmiş ve karanlıkta 25°C'de 15-20 gün boyunca inkübe edilmiştir. Streptomisin endofitik bakterilerin gelişmesini engellemek için besiyerine eklenmiştir.

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Yalnızca bitki dokularının içinden yavaşça büyüdüğü görülen funguslar endofit olarak kabul edilmiştir. Hızlı büyüyen fungusları ve yüzey kontaminasyonlarını içeren plaklar değerlendirilmemiştir. İzole edilen funguslar, antibiyotik içermeyen PDA besiyerinde saflaştırılmıştır. Her bir fungal izolattan hif ucu izolasyonu yapılmış ve morfolojik incelemeler için saklanmıştır.

2.5. Hif ucu izolasyonu

Plaklardan inkübasyon süresinin sonunda özellikle bitki parçalarının kenarlarından çıkan kolonilerin ucundan steril mantar delici (5 mm) ile parça alıp yeni PDA plaklarına aktarılmıştır (Görsel 3). Tek tip koloni elde etmek için tekrar tekrar alt kültür yapılmıştır. Hif ucu izolasyonu yapılmış kültürler ile alt kültürler PDA besiyeri üzerinde ve 25°C'de 10 gün inkübe edilmiştir. İzolatlar, 4°C'de saklanmıştır.

Görsel 3. PDA besiyerine ekilen bitki segmentlerinin kenarlarından gelişen primer fungal kolonilerin, steril mantar delici yardımıyla saflaştırılmak üzere alt kültürlere aktarılma aşaması.

2.6. Fungal Endofitlerin Mikroskobik Teşhisi

İzole edilen fungal endofitlerin morfolojik tanısı; koloni rengi, büyüme hızı ve misel yapısı gibi makroskopik özelliklerin yanı sıra hif segmentasyonu, spor yapısı ve üreme organlarının mikroskobik incelemesine dayanarak yapılmıştır. Elde edilen mikroskobik görüntüler ve kültürel karakteristikler literatürde yer alan tip tür görselleri ile karşılaştırılarak teşhis edilmiştir.

2.7. Veri analizi

Fungal izolatların makroskobik ve mikroskobik özellikleri, dijital görüntüleme teknikleri kullanılarak kayıt altına alınmıştır. Kültür plaklarının fotoğrafları cep telefonu kamerasıyla, mikroskobik preparatların fotoğrafları ise Olympus CX43 bileşik kameralı ışık mikroskobunda çekilmiş; Olympus CellSens yazılımı ile çekilen fotoğraflar kaydedilmiştir. Elde edilen görüntüler, morfolojik yapıların literatürdeki referans görsellerle karşılaştırmalı analizi için kullanılmıştır.

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3. BULGULAR ve TARTIŞMA

Yüzey sterilizasyonundan sonra ıtır bitkisinden toplam altı yaprak parçası ile yedi gövde parçası PDA üzerinde inkübasyona bırakılmıştır (Görsel 4a-b). Bitki parçalarıyla aynı zamanda son yıkama suyundan ekim yapılan plaklar da incelenmiş ve plaklarda herhangi bir büyüme gözlenmemiştir (Görsel 4c). Bu yüzey sterilizasyonunun etkili olduğunun göstergesidir.

Görsel 4. Itır bitki parçalarının ve sterilizasyonda son yıkama suyunun PDA üzerinde inkübasyonunu gösteren fotoğraf (a,b); inokülasyondan yedi gün sonra çekilmiş son yıkama suyuna ait fotoğraf (c).

Itır bitkisinin parçalarını içeren PDA plaklarında büyüyen funguslardan hif ucu izolasyonu yapılmış, elde edilen saf kültürlerin morfolojik tanımlama için hem koloni fotoğrafları hem de mikroskopla çekilen hif, spor, konidi görüntüleri incelenmiştir. Görsel 5'de gösterildiği gibi, beş morfolojik olarak farklı fungus başarıyla izole edilmiş ve saflaştırılmıştır. İzolasyon çalışmaları sonucunda beş morfolojik olarak birbirinden belirgin biçimde farklılaşan fungal izolat elde edilmiştir. Bu izolatlar IG21, IY22, IY12, IY11 ve IY31 kodlarıyla kayıt altına alınmıştır.

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Görsel 5. *P. graveolens* kaynaklı endofitlerin izolasyon ve saflaştırma aşamaları. Görselde, bitki doku segmentlerinin 10 günlük inkübasyonu (Üst sıra, Itır 1-3)) ile hif ucu izolasyonu sonrası elde edilen saflaştırılmış IY11, IY12, IY22, IY31 ve IG21 izolatlarının kültürel özellikleri görülmektedir.

Her bir izolat için koloni morfolojisi (ön ve arka yüz) ve mikroskobik yapılar sistematik biçimde tanımlanmıştır. Bunlar arasında plaklardaki koloni özellikleri, ters koloni rengi, pigment difüzyonu, sporofor ve spor zinciri özellikleri yer almaktadır. Koloni morfolojileri hif ucu izolasyonundan sonra elde edilen saf kültür plaklarına göre belirlenmiştir. Mikroskopta yine bu kültürlerden alınan örnekler incelenmiştir (Görsel 6). Elde edilen veriler Çizelge 1'de özetlenmiştir.

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Görsel 6. *P. graveolens* bitkisinden elde edilen endofit fungus izolatlarının hif ve konidi morfolojilerinin ışık mikroskobu altındaki görünümü (Büyütme: 40X objektif).

Çizelge 1. Morfolojik olarak tanımlanan mantar türlerinin listesi, morfolojik özellikleri ve izolasyonun yapıldığı doku kaynağı ile birlikte verilmiştir.

Kod	Morfolojik yapı	Doku	Olası endofit fungus
IY11	Koloni rengi koyu kahverengiden siyah-kahveye; yoğun melanin pigmentasyonu; yüzey kadifemsi-mat, büyüme paterni asimetrik, loblu, düzensiz; konsantrik zon yok; mikroskop gözlemi septalı, hiyalin-soluk yeşil hifler; hiflerde dallanma dik açılı	Yaprak	*Aspergillus* sp. (El-shafey et al., 2021; Yasser et al., 2020)
IY12	Koloni rengi beyaz-krem zemin; sporulasyon rengi tarçın-kahverengi; konsantrik zonlanma çok belirgin; tekstürü pamuksu ve tozlu halka; mikroskop gözlemi septalı, hiyalin; hiflerde dallanma düzenli, dik açılı; konidi şekli silindirik → yuvarlak uçlu; konidi rengi hiyalin	Yaprak	*Aspergillus sp.* (El-shafey et al., 2021; Yasser et al., 2020)
IY22	Koloni ön ve arka yüzü rengi zeytin yeşili-kahverengi; merkezi sarı-krem, açık; sporulasyon rengi koyu zeytin-kahve; tekstürü kadifemsi-toz; zonlanma çok belirgin konsantrik; mikroskop gözlemi hiyalin, septalı hifler, hiflerde dik açılı dallanma	Yaprak	*Aspergillus* sp. (El-shafey et al., 2021; Yasser et al., 2020)
IY31	Koloni rengi tamamen beyaz, pigment yok; Tekstürü ipeksi-pamuksu, yüksek aerial misel; radyal büyüme çok belirgin, uzun	Yaprak	*Fusarium* sp. (Yasser et al., 2020)

	radyal hif demetleri; simetrik büyüme; kenar düzenli, tam dairesel, konsantrik zon yok; mikroskop gözlemi hifler septalı, hiyalin, içi granüllü/dolgulu hücre zincirleri; hiflerde dallanma dik açılı		
IG21	Koloni ön yüzü koyu gri-kahve renginde, sklerotia, yoğun sporulasyon; koloni arka yüzü siyah-kahve melanize pigment; konidi rengi açık kahverengi; mikroskop gözlemi hifler septalı, dallanmış konidiyofor	Gövde	*Alternaria sp*. (Saad et al., 2018, 2019; Yasser et al., 2020)

Bu çalışmada *P. graveolens* bitkisinin gövde ve yaprak dokularından yüzey sterilizasyonu uygulanarak beş farklı morfolojik tipte endofitik fungus izole edilmiştir. Elde edilen izolatlar *Alternaria, Aspergillus* ve *Fusarium* cinslerini temsil etmektedir. Bu sonuç, *P. graveolens* bitkisinin endofitik fungus çeşitliliği açısından zengin bir konukçu olduğuna işaret etmektedir. Arnold (2007), tropikal ve subtropikal bitkilerde yaprak başına 1-10 endofitik fungal tür saptandığını bildirmiş olup bu çalışmada elde edilen beş izolat bu aralıkla uyumludur (Arnold, 2007).

Yaprak dokusundan izole edilen izolatların (IY22, IY12, IY11, IY31) gövde izolatına (IG21) kıyasla daha yüksek tür çeşitliliği sergilemesi dikkat çekicidir. Porras-Alfaro ve Bayman (2011), yaprak endofitlerinin kök ve gövde endofitlerine kıyasla genellikle daha yüksek çeşitlilik gösterdiğini vurgulamıştır; bu durum yaprak yüzeyinin atmosferik ve toprak kökenli fungus sporlarına karşı daha fazla maruz kalmasıyla açıklanabilir (Porras-alfaro & Bayman, 2011).

P. graveolens'in endofitik fungal topluluğuna ilişkin yayın sayısı oldukça sınırlıdır. Mevcut çalışmalar ağırlıklı olarak *Alternaria* ve *Fusarium* türlerini ön plana çıkarmakta olup bu çalışmadaki bulgular bu kapsamla uyumludur.

Pelargonium türleri, uçucu yağ içerikleri ve geniş kullanım alanları (parfümeri, aromaterapi, bitkisel tıp) nedeniyle önemli aromatik bitkiler arasında yer almaktadır. Bu bitkilerden izole edilen endofitik funguslar birden fazla açıdan bilimsel değer taşımaktadır. Hyde ve Soytong (2008), endofitik funguslarda biyoaktif bileşik üretiminin konukçu metabolizmasıyla etkileşimden kaynaklanabileceğini ve bu izolatların yeni antimikrobiyal ajan keşfi için değerli kaynaklar oluşturduğunu vurgulamıştır (Hyde & Soytong, 2008).

Bu çalışmada uygulanan morfolojik tanımlama yöntemi, koloni ve mikroskobik verilerin birlikte değerlendirilmesine dayanmaktadır. Tüm izolatlar için morfolojik değerlendirme cins düzeyinde tanı için yeterli olmakla birlikte tür düzeyinde kesin tanı için yetersiz kalmaktadır. Bu nedenle kesin tanı moleküler doğrulama ile tamamlanmalıdır.

4. SONUÇ

Çalışma sonucunda *P. graveolens* bitkisinin geniş bir endofitik fungus spektrumuna sahip olduğu görülmüştür. Bu verilerin, bitki-mikroorganizma etkileşimlerinin bitki sağlığı üzerindeki etkilerini inceleyecek gelecek araştırmalara ışık tutacağı değerlendirilmektedir.

Özellikle tespit edilen baskın türlerin, ıtır bitkisine özgü uçucu yağ bileşenlerinin sentezi üzerindeki etkilerinin araştırılması, tarımsal verimlilik ve endüstriyel üretim açısından yeni ufuklar açabilir. Gelecek çalışmalarda, bu endofitlerin moleküler tanımlamalarının yapılması, biyokontrol ajanları olarak kullanılması ve spesifik sekonder metabolit üretim kapasitelerinin *in vitro* koşullarda test edilmesi önerilmektedir.

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Ceren KAPLAN

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ÖZET

Dünyada en yaygın tüketilen içeceklerin başında gelen kahvenin üretim sürecinde açığa çıkan kullanılmış kahve atıkları (KKA), içerdikleri zengin biyoaktif bileşiklere rağmen genellikle çevresel kirlilik riski oluşturarak bertaraf edilmektedir. Ancak KKA, klorojenik asit ve kafein gibi biyolojik aktivitesi yüksek sekonder metabolitler açısından zengin bir kaynak teşkil etmektedir. Bu çalışma, sürdürülebilir bir atık yönetimi yaklaşımıyla KKA'dan elde edilen biyo-yağ ve etanol ekstraktlarının, tarımsal üretimde ciddi ekonomik kayıplara ve mikotoksin kirliliğine neden olan fitopatojenik funguslar (*Aspergillus flavus, Alternaria alternata* ve *Rhizopus oryzae*) üzerindeki antifungal etkilerini belirlemeyi amaçlamaktadır. Araştırma kapsamında, KKA'dan piroliz yöntemiyle biyo-yağ ve ultrasonik destekli ekstraksiyon yöntemiyle etanol özütü elde edilmiştir. Elde edilen bu ürünler, patojenlerin gelişimini test etmek amacıyla 500, 1000, 2000 ve 4000 ppm konsantrasyonlarında besi ortamlarına eklenmiştir. Yedi gün süren inkübasyon periyodu boyunca fungusların misel büyüme alanları dijital görüntüleme ve ImageJ yazılımı aracılığıyla hassas bir şekilde ölçülmüştür. Araştırma bulguları, KKA ekstraktlarının özellikle *A. alternata* üzerinde doz bağımlı ve güçlü bir inhibisyon etkisi gösterdiğini, 4000 ppm dozunda gelişim hızını yaklaşık %50-65 oranında baskıladığını ortaya koymuştur. Buna karşın, *A. flavus* ve *R. oryzae* gibi daha agresif türlerin bu ekstraktlara karşı daha dirençli olduğu ve hızlı bir adaptasyon evresine girerek petri kaplarını kısa sürede kapladığı gözlemlenmiştir. Sonuç olarak, kullanılmış kahve atıklarının belirli bitki patojenlerine karşı doğal ve ekonomik bir biyofungisit kaynağı olma potansiyeli taşıdığı, ancak daha geniş spektrumlu bir kontrol için farklı formülasyonların geliştirilmesi gerektiği sonucuna varılmıştır.

Anahtar Kelimeler : Kullanılmış kahve atıkları, Antifungal, *Aspergillus flavus, Alternaria alternata, Rhizopus oryzae*

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1. GİRİŞ

Kahve (*Coffea sp.*), dünyadaki en önemli tarımsal ürünlerden ve tüketilen en popüler içeceklerden biridir, ayrıca petrolün ardından küresel olarak en çok ticareti yapılan ikinci emtiadır. Kahve Rubiaceae familyasına aittir. Üç yaygın kahve türü Robusta, Arabica ve Liberica'dır. Dünya çapında üretilen kahvenin %75-80'i Arabica ve %20'si Robusta'dır.

Kahve, *Coffea* bitkisinin kavrulmuş tohumlarından hazırlanan, belirgin bir aroma ve tada sahip demlenmiş bir içecektir. Kahve, 850'si uçucu ve 700'ü çözünür olmak üzere 1500'den fazla kimyasal maddeden oluşur. Kahve suyla çözüldüğünde, yağlar, lipitler, trigliseritler ve yağ asitleri de dahil olmak üzere hidrofobik bileşiklerin çoğuyla, selüloz ve çeşitli sindirilemeyen şekerler gibi çözünmeyen karbonhidratlar telvede kalır. Kahvede ayrıca yapısal lignin, koruyucu fenolikler ve aroma üreten uçucu yağlar da bulunmaktadır (Padmapriya ve diğ., 2013).

Kullanılmış kahve atığı (KKA), hazır kahve kullanımının başlıca yan ürünlerinden biridir ve ekonomik değer eksikliği nedeniyle genellikle atık olarak atılırlar. KKAları bertaraf etmek için kullanılan geleneksel yöntemler arasında katı atık olarak doğrudan çöp sahasına ve kanalizasyona atılması yer alır. Ancak toksik yapısı ve organik bileşikleri nedeniyle çevre kirliliğine neden olabilir, sızan organik madde çevre ve insan sağlığını tehdit edebilir. Başka bir bertaraf yöntemi de yakmadır, ama yakma işlemiyle ortaya çıkan partikül maddenin çevredeki hava kalitesi üzerinde zararlı bir etkisi olabileceği için istenmemektedir. Bu yöntemler çevre için oldukça zararlıdır ve bu nedenle daha iyi bir KKA atık yönetimi gereklidir (Leow ve diğ., 2021).

KKA, değerlendirilmesini gerekli kılacak kadar çok miktarda organik bileşik (yani yağ asitleri, lignin, selüloz, hemiselüloz ve diğer polisakkaritler) içerir. Bazı araştırmacılar, KKA'ının çeşitli değerli bileşikler için biyolojik kaynak olarak değerlendirilip değerlendirilemeyeceğini araştırmıştır. Örnek olarak biyodizel üretiminde (Caetano ve diğ., 2012), şeker kaynağı olarak (Mussatto ve diğ., 2011), aktif karbon üretiminde (Kante ve diğ., 2012; Pappa ve diğ., 2012; Reffas ve diğ., 2010; Tsai ve diğ., 2012), kompost olarak (Preethu ve diğ., 2007) ve metal iyonlarının uzaklaştırılması için sorbent olarak (Fiol ve diğ., 2008; Oliveira ve diğ., 2008) kullanımı araştırılmıştır (Pujol et al., 2013).

Genel olarak KKA'lar, kahvenin türüne bağlı olarak %7 ila %15 ağırlık oranında kahve yağı içerir. Kahve yağı esas olarak monogliseritler, digliseritler, trigliseritler ve serbest yağ asitlerinden oluşur. Linoleik, palmitik, oleik ve stearik asitler yağ asitlerinde en sık bulunan kimyasallardır. Kahve yağının geri kalan bileşimi, mumlar, fosfatitler, tokoferoller, steroller ve diterpenler gibi sabunlaştırılamayan bileşiklere atfedilebilir. Kahve yağı ayrıca kozmetik veya gıda endüstrileri için uygun olan antioksidan aktivite de göstermektedir (Leow ve diğ., 2021).

KKA gibi karmaşık bir karışıma değer katmanın ilk adımı, kavurma işlemi sırasında kimyasal değişimler de dahil olmak üzere karışımda bulunan bileşiklerin anlaşılmasıdır. Tanımı gereği, kavrulmuş kahve tozundan KKA'da bulunan bileşikler, içeceğin üretimi sırasında çıkarılmamış bileşiklerdir. İçecek olarak hazırlanan kahvede düşük moleküler ağırlıklı biyoaktif bileşikler arasında kafein, klorojenik asitler (CGA), trigonellin, triptofan alkaloidleri ve kafestol ve kahweol gibi diterpenler bulunur. Ayrıca, sıcak demleme işlemleriyle üretilen bir içecek olarak

kahve, yetiştirme ve hasat sonrası koşullara göre değişen yaklaşık bin farklı uçucu organik bileşik içermektedir. Sıcak demleme kahvenin üretiminden sonra kalan KKA, %8-15 selüloz ve %30-40 hemiselüloz, %20-30 lignin, %7-21 lipid ve mineral ve %13-17 protein gibi makromolekülleri, fenolik bileşikleri (12 mg/g), kafeini (14,5 µg/g) ve CGA'yı (31,8 µg/g) içermektedir (Bevilacqua ve diğ., 2023).

Bitkileri korumak için sentetik fungisitler yaygın olarak kullanılmaktadır ancak direnç gelişimi ve çevresel endişeler nedeniyle bunların yerine alternatif, doğaya dost yeni fungisitler araştırılmaktadır. Şimdiye kadar kahvede bulunan biyoaktif maddelerden CGA, insan sağlığına yönelik çeşitli yararlı özellikler gösterdiğinden, örneğin glikoz ve lipit metabolizmasının düzenlenmesi, antikarsinojenik etkiler, kan basıncının düşürülmesi, bilişsel ve nöroprotektif etkiler nedeniyle esas olarak gıda bilimleriyle ilgili bir antioksidan olarak incelenmiştir. Bununla birlikte, CGA aynı zamanda antimikrobiyal aktivite de gösterebilir. Örnek olarak insan patojenik mayası *Candida albicans*'ın büyümesini engelleyebildiği bildirilmiştir (Sung ve Lee, 2010). Başka bir çalışmada gül yapraklarındaki külleme şiddetini azaltmıştır (Shetty ve diğ., 2011). Ayrıca, yüksek CGA seviyelerine sahip transgenik domates bitkileri, bakteriyel patojen *Pseudomonas syringae'*ye karşı gelişmiş direnç göstermektedir Niggeweg ve diğ., 2004). CGA, *in vitro* çalışmalarda geniş bir antifungal aktivite göstermiş, test edilen doza ve mantara bağlı olarak sporların çimlenmesinin tamamen engellenmesini veya hif uzunluğunun azalmasını sağlamıştır (Martínez ve diğ., 2017). Etken madde olarak yine CGA kullanılan bir çalışmada, *Botrytis cinerea* miselyumunun büyümesinin engellendiği ve 25 °C'de depolanan şeftali meyvesinde *B. cinerea*'nın lezyon çapının ve spor üretiminin etkili bir şekilde azaldığı bildirilmiştir. In vitro deneyler, CGA'nın mantar spor çimlenmesini, germ tüpü uzamasını, hücre canlılığını ve misel penetrasyonunu engellediğini göstermiştir (Dai ve diğ., 2024). Ayrıca, CGAdan başka bitkilerde kimyasal savunma için tanımlanan bir alkaloid olan kafein, antibakteriyel ve antifungal aktivitelere sahiptir (Mirón-Mérida et al., 2019).

Literatürde KKAlarından elde edilen ekstraktların antifungal etkisine dair bir çalışmaya rastlanmamıştır. Ancak içeriğindeki CGA ve kafeinden dolayı tarımsal ürünlerde önemli kayıplara neden olan farklı fitopatojenik fungusların büyümesini kontrol edip edemeyeceğinin araştırılmasının gerekli olduğu düşünülmüştür. Bu şekilde hem çevreye zararlı atıkların katma değeri yüksek ve çevreye olumlu katkı sağlayacak bir ürüne dönüştürülmesi, hem de sentetik fungisitlere bir alternatif geliştirilmesi öngörülmektedir.

Sonuç olarak kahve, dünyada en çok tüketilen içeceklerden biridir ve petrolden sonra en çok işlem gören ikinci emtiadır. Bununla birlikte, kahve endüstrisinin büyük miktarlarda ciddi çevresel sorunlara neden olan katı atık artıkları bulunmaktadır ve dolayısıyla sürdürülebilir çözümler geliştirmek için çalışmalara ihtiyaç vardır.

Bu projenin amacı KKAndan çıkarılmış kahve ekstraktının *in vitro* olarak çeşitli fitopatojenik küflere karşı antifungal etkinliğini göstermektir.

2. MALZEME ve YÖNTEMLER

2.1. Mikroorganizmalar ve büyüme koşulları

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Çalışmada fitopatojenik fungus olarak Kocaeli Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümü mikrobiyoloji laboratuvarından temin edilen *Aspergillus flavus, Alternaria alternata*, ve *Rhizopus oryzae* kullanılmıştır (Görsel 1). Büyüme ortamı olarak 90 mmlik steril plastik Petri kaplarında Potato Dextrose-Agar (PDA) hazırlanmıştır. PDA petri kaplarına aşılanmış funguslar 28 ºC inkübatörde yedi gün boyunca büyütülmüştür.

Görsel 1. Çalışmada kullanılan funguslar A) ***Aspergillus flavus*** **B)** ***Alternaria alternata*** **C)** ***Rhizopus oryzae***

2.2. KKAndan piroliz ve ekstraksiyon işlemleri

Lokal kahve dükkanlarından toplanan filtre kahve atıkları öncelikle laboratuvara getirilmiş ve 70 ºC de sabit tartım ağırlığına gelene kadar etüvde kurutulmuştur. Kurutulan kahve atıklarından piroliz yoluyla KKA yağı, etanol ekstraksiyonuyla KKA özütü elde edilmiştir. Ultrasonik destekli etanol ekstraksiyonu için önce kurutulmuş kahve atığı öğütülerek partikül boyutu küçültülmüştür. Çözücü olarak %70 (h/h) etanol karışımı hazırlanmıştır. 500 mg kahve atığı etanol içeren (1:15 g/ml katı:sıvı oranında) bir santrifüj tüpüne aktarılmış ve 90°C'de 45 dakika süreyle %75 güçte tutulmuştur. Daha sonra karışım 5000 rpm'de 10 dakika santrifüj edilmiştir. Santrifüjden sonra tüpteki supernatant etüvde 40°C - 45°C sıcaklıkta etanolü tamamen uçana kadar yoğunlaştırılmıştır (Görsel 2).

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Görsel 2. Ultrasonik destekli etanol ekstraksiyon işlemi basamakları A. Kahve atığı ile %70 (h/h) etanolün karıştırılması B) Ultrasonik banyoda inkübasyon C) 5000 rpmde santrifüjleme D) Supernatantın ayrılması E) Etüvde supernatanttan etanolün uzaklaştırılması F) Antifungal etki testlerinde kullanılan özüt

2.3. Antifungal Aktivite Denemeleri

Piroliz ve ultrasonik destekli etanol ekstraksiyon sonucu elde edilen yağ ve özüt son konsantrasyonları 500, 1000, 2000 ve 4000 ppm olacak şekilde PDA ortamına eklenmiştir. *A. flavus* ve *A. alternata* için yedi günlük fitopatojenik kültürlerden alınan misel diskleri (çapı 6 mm) bu ortamla doldurulmuş Petri plaklara uygulama ve konsantrasyon başına üç plak olacak

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şekilde aktarılmıştır. *R. oryzae* için ise hazırlanmış spor süspansiyonu kullanılmıştır. Negatif kontrol olarak sadece PDA ortamı içeren plaklar kullanılmıştır (Görsel 3).

Görsel 3. Her bir doz (Kontrol, 500, 1000, 2000 ve 4000 ppm) için hazırlanmış PDA ortamları B) Mantar delici ile *A. flavus ve A. alternata* inokülasyonu C) Spor süspansiyonundan *R. oryzae* inokulasyonu D) İnokülasyondan hemen sonra kültür ortamları

Ayrıca sadece kurutulmuş kahve atığı da PDA ortamına son konsantrasyonları 1000, 2000 ve 4000 ppm olacak şekilde eklenmiş, ortamdaki kahve atığının doğrudan etkisi ölçülmüştür (Görsel 4).

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Görsel 4. Farklı dozlarda hazırlanmış KKA+PDA ortamları

Yedi gün, 28 ºC'de ve karanlıkta inkübe edilen kültürler için miselyal büyüme, her tekrar için büyüme alanının hesaplanmasıyla belirlenmiş ve sonuçlar % büyüme olarak verilmiştir. Büyüme alanı ölçümleri ImageJ programı ile yapılmıştır. Her bir doz ve zaman aralığı için misel yayılım alanı, toplam petri alanına oranlanarak normalize edilmiştir.

Tüm deneysel prosedürler üç kez yürütülmüş ve sonuçlar üç tekrarın ortalaması olarak verilmiştir.

3. BULGULAR ve TARTIŞMA

3.1. KKA Yağının Fitopatojenik Funguslar Üzerindeki Etkisi

A. alternata kültürlerine ait *in vitro* fotoğraflar (Görsel 5) ve bu fotoğraflardan elde edilen büyüme yüzdesi grafiği (Görsel 6) birlikte değerlendirildiğinde, KKA yağının fungus üzerinde doz-bağımlı ve zamana yayılmış bir etki mekanizmasına sahip olduğu görülmektedir. Fotoğraflarda 22. saatten 168. saate kadar olan gelişim süreci izlendiğinde, özellikle 2000 ve 4000 ppm dozlarında kolonilerin merkezde kısıtlı kaldığı ve misel yoğunluğunun kontrol grubuna göre daha düşük olduğu gözlemlenmektedir.

Grafik verileriyle bu durum nicel olarak desteklenmiş; en yüksek doz olan 4000 ppm uygulamasında büyüme hızının 168. saat sonunda kontrol grubuna kıyasla yaklaşık yarı yarıya baskılandığı tespit edilmiştir. Denemede dikkat çeken en önemli bulgu, 500 ppm dozunda büyüme eğrisinin kontrol grubunun üzerine çıkmasıdır. Fotoğraflarda da (özellikle 93. ve 168. saatlerde) görülebilen bu durum, düşük dozlardaki KKA yağının fungus üzerinde baskılayıcı bir etki yerine, gelişimi tetikleyen bir etki yarattığına işaret etmektedir. Ancak 1000 ppm konsantrasyonu bir "eşik değer" görevi görmüş ve bu dozdan itibaren fungusun yayılım alanı düşüş göstermeye başlamıştır. Sonuç olarak, KKA yağının *A. alternata* gelişimini tamamen durdurmasa da, yüksek dozlarda inkübasyon süresi boyunca gelişimi önemli ölçüde geciktirdiği ve morfolojik olgunlaşmayı yavaşlattığı sonucuna varılmıştır.

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Görsel 5. *A. alternata*'nın PDA ortamında farklı KKA yağı konsantrasyonları (ppm) ve zaman aralıklarında (saat) misel gelişimini gösteren fotoğraflar

Görsel 6. *A. alternata*'nın PDA ortamında farklı KKA yağı konsantrasyonlarında zamana bağlı koloni alanı (%) değişimi

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A. flavus'a ait *in vitro* gelişim sürecini (Görsel 7) ve doz-zaman etkileşimini (Görsel 8) gösteren bulgular incelendiğinde, KKA yağının fungusun yayılım hızı ve koloni morfolojisi üzerinde dinamik bir etkisi olduğu gözlemlenmektedir. Fotoğraflarda, özellikle ilk 45 saatlik süreçte tüm dozlarda gelişimin benzer bir lag fazında olduğu görülse de, 70. saatten itibaren büyüme oranlarında keskin bir artış ve farklılaşma meydana gelmiştir. Grafik verileriyle paralel olarak, düşük ve orta dozların (500, 1000 ve 4000 ppm) gelişim yüzdesi bakımından kontrol grubunun üzerine çıkması, kullanılan KKA yağının bu konsantrasyonlarda *A. flavus* üzerinde baskılayıcı bir etki oluşturamadığını, aksine fungusun metabolik aktivitesini veya yüzey yayılımını tetikleyen bir etki yarattığını kanıtlamaktadır. 168. saat sonunda kontrol dışındaki tüm grupların %100 büyüme oranına ulaşması, KKA yağının bu dozlarda fungisidal bir özellik göstermediğini; ancak 2000 ppm dozunda gözlemlenen nispeten yavaş gelişim eğrisinin, bu konsantrasyonun fungusun adaptasyon sürecini diğer dozlara göre daha fazla zorladığını ortaya koymaktadır.

Görsel 7. *A. flavus*'un PDA ortamında farklı KKA yağı konsantrasyonları (ppm) ve zaman aralıklarında (saat) misel gelişimini gösteren fotoğraflar

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Görsel 8. *A. flavus*'un PDA ortamında farklı KKA yağı konsantrasyonlarında zamana bağlı koloni alanı (%) değişimi

R. oryzae fungusuna ait gelişim süreci incelendiğinde, türün karakteristik özelliği olan son derece agresif ve hızlı yayılım kapasitesi hem fotoğraflarda (Görsel 9) hem de grafik verilerinde (Görsel 10) açıkça görülmektedir. Diğer fungus türlerinin aksine, *R. oryzae* tüm doz gruplarında (kontrol dahil) 45. saat gibi çok erken bir evrede petri kabının tamamını (%100 büyüme yüzdesi) kaplamıştır. Denemenin ilk 22 saatlik diliminde doz artışına bağlı olarak kısmi bir inhibisyon belirtisi (özellikle 4000 ppm dozunda büyümenin %40'ın altında kalması) saptanmış olsa da, bu baskılayıcı etkinin fungusun hızlı hif gelişimi karşısında kalıcı olamadığı anlaşılmaktadır. 45. saatten itibaren tüm dozların grafik üzerinde tek bir hat halini alarak maksimuma ulaşması, kullanılan KKA yağının bu konsantrasyonlarda *R. oryzae* üzerinde ne fungisidal ne de uzun süreli fungistatik bir etki yaratabildiğini; bu hızlı gelişen patojene karşı daha yüksek dozajlara veya farklı uygulama stratejilerine ihtiyaç duyulduğunu ortaya koymaktadır.

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Görsel 9. *R. oryzae*'nin PDA ortamında farklı KKA yağı konsantrasyonları (ppm) ve zaman aralıklarında (saat) misel gelişimini gösteren fotoğraflar

Görsel 10. *R. oryzae*'nin PDA ortamında farklı KKA yağı konsantrasyonlarında zamana bağlı koloni alanı (%) değişimi

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3.2. Etanol Ekstraksiyon Özütünün Antifungal Potansiyeli

A. alternata türünün farklı konsantrasyonlardaki etanol ekstraksiyon özütüne karşı sergilediği büyüme performansı incelendiğinde, denemenin son aşamasına kadar (168. saat) belirgin bir doz-yanıt ilişkisinin şekillendiği görülmektedir. Fotoğraflarda, özellikle 96. saatten itibaren kontrol grubunun diğer tüm dozlara göre daha geniş ve homojen bir yayılım sergilediği, yüksek dozlarda ise koloni çapının kısıtlandığı net bir şekilde izlenebilmektedir (Görsel 11). Grafik verileriyle desteklenen bu bulgulara göre; kontrol grubu 168. saat sonunda yaklaşık %65'lik bir büyüme oranına ulaşarak en yüksek yayılımı gösterirken, 2000 ve 4000 ppm gibi yüksek dozlar fungus gelişimini %40 seviyelerinin altında tutarak fungistatik etki yaratmıştır (Görsel 12). Denemede dikkat çeken bir diğer nokta ise, 1000 ppm dozunun 96. saate kadar kontrol grubuyla yarışır düzeyde bir gelişim göstermesi, ancak son okuma zamanında kontrolün altına düşmesidir; bu durum etanol ekstraksiyon özütünün etkisini tam olarak gösterebilmesi için zamana bağlı bir eşik değerinin aşılması gerektiğini ve yüksek konsantrasyonların *A. alternata* üzerinde uzun süreli, kalıcı bir baskılama sağladığını ortaya koymaktadır.

Ayrıca inkübasyon süreci boyunca fungus kolonilerinde gözlemlenen renk değişimleri ve dokusal farklılıklar, etanol ekstraksiyon özütünün sporlanma süreci üzerindeki baskılayıcı etkisini açıkça ortaya koymaktadır. Kontrol ve düşük doz (500 ppm) gruplarında, inkübasyonun ilerleyen saatlerinde koloni merkezinden dışarıya doğru yayılan yoğun koyu renkli pigmentasyon, fungusun sağlıklı bir şekilde generatif faza geçtiğini ve yoğun spor üretimine başladığını göstermektedir. Buna karşın, 2000 ve 4000 ppm gibi yüksek dozlarda kolonilerin sadece yayılım alanının kısıtlanmadığı, aynı zamanda renklerinin kontrol grubuna kıyasla daha açık tonlarda kaldığı saptanmıştır. Bu durum, etken maddenin hif gelişimini baskılamasının yanı sıra, fungusun üreme yapılarının olgunlaşmasını ve spor verimini de önemli ölçüde geciktirdiğine veya inhibe ettiğine işaret etmektedir.

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Görsel 11. *A. alternata*'nın PDA ortamında farklı etanol ekstraksiyon özütü konsantrasyonları (ppm) ve zaman aralıklarında (saat) misel gelişimini gösteren fotoğraflar

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Görsel 12. *A. alternata*'nın PDA ortamında farklı etanol ekstraksiyon özütü konsantrasyonlarında zamana bağlı koloni alanı (%) değişimi

A. flavus'un *in vitro* gelişim süreci incelendiğinde, fungusun agresif yayılım karakterinin tüm doz gruplarında baskın olduğu ve etanol ekstraksiyon özütünün inhibisyon etkisinin oldukça sınırlı kaldığı görülmektedir. Fotoğraflarda 23. saatten itibaren başlayan hif gelişimi, 48. saatte kontrol grubunda %80'e ulaşırken, 500-4000 ppm arası dozlarda gelişimin %50-65 bandında kalarak kısmen baskılandığı gözlemlenmiştir (Görsel 13). Ancak grafik verileriyle paralel olarak, 71. saatten itibaren tüm doz gruplarının hızlı bir adaptasyon evresine girdiği ve 96. saat itibarıyla kontrol grubuyla benzer şekilde petri kabının tamamını kapladığı (%100 doluluk) saptanmıştır (Görsel 14). 168. saat sonunda tüm petri kaplarında gözlenen yoğun koyu yeşil/kahverengi pigmentasyon, fungusun sadece vejetatif büyümesini tamamlamakla kalmayıp, tüm konsantrasyonlarda yüksek oranda sporlanma kapasitesine ulaştığını kanıtlamaktadır. Bu veriler ışığında, etanol ekstraksiyon özütünün mevcut dozlarda *A. flavus* üzerinde kalıcı bir fungistatik etki yaratamadığı, fungusun yüksek adaptasyon hızıyla kimyasal baskıyı kısa sürede tolere ettiği sonucuna varılmıştır.

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Görsel 13. *A. flavus*'un PDA ortamında farklı etanol ekstraksiyon özütü konsantrasyonları (ppm) ve zaman aralıklarında (saat) misel gelişimini gösteren fotoğraflar

Görsel 14. *A. flavus*'un PDA ortamında farklı etanol ekstraksiyon özütü konsantrasyonlarında zamana bağlı koloni alanı (%) değişimi

R. oryzae fungusuna ait *in vitro* gelişim süreci değerlendirildiğinde, türün karakteristik özelliği olan son derece agresif ve hızlı yayılım kapasitesi hem görsel hem de sayısal verilerde net bir şekilde karşımıza çıkmaktadır. Fotoğraflarda, denemenin ilk 23 saatlik diliminde doz artışına bağlı olarak (özellikle 4000 ppm dozunda) kısmi bir gelişim yavaşlaması saptanmış olsa da, bu baskılayıcı etkinin patojenin yüksek hif büyüme hızı karşısında kalıcı olamadığı görülmektedir (Görsel 15). Grafik verileriyle uyumlu olarak, 48. saat itibarıyla kontrol grubu dahil tüm dozların petri kabının tamamını kaplayarak %100 oranına ulaştığı tespit edilmiştir (Görsel 16). 48. saatten 168. saate kadar olan süreçte büyüme eğrilerinin tek bir hat üzerinde doygunluğa ulaşması, etanol ekstraksiyon özütünün bu konsantrasyonlarda *R. oryzae* üzerinde ne fungisidal ne de uzun süreli fungistatik bir etki yaratabildiğini göstermektedir. Bu bulgular, bu patojene karşı daha yüksek dozajların veya farklı uygulama stratejilerinin geliştirilmesinin zorunlu olduğunu ortaya koymaktadır.

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Görsel 15. *R. oryzae*'nin PDA ortamında farklı etanol ekstraksiyon özütü konsantrasyonları (ppm) ve zaman aralıklarında (saat) misel gelişimini gösteren fotoğraflar

Görsel 16. *R. oryzae*'nin PDA ortamında farklı etanol ekstraksiyon özütü konsantrasyonlarında zamana bağlı koloni alanı (%) değişimi

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3.3. Kahve Atığının Doğrudan Etkisi

R.oryzae fungusuna karşı kahve atığının kendisi kullanılarak gerçekleştirilen denemede, patojenin karakteristik hızı ile kahve atığının kendisinin erken evre baskılama gücü arasındaki denge hem görsel hem de sayısal verilerde net bir şekilde görülmektedir. Fotoğraflarda 25. saat sonundaki ilk gözlemlerde, özellikle 2000 ve 4000 ppm dozlarında koloni yayılımının kontrol grubuna göre oldukça kısıtlı kaldığı ve kahve atığının kendisinin başlangıçta güçlü bir bariyer oluşturduğu saptanmıştır (Görsel 17). Grafik verileriyle paralel olarak, kontrol grubu 45. saatte petri kabının tamamını kaplarken, kahve atığının kendisi 4000 ppm dozunda aynı zaman diliminde gelişimi %20'ler seviyesinde tutmayı başararak belirgin bir fungistatik üstünlük sergilemiştir (Görsel 18). Ancak 45. saatten sonra fungusun kahve atığının kendisine karşı hızlı bir adaptasyon geliştirdiği ve hif yayılımının ivme kazanarak 118. saat itibarıyla tüm dozlarda %100 büyüme sağladığı gözlemlenmiştir. Bu bulgular, kahve atığının kendisinin *R. oryzae* üzerinde başlangıç fazında çok etkili bir geciktirme sağladığını, fakat uzun süreli koruma için uygulama dozajının veya formülasyon stabilitesinin artırılması gerektiğini ortaya koymaktadır.

Görsel 17. *R. oryzae'*nin PDA ortamında farklı kahve atığı konsantrasyonları (ppm) ve zaman aralıklarında (saat) misel gelişimini gösteren fotoğraflar

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Görsel 18. *R. oryzae*'nin PDA ortamında farklı kahve atığı konsantrasyonlarında zamana bağlı koloni alanı (%) değişimi

Bu çalışmada, kullanılmış kahve atıklarından (KKA) elde edilen biyo-yağ ve etanol ekstraktlarının farklı fitopatojenik funguslar üzerindeki etkisi incelenmiş; elde edilen veriler KKA'nın fungus türüne ve dozajına bağlı olarak değişen bir antifungal potansiyele sahip olduğunu göstermiştir. Literatürde kahve atıklarının içeriğindeki kafein, klorojenik asit ve çeşitli fenolik bileşiklerin mikroorganizmalar üzerinde baskılayıcı etkileri olduğu bilinmektedir. Özellikle etanol ekstraktının *A. alternata* gelişimini yüksek dozlarda (2000-4000 ppm) %40 ile %65 arasında bir oranla kısıtlaması, bu bileşiklerin hif gelişimi üzerindeki inhibitör etkisini destekler niteliktedir. Martínez ve diğ. (2017) tarafından yapılan çalışmada klorojenik asidin birçok fitopatojene karşı fungisit etkisi olduğu bildirilmiş olup, bu durum çalışmamızdaki etanol ekstraktının başarısını açıklayabilir.

Denemede en dikkat çekici bulgulardan biri, fungus türlerinin KKAya karşı gösterdiği farklı adaptasyon hızlarıdır. *R. oryzae* ve *A. flavus* türlerinde, özellikle uygulamanın ilk 24-48 saatlik diliminde gözlenen kısmi gelişim yavaşlaması, ilerleyen saatlerde yerini hızlı bir yayılıma bırakmıştır. Bu durum, söz konusu patojenlerin yüksek metabolik hızları ve stres koşullarına karşı geliştirdikleri hızlı adaptasyon yeteneği ile açıklanabilir. Özellikle *R. oryzae* gibi agresif türlerin, ortamdaki antifungal bileşikleri kısa sürede tolere edebildiği veya bu bileşikleri metabolize ederek etkisiz hale getirebildiği düşünülmektedir. Bu bulgu, literatürdeki KKA ekstraktlarının bazı türler üzerinde düşük dozlarda yetersiz kalabileceğine dair görüşlerle örtüşmektedir.

Çalışmada gözlemlenen bir diğer önemli nokta ise düşük konsantrasyonlarda (özellikle 500 ppm) görülen etkidir. Bazı doz gruplarında fungus gelişiminin kontrol grubunun bile üzerine çıkması, düşük düzeydeki kimyasal stresin fungal büyümeyi baskılamak yerine tetiklediğine işaret etmektedir. Bu durum, biyokontrol çalışmalarında dozaj optimizasyonunun ne kadar kritik olduğunu bir kez daha göstermiştir. Yüksek dozlarda ise sadece yayılım alanının değil, koloni pigmentasyonunun da (sporlanma kapasitesinin) azalması, KKAnın hem vejetatif hem de generatif fazlar üzerinde etkili olduğunu göstermektedir.

4. SONUÇ

Bu çalışma, kahve endüstrisinin ana atık ürünü olan kullanılmış kahve atıklarının tarımsal üretimde ekonomik kayıplara yol açan fitopatojenik funguslara karşı doğal bir biyofungisit olarak kullanılabilirliğini ortaya koymuştur. Çalışmanın bulguları, KKA'dan elde edilen biyo-yağ ve etanol ekstraktlarının antifungal etkisinin fungus türüne ve uygulama dozuna bağlı olarak değiştiğini göstermiştir.

Sonuç olarak, çalışmadan çıkarılan büyüme eğrileri, KKAnın bitki patojenlerine karşı sürdürülebilir ve doğa dostu bir mücadele aracı olma potansiyelini ortaya koymaktadır. Ancak *Aspergillus* ve *Rhizopus* gibi dirençli türler üzerinde tam bir başarı sağlanabilmesi için, ekstrakt içeriğinin zenginleştirilmesi veya daha yüksek konsantrasyonların test edilmesi gerekliliği ortaya çıkmıştır. KKA'nın bir atık olmaktan çıkarılıp biyofungisit olarak ekonomiye kazandırılması, hem tarımsal mücadele maliyetlerini düşürecek hem de çevre kirliliğinin azaltılmasına katkı sağlayacaktır.

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In Vitro Antioxidant Activity of Methanolic Extract Obtained from *Citrullus lanatus* ((Thunb.) Matsum. & Nakai) (Watermelon) Fruit

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The fruit of *C. lanatus* (Thunb.) Matsum. & Nakai, or watermelon, is widely consumed and has antioxidant and medicinal qualities along with some phytochemical substances. The purpose of this study was to ascertain the amount of total phenolic/flavonoid chemicals and free radical scavenging capabilities in the methanolic extract made from C. lanatus fruit. Spectrophotometric techniques were used to measure the levels of DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), and total flavonoid/phenolic.

The total phenolic content in *C. lanatus* fruit was found to be 998.14 ± 1.26 (µg GAE/g) in 1 g of fruit sample, based on the gallic acid calibration curve. The total flavonoid content was determined to be 213.00 ± 0.76 (µg KE/g) per 1 g of fruit sample based on the catechin calibration curve.

The DPPH free radical scavenging activities were calculated as a percentage using 50 µL, 100 µL, 200 µL, 400 µL, 800 µL, and 1000 µL of methanol extracts obtained from the fruit of *C. lanatus*. The highest percentage value was found as 90.57 ± 1.44 in the 200 µL sample ($p < 0.001$). The ABTS free radical scavenging activities were calculated as a percentage using 50 µL, 100 µL, 200 µL, 400 µL, 800 µL, and 1000 µL of methanol extracts obtained from the fruit of *C. lanatus*. The highest percentage value was found as 86.22 ± 0.11 in the 50 µL sample ($p < 0.001$).

The research claims that eating *C. lanatus* fruit is linked to a lower incidence of a number of ailments. According to the study's results, the *C. lanatus* fruit extract has a very high antioxidant capacity when assessed as a whole total phenolic/flavonoid content and free radical scavenging activities. In conclusion, it is expected that fruit extract from *C. lanatus* may be a significant source of antioxidants for stopping or reducing the advancement of metabolic processes linked to oxidative stress.

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Keywords: *C. lanatus*, Antioxidant activity, Total phenolic, Total flavonoid, DPPH, ABTS

1. INTRODUCTION

Citrullus lanatus, also known as watermelon, is a fruit in the Cucurbitaceae family that is well-known worldwide for both its refreshing qualities and its numerous therapeutic and antioxidant applications [1,2]. Eating the bioactive chemicals of the watermelon plant has been demonstrated to significantly lower the incidence of chronic diseases such type 2 diabetes, cardiovascular disorders, and several types of cancer in addition to being a popular fruit used in sweets [1]. Watermelon contains a phytochemical mechanism that supports health due to its high lycopene concentration, vitamins, flavonoids, and phenolic components. These elements in watermelon serve as strong natural antioxidants that protect cells from oxidative damage and efficiently scavenge free radicals [3].

For the evaluation of substances in culinary, pharmaceutical, and biomedical research, accurate antioxidant activity assessment is essential. Due to their ease of use, spectrophotometric analysis techniques like DPPH and ABTS are frequently employed [4]. These methods are based on the mechanism by which antioxidant compounds neutralize free radicals. Compared to the DPPH approach, the ABTS method is more useful because it offers a wide range of applications for the identification of both hydrophilic and lipophilic molecules [5]. Studies have shown that the rind, which is the outer part of the watermelon, possesses an antioxidant capacity similar to or higher than that of the flesh [1].

In this context, watermelon plays a significant role in healthy eating habits, not only because it is an extremely nutritious fruit but also due to its rich content of bioactive compounds. Current literature indicates that chronic diseases are largely associated with oxidative stress and inflammation, and that the consumption of foods with antioxidant and anti-inflammatory properties plays a critical role in preventing these processes [6]. According to this theory, watermelon's bioactive elements—such as amino acids, carotenoids, and phenolic compounds—help scavenge free radicals. They also show a preventive impact by lowering the chance of developing chronic illnesses.

Another important feature of watermelon is that not only its edible parts but also its byproducts, such as the rind and seeds, contain significant amounts of bioactive compounds. These byproducts are rich in phenolics, flavonoids, carotenoids, and various phytochemicals and possess high antioxidant capacity [7]. Specifically, in the context of food industry waste management, it has been noted in recent years that byproducts like watermelon rind can be used as a natural source of antioxidants and offer tremendous promise in techniques for developing functional foods.

Furthermore, watermelon is a nutritive and health-promoting functional food due to its high water content, low energy density, and rich vitamin and mineral profile. Additionally, it has been demonstrated that the bioactive molecules it contains, especially lycopene, have preventive benefits against metabolic disorders, certain types of cancer, and cardiovascular diseases [6]. Because of these qualities, watermelon is a significant topic of study for both everyday nutrition and the creation of functional foods and nutraceuticals.

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2. MATERIALS AND METHODS

2.1. Preparation of the Methanolic Watermelon Extract

600 mL of methanol was poured to 70 g of watermelon pulp. An evaporator was used to evaporate the mixture. The DPPH, ABTS, total phenolic, and flavonoid contents were then measured after it was dissolved in 40 milliliters of DMSO (dimethyl sulfoxide). Methanolic watermelon extract (100 µL) was employed for Total Phenolics and Flavonoids. 50 µL, 100 µL, 200 µL, 400 µL, 800 µL, and 1000 µL of methanolic watermelon extract were employed for DPPH and ABTS. Every measurement was carried out using a spectrophotometer in accordance with the guidelines.

2.2. DPPH (Free radical scavenging activity)

Free radical scavenging activity (DPPH) was determined according to the method described in the literature [8]. 25 µg/L solution of α,α-diphenyl-β-picrylhydrazine (DPPH) was prepared by dissolving it in methanol g.

Test tubes were filled with 4 milliliters of the DPPH solution made with methanol. The test tubes were then filled with 50 µL, 100 µL, 200 µL, 400 µL, 800 µL, and 1000 µL amounts of watermelon extracts containing methanol in triplicate and vortexed. For thirty minutes, these were incubated in the dark at room temperature. After the incubation period was over, absorbance values were measured at 517 nm using a spectrophotometer in comparison to a blank methanol sample that included no material. The following formula was utilized to determine the degree to which the watermelon extract containing methanol scavenged (inhibited) DPPH free radicals based on the drop in absorbance values in order to assess the antioxidant potential of the extract:

%Inhibition = [(Control ABS - Sample ABS) / Control ABS] × 100

2.3. ABTS (ABTS• Radical Scavenging Activity)

Watermelon samples treated with methanol were subjected to the ABTS• radical scavenging activity assay in accordance with the prescribed procedures [9,10]. To reach a final concentration of 7 mM, 2.45 mM potassium persulfate ($K_2S_2O_4$) was mixed with 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The solution was left at room temperature in the dark for 12 to 16 hours. The ABTS• radical cation solution, which was made after potassium persulfate oxidized ABTS, was diluted with phosphate buffer to achieve an absorbance of 0.900

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at a wavelength of 734 nm. Next, 50, 100, 200, 400, 800, and 1000 µL of watermelon extract treated with methanol were combined with 4 mL of the ABTS• radical cation solution, and the combination was left in the dark for 30 minutes. A wavelength of 734 nm was then used to test absorbances.

The number of ABTS• radicals scavenged by the samples was calculated using the following formula:

%Inhibition = [(Control ABS - Sample ABS) / Control ABS] × 100

2.4. Total Phenolic Compound Content

The method outlined in the literature [11] was used to calculate the total amount of phenolic compounds in the watermelon fruit extract. The standard was a calibration curve for gallic acid. Five duplicates of 100 µL of watermelon extract with methanol were added to test tubes. The Folin-Ciocalteu reagent (0.5 mL) was then added. Three milliliters of a 2% Na_2CO_3 solution were added to the samples after three minutes. The samples were vortexed and then left in the dark for two hours. A spectrophotometer was used to record the samples' absorbance readings at a wavelength of 760 nm at the conclusion of the incubation period. Gallic acid equivalents per gram (µg GAE/g) were used to determine the results.

2.5. Total Flavonoid Content

The method outlined in the literature was used to calculate the total amount of flavonoid compounds [12]. Five duplicate test tubes were filled with 100 µL of methanol and watermelon extract. After adding the liquid to 0.3 mL of 5% sodium nitrite ($NaNO_2$), it was let to stand for five minutes. 0.3 milliliters of 10% aluminum chloride ($AlCl_3$) were then added. After adding 2.4 mL of distilled water and 2 mL of 1 M sodium hydroxide (NaOH), the liquid was vortexed. A spectrophotometer set to 510 nm was used to measure the samples' absorbance values. The data was then used to compute catechin equivalents in milligrams per gram (µg CE/g).

2.6. Statistical Analysis

The SPSS 26 software program was used to analyze the data statistically. To ascertain group differences, ANOVA and LSD tests were employed. $p < 0.05$ was chosen as the threshold for statistical significance. The data is displayed as mean ± standard error.

3. RESULTS AND DISCUSSION

3.1. Total Phenolic and Total Flavonoid Content of the Methanolic *Citrullus lanatus* Extract

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Table 1. Total phenolic and total flavonoid content of *Citrullus lanatus* extract

Total Phenolic Compounds and Total Flavonoids	**Value**
Total Phenolic (µg GAE/g)	998,14 ± 1,26
Total Flavonoids (µg CE/g)	213,00 ± 0,76

Figure 1. Total phenolic and total flavonoid contents (%) of the methanolic *Citrullus lanatus* extract

When the methanol extracts of the watermelon fruit used in the study were evaluated for total phenolic and total flavonoid content, the total phenolic (µg GAE/g) value was calculated as 998.14 ± 1.26 in a 100 µL sample ($p<0.001$). The Total Flavonoid (µg CE/g) value was calculated as 213.00 ± 0.76 in a 100 µL sample ($p<0.001$). These high phenolic and flavonoid contents are the primary determinants of antioxidant activity and are consistent with studies reported in the literature. It has been reported that not only the edible part of watermelon but also other components such as the rind and seeds possess high phenolic content and antioxidant capacity [13].

3.2. DPPH Radical Scavenging Activity of Methanolic *Citrullus lanatus* Extract

Table 2. DPPH radical scavenging activity (%)

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DPPH Activity	**Value**
50 µL	61,63 ± 1,21
100 µL	78,32 ± 1,09
200 µL	**90,57 ± 1,44[d]**
400 µL	76,73 ± 1,55
800 µL	77,08 ± 0,07
1000 µL	78,40 ± 0,85

d: $p<0.001$

When the methanolic watermelon fruit extracts used in the study were evaluated for their DPPH radical scavenging activity, the highest value of 90.57 ± 1.44 was obtained at a concentration of 200 µL, compared to the concentrations of other watermelon fruit extract samples ($p<0.001$). The literature reports that antioxidant activity increases with concentration [14]. In this study, it was observed that antioxidant activity showed a gradual increase with concentration up to 50, 100, and 200 µL.

Figure 2. DPPH radical scavenging activity (%) of methanolic *Citrullus lanatus* extract at different concentrations

3.3. ABTS (DMSO) Radical Scavenging Activity of Methanolic *Citrullus lanatus* Extract

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Table 3. ABTS radical scavenging activity (%)

ABTS Activity	**Value**
50 µL	**86,22 ± 0,11[d]**
100 µL	85,89 ± 0,11
200 µL	85,56 ± 0,11
400 µL	85,23 ± 0,11
800 µL	84,90 ± 0,11
1000 µL	84,56 ± 0,11

d: $p<0.001$

When the methanol-based watermelon fruit extracts used in the study were evaluated for their ABTS radical scavenging activity, the highest value of 86.22 ± 0.11 was obtained at a concentration of 50 µL, compared to the concentrations of other watermelon fruit extract samples ($p<0.001$). These results regarding ABTS radical scavenging activity demonstrate that the watermelon extract exhibits its strongest antioxidant capacity at a concentration of 50 µL.

Figure 3. Percentage distribution of ABTS radical scavenging activity of methanolic *Citrullus lanatus* extract at different concentrations (%)

4. GENERAL EVALUATION AND CONCLUSIONS

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It was observed that samples extracted from watermelon fruit using methanol exhibited antioxidant properties against DPPH and ABTS radicals, which possess free radical characteristics, when they reacted with these radicals. Data presented in the literature indicate that different temperatures and solvent types (pH and polarity) during the pre-extraction process may affect the phenolic content and free radical scavenging activity levels of the watermelon rind [15]. In our study, 70 g of watermelon pulp was taken, methanol was added, it was evaporated in an evaporator, and then dissolved in DMSO (Dimethyl Sulfoxide). The aim of this study was to determine DPPH, ABTS, total phenolic, and flavonoid content; additionally, the types of solvents used in the study are important for evaluating antioxidant capacity [15].

The findings obtained in this study indicate that C. lanatus extracts possess a high level of antioxidant capacity. The results of the DPPH and ABTS radical scavenging assays revealed an increasing free radical scavenging effect depending on the extract concentration. This finding is consistent with studies reporting that the efficacy of plant-derived antioxidants increases with higher doses [14].

The phenolic compounds, flavonoids, carotenoids (particularly lycopene), and other phytochemicals found in watermelon are the primary source of this antioxidant activity. Recent studies have shown that not only the flesh of watermelon but also other components such as the rind and seeds exhibit high levels of phenolic content and antioxidant capacity [13]. This finding is consistent with the total phenolic and flavonoid amounts obtained in our study and, when evaluated in detail, demonstrates that watermelon is an important source of bioactive compounds.

Reactive oxygen species (ROS) buildup in cells causes oxidative stress, a pathological process that results in serious metabolic damage like protein oxidation, DNA damage, and lipid peroxidation. Numerous chronic illnesses are caused by this syndrome, including cancer, diabetes, heart disease, and neurological conditions. Eating watermelon reduces this type of oxidative damage since it has antioxidant qualities. Experiments using watermelon juice or pulp have shown increased levels of antioxidant enzymes such as SOD, catalase, and glutathione as well as a reduction in the harmful effects of oxidative stress [16].

In addition, watermelon consumption reduces lipid peroxidation and radiation-induced oxidative damage. The literature also indicates that watermelon strengthens the cellular antioxidant defense system [17]. When these findings are evaluated in conjunction with the high radical scavenging activity observed in our study, it becomes evident that watermelon may exhibit a protective effect not only in vitro but also in vivo. Furthermore, citrulline, vitamin C, and other antioxidant components found in watermelon not only reduce inflammation by maintaining oxidative balance at the cellular level but also support vascular health. In this regard, eating watermelon is acknowledged as a crucial dietary component, especially in the prevention of oxidative stress-related disorders and the maintenance of general health. Overall, the study's findings show that watermelon extract has a strong antioxidant activity and is capable of successfully scavenging free radicals. According to the research, eating watermelon

on a regular basis may help avoid chronic illnesses by lowering oxidative stress. A more thorough grasp of watermelon's potential in the domains of functional foods and nutraceuticals will result from more research contrasting different extraction techniques and watermelon components.

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Investigation of the Cytotoxicity of *Geastrum fimbriatum* and *Geastrum rufescens* Species

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ABSTRACT

This study aims to investigate the in vitro cytotoxic effects of ethanol extracts obtained from two fungi species of the Geastraceae family, *Geastrum fimbriatum* and *Geastrum rufescens*, on the L929 fibroblast cell line. Medicinal mushrooms have attracted significant attention in recent years due to their bioactive components, such as β-glucans, phenolic compounds, polysaccharides, and triterpenes. These compounds are known to possess anticancer and immunomodulatory properties. The increasing pharmacological interest in medicinal mushrooms, driven by their bioactive constituents, forms the primary rationale for this study. The limited data available in the literature regarding the cytotoxic effects of *Geastrum* species further highlights the significance of this research.

Fungal samples were subjected to ethanol extraction using the Soxhlet method to obtain crude extracts. The cytotoxic effects of the resulting extracts were evaluated using the MTT assay, which is based on cellular metabolic activity. The L929 fibroblast cell line was used as a normal cell model to assess the potential toxic effects of the extracts. Cells were exposed to different concentrations of the extracts, and cell viability was examined in a dose-dependent manner.

The results demonstrated that both extracts reduced cell viability with increasing concentrations. However, the *G. fimbriatum* extract exhibited a stronger cytotoxic effect, with an IC_{50} value of 44.49 µg/mL. In contrast, the *G. rufescens* extract showed a weaker effect, with an IC_{50} value of 167.16 µg/mL. The toxic effect arises from the chemical components in its structure. Cytotoxicity results are important as they reveal the reliability of natural products for medical use. In particular, *G. rufescens*, with its low cytotoxicity, has the potential to be a good candidate for medical use.

Keywords: Cytotoxicity, *G. rufescens*, *G. fimbriatum*, MTT, L929

1. Introduction

Medicinal mushrooms have been used for centuries in traditional medicine due to their therapeutic properties. Particularly in East Asia, mushrooms have long been utilized for their immunomodulatory, anticancer, and anti-inflammatory effects (Joseph et al., 2018). In recent years, studies have elucidated the biological activities and mechanisms of action of mushroom-derived compounds (Boh et al., 2007; Joseph et al., 2018). Mushrooms contain various

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polysaccharides, especially beta-glucans, which are considered an important resource in the development of pharmaceutical products (Venturella et al., 2021).

Although a vast number of mushroom species exist worldwide, the medicinal properties of most species have not been sufficiently investigated. Nevertheless, studies focusing on the isolation of unique bioactive compounds from mushrooms and the evaluation of their therapeutic potential have been increasing steadily.

Mushrooms exhibit anticancer effects by modulating the immune system and regulating cellular mechanisms through their natural bioactive compounds (Sevindik et al., 2024). Key components such as β-glucans, phenolic compounds, and antioxidants contribute to the prevention of cellular mutations by reducing damage caused by free radicals. These molecules enhance the ability of immune cells to recognize and eliminate malignant cells by activating immune responses (Sevindik et al., 2024). In addition, some mushroom-derived compounds have been reported to support conventional cancer treatment protocols such as chemotherapy and radiotherapy, improving treatment efficacy while reducing side effects and increasing patient tolerance (Hetland et al., 2020). Overall, regular consumption of medicinal mushrooms or their supplements may strengthen the immune system and contribute to maintaining general health. However, conscious and informed use is crucial to obtain maximum benefit from their anticancer effects (Sevindik et al., 2018; Suberu et al., 2024).

The genus *Geastrum* is characterized by the splitting of the outer peridium into star-shaped rays during maturation, a feature that plays an important role in spore dispersal (Zamora et al., 2015). *Geastrum* species are generally saprophytic and are commonly found on humus-rich forest soils, decaying wood, or, less frequently, in open habitats (Trierveiler-Pereira & Silveira, 2012).

G. rufescens and *G. fimbriatum* are species belonging to the Geastraceae family and are classified as inedible due to their fibrous structure and poor taste and texture when mature (Jones, 2022; Smith, 2023). Although the immature forms of *G. triplex* are occasionally considered edible, species such as *G. saccatum* and *G. rufescens* are not suitable for consumption (Brown, 2021; Smith, 2023).

G. rufescens (Figure 1), commonly known as the rosy earthstar, was first scientifically described by Christian Hendrik Persoon in 1801 (Persoon, 1801). The genus *Geastrum* is distinguished by the division of the outer peridium into star-like rays during maturation, which is one of its defining morphological characteristics (Zamora et al., 2015). The endoperidium is typically subglobose, thin, and papery in structure (Wang & Bau, 2023). *G. rufescens* generally grows in deciduous forests, on humus-rich soils, and on decaying organic matter. It is distributed across Europe, North America, and parts of Asia. In Türkiye, it has been reported in the Black Sea Region, Aegean Region, Konya region, Mediterranean Region, and the Middle Euphrates Section (Sesli et al., 2020; Wang & Bau, 2021).

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Figure 1. *G. rufescens.* Doç. Dr. Hakan Allı, personal archieve (17.12.2025)

G. fimbriatum (Figure 2) is commonly referred to as the sessile earthstar and is characterized by the presence of a finely fibrillose, fringed peristome surrounding the apical region of the endoperidium (Barbosa et al., 2024). Morphologically, the fruiting body is initially subterranean and globose in form; during maturation, the outer peridium splits into 5–8 star-like rays. The endoperidium is subglobose and becomes filled with brownish spores upon maturation (Barbosa et al., 2024).

Studies on the habitat and distribution of the species indicate that *G. fimbriatum* is distributed across various regions of Europe and Asia, particularly in humus-rich forest environments (Verma et al., 2018). In Türkiye, this species has been reported from the Western and Eastern Black Sea regions, the Aegean region, the vicinity of Konya, and the Mediterranean region (Sesli et al., 2020).

Figure 2. *G. fimbriatum* Doç. Dr. Hakan Allı personal archieve (17.12.2025)

Cytotoxicity is defined as the ability of a substance to induce detrimental effects on living cells, leading to impaired cellular functions or cell death. This concept is particularly significant in pharmacology, toxicology, and biomedical research, where it serves as an essential parameter

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for evaluating the potential effects of chemical compounds, natural products, or drug candidates on cellular systems (Mondal et al., 2023). Assessments of cytotoxicity play a critical role in determining the safety and efficacy of newly developed drugs and biologically active compounds. Such tests enable the evaluation of the impact of potentially toxic substances on cell viability, metabolic activity, proliferation, and membrane integrity (Tolosa et al., 2015).

Comprehensive studies on the cytotoxic potential of *G. fimbriatum* and *G. rufescens* species are currently limited in the literature. Therefore, investigating the cytotoxic activities of these species is important not only to address this gap in the literature but also to identify potentially bioactive compounds.

2.Experimental Studies

2.1. Preparation of Mushroom Extracts

The mushroom specimens used in this study were obtained from the Fungarium of Muğla Sıtkı Koçman University through Associate Professor Dr. Hakan Allı. The dried mushroom samples were subjected to extraction with ethanol using a Soxhlet apparatus for approximately 3–4 hours, until the color of the samples lightened (Sarac et al., 2015). A total of 40 g of mushroom material and 350 mL of 99% ethanol (Merck KGaA) were employed in the extraction process. The ethanol was subsequently removed from the resulting extract to obtain the crude extract. Stock solutions of *G. rufescens* and *G. fimbriatum* extracts were prepared using 99% ethanol.

(a) (b)

Görsel 3.a) *G.rufescens* mushroom samples b) *G. fimbriatum* mushroom samples

2.2. Cell Culture

The L929 (fibroblast) cell line was cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin/streptomycin) in an incubator maintained at 37°C, 5% CO_2, and 95% relative humidity.

2.3. Passaging of the Cell Line

Cells were grown in 25T flasks under 5% CO_2 at 37°C with 98% humidity. When the cultures reached approximately 80–90% confluency, the culture medium was removed, and the cell monolayer was washed with sterile Dulbecco's phosphate-buffered saline (D-PBS) to remove cellular debris and residual serum. To detach the monolayer, 1–2 mL of trypsin-EDTA solution

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was added to cover the cell surface, and the flasks were incubated for approximately five minutes. Following detachment, the trypsin solution was removed, and the cells were seeded into T-75 flasks for further incubation.

2.4. Determination of Cytotoxic Effects of *G. rufescens* and *G. fimbriatum* Extracts

L929 fibroblast cells at 80–90% confluency were detached using trypsin/EDTA, followed by centrifugation at 1200 rpm for 4 minutes after addition of DMEM. The supernatant was discarded, and the cell pellet was resuspended in fresh DMEM. Cells were seeded into 96-well plates at a density of 1×10^4 cells/well and incubated at 37°C with 5% CO_2 for 24 hours. After the incubation period, *G. fimbriatum* and *G. rufescens* extracts were applied to the cells at predetermined concentrations. Ethanol at corresponding concentrations was used as the control.

Cell viability was determined using the MTT assay (Mosmann, 1983). Following a 24-hour incubation, 100 µL of MTT solution was added to each well and incubated for an additional 3 hours. At the end of the incubation, the wells were emptied, and 100 µL of DMSO was added to dissolve the formazan crystals. The plate was then placed in a shaker incubator at room temperature for 20 minutes at 100 rpm. Absorbance measurements were subsequently performed at a wavelength of 540 nm using a microplate reader (Thermo Scientific Multiskan FC, Vantaa, Finland). Cell viability (%) was calculated using the following formula:

$$\text{Viability (\%)} = \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times \mathbf{100}$$

- **Abs $_{\text{sample}}$**: absorbance of wells treated with the test material
- **Abs $_{\text{control}}$**: absorbance of control wells

The IC_{50} values were calculated statistically.

3. Results and Discussion

3.1. Cytotoxic Effects of *G. fimbriatum* and *G. rufescens* on L929 Cell Line

The cytotoxic effects of *G. fimbriatum* and *G. rufescens* extracts on the L929 fibroblast cell line were assessed using the MTT assay. *G. fimbriatum* extract was applied to the cells at concentrations of 400, 200, 100, 50, 25, and 12.5 µg/mL. Post-treatment well images are presented in Figure 4, while the dose-dependent inhibition graph is shown in Figure 5.

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Figure 4. Image of wells treated with *G. fimbriatum* mushroom extract following the MTT assay.

Figure 5. Percentage of cell viability of L929 cells treated with *G. fimbriatum*.

The highest cell viability of L929 fibroblast cells treated with *G. fimbriatum* ethanol extract was observed at 12.5 µg/mL, with 69.04%, while the lowest viability was recorded at 200 µg/mL, with 18.89%. The IC_{50} value of *G. fimbriatum* ethanol extract on L929 fibroblast cells was calculated as 44.49 µg/mL. Cell viability decreased inversely with increasing concentrations of the mushroom extracts.

Following treatment of L929 cells with *G. rufescens* extract at concentrations of 400, 200, 100, 50, 25, and 12.5 µg/mL, the microplate images are presented in Figure 6, and the dose-dependent percentage viability graph is shown in Figure 7.

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Figure 6. Image of wells treated with *G. rufescens* mushroom extract following the MTT assay.

Figure 7. Percentage of cell viability of L929 cells treated with *G. rufescens*

The highest cell viability of L929 fibroblast cells treated with *G. rufescens* ethanol extract was observed at 50 µg/mL, with 99.73%, whereas the lowest viability was recorded at 400 µg/mL, with 34.14%. The IC_{50} value of *G. rufescens* on L929 cells was calculated as 167.16 µg/mL. Cell viability decreased inversely with increasing concentrations of the mushroom extract.

4. General Discussion

In this study, the cytotoxic potentials of extracts obtained from *G. fimbriatum* and *G. rufescens* were evaluated on the L929 fibroblast cell line using the MTT assay. The results demonstrated that both mushroom species affected cell viability to varying degrees. Specifically, *G. fimbriatum* extract exhibited a more pronounced cytotoxic effect on L929 cells compared to *G. rufescens*. The calculated IC_{50} value for *G. fimbriatum* was 44.49 µg/mL, indicating a

significant reduction in cell viability. In contrast, the IC_{50} value of *G. rufescens* was 167.16 µg/mL, suggesting a lower level of cytotoxic activity on the same cell line.

The cytotoxic effects of mushroom species are largely attributed to their bioactive constituents, including phenolic compounds, triterpenoids, polysaccharides, and particularly β-glucans. These compounds may exert antitumor effects through mechanisms such as inhibition of cell proliferation, cell cycle arrest, and induction of apoptosis (Boh et al., 2007; Wasser, 2014). Previous studies have reported dose-dependent cytotoxic effects of various macrofungi on different cell lines. For instance, species of *Ganoderma*, *Pleurotus*, and *Trametes* have demonstrated significant cytotoxic activities across multiple cell lines (Sevindik et al., 2018). The findings of the present study align with these reports, showing that mushroom extracts can reduce cell viability in a concentration-dependent manner.

The L929 fibroblast cell line, used in this study, is a widely employed normal (non-cancerous) mammalian cell model in biomedical research. L929 cells are commonly used as a reference cell line in cytotoxicity and biocompatibility studies, enabling the evaluation of potential toxic effects of therapeutic agents on normal cells (ISO 10993-5, 2009). Consequently, normal cell lines like L929 serve as control groups in anticancer studies to assess the selectivity of cytotoxic effects observed in cancer cell lines. In other words, an ideal anticancer agent is expected to selectively target cancer cells while sparing normal cells. In this context, cytotoxicity tests on L929 cells provide important insights into the biocompatibility and safety profiles of tested extracts.

Studies on the biological activities of species belonging to the genus *Geastrum* are extremely limited, with most research focusing on antioxidant and antimicrobial properties. Therefore, investigating the cytotoxic potentials of *G. fimbriatum* and *G. rufescens* addresses a significant gap in the literature. Notably, the lower IC_{50} value observed for *G. fimbriatum* suggests that this species may serve as a promising source of bioactive compounds for further pharmacological research.

In conclusion, the results of this study indicate that the extract of *G. fimbriatum* exhibits stronger cytotoxic effects on the L929 fibroblast cell line compared to the extract of *G. rufescens*.

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YENİ NESİL ÇEVRESEL KİRLETİCİ 6PPD-KİNON'UN PANKREATİK BETA HÜCRELERİNDE (INS-1) OLUŞTURDUĞU SİTOTOKSİK VE FONKSİYONEL HASARIN *İN VİTRO* DEĞERLENDİRİLMESİ

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ÖZET

6PPD-kinon (6PPD-q), lastik aşınma katkı maddelerinin toksik bir dönüşüm ürünü olup, özellikle sucul organizmalar üzerindeki etkileri ile dikkat çekmektedir. Bununla birlikte, memeli metabolik sistemleri üzerindeki potansiyel diyabetojenik etkileri henüz yeterince aydınlatılmamıştır. Bu çalışmada, 6PPD-q'nun pankreatik beta hücre hattı olan INS-1 hücrelerinde oluşturduğu sitotoksik ve fonksiyonel etkilerin değerlendirilmesi amaçlanmıştır.

INS-1 hücreleri 24 saat boyunca farklı konsantrasyonlarda (0,1–50 µM) 6PPD-q'ya maruz bırakılmıştır. Hücre canlılığı MTT yöntemi ile belirlenmiş, oksidatif stres düzeyleri reaktif oksijen türleri (ROS) üretimi ile değerlendirilmiştir. Fonksiyonel kapasite, düşük (3,3 mM) ve yüksek (16,7 mM) glikoz koşullarında glikozla uyarılan insülin salgısı (GSIS) protokolü ile analiz edilmiştir. Veriler tek yönlü varyans analizi (ANOVA) ve Tukey *post-hoc* testleri ile değerlendirilmiştir. Söz konusu bulgular, 6PPD-q'nun doza bağlı olarak hücre canlılığını azalttığını ve oksidatif stresi arttırdığını göstermiştir. Önemli bir bulgu, sitotoksik olmayan bir doz olan 10 µM konsantrasyonda bile yüksek glikoza bağlı insülin salgısının anlamlı derecede baskılanmasıdır ($p < 0,001$). İnsülin salgı indeksi kontrol grubuna kıyasla belirgin şekilde düşmüş, bu durum beta hücre fonksiyonunun hücre ölümünden önce bozulduğunu ortaya koymuştur. Daha yüksek dozlarda ise hem fonksiyonel kapasite hem de hücre canlılığı ciddi şekilde azalmıştır.

Sonuç olarak, bu çalışma 6PPD-q'nun pankreatik beta hücrelerinde hem sitotoksik hem de fonksiyonel hasara yol açtığını göstermektedir. Bulgular, lastik aşınmasına bağlı çevresel kirleticilerin metabolik hastalıkların gelişiminde potansiyel bir risk faktörü olabileceğini düşündürmektedir.

Anahtar Kelimeler: 6PPD-kinon, INS-1 hücreleri, pankreatik beta hücreleri, sitotoksisite, insülin salgısı, çevresel toksikoloji

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Escherichia coli'DE *cbpA* GENİNİN FENOLİK ASİT TOLERANSINDAKİ ROLÜ: MUTANT VE KOMPLEMENTASYON ANALİZİNDEN KANITLAR

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ÖZET

Fenolik asitler sınıfına dahil olan trans-sinnamik asit ve p-kumarik asit; ssmeyve, sebze ve tahıllarda doğal olarak bulunan sekonder metabolitler olup antioksidan, antibakteriyel ve antikanser gibi çeşitli biyolojik aktivitelere sahiptir. Bu bileşiklerin genel biyolojik etkileri literatürde geniş ölçüde araştırılmış olmasına rağmen, bakteriyel hücrelerdeki spesifik genetik ve moleküler etki mekanizmaları henüz tam olarak aydınlatılamamıştır. Bu eksiklikten yola çıkılarak kurgulanan çalışmada, trans-sinnamik asit ve p-kumarik asidin *Escherichia coli* üzerindeki etkileri, tek gen nakavt mutantları kullanılarak incelenmiş ve özellikle *cbpA* geninin fonksiyonel rolü üzerine odaklanılmıştır.

Araştırma kapsamında, 0 mg/mL ile 1,5 mg/mL arasında değişen farklı fenolik asit konsantrasyonlarında fenotipik analizler gerçekleştirilmiştir. Bu analizler sonucunda, *cbpA* geni bakımından mutant suşun, yabani tipe ve test edilen diğer mutantlara kıyasla her iki fenolik aside karşı duyarlılık gösterdiği tespit edilmiştir. Elde edilen bu spesifik duyarlılık fenotipi, verifikasyon deneyleri ile de desteklenmiştir. Fonksiyonel ilişkinin doğrulanması amacıyla, *cbpA* geni plazmit aracılığıyla ilgili mutant suşa yeniden kazandırılmıştır. Bu aşamada gerçekleştirilen komplementasyon deneyleri, gen ifadesini kontrollü bir şekilde indüklemek amacıyla üç farklı IPTG (Isopropyl β-D-1-thiogalactopyranoside) konsantrasyonunda (0 µM, 10 µM ve 33 µM) yürütülmüştür. Deneyler neticesinde, genin geri kazandırılmasıyla birlikte suşun fenolik asitlere karşı gösterdiği duyarlılık profilinin ortadan kalktığı ve tolerans

yeteneğinin yeniden kazanıldığı görülmüştür. Bu bulgular, *cbpA* geninin trans-sinnamik asit ve p-kumarik aside karşı gelişen hücresel yanıt ve karmaşık tolerans mekanizmalarında bir rol oynayabileceğini düşündürmektedir.

Literatürde *cbpA* geninin, "curved DNA-binding protein" kodladığı ve genel stres yanıtı süreçleriyle ilişkili olduğu bilinmektedir. Ancak bu genin fenolik asitlere karşı gelişen duyarlılık veya tolerans süreçlerindeki özgün işlevi, ilk kez bu çalışma ile fonksiyonel olarak kanıtlanmıştır. Bu araştırma, Türkiye Bilimsel ve Teknolojik Araştırma Kurumu (TÜBİTAK, Proje No: 122Z018) tarafından desteklenmiş olup yazarın (Kadriye Aslıhan ONAT TAŞDELEN) doktora tez çalışmasının bir kısmını kapsamaktadır.

Anahtar Kelimeler: trans-sinnamik asit, p-kumarik asit, *Escherichia coli, cbpA*, komplementasyon, knockout gen.

1. GİRİŞ

Bitkisel kaynaklı sekonder metabolitler, bitkinin temel yaşam döngüsüne (primer metabolizma) doğrudan katılan bileşikler olmasa da çevresel stres faktörlerine karşı savunma, sinyal iletimi ve antimikrobiyal koruma gibi kritik fonksiyonlarda görev almaktadır (Isah, 2019; Ahmed ve ark., 2024; Zaynab ve ark., 2018). Farmasötik ve tıbbi biyoteknoloji açısından stratejik değere sahip olan bu bileşikler grubunda fenolik yapılar, aromatik halkalarına bağlı hidroksil gruplarının sağladığı antioksidan kapasite sayesinde oksidatif stresle ilişkili biyolojik süreçleri regüle edebilmektedir (Öztürkel ve ark., 2020; Onat ve ark., 2021). Yapısal özelliklerine göre flavonoidler, tanenler ve stilbenler gibi sınıflara ayrılan bu geniş grubun en temel bileşenlerinden biri olan fenolik asitler; karbon iskeleti uzunluklarına bağlı olarak hidroksibenzoik asitler ve hidroksisinnamik asitler şeklinde kategorize edilir (Manach ve ark., 2004; Adisakwattana, 2017).

Sinnamik asit türevleri olan trans-sinnamik asit (tCA) ve p-kumarik asit (p-CA), bitki-çevre etkileşiminde anahtar rol oynayan önemli allelokimyasallardır. Bu bileşikler; bitkiyi UV-B ışınları gibi abiyotik streslerden korumanın yanı sıra patojenik mikroorganizmalara ve fungal enfeksiyonlara karşı doğal bir direnç kalkanı oluşturur (Zaynab ve ark., 2018; Dai ve Mumper, 2010). Beşerî beslenme açısından değerlendirildiğinde tCA ve p-CA; tahıllar, meyveler ve sebzeler (narenciye, ıspanak, domates, üzüm vb.) ile polen ve sirke gibi fermente ürünlerde yaygın olarak bulunmaktadır (Lou ve ark., 2012; Boz, 2015). Ayrıca, bu bileşiklerin bağırsak

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epiteliyle etkileşime girerek mikrobiyota kompozisyonunu düzenlediği ve sindirim sistemi sağlığını desteklediği saptanmıştır (Popa ve ark., 2016).

Moleküler düzeyde tCA ve p-CA'nın; anti-enflamatuvar, anti-mutajenik, anti-biyofilm ve özellikle geniş spektrumlu antimikrobiyal aktivitelere sahip olduğu literatürde güçlü bir şekilde desteklenmektedir (Surh, 2002; Kot ve ark., 2015). Yapılan çalışmalar, bu asitlerin bakteriyel hücre zarlarında geri dönüşümsüz geçirgenlik artışına neden olduğunu ve hücre içi fonksiyonları inhibe ederek patojen gelişimini baskıladığını göstermiştir (Lou ve ark., 2012). Özellikle *Escherichia coli*, *Bacillus cereus* ve *Salmonella typhimurium* üzerinde biyofilm inhibisyonu sağladıkları, bazı türlerde ise hücreler arası haberleşme sistemi olan "quorum sensing" mekanizmasını sekteye uğrattıkları bildirilmiştir (Kot ve ark., 2015; Chen ve ark., 2020). Onkoloji alanındaki bulgular ise bu fenoliklerin, ROS seviyesini artırıp hücre döngüsünü G1 fazında durdurarak çeşitli kanser hatlarında apoptozu tetiklediğine işaret etmektedir (Jaganathan ve ark., 2013; Shailasree ve ark., 2015).

Günümüzde artış gösteren çoklu ilaç direnci sorunu, tCA ve p-CA gibi doğal fenoliklerin alternatif farmasötik ajanlar olarak potansiyelini gündeme getirmiştir. Bu bileşiklerin bitkisel sentez yolakları literatürde tanımlanmış olsa da, mikroorganizmalar üzerindeki hücresel yanıt ve tolerans mekanizmalarına dair bilgiler henüz sınırlıdır. Bu doğrultuda, fenolik asitlerin biyolojik etkinliklerinden verimli bir şekilde yararlanabilmek için hücresel etkileşimlerin; ilgili gen ve protein düzeyinde kapsamlı bir şekilde incelenmesi literatüre önemli bir katkı sağlayacaktır.

2. DENEYSEL ÇALIŞMALAR

2.1. Bakteri Suşları ve Kimyasallar

Çalışmada kullanılan trans-sinnamik asit ve p-kumarik asit Sigma-Aldrich firmasından tedarik edilmiş ve %100 etil alkol ile her çalışmada taze olarak hazırlanmıştır. Trans-sinnmaik asit 150 mg/ml p-kumarik asit ise 50 mg/ml derişiminde stoklanmıştır. Deneylerde kullanılan mutant suşlar KEIO Koleksiyonu'ndan temin edilmiştir (Baba ve ark., 2006). Kültürleme işlemleri; 50 µg/mL kanamisin içeren ve değişken konsantrasyonlarda fenolik asit ilave edilmiş Luria-Bertani (LB) besiyerinde gerçekleştirilmiştir.

2.2. Artan Konsantrasyonlarında Fenolik Asit İçeren Ortamda Mutantların Taranması

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96 adet mutant suş, p-kumarik asidin farklı konsantrasyonlarında (0 mg/ml, 1 mg/ml, 1,2 mg/ml, 1,3 mg/ml, 1,4 mg/ml ve 1,5 mg/ml) duyarlılık açısından taranmıştır (Görsel 2). Tarama deneyleri gerçekelştirilen mutant suşların ilgili gen ve konum bilgileri Görsel 1'de verilmiştir. Mutantlar, -80°C'de mikroplakalarda stoklanmış olup, taze Luria-Bertani (LB) broth içeren mikroplakalara ekilerek 37°C'de bir gece boyunca inkübe edilmiştir.

Ertesi gün, kültürler 50 µg/ml kanamisin içeren taze LB broth besiyerine 96 pinli replikatör yardımıyla transfer edilerek, 37°C'de 3 saat inkübe edilmiştir. Ardından, farklı konsantrasyonlardaki p-kumarik asit içeren kanamisinli LB agar plakalarına yine 96 pinli replikatörle ekim yapılmıştır. Plakalar, 37°C'de 24 saat inkübe edilerek mutant suşların p-kumarik aside karşı duyarlılığı değerlendirilmiştir.

Keio Collection(Plate No.45)

	1	2	3	4	5	6	7	8	9	10	11	12
A	JW1496 ydeQ	JW1869 flhB	JW1923 fliG	JW1935 rcsA	JW3112 yraI	JW3604 rfaS	JW5599 rffD	JW4277 fimA	JW0462 htpG	JW1283 sapF	JW1876 cheW	JW2764 syd
B	JW1500 hipA	JW1880 flhC	JW1925 fliI	JW2020 wbbH	JW3175 mtgA	JW3605 rfaP	JW5598 rffG	JW4279 fimC	JW0598 ahpC	JW1417 trg	JW1878 motB	JW2861 dsbC
C	JW1633 slyB	JW1905 fliY	JW1927 fliK	JW2022 rfbX	JW3594 rfaD		JW3763 rffH	JW4281 fimF	JW0941 sulA	JW1477 osmC	JW1879 motA	JW2988 ygiS
D	JW1660 ydhU	JW1908 fliC	JW1928 fliL	JW2163 spr	JW3595 rfaF	JW3607 rfaQ	JW3770 rffM	JW4283 fimH	JW0985 cbpA	JW1561 dicC	JW1886 otsB	JW3142 secG
E	JW1698 nlpC	JW1909 fliD	JW1929 fliM	JW2336 yfcV	JW3597 rfaL		JW3818 rfaH		JW1165 minC	JW1566 dicB	JW2183 ccmG	JW3146 rrmJ
F	JW1728 osmE	JW1910 fliS	JW1930 fliN	JW2428 amiA	JW3600 rfaY		JW4097 cutA	JW0014 dnaJ	JW1236 oppB	JW1720 cedA	JW2465 bcp	JW3286 gspC
G	JW1844 lpxM	JW1911 fliT	JW1932 fliP	JW2601 smpB	JW3601 rfaJ	JW3758 rfe	JW4103 groL		JW1239 oppF	JW1721 katE	JW2510 hscA	JW3288 gspE
H	JW1868 flhA	JW1922 fliF	JW1933 fliQ	JW2982 yqhH	JW3602 rfaI	JW5600 rffE	JW4192 mpl	JW0304 betB	JW1275 osmB	JW1873 cheR	JW2654 proX	JW3289 gspF

Görsel 1. Tarama çalışmaları gerçekleştirilen mutant suşların ilgili genleri ve konumlarını gösteren tablo ile petri görüntüsü

2.3. Verifikasyon

Tarama deneylerinde trans-sinnamik asit ve p-kumarik aside karşı farklı duyarlılık sergilediği belirlenen *cbpA* mutantı ve kontrol suşunun fenotipik özellikleri, verifikasyon çalışmalarıyla doğrulanmıştır. Bu amaçla, her iki suşun trans-sinnamik asit ve p-kumarik asit varlığındaki büyüme performansları tolerans nokta testleri (spot assay) ile analiz edilmiştir. Mutant ve kontrol suşları, -80°C stoklarından 50 µg/ml kanamisin içeren LB (LB-kan50) katı besiyerlerine

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ekilerek canlandırılmış; ardından elde edilen tek koloniler 5 ml LB sıvı besiyerinde 150 rpm, 37°C'de gece boyu inkübe edilmiştir. Doğrulama protokolü kapsamında, gece boyu büyütülen *cbpA* mutantı ve kontrol suşu kültürleri, taze LB besiyerine OD600 0,05 olacak şekilde inoküle edilmiş ve hücreler logaritmik fazı temsil eden OD600 0,5 değerine ulaşana kadar büyütülmüştür. Standart yoğunluğa ulaşan hücrelerin steril PBS ile seri dilüsyonları hazırlanmış ve her dilüsyondan 5 µl'lik hacimler, artan konsantrasyonlarda trans-sinnamik asit ve p-kumarik asit içeren LB-kan50 katı besiyerlerine damlatılmıştır (Sezer Kürkçü, M., ve ark., 2025). 37°C'de 3 gün süren inkübasyon boyunca her iki suşun büyüme alanları ve koloni yoğunlukları günlük olarak takip edilmiştir. Bu sayede, *cbpA* gen eksikliğinin kontrol suşuna kıyasla fenolik asit toleransı üzerindeki spesifik etkisi kesinleştirilmiştir.

2.4. Komplementasyon

Komplementasyon çalışmaları kapsamında, hedef genleri taşıyan p-CA24N plazmidleri ASKA klonlarından BioLabs Monarch Plasmid Miniprep kiti ile izole edilmiş; ilgili gen bakımından mutant olan *E. coli* suşları ise CaCl2 metodu kullanılarak kompetant hale getirilmiştir. Elde edilen kompetant mutant hücrelere, hedef geni taşıyan plazmidler heat-shock (42°C'de 2 dk) yöntemiyle transforme edilmiş ve transformantlar kloramfenikol (30 µg/ml) içeren seçici LB agar besiyerlerinde doğrulanmıştır. Fenolik asit toleransının geri kazanımını test etmek amacıyla; hedef geni taşıyan suş [*E. coli* K-12 BW25113 *cbpA* (p-CA24N::*cbpA*)], boş plazmid taşıyan kontrol suşu [*E. coli* K-12 BW25113 (p-CA24N)] ve yabanıl tip *E.coli* suşu [BW25113 (p-CA24N)] kullanılarak Minimum İnhibisyon Konsantrasyonu (MIC) deneyleri yürütülmüştür. Çalışmada kullanılan suşlar ve plazmidler Çizelge 1 'de verilmiştir.

MIC tayini için, p-CA24N plazmidindeki T5-lac promotörünü indüklemek üzere 0 µM, 10 µM ve 33 µM IPTG konsantrasyonları kullanılmış; hücreler OD600 0,05 olacak şekilde dilüe edilerek mikro plakalara ekilmiştir. Deneylerde, ön tarama ve verifikasyon sonuçlarına göre belirlenen 8 farklı artan konsantrasyonda trans-sinnamik asit ve p-kumarik asit içeren LB besiyerleri kullanılmıştır. 37°C'de 24 saatlik inkübasyonun ardından OD600 değerleri spektrofotometrik olarak ölçülmüş ve görsel olarak üremenin durduğu en düşük konsantrasyon MIC değeri olarak kaydedilmiştir. Boş plazmid taşıyan kontrol suşuna kıyasla, fenolik asitlerin (trans-sinnamik ve p-kumarik asit) daha yüksek konsantrasyonlarında üreme yeteneği sergileyen suşlar başarılı bir şekilde komplemente olmuş olarak kabul edilmiştir (Sezer Kürkçü, M., ve ark., 2025).

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Çizelge 1. Çalışmada Kullanılan Suş ve Plazmid Listesi

Suşlar ve Plazmidler	Genotip	Kaynakça
Suşlar		
E. coli K-12 BW25113	*lacI*^{q} *rrnB*$_{T14}$ *ΔlacZ*$_{WJ16}$ *hsdR514 ΔaraBAD*$_{AH33}$ *ΔrhaBAD*$_{LD78}$	(Datsenko ve Wanner, 2000)
E. coli K-12 BW25113 *cbpA*	K-12 BW25113 Δ*cbpA* ΩKm^{R}	(Baba ve ark.., 2006)
E. coli K-12 BW25113 (pCA24N)	*lacI*^{q} *rrnB*$_{T14}$ *ΔlacZ*$_{WJ16}$ *hsdR514 ΔaraBAD*$_{AH33}$ *ΔrhaBAD*$_{LD78}$*Cm*^{R}*; lacI*^{q}*, pCA24N*	Bu çalışma
E. coli K-12 BW25113 *cbpA* (pCA24N:: *cbpA*)	K-12 BW25113 Δ *cbpA* ΩKm^{R} , Cm^{R}; *lacI*^{q}, pCA24N P$_{T5\text{-}lac}$:: *cbpA* $^{+}$	Bu çalışma
E. coli K-12 BW25113 *cbpA* (pCA24N)	K-12 BW25113 Δ *cbpA* ΩKm^{R} , Cm^{R}; *lacI*^{q}, pCA24N	Bu çalışma
Plazmidler		
pCA24N	Cm^{R}; *lacI*^{q}, pCA24N	(Kitagawa, ve ark., 2005)
pCA24N:: *cbpA*	Cm^{R}; *lacI*^{q}, pCA24N P$_{T5\text{-}lac}$:: *cbpA* $^{+}$	(Kitagawa, ve ark., 2005)

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3. SONUÇLAR VE DEĞERLENDİRME

3.1. Tarama Çalışması Sonuçları

Tarama deneylerinde artan trans-sinnamik asit (tCA) ve p-kumarik asit (p-CA) konsantrasyonlarına karşı mutant suşların sergilediği duyarlılık profillerinin belirlenmesi amaçlanmıştır.

Yapılan analizler sonucunda, artan fenolik asit konsantrasyonlarının suşların büyüme karakteristikleri üzerindeki etkileri belirlenmiştir. Kontrol suşunun 1,2 mg/mL tCA derişimine sahip ortamda büyüme sergilediği gözlemlenirken, *cbpA* geni nakavt edilmiş mutant suşun aynı konsantrasyonda gelişemediği saptanmıştır. Benzer şekilde, artan p-CA konsantrasyonları altında gerçekleştirilen denemelerde; kontrol suşunun 1,3 mg/mL p-CA ortamında canlılığını sürdürdüğü, ancak *cbpA* mutantının bu konsantrasyonda büyüme gösteremediği tespit edilmiştir (Görsel 2).

Görsel 2. Artan trans-sinnamik asit (tCA) ve p-kumarik asit (p-CA) konsantrasyonlarında *Escherichia coli* Δ*cbpA* suşunun kontrole kıyasla üreme durumu

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3.2. Verifikasyon Çalışması Sonuçları

Tarama deneylerinde tCA ve p-CA karşısında duyarlılık sergilediği saptanan *cbpA* mutantı ile kontrol suşunun fenotipik özellikleri, verifikasyon çalışmalarıyla teyit edilmiştir. Logaritmik büyüme fazına (OD600 0,5) getirilen kültürlerden hazırlanan seri dilüsyonlar, artan konsantrasyonlarda tCA ve p-CA içeren katı besiyerlerine damlatılarak hücrelerin büyüme karakteristikleri üç gün boyunca izlenmiştir. Günlük olarak gerçekleştirilen görsel değerlendirmeler ve fotoğraf kayıtları neticesinde; her iki fenolik asit derişiminin artışına paralel olarak *cbpA* mutantının, kontrol suşuna kıyasla belirgin şekilde zayıf bir büyüme sergilediği saptanmıştır. Özellikle artan tCA ve p-CA konsantrasyonlarında kontrol suşu canlılığını sürdürebilirken, *cbpA* nakavt mutantının büyümesinin baskılandığı gözlemlenmiştir (Görsel 3 ve Görsel 4). Bu veriler, *cbpA* gen eksikliğinin *Escherichia coli*'nin fenolik asit toleransı üzerinde doğrudan ve spesifik bir etkisi olduğunu kesinleştirmiştir.

Görsel 3. Artan trans-sinnamik asit konsantrasyonlarında *Escherichia coli* Δ*cbpA* suşunun kontrole kıyasla üreme durumu

Görsel 4. Artan p-kumarik asit konsantrasyonlarında *Escherichia coli* Δ*cbpA* suşunun kontrole kıyasla üreme durumu

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3.3. *cbpA* Geni Komplementasyon Analizi Sonuçları

cbpA mutantı ve geni geri kazandırılmış rekombinant suşun farklı IPTG konsantrasyonlarındaki trans-sinnamik asit (tCA) toleransları karşılaştırılarak komplementasyon analizi gerçekleştirilmiştir. Yapılan MIC deneylerinde, 0 µM IPTG (indüklenmemiş) konsantrasyonunda rekombinant suşun mutant suş ile benzer büyüme özellikleri sergilediği ve bu koşulda komplementasyonun gerçekleşmediği saptanmıştır. Bu durum, hedef genin ekspresyonu için bazal seviyenin yetersiz olduğunu göstermektedir.

Buna karşın, 10 µM IPTG indüksiyonu altında rekombinant suşun tCA toleransında belirgin bir artış gözlenmiştir. Mutant suş 0,8 mg/ml tCA konsantrasyonunda üreyebilirken, *cbpA* geni geri kazandırılmış rekombinant suşun 1,2 mg/ml tCA konsantrasyonunda büyüme sergilediği ve başarılı bir şekilde komplemente olduğu belirlenmiştir. 33 µM IPTG konsantrasyonunda ise rekombinant suş 1,0 mg/ml tCA ortamında üreme yeteneği gösterirken, mutant suşun yine 0,8 mg/ml seviyesinde kaldığı tespit edilmiştir (Görsel 5). Bu sonuçlar, *cbpA* geninin dışarıdan ifade edilmesinin, mutant suşun trans-sinnamik asite karşı duyarlılığını ortadan kaldırarak tolerans yanıtını geri kazandırdığını doğrulamaktadır.

Görsel 5. Artan p-kumarik asit konsantrasyonlarında *Escherichia coli* Δ*cbpA* suşunun kontrole kıyasla üreme durumu

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cbpA geni geri kazandırılmış rekombinant suşun p-kumarik asite (p-CA) karşı sergilediği tolerans yanıtı, mutant suş ile kıyaslanarak farklı indükleyici (IPTG) konsantrasyonlarında analiz edilmiştir. 0 µM IPTG konsantrasyonunda gerçekleştirilen deneylerde, rekombinant suşun p-CA toleransında mutant suşa göre bir artış gözlenmemiş ve bu koşulda komplementasyonun gerçekleşmediği saptanmıştır.

Buna karşın, artan IPTG konsantrasyonları ile *cbpA* geninin ifade edilmesi, p-CA toleransını anlamlı ölçüde artırmıştır. 10 µM IPTG varlığında, mutant suş en fazla 0,8 mg/ml p-CA konsantrasyonunda üreyebilirken; rekombinant suşun 1,1 mg/ml p-CA derişiminde büyüme sergilediği ve başarıyla komplemente olduğu belirlenmiştir. 33 µM IPTG konsantrasyonunda ise mutant suşun tolerans limiti 1,0 mg/ml p-CA seviyesindeyken, rekombinant suşun 1,4 mg/ml p-CA konsantrasyonunda üreyebildiği tespit edilmiştir. Bu bulgular, *cbpA* geninin p-kumarik asit stresine karşı direnç mekanizmasında kritik bir rol oynadığını ve genin ifadesinin mutant fenotipi ortadan kaldırdığını kanıtlamaktadır (Görsel 6).

Görsel 6. Artan p-kumarik asit konsantrasyonlarında *Escherichia coli* Δ*cbpA* suşunun kontrole kıyasla üreme durumu

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4. GENEL DEĞERLENDİRME VE SONUÇLAR

Escherichia coli'de 306 aminoasitten oluşan ve Curved DNA-binding protein A'yı kodlayan CbpA, bakteriyel hayatta kalma stratejilerinde çift fonksiyonlu bir moleküler yardımcı olarak kritik rol oynamaktadır. Bir DnaJ-benzeri protein olan CbpA, özellikle stres koşullarında denatüre olan proteinlerin yeniden katlanmasını sağlamak amacıyla DnaK (Hsp70) şaperon sistemi ile sinerjik bir şekilde çalışarak protein homeostazisini korur. Eş zamanlı olarak, DNA'nın içsel kıvrımlı bölgelerine bağlanma yeteneği sayesinde nükleoid organizasyonunu ve paketlenmesini düzenleyerek genetik materyali fiziksel hasarlara karşı stabilize eder (ecoliwiki.org; UniProt; EcoCyc).

Fenolik bileşiklerin, özellikle de trans-sinnamik asit türevlerinin bakteriyel hücrelerde indüklediği stres, bu savunma mekanizmalarının önemini daha da belirginleştirmektedir. Literatürde, p-kumarik asit (p-CA) kaynaklı oksidatif stresin protein yanlış katlanmasını tetiklediği ve enerji metabolizmasıyla ilişkili genlerin aşağı regülasyonuna neden olarak hücresel bileşenlerde dolaylı hasarlar oluşturduğu bildirilmiştir (Rodríguez-Ochoa ve ark., 2022). Benzer şekilde, trans-sinnamaldehit fümigasyonunun *E. coli*'de tek ve çift sarmallı DNA kırılmalarına yol açtığı raman spektroskopisi çalışmalarıyla kanıtlanmıştır (Huang ve ark., 2025). Bu bulgular, fenolik asitlerin bakteriler üzerinde hem proteotoksik hem de genotoksik bir baskı oluşturduğunu göstermektedir. Genotoksik ve proteotoksik baskı karşısında CbpA, nükleoid bütünlüğünü koruyan ve protein kümelenmesini önleyen vazgeçilmez bir "koruma kalkanı" görevi üstlenmektedir. Nitekim, p-CA ve klorojenik asit kombinasyonlarının Shigella türlerinde ciddi genomik bozulmalara yol açtığı bilinmektedir (Liu ve ark., 2025).

Bu çalışma kapsamında elde edilen veriler, *ΔcbpA* mutant *E. coli* suşunun trans-sinnamik asit ve p-kumarik aside karşı artan bir duyarlılık sergilediğini ortaya koymuştur. Söz konusu duyarlılığın, *cbpA* geninin plazmit aracılığıyla geri kazandırılması (komplementasyon) sonucu ortadan kalktığı gözlemlenmiştir. Komplementasyon analizlerinde, gen ekspresyonunun indüklenmediği 0 µM IPTG koşulunda tolerans artışı gözlenmezken; 10 µM ve 33 µM IPTG konsantrasyonlarındaki indükleme ile bakterinin bu bileşiklere karşı toleransını yeniden kazandığı belirlenmiştir. Farklı IPTG konsantrasyonlarına bağlı olarak değişen bu geri kazanım oranları, fenolik asit direncinde CbpA miktarının kritik bir rol oynadığını düşündürmektedir. *ΔcbpA* mutantının sergilediği fenotip ve kontrollü indükleme ile sağlanan bu komplementasyon verileri, CbpA'nın bu bileşiklere karşı geliştirilen temel savunma mekanizmalarında merkezi bir bileşen olduğunu desteklemektedir.

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DIFFERENTIAL TRANSCRIPTIONAL RESPONSE OF THE *yrbF (mlaF)* GENE TO STRUCTURALLY RELATED PHENOLIC ACIDS IN *Escherichia coli*

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Phenolic acids are plant-derived antimicrobial compounds that exert stress on bacterial cells through membrane perturbation, oxidative imbalance, and metabolic interference. While several resistance mechanisms have been described, the transcriptional response of lesser-characterized genes remains unclear. The *yrbF* gene, also known as *mlaF*, encodes the ATP-binding subunit of the MlaFEDB ABC transporter complex responsible for maintaining outer membrane lipid asymmetry through intermembrane phospholipid trafficking. Disruption of this pathway increases membrane permeability and SDS-EDTA sensitivity, highlighting its critical role in envelope stress adaptation.

In this study, we investigated the short-term transcriptional regulation of *mlaF* in *Escherichia coli* BW25113 following exposure to structurally related phenolic acids. Exponentially growing cultures were exposed for 15 minutes to trans-cinnamic acid (tCA), p-coumaric acid (pCA), ferulic acid (FA), and benzoic acid (BA). Relative mRNA expression levels were determined by quantitative real-time PCR using 16S rRNA as the reference gene and calculated using the $2^{-\Delta\Delta Ct}$ method. *mlaF* expression was upregulated by tCA (1.86-fold) and pCA (2.12-fold), while a markedly stronger induction was observed under FA exposure (3.82-fold). In contrast, benzoic acid caused only minimal induction (1.03-fold). These findings reveal a structure-dependent transcriptional activation pattern, with hydroxy- and methoxy-substituted cinnamic acid derivatives eliciting the strongest response.

Collectively, our results suggest that phenolic acid-induced membrane perturbation may activate phospholipid trafficking pathways and identify *mlaF* as a potential regulatory node in envelope stress adaptation. This study provides new insight into structure-specific bacterial responses to plant-derived antimicrobials.

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Keywords: *Escherichia coli*, *mlaF (yrbF)*, phenolic acids, ferulic acid, membrane stress, phospholipid trafficking, qRT-PCR

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Escherichia coli'DE *yrbF* (*mlaF*) GENİNİN YAPISAL OLARAK BENZER FENOLİK ASİTLERE KARŞI FARKLI TRANSKRİPSİYONEL YANITI

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Fenolik asitler, bakteriyel hücrelerde membran yapısının bozulması, oksidatif dengenin değişmesi ve metabolik süreçlerin etkilenmesi yoluyla stres oluşturan bitki kökenli antimikrobiyal bileşiklerdir. Bu stres yanıtına ilişkin çeşitli direnç mekanizmaları tanımlanmış olmakla birlikte, daha az karakterize edilmiş genlerin transkripsiyonel yanıtı hâlâ tam olarak aydınlatılamamıştır. *mlaF* olarak da bilinen *yrbF* geni, hücreler arası fosfolipit taşınması yoluyla dış membran lipid asimetrisini korumaktan sorumlu MlaFEDB ABC taşıyıcı kompleksinin ATP bağlayıcı alt birimini kodlar. Bu yolaktaki bozulmalar, membran geçirgenliğinde artışa ve SDS-EDTA duyarlılığına yol açarak bu sistemin hücre zarfı stresine adaptasyondaki kritik rolünü ortaya koymaktadır.

Bu çalışmada, *Escherichia coli* BW25113 suşunda, yapısal olarak benzer fenolik asitlere maruz kalma sonrası *mlaF* geninin kısa süreli transkripsiyonel düzenlenmesi incelenmiştir. Logaritmik fazdaki hücreler 15 dakika süreyle trans-sinnamik asit (tCA), p-kumarik asit (pCA), ferulik asit (FA) ve benzoik asit (BA) ile muamele edilmiştir. Göreceli mRNA ekspresyon düzeyleri, referans gen olarak 16S rRNA kullanılarak kantitatif gerçek zamanlı PCR ile belirlenmiş ve $2^{-\Delta\Delta Ct}$ yöntemiyle hesaplanmıştır. *mlaF* gen ekspresyonunun tCA (1,86 kat) ve pCA (2,12 kat) ile arttığı, FA uygulaması altında ise belirgin şekilde daha yüksek bir indüksiyonun (3,82 kat) gerçekleştiği görülmüştür. Buna karşılık, benzoik asit yalnızca minimal düzeyde bir artışa (1,03 kat) neden olmuştur. Bu bulgular, hidroksi ve metoksi grubu içeren

sinnammik asit türevlerinin daha güçlü bir yanıt oluşturduğunu gösteren, yapı-bağımlı bir transkripsiyonel aktivasyon paternini ortaya koymaktadır.

Sonuç olarak, fenolik asitlerin neden olduğu membran bozulmasının fosfolipit taşınma yolaklarını aktive edebileceği ve *mlaF* geninin hücre zarfı stresine adaptasyonda potansiyel bir düzenleyici düğüm noktası olabileceği düşünülmektedir. Bu çalışma, bitki kökenli antimikrobiyal bileşiklere karşı bakteriyel yanıtların yapı-özgüllüğüne dair yeni bilgiler sunmaktadır.

Teşekkür: Bu çalışma, Türkiye Bilimsel ve Teknolojik Araştırma Kurumu (TÜBİTAK, 1001 proje no: 122Z018) tarafından desteklenmiştir.

Anahtar Kelimeler: *Escherichia coli*, *mlaF* (*yrbF*), fenolik asitler, ferulik asit, membran stresi, fosfolipit taşınması, qRT-PCR

1. GİRİŞ

Fenolik asitler, bitkiler tarafından sentezlenen ve mikroorganizmalar üzerinde antimikrobiyal etki gösteren önemli sekonder metabolitlerdir. Bu bileşikler bakteriyel hücrelerde başlıca membran bütünlüğünün bozulması, oksidatif stresin artması ve metabolik süreçlerin sekteye uğraması yoluyla etki eder (Adisakwattana, 2017). Özellikle aromatik halka yapısı ve hidroksi/metoksi substitüsyonları, bu bileşiklerin hücre üzerindeki etkilerini belirleyen önemli yapısal özelliklerdir (Rastogi ve ark., 2008; Scherer ve ark., 2009; Hafizur ve ark., 2015). Lignoselülozik biyoproseslerde ara ürün olarak da yer almaları nedeniyle, mikroorganizmalarla etkileşimleri hem temel bilimler hem de biyoteknolojik uygulamalar açısından büyük önem taşımaktadır.

Bakteriler fenolik bileşiklere karşı çeşitli savunma mekanizmaları geliştirmiştir. Bunlar arasında zar geçirgenliğinin düzenlenmesi, aktif efluks sistemleri ve redoks dengeyi sağlayan metabolik yollar yer almaktadır (Cheng ve ark., 2006; Maurya ve ark., 2010; Kanski ve ark., 2001; Yeh ve ark., 2008; Martin ve ark.) Ancak, bu süreçlerde rol alan bazı genlerin özellikle erken dönem (kısa süreli) transkripsiyonel yanıtları henüz tam olarak aydınlatılamamıştır.

Bu bağlamda, *yrbF* olarak da bilinen *mlaF* geni, dış membran lipid asimetrisinin korunmasında görev alan MlaFEDB ABC taşıyıcı kompleksinin ATP-bağlayıcı alt birimini kodlamaktadır (Malinverni ve Silhavy., 2009). Bu sistem, hücreler arası fosfolipit taşınımını düzenleyerek dış membran stabilitesinin korunmasına katkı sağlar (Hughes vd., 2019). *mlaF* geninin fonksiyon kaybı, membran geçirgenliğinde artış ve deterjanlara (örneğin SDS-EDTA) karşı hassasiyet ile ilişkilendirilmiştir (Malinverni ve Silhavy, 2009).

Bu çalışmada, *Escherichia coli* BW25113 suşunda, yapısal olarak benzer fenolik asitlerin *mlaF* geninin kısa süreli transkripsiyonel düzenlenmesi üzerindeki etkileri araştırılmıştır. Çalışmanın temel amacı, fenolik bileşiklerin yapısal özellikleri ile bakteriyel yanıt arasındaki ilişkiyi ortaya

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koymak ve membran stresine adaptasyonda rol oynayan genlerin düzenlenmesini değerlendirmektir.

2. DENEYSEL ÇALIŞMALAR

2.1. *E. coli* bakterisinin yetiştirilmesi ve fenolik bileşiklere maruz bırakılması

Bu çalışmada *Escherichia coli* BW25113 suşu kullanılmıştır. Bakteriyel kültürler Luria-Bertani (LB) besiyerinde 37°C'de, 150 rpm çalkalama koşullarında büyütülmüştür. Hücreler logaritmik büyüme fazına ulaştığında deneysel uygulamalara geçilmiştir. Fenolik bileşikler olarak trans-sinnamik asit (tCA), p-kumarik asit (pCA), ferulik asit (FA) ve benzoik asit (BA) kullanılmıştır. Her bir bileşik uygun çözücülerde hazırlanarak kültür ortamına eklenmiş ve bakteriler 15 dakika süreyle bu bileşiklere maruz bırakılmıştır. Kontrol grubu olarak fenolik bileşik içermeyen kültürler kullanılmıştır.

2.2. RNA izolasyonu

Fenolik bileşik uygulamasını takiben hücreler hızlı bir şekilde hasat edilmiştir. Toplam RNA izolasyonu, üretici firma protokolüne uygun olarak ticari bir RNA izolasyon kiti kullanılarak gerçekleştirilmiştir. Elde edilen RNA örneklerinin saflığı ve konsantrasyonu spektrofotometrik olarak değerlendirilmiş, ayrıca RNA bütünlüğü agaroz jel elektroforezi ile kontrol edilmiştir.

2.3. cDNA eldesi

İzole edilen toplam RNA'dan cDNA sentezi, ters transkripsiyon reaksiyonu kullanılarak gerçekleştirilmiştir. Bu amaçla, uygun miktarda RNA, random primerler ve reverse transkriptaz enzimi içeren reaksiyon karışımı hazırlanmış ve üretici firma talimatlarına göre inkübasyon gerçekleştirilmiştir. Elde edilen cDNA örnekleri, real-time PCR analizlerinde şablon olarak kullanılmıştır.

2.4. Real-time PCR reaksiyonunun gerçekleştirilmesi

Gen ekspresyon düzeylerinin belirlenmesi amacıyla kantitatif gerçek zamanlı PCR (qRT-PCR) yöntemi kullanılmıştır. Reaksiyonlar, uygun primer çiftleri, SYBR Green içeren master mix ve cDNA şablonu kullanılarak gerçekleştirilmiştir. Primer çifti olarak GAACATACCCAACTTCCCGC ve CATGGTGATGGGATCTTGCC sekansına sahip forward ve reverse primerleri kullanılmıştır. *mlaF* geninin ekspresyon düzeyleri, referans gen olarak kullanılan 16S rRNA geni ile normalize edilmiştir. Göreceli gen ekspresyon düzeyleri, $2^{-\Delta\Delta Ct}$ yöntemi kullanılarak hesaplanmıştır. Tüm deneyler en az üç teknik tekrar ile yürütülmüştür.

3. SONUÇLAR VE DEĞERLENDİRME

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Fenolik asitlere maruz bırakılan *E. coli* hücrelerinde *mlaF* geninin ekspresyon düzeylerinde belirgin değişiklikler gözlenmiştir. Elde edilen sonuçlara göre, trans-sinnamik asit (tCA) uygulaması *mlaF* ekspresyonunda 1,86 katlık bir artışa neden olurken, p-kumarik asit (pCA) ile bu artış 2,12 kat olarak belirlenmiştir. Ferulik asit (FA) uygulaması ise en yüksek indüksiyonu oluşturmuş ve *mlaF* ekspresyonunun 3,82 kat arttığı tespit edilmiştir. Buna karşılık, benzoik asit (BA) uygulaması yalnızca minimal düzeyde bir değişime (1,03 kat) yol açmıştır (Görsel 1).

Görsel 1. *Escherichia coli* BW25113 suşunda farklı fenolik asitlere maruz kalma sonrası *mlaF (yrbF)* geninin relatif ekspresyon düzeyleri. Hücreler 15 dakika süreyle fenolik bileşiklere maruz bırakılmış ve gen ekspresyonu qRT-PCR ile analiz edilmiştir. Ekspresyon düzeyleri, referans gen olarak kullanılan 16S rRNA'ya normalize edilmiş ve 2^−ΔΔCt yöntemi ile hesaplanmıştır. (A) trans-sinnamik asit (tCA), (B) p-kumarik asit (pCA), (C) ferulik asit

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(FA), (D) benzoik asit (BA) uygulamaları sonrası elde edilen sonuçlar gösterilmektedir. Veriler ortalama ± standart hata (SE) olarak sunulmuştur.

Bu sonuçlar, fenolik asitlerin bakteriyel yanıtı üzerinde yapı-bağımlı bir etki oluşturduğunu göstermektedir. Özellikle hidroksi ve metoksi grubu içeren sinnammik asit türevlerinin, benzoik asite kıyasla daha güçlü bir transkripsiyonel yanıt oluşturduğu görülmektedir. Bu durum, substitüsyon paterninin membran ile etkileşimi ve hücre zarfı üzerinde oluşturduğu stres düzeyi ile ilişkili olabilir. Daha kompleks yapıya sahip fenolik bileşiklerin, dış membranda daha fazla bozulmaya yol açarak Mla sistemi gibi fosfolipit taşınım mekanizmalarını aktive ettiği düşünülmektedir.

4. GENEL DEĞERLENDİRME VE SONUÇLAR

Bu çalışma, yapısal olarak benzer fenolik asitlerin *mlaF* geninin ekspresyonu üzerinde farklı etkiler oluşturduğunu ortaya koymuştur. Elde edilen bulgular, fenolik bileşiklerin bakteriyel hücrelerde oluşturduğu stresin yalnızca varlığına değil, aynı zamanda kimyasal yapılarına da bağlı olduğunu göstermektedir. Özellikle ferulik asidin güçlü indükleyici etkisi, bu bileşiğin membran bütünlüğü üzerinde daha belirgin bir etki oluşturduğunu ve fosfolipit taşınım sistemlerinin aktivasyonunu tetiklediğini düşündürmektedir.

Sonuç olarak, *mlaF* geninin fenolik asitlere karşı gelişen hücre zarfı stres yanıtında önemli bir rol oynadığı ve potansiyel bir düzenleyici düğüm noktası olduğu ortaya konulmuştur. Bu bulgular, fenolik bileşiklerin antimikrobiyal mekanizmalarının anlaşılmasına katkı sağlamakta ve aynı zamanda bakteriyel direnç mekanizmalarının hedeflenmesine yönelik yeni yaklaşımlar için temel oluşturmaktadır.

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THE MULTIFACETED EFFECTS OF PROBIOTICS ON HEALTH: A CURRENT ASSESSMENT OF IMMUNE REGULATION, INFLAMMATION SUPPRESSION, AND ANTICANCER POTENTIAL

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ABSTRACT

Probiotics are live microorganisms that, when consumed in sufficient quantities, can have positive effects on host health and have attracted increasing attention in recent years, particularly with advances in microbiota research. It is becoming increasingly clear that these microorganisms can influence not only gut balance but also the immune system, inflammatory processes, and even cancer development. Current findings indicate that probiotics can influence both innate and adaptive immune responses through various mechanisms. Increased macrophage and natural killer cell activity, along with regulation of cytokine balance (e.g., IL-10 and TNF-α levels), are key components of this effect. Furthermore, some species are known to limit colonization by competing with pathogenic microorganisms or producing antimicrobial compounds. The anti-inflammatory effects of probiotics are mostly associated with the suppression of inflammatory signaling pathways and the rebalancing of the gut microbiota. The production of metabolites such as short-chain fatty acids also plays a significant role in this process. These characteristics are noteworthy considering that chronic inflammation is thought to be a contributing factor in the development of many diseases. Furthermore, some studies show that probiotics can trigger apoptosis in tumor cells, limit cell proliferation, and play a supportive role in treatment processes. This review aims to provide a holistic assessment of the current literature on the immunomodulatory, antimicrobial, anti-inflammatory, and anticancer effects of probiotics. In our study, the fundamental mechanisms underlying these effects will be discussed in detail, particularly focusing on strain-specific differences and potential clinical applications. In this respect, the review aims to contribute to future research on the therapeutic use of probiotics.

Keywords: Probiotics, Immune response, Anti-inflammatory effect, Anticancer potential, Microbiota

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1. INTRODUCTION

The human gastrointestinal tract hosts a vast ecosystem of approximately 100 trillion (10^{14}) microorganisms that play a crucial role in the host's physiology, metabolism, and immune system. Probiotics are defined by FAO/WHO as "live microorganisms that, when administered in sufficient quantities, provide a health benefit to the host." While the scientific basis for this concept dates back to Elie Metchnikoff's 1907 report on the association between lactic acid-producing bacteria and longevity, modern medicine now considers these microorganisms not merely as digestive aids but as complex immunomodulatory agents (Hill et al., 2014). The gut microbiota provides critical signals for "training" the immune system, as well as metabolic functions such as the digestion of nutrients, the synthesis of vitamins K and B, and the production of short-chain fatty acids (SCFAs). The gastrointestinal system is the largest immunological organ in the body, housing approximately 70-80% of the total immune capacity through the gut-associated lymphoid tissue (GALT). Here, the host must maintain an active defense mechanism against pathogens while developing "immune tolerance" to harmless commensal bacteria. Probiotics play a vital role in maintaining this delicate balance by strengthening the intestinal barrier, preventing pathogen attachment, and modulating cytokine release (Mazziotta et al., 2023). *Lactobacillus* and *Bifidobacterium* genera, in particular, stand out as the strains with the broadest clinical evidence. Today, the effects of probiotics are not limited to the gut but offer systemic health improvement potential through gut-brain, gut-lung, and gut-liver axes. This article aims to analyze the molecular mechanisms of these interactions and their place in disease management in light of current literature (Adejumo et al., 2023).

2. Intestinal Barrier Function and Metabolically Related Fatty Liver Disease (MAFLD)

The intestinal epithelium acts as both a physical and chemical barrier between antigens within the lumen and host tissue, forming the body's first line of defense. The structural integrity of this barrier is maintained by tight junction proteins (ZO-1, occludin, claudin) between epithelial cells. In cases of dysbiosis (microbial imbalance), the expression of these proteins decreases, leading to the development of the "leaky gut" phenomenon. Increased intestinal permeability initiates gut-liver axis dysfunction, allowing bacterial components (especially lipopolysaccharides - LPS) to pass into the portal circulation and triggering chronic subclinical inflammation in the liver (De Heer 2024). Molecular analyses in patients with metabolic fatty liver disease (MAFLD) and type 2 diabetes have shown increased synthesis of zonulin, a protein that regulates the intestinal barrier, and elevated levels of Gram-negative bacteria (Bacteroidetes) compared to Gram-positive bacteria. Increased endotoxemia stimulates Toll-like receptors (TLR4, TLR9) on Kupffer cells in the liver, leading to overproduction of pro-inflammatory cytokines (IL-6, TNF-α) and ultimately liver damage with fibrosis (Fasano 2020). A randomized clinical trial cited in the literature investigated the use of rifaximin (antibiotic) in combination with multi-species probiotics (*Lactobacillus*, *Bifidobacterium*, and *Saccharomyces Boulardii*) and prebiotics (inulin, pectin) for a period of 6 months. The study results demonstrated that this combined treatment significantly reduced serum zonulin and IL-

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6 levels, stabilized the intestinal barrier, and normalized liver enzymes (ALT, AST), reducing the steatosis stage from severe (S3) to moderate (S2). These data highlight the potential of microbiota modulation as a pharmacological strategy in the treatment of MAFLD (Didyk et al., 2024).

3. Short-Chain Fatty Acids (SCFAs): The Power of Microbial Metabolites

Short-chain fatty acids (SCFAs), produced by the anaerobic fermentation of dietary fiber by the gut microbiota and probiotics, consist primarily of acetate, propionate, and butyrate and exert multifaceted effects on host health. These small 2-4 carbon molecules are central to energy metabolism in the colon. Butyrate, in particular, is the primary energy source for colonocytes and accelerates the assembly of tight junction proteins by activating the AMP-activated protein kinase (AMPK) pathway in epithelial cells, thus physically strengthening intestinal barrier integrity (Czachajda et al., 2024). The immunomodulatory effects of SCFAs occur through signaling via G-protein coupled receptors (GPR41, GPR43, GPR109A). These molecules exert an anti-inflammatory effect on macrophages and dendritic cells, increasing IL-10 production while suppressing the NF-κB signaling pathway and reducing the synthesis of pro-inflammatory mediators such as TNF-α and IL-6. Furthermore, acetate has been found to alleviate neutrophilic inflammation by increasing anexi A1 synthesis and showing a negative correlation with pro-inflammatory interferon-gamma (IFN-γ) (Adejumo et al., 2023). Metabolically, propionate has the potential to suppress cholesterol synthesis (via HMG-CoA reductase inhibition) by entering the hepatic portal circulation. SCFAs also regulate satiety signals (leptin, GLP-1, PYY), positively affecting appetite control and insulin sensitivity. Clinical studies show significantly lower fecal and serum SCFA levels in patients with IBD, obesity, and type 2 diabetes. This situation reinforces the importance of probiotic and high-fiber dietary supplementation in the management of metabolic diseases (Didyk et al., 2024).

4. T-Cell Immunity and Therapeutic Potential

T cells are considered the conductors of the adaptive immune system and can be directly modulated by probiotics at both developmental and functional stages. Naive T cells, after encountering antigens presented by dendritic cells, differentiate into Th1 (cellular response), Th2 (humoral response), Th17 (inflammatory), or Treg (regulatory) subgroups depending on the cytokine environment (Liu et al., 2025). For a healthy immune system, the balance between Th17 cells, which cause tissue damage, and Treg cells, which limit the immune response, is vital. In diseases such as IBD, rheumatoid arthritis, and multiple sclerosis, this balance appears to be disrupted in favor of Th17. There is strong mechanistic evidence that probiotics correct this balance (De Heer 2024). For example, the *Lactobacillus rhamnosus* GG (LGG) strain has been shown to reduce inflammatory damage in bone tissue by decreasing RORγt expression and increasing Foxp3+ Treg cells in osteoporosis models. Furthermore, perinatal supplementation of the mother with *L. acidophilus* and *B. bifidum* has been found to support thymus development in newborns and strengthen the immune system early in life by accelerating CD4+/CD8+ T cell maturation. In the oncological field, the I3A (indol-3-aldehyde) molecule produced by L. reuteri has been observed to enhance the effectiveness of immune checkpoint inhibitors (anti-PD-1) by increasing the cytotoxic activity (IFN-γ production) of

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CD8+ T cells in the tumor microenvironment. Strains such as B. pseudolongum have been reported to provide long-term antitumor protection by promoting the conversion of CD8+ T cells into memory cells via L-arginine metabolism. These findings demonstrate that probiotics are powerful adjuvants in both chronic inflammatory diseases and cancer immunotherapy (Mazziotta et al., 2023).

5. Oncological Processes and Probiotics

The high cytotoxicity and side effects of chemotherapeutic agents used in cancer treatments have increased interest in natural and safe adjuvant therapies such as probiotics. Probiotics exhibit antitumor effects in various cancer types, especially colorectal cancer (CRC), through mechanisms such as cell cycle arrest, triggering apoptosis, and strengthening immune surveillance. At the molecular level, probiotics inhibit the proliferation of cancer cells through genetic programming. For example, the p8 protein derived from *L. rhamnosus* suppresses the cyclin B1/CDK1 complex by increasing p21 expression and arrests the cell cycle in the G2/M phase. Similarly, *L. plantarum* provides inhibition on the cell cycle through p53 activation, while *L. paracasei* does so via the mTOR/4EBP1 signaling pathway. The ability of probiotics to trigger apoptosis is associated with the downregulation of survival genes Bcl-2 and Bcl-xL and the inactivation of the NF-κB signaling pathway. Furthermore, metabolites such as ferrichrome and butyrate produced by these microorganisms prevent DNA damage by scavenging reactive oxygen species (ROS) and trigger programmed cell death in cancer cells (Chung et al., 2020). Current research focuses on the critical role of probiotics in enhancing the efficacy of immune checkpoint inhibitors (ICBs). I3A (indole-3-aldehyde) secreted by L. reuteri or butyrate from Roseburia intestinalis directly stimulate the cytotoxic activity (IFNγ and Granzyme B production) of CD8+ T cells in the tumor microenvironment, thereby suppressing tumor growth. Additionally, strains such as *B. pseudolongum* have been observed to provide long-term antitumor protection by promoting the differentiation of CD8+ T cells into memory cells. Biotechnological advancements have paved the way for the use of engineered probiotics as "smart drug delivery vehicles." For example, genetically modified E. coli Nissle 1917 can be engineered to produce L-arginine within tumors, thereby enhancing T cell infiltration and activity. These synthetic biology approaches demonstrate that probiotics can be used to direct CAR-T cell therapies targeting tumors or to deliver immune-stimulating nanobodies directly to the tumor core. This data confirms the potential for probiotics to become an integral part of modern oncological strategies (Liu et al., 2025).

6. Effects on Cholesterol Metabolism

The hypocholesterolemic effects of probiotics have attracted significant attention in recent years as a "natural and safe alternative" in cardiovascular protection strategies. This effect occurs not through a single pathway, but through the synergy of a number of complex biochemical mechanisms. The best-characterized mechanism is the Bile Salt Hydrolase (BSH) enzyme activity possessed by some probiotics (particularly *L. plantarum* and *Bifidobacterium* strains). The BSH enzyme deconjugates bile salts conjugated with glycine or taurine, converting them into secondary bile acids with low water solubility. Since these acids cannot be reabsorbed from the small intestine, they are excreted in the feces. The body is forced to use serum

cholesterol as a raw material to replenish the lost bile salt pool; this "compensatory synthesis" process leads to a significant decrease in serum total cholesterol and LDL levels (Çavdar et al., 2025). Another mechanism is the direct incorporation of cholesterol into the cell membranes of probiotics or their assimilation within the lumen. Strains with high hydrophobicity have been shown to inhibit cholesterol absorption by integrating it into phospholipid tails. Furthermore, some probiotics, due to the cholesterol reductase enzyme they structurally possess, have been found to convert cholesterol into coprostanol, an unabsorbable form, facilitating its excretion in the feces. SCFAs, such as propionate produced by the fermentation of prebiotics, also balance systemic cholesterol levels by inhibiting cholesterol biosynthesis in the liver. While clinical meta-analyses report that probiotic consumption can lead to an average decrease of 8.40 mg/dl in serum cholesterol and 6.63 mg/dl in LDL, it is emphasized that this effect is highly strain-specific and varies depending on the dose and duration of administration (Czachajda et al., 2024).

7. Clinical Application, Safety, and Industrial Challenges

Although probiotics are generally considered "safe" (GRAS/QPS), the inability to generalize strain-specific efficacy in clinical applications is one of the biggest challenges. The anti-inflammatory effect of one strain may not be present in another strain of the same genus. In addition, the use of probiotics in severely immunocompromised individuals, premature infants, or critically ill patients with vascular catheters may, albeit rarely, carry risks of sepsis, bacteremia, or probiotic-derived infections. Another concern is the potential of probiotics to transfer antibiotic resistance genes to pathogenic bacteria (mutagenesis); this requires rigorous research, especially regarding the safety of genetically modified strains (Adejumo et al., 2023). Industrial-scale probiotic production faces significant technical obstacles in terms of preserving the viability and physiological properties of microorganisms. Bioreactor conditions, freezing, and lyophilization (freeze-drying) stages in the production process can damage cell viability. To prevent this damage, cryoprotectants are being used and microencapsulation technologies that enhance cell stability are being developed (Hill et al., 2014). Microencapsulation increases the resistance of the probiotic to stomach acid and bile salts, ensuring it reaches the target region, the intestines, alive. Current regulations vary worldwide; in Europe (EFSA), probiotics are considered "functional foods," while in the US (FDA), they may fall into the category of dietary supplements or medicinal products depending on their intended use. In the future, "personalized probiotic therapies" based on the host's microbiota composition and genetic makeup, and genetically engineered "smart probiotics," will be an integral part of precision medicine practices (Mazziotta et al., 2023).

CONCLUSION

The findings discussed in this review reveal that probiotics are not only microorganisms that balance the gut microbiota, but also important regulators exhibiting multifaceted biological effects. Their influence on different components of the immune system, their role in controlling inflammatory processes, and their potential to intervene in various disease mechanisms have made these microorganisms clinically noteworthy. The current literature shows that probiotics are effective primarily through key mechanisms such as maintaining intestinal barrier integrity,

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producing metabolites like short-chain fatty acids, and balancing the immune response. Furthermore, they appear to play an active role in processes such as regulating T cell responses and maintaining the balance between pro-inflammatory and anti-inflammatory cytokines. These effects offer significant advantages in the prevention and management of diseases associated with chronic inflammation. From an oncological perspective, probiotics appear to be potential supportive agents through mechanisms such as suppressing the cell cycle, triggering apoptosis, and influencing the tumor microenvironment. Furthermore, findings regarding the enhancement of the effectiveness of immunotherapies suggest that research in this area will intensify in the future. However, the fact that the effects of probiotics are largely strain-specific, that clinical outcomes are not always consistent, and that standard usage protocols are not yet clearly defined are significant limitations. Moreover, safety, especially in immunocompromised individuals, needs to be carefully considered. Maintaining viability and stability in industrial production processes is also a separate technical challenge. In future studies, it is important to focus on clinical trials with larger sample groups, to more clearly elucidate the mechanisms of action of probiotics, and to standardize their dosages and durations. In addition, the development of approaches specific to the individual's microbiota and the use of genetically engineered next-generation probiotics will be decisive in the development of this field. In conclusion, probiotics offer an increasingly important area of research due to their effects on the immune system, inflammation, and disease processes. For this potential to be effectively reflected in clinical applications, existing knowledge needs to be supported by further studies.

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DETERMINATION OF ANTICANCER ACTIVITY AND BIOCOMPATIBILITY OF COMMERCIAL AND SYNTHESIZED PLA-PEG-PLA BLOCK COPOLYMERS

SENTEZ VE TİCARİ PLA-PEG-PLA BLOK KOPOLİMERLERİN ANTİKANSER ETKİNLİĞİ VE BİYOUYUMLULUKLARININ TAYİN EDİLMESİ

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ABSTRACT

In recent years, the disadvantages of non-biodegradable synthetic particularly accumulation in nature, have led to a significant increase in interest in biodegradable polymers. It has been reported that the PLA-PEG block copolymers obtained via copolymerization combine the advantageous properties of both individual polymers. A review of the literature reveals substantial data on the cytotoxicity of drug delivery systems prepared from physically or covalently assembled PLA–PEG and PLA–PEG–PLA block copolymers; however, limited information is available regarding the cytotoxic effects of PLA–PEG and/or PLA–PEG–PLA alone. Based on this knowledge, the anticancer activities of a synthesized PLA–PEG–PLA block copolymer, designed as a drug carrier system with an optimized molecular weight, were compared with those of a commercial block copolymer.

The block copolymer was synthesized via cationic ring-opening polymerization using HO-PEG-OH. Different characterization techniques were carried out to determine the chemical structure and composition of the synthesized block copolymer. The results demonstrated that the synthesized copolymer exhibited physicochemical properties comparable to those of the commercial polymer. Biocompatibility was evaluated using the murine fibroblast cell line L929. The anticancer activities of the synthesized and commercial polymers at different concentrations were assessed in Caco-2 cells (human epithelial colorectal adenocarcinoma) and MCF-7 cells (human breast cancer cell line). Anticancer activity results revealed that the synthesis and commercial polymer exhibited similar anticancer activity and biocompatibility. These results demonstrated that the synthesized polymer exhibited properties comparable to those of the commercial polymer and were consistent with the initial objectives of the study.

Anahtar Kelimeler : Anticancer, biocompatibility, PLA-PEG-PLA block copolymer

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MICROBIAL DIVERSITY AND FUNCTIONAL ANALYSIS IN TAIWAN HIGH-MOUNTAIN HOT SPRINGS

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ABSTRACT

High-mountain hot springs in Taiwan provide extreme ecological niches where temperature, mineral composition, and fluctuating geochemical conditions shape distinctive microbial communities. This study investigates microbial diversity and functional potential in selected hot spring systems located in alpine regions of Taiwan, with attention to how microbial assemblages adapt to thermal stress and nutrient limitation. Using culture-independent molecular methods, community profiling, and functional annotation, the research reveals that these ecosystems harbor diverse bacteria and archaea with specialized metabolic strategies. Thermophilic lineages dominate the high-temperature zones, while microhabitats with reduced thermal intensity support broader taxonomic variation and greater functional complexity. The findings indicate that microbial composition is strongly influenced by environmental gradients, particularly temperature, pH, and sulfur availability. Functional analysis suggests active roles in sulfur cycling, organic matter turnover, and stress tolerance, highlighting the ecological importance of these communities in sustaining geochemical processes within hot spring habitats. The study also underscores the biotechnological relevance of thermophilic microorganisms, which may yield enzymes with industrial applications in food processing, bioenergy, and environmental remediation. Beyond their applied value, these microbial systems offer insight into life adaptation under extreme conditions and contribute to broader discussions of microbial resilience in mountainous geothermal environments. The research emphasizes the need for conservation-oriented management of Taiwan's hot spring ecosystems, as human disturbance and tourism pressure may threaten microbial biodiversity before its ecological and scientific potential is fully understood. Overall, the study provides a comprehensive view of microbial diversity and ecosystem function in one of Taiwan's most distinctive natural environments.

Key words: microbial diversity, hot springs, thermophiles, functional analysis, Taiwan

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PHYTOREMEDIATION POTENTIAL OF TAIWANESE NATIVE PLANTS FOR INDUSTRIAL WASTEWATER TREATMENT

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ABSTRACT

Industrial wastewater continues to pose a significant environmental challenge in Taiwan, particularly in regions where manufacturing activities generate complex mixtures of heavy metals, dyes, and organic pollutants. This study examines the phytoremediation potential of selected native Taiwanese plants as sustainable agents for wastewater treatment. Native species were evaluated for their ability to tolerate contaminated conditions, accumulate pollutants in plant tissues, and support rhizosphere processes that promote contaminant reduction. The research shows that several plant species native to wetland and riparian habitats exhibit strong resilience under polluted conditions and can contribute to the stabilization, uptake, and transformation of harmful substances. In addition to direct absorption of contaminants, the plants stimulate microbial activity in the root zone, improving the breakdown of organic compounds and the immobilization of metals. The findings suggest that phytoremediation can function as a low-cost, ecologically compatible complement to conventional treatment technologies, especially in smaller industrial zones where infrastructure investments may be limited. The study further highlights the importance of species selection, seasonal growth patterns, and site-specific environmental conditions in determining treatment efficiency. Because native plants are already adapted to local climate and soil characteristics, they offer practical advantages over introduced species. This research supports the integration of ecological restoration principles into wastewater management and argues for broader use of phytotechnology in Taiwan's industrial landscapes. The results provide a useful basis for designing sustainable treatment systems that combine environmental protection with landscape recovery and biodiversity conservation.

Key words: phytoremediation, native plants, industrial wastewater, Taiwan, environmental restoration

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GENETIC ADAPTATION MECHANISMS OF ETHIOPIAN COFFEE ARABICA VARIETIES TO DROUGHT STRESS

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ABSTRACT

Arabica coffee is one of Ethiopia's most important biological and economic resources, yet its long-term sustainability is increasingly threatened by drought stress associated with climate variability. This study explores the genetic adaptation mechanisms that enable Ethiopian coffee Arabica varieties to survive under water-limited conditions. By comparing drought-tolerant and drought-sensitive landraces, the research identifies physiological and molecular traits associated with resilience. The analysis shows that adapted varieties maintain stronger stomatal regulation, better leaf water retention, and more stable photosynthetic activity during periods of limited moisture. At the molecular level, stress-responsive genes involved in osmotic adjustment, antioxidant defense, and root development are more actively expressed in tolerant plants. These responses suggest that adaptation in Ethiopian coffee is not based on a single mechanism but on a coordinated set of physiological and genetic traits shaped by long-term exposure to diverse highland environments. The study also highlights the value of Ethiopia's traditional coffee genetic resources as a reservoir of climate resilience for future breeding programs. Because many local varieties have evolved within complex agro-ecological systems, they provide important models for understanding natural adaptation to drought. The findings support conservation of coffee genetic diversity and encourage breeding strategies that preserve locally adapted traits while improving productivity. In a broader sense, the research demonstrates how indigenous crop diversity can contribute to food security and climate adaptation in vulnerable agricultural regions. The results offer both scientific and practical relevance for sustainable coffee production under changing environmental conditions.

Key words: Arabica coffee, drought stress, genetic adaptation, Ethiopia, crop resilience

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BIODIVERSITY CONSERVATION STRATEGIES FOR ETHIOPIAN HIGHLAND FROG SPECIES

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ABSTRACT

Ethiopian highland frog species represent an essential component of montane biodiversity, yet many populations are increasingly threatened by habitat loss, climate change, agricultural expansion, and disease. This study examines conservation strategies designed to support the survival of endemic frog species in Ethiopian highland ecosystems. Through field surveys, habitat assessments, and species distribution analysis, the research identifies the ecological conditions most critical for frog persistence. Moisture availability, stream integrity, vegetation cover, and elevation emerge as key determinants of population stability. The study also reveals that land-use change and habitat fragmentation have reduced connectivity between breeding sites, increasing isolation and vulnerability. In addition, environmental pressures associated with warming temperatures appear to be shifting suitable habitat ranges upward, placing further stress on already restricted populations. Conservation strategies proposed in the study include habitat restoration, protection of breeding wetlands, community-based monitoring, and stronger integration of amphibian conservation into regional land management planning. The findings emphasize that effective protection of highland frogs requires not only biological knowledge but also collaboration with local communities and policy institutions. Because amphibians function as sensitive indicators of ecosystem health, their conservation has broader implications for water resources, biodiversity integrity, and climate adaptation in Ethiopia's highland landscapes. The study concludes that proactive conservation planning is essential to prevent further decline of these ecologically valuable species and to preserve the unique biological heritage of Ethiopian mountain systems.

Key words: amphibian conservation, Ethiopian highlands, habitat loss, biodiversity, climate change

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MOLECULAR IDENTIFICATION OF IRANIAN PISTACHIO PATHOGENS AND RESISTANCE GENES

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ABSTRACT

Pistachio cultivation is a major agricultural activity in Iran, but its productivity is increasingly affected by fungal and bacterial pathogens that reduce yield and compromise tree health. This study focuses on the molecular identification of major pistachio pathogens and the characterization of resistance genes associated with host defense responses. Samples collected from orchards in different pistachio-growing regions were analyzed using molecular diagnostics to determine pathogen identity and prevalence. The results show that several pathogenic species are consistently associated with leaf spots, branch dieback, and fruit deterioration, indicating a complex disease profile rather than a single causal agent. Molecular screening of host material also reveals the presence of defense-related genes linked to pathogen recognition, signal transduction, and stress response. These findings suggest that resistance in pistachio is influenced by a combination of genetic factors and environmental conditions. The study highlights the importance of accurate pathogen detection for effective disease management, particularly in orchards where symptoms may overlap and visual diagnosis alone is unreliable. It also emphasizes the value of resistance gene analysis in breeding programs aimed at developing more resilient cultivars. By combining pathogen identification with host molecular profiling, the research contributes to improved understanding of pistachio disease ecology and provides a foundation for integrated management strategies. The findings are relevant not only for Iranian agriculture but also for other pistachio-producing regions facing similar phytosanitary challenges. The study ultimately supports the use of molecular tools as a practical framework for enhancing crop protection and sustaining long-term orchard productivity.

Key words: pistachio, molecular identification, plant pathogens, resistance genes, Iran

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PHYTOCHEMICAL SCREENING OF MEDICINAL HERBS FROM IRANIAN ZAGROS MOUNTAINS

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ABSTRACT

The Zagros Mountains contain a rich diversity of medicinal herbs that have long been used in traditional healing systems, yet many of these species remain insufficiently studied at the phytochemical level. This research investigates selected medicinal herbs from the Iranian Zagros region in order to identify their major bioactive constituents and assess their potential therapeutic relevance. Using extraction and screening methods, the study evaluates the presence of phenolics, flavonoids, terpenoids, alkaloids, and other secondary metabolites known for pharmacological activity. The results indicate that several species contain high concentrations of compounds associated with antioxidant, anti-inflammatory, and antimicrobial properties. Variation in chemical composition appears to be influenced by altitude, habitat type, and seasonal collection time, suggesting that ecological conditions play an important role in metabolite production. The study also highlights the significance of traditional knowledge in guiding plant selection, as local communities often identify species with strong medicinal value based on long-standing empirical use. These findings support the idea that the Zagros flora represents an important reservoir for natural product research and future drug discovery. At the same time, they underscore the need for sustainable harvesting practices, since increasing demand for herbal remedies may place pressure on wild populations. The research contributes to the documentation of Iran's medicinal plant heritage and provides a scientific basis for further pharmacological and conservation studies. Overall, the study shows that phytochemical screening can serve as a bridge between ethnobotanical tradition and modern biomedical research.

Key words: medicinal herbs, phytochemistry, Zagros Mountains, bioactive compounds, Iran

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ENDANGERED BIRD POPULATION DYNAMICS IN GEORGIAN CAUCASUS MOUNTAIN ECOSYSTEMS

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ABSTRACT

The Caucasus mountain ecosystems of Georgia support a unique avian fauna, including several endangered bird species whose populations are increasingly influenced by habitat change and human activity. This study examines population dynamics, habitat use, and conservation pressures affecting endangered birds in selected mountain regions of Georgia. Through field observations, nesting surveys, and habitat assessment, the research identifies key factors shaping population stability. The findings show that breeding success is closely linked to intact forest cover, limited disturbance, and access to high-altitude feeding areas. In contrast, grazing pressure, tourism expansion, and forest degradation contribute to habitat fragmentation and reduced reproductive success. Seasonal movement patterns also indicate that these birds depend on a network of connected habitats rather than isolated protected areas. The study suggests that population trends are sensitive to both environmental variability and land-use change, making long-term conservation planning essential. Conservation strategies proposed include the protection of nesting zones, improved monitoring of population trends, and integration of bird conservation into mountain land management policies. The research also emphasizes the role of local communities in protecting biodiversity through sustainable land use. Because endangered birds are important indicators of ecosystem integrity, their decline reflects broader ecological risks in the Caucasus region. The study concludes that effective conservation must combine scientific monitoring with practical habitat protection and regional cooperation. These findings contribute to the broader understanding of mountain biodiversity conservation in Georgia and the Caucasus.

Key words: endangered birds, population dynamics, Caucasus Mountains, biodiversity conservation, Georgia

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FUNGAL DIVERSITY SURVEY IN GEORGIAN BLACK SEA COASTAL FORESTS

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ABSTRACT

Georgian Black Sea coastal forests represent biologically rich but ecologically sensitive ecosystems where fungal communities play a crucial role in nutrient cycling, decomposition, and plant health. This study surveys fungal diversity in selected coastal forest areas along Georgia's Black Sea region, aiming to document species composition and evaluate ecological functions. Field sampling and laboratory identification reveal a broad spectrum of fungal taxa associated with leaf litter, soil, decaying wood, and living plant tissues. The results indicate that fungal diversity is strongly influenced by humidity, forest structure, and plant community composition. Saprotrophic fungi dominate decomposer functions, while mycorrhizal species contribute to forest productivity and tree resilience. The study also identifies fungal taxa that may serve as indicators of environmental change, particularly in areas affected by tourism, fragmentation, and altered microclimates. Because coastal forests are under increasing pressure from land conversion and human disturbance, documenting fungal diversity is important for understanding ecosystem stability and hidden biodiversity. The findings suggest that fungal communities are not only functionally essential but also highly responsive to local environmental conditions. The research contributes to baseline biodiversity knowledge and supports future conservation planning in Georgia's coastal regions. It also demonstrates that fungi should be considered integral components of forest management rather than secondary or overlooked organisms. Overall, the study provides a foundation for ecological monitoring and highlights the need to preserve fungal habitats as part of broader forest conservation efforts.

Key words: fungal diversity, coastal forests, Black Sea, forest ecology, Georgia

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GENETIC DIVERSITY ASSESSMENT OF GEORGIAN WINE GRAPE CULTIVARS

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ABSTRACT

Georgia is recognized as one of the world's oldest centers of viticulture, and its traditional wine grape cultivars represent an important part of both agricultural heritage and genetic resources. This study assesses the genetic diversity of selected Georgian wine grape cultivars in order to better understand their relationships, conservation value, and potential for future breeding. Molecular marker analysis reveals substantial variation among cultivars, confirming that Georgian vineyards contain a rich and differentiated genetic pool. Some cultivars show close genetic relationships, likely reflecting historical propagation and regional selection, while others appear to preserve distinct lineages associated with local adaptation. The study also suggests that traditional cultivars may possess traits linked to climate tolerance, disease resistance, and wine quality, making them valuable for both conservation and agricultural innovation. Because modern viticulture increasingly favors a limited number of commercial varieties, the preservation of local grape diversity is essential to maintain resilience and cultural identity. The findings support the importance of in situ and ex situ conservation programs and highlight the need for continued molecular characterization of indigenous cultivars. They also reinforce the role of Georgia as a key center of grape domestication and genetic heritage. By documenting and comparing the diversity of traditional cultivars, the study provides useful information for breeders, growers, and policy makers seeking to strengthen sustainable viticulture. Overall, the research demonstrates that genetic diversity is not only a biological resource but also a foundation for the continuity of Georgian wine culture.

Key words: grape cultivars, genetic diversity, viticulture, molecular markers, Georgia

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ENZYMATIC SYNTHESIS OF NOVEL GLYCOLIPIDS FOR BIOMEDICAL APPLICATIONS USING ENGINEERED LIPASES

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ABSTRACT

This study explores the enzymatic synthesis of novel glycolipids using engineered lipases and evaluates their potential biomedical applications. Glycolipids are increasingly recognized as functional biomolecules with promising roles in drug delivery, membrane interaction, antimicrobial activity, and biocompatible material design. In this research, a series of lipase variants were engineered to improve regioselectivity, catalytic stability, and substrate tolerance in non-aqueous synthesis systems. Using sugar donors and long-chain fatty acid derivatives, the optimized enzymes successfully produced several glycolipid structures under mild reaction conditions. Structural verification and purity assessment confirmed the formation of target compounds with favorable physicochemical properties. Comparative analysis showed that engineered lipases exhibited stronger catalytic efficiency than the wild-type enzyme and maintained activity over prolonged reaction periods. The resulting glycolipids demonstrated improved emulsifying behavior, enhanced surface activity, and good compatibility with biological environments. Preliminary bioassays indicated potential applications in antimicrobial formulations and controlled-release systems, suggesting that these molecules may serve as useful candidates for future pharmaceutical and biomedical development. The study also highlights the advantages of biocatalysis over conventional chemical synthesis, including reduced solvent use, lower energy demand, and fewer unwanted by-products. Overall, the findings support the use of protein engineering as an effective strategy for designing sustainable catalytic platforms for the production of value-added biomolecules. This work contributes to the growing field of enzyme-mediated lipid chemistry and provides a foundation for further optimization of glycolipid synthesis for therapeutic and diagnostic applications.

Keywords: glycolipids, engineered lipases, biocatalysis, biomedical applications, enzyme engineering

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MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS BIOMARKERS IN NEURODEGENERATIVE DISEASE MODELS

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ABSTRACT

This study investigates the relationship between mitochondrial dysfunction and oxidative stress biomarkers in experimental models of neurodegenerative disease. Mitochondrial impairment is widely regarded as a central mechanism in the progression of disorders such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. In this research, biochemical and cellular markers associated with mitochondrial respiration, membrane potential, reactive oxygen species formation, and antioxidant defense were evaluated in disease-relevant models. The findings indicate that mitochondrial damage is accompanied by elevated oxidative stress, disrupted ATP production, and altered expression of enzymes responsible for maintaining cellular redox balance. Several biomarkers, including lipid peroxidation products, protein carbonyls, and glutathione-related indices, showed consistent association with neuronal injury and functional decline. The study also observed that oxidative stress can amplify mitochondrial defects, creating a self-reinforcing cycle that accelerates neurodegeneration. These results underline the importance of early detection of mitochondrial and oxidative abnormalities for disease monitoring and therapeutic intervention. In addition, the data suggest that targeting mitochondrial quality control, antioxidant defense systems, and energy metabolism may offer promising strategies for neuroprotection. The present work contributes to a deeper understanding of disease mechanisms and supports the development of biomarker-based approaches for diagnosis and treatment evaluation in neurodegenerative conditions.

Keywords: mitochondrial dysfunction, oxidative stress, neurodegenerative disease, biomarkers, neuronal injury

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BIOCHEMICAL CHARACTERIZATION OF DATE PALM PHOSPHATASES UNDER DROUGHT STRESS CONDITIONS

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ABSTRACT

This research examines the biochemical characteristics of phosphatase enzymes extracted from date palm tissues exposed to drought stress conditions. Date palm is a highly drought-tolerant crop, and its adaptive responses provide an important model for understanding enzymatic regulation under environmental stress. The study focused on enzyme activity, pH and temperature sensitivity, substrate preference, and stress-related stability in samples obtained from plants subjected to varying water availability. The results showed that drought stress significantly altered phosphatase activity, reflecting changes in phosphorus metabolism and cellular signaling pathways. Certain phosphatase isoforms demonstrated increased stability under dehydration, suggesting a protective role in maintaining metabolic balance during water limitation. The analysis also indicated that enzyme activity was closely linked to osmotic adjustment, membrane integrity, and stress-induced phosphate recycling. These findings support the view that phosphatases contribute to drought adaptation by regulating nutrient mobilization and intracellular homeostasis. Understanding these biochemical responses may help improve crop resilience through breeding or biotechnological approaches. The study provides useful insights into plant stress physiology and highlights the broader agricultural significance of enzyme-level adaptations in arid-region species. Overall, the results contribute to the characterization of molecular mechanisms underlying drought tolerance in date palm and may inform future strategies for sustainable crop production in water-scarce environments.

Keywords: date palm, phosphatase, drought stress, enzyme characterization, plant physiology

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METABOLIC PATHWAY ANALYSIS OF ANTIBIOTIC-PRODUCING ENDOPHYTES FROM EGYPTIAN DESERT PLANTS

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ABSTRACT

This study investigates the metabolic pathways of antibiotic-producing endophytic microorganisms isolated from medicinal plants native to Egyptian desert ecosystems. Endophytes associated with harsh and nutrient-limited environments are known to produce bioactive metabolites with strong antimicrobial potential. In this research, selected isolates were screened for antimicrobial activity, and their metabolic profiles were analyzed to identify key biosynthetic pathways involved in secondary metabolite production. The findings indicate that several isolates possessed distinct enzymatic and metabolic capabilities linked to polyketide, non-ribosomal peptide, and hybrid metabolite synthesis. These pathways were associated with the production of compounds showing inhibitory effects against clinically relevant bacterial strains. The study also suggests that desert plant endophytes activate specialized metabolic routes as survival strategies in stressful ecological conditions, which may explain their capacity to generate structurally diverse antibiotics. Comparative analysis revealed that isolates with stronger bioactivity also exhibited broader metabolic flexibility and enhanced resistance to environmental stress. These results emphasize the value of desert biodiversity as a source of novel microbial resources for drug discovery. The work contributes to the understanding of endophyte metabolism and supports the development of natural product-based strategies for addressing antibiotic resistance. Future research may focus on purification, structural elucidation, and biosynthetic gene cluster analysis to advance the pharmaceutical potential of these microorganisms.

Keywords: endophytes, antibiotic production, metabolic pathways, Egyptian desert plants, natural products

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INVESTIGATION OF LIPID PEROXIDATION PRODUCTS IN CARDIOVASCULAR DISEASE PATIENTS

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ABSTRACT

This study investigates lipid peroxidation products as biochemical indicators in patients with cardiovascular disease. Oxidative stress has been widely implicated in the development and progression of atherosclerosis, myocardial injury, and endothelial dysfunction. In this work, biomarkers associated with lipid oxidation were analyzed in clinical samples to evaluate their diagnostic and pathological relevance. The results demonstrated elevated levels of lipid peroxidation products in cardiovascular patients compared with healthy controls, indicating increased oxidative damage to cell membranes and circulating lipids. These changes were accompanied by reduced antioxidant capacity, suggesting an imbalance between reactive oxygen species production and protective defense mechanisms. The findings also showed that oxidative stress markers were more pronounced in patients with advanced clinical symptoms, supporting their possible use as indicators of disease severity. The study highlights the role of lipid peroxidation not only as a consequence of cardiovascular pathology but also as a contributing factor to inflammatory and degenerative processes. These results underline the importance of oxidative biomarkers in risk assessment, early diagnosis, and treatment monitoring. The data further suggest that antioxidant-targeted interventions may support cardiovascular protection. Overall, the study contributes to a better biochemical understanding of cardiovascular disease and supports the inclusion of lipid peroxidation markers in future diagnostic and prognostic strategies.

Keywords: lipid peroxidation, cardiovascular disease, oxidative stress, biomarkers, antioxidant defense

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PROTEOMIC PROFILING OF STRESS-INDUCED CHANGES IN AZERBAIJANI GRAPE CULTIVARS

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ABSTRACT

This research examines proteomic changes in Azerbaijani grape cultivars exposed to environmental stress conditions. Grapevine productivity and fruit quality are strongly influenced by temperature fluctuations, water deficit, and other climatic pressures, making molecular-level stress analysis essential for crop improvement. In this study, comparative proteomic profiling was performed to identify proteins associated with stress response, cellular protection, and metabolic regulation. The results showed significant changes in proteins linked to photosynthesis, carbohydrate metabolism, antioxidant defense, and signal transduction. Stress-tolerant cultivars displayed stronger activation of protective proteins and more stable expression patterns under adverse conditions. These findings suggest that proteomic adaptation plays a central role in maintaining vine resilience and supporting fruit development under environmental stress. The study also identified cultivar-specific protein signatures that may serve as useful biomarkers for breeding programs aimed at improving stress tolerance and agronomic performance. By connecting protein expression patterns with physiological responses, the research provides a clearer understanding of how grape cultivars respond to changing climatic conditions. The findings may support future strategies for vineyard management, cultivar selection, and molecular breeding in Azerbaijan and similar viticultural regions. Overall, the study contributes to crop proteomics and offers practical insights for sustaining grape production in challenging environments.

Keywords: proteomics, grape cultivars, stress response, Azerbaijan, plant resilience

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GLYCOGENOLYSIS REGULATION IN HIGH-ALTITUDE ADAPTED LIVESTOCK FROM KAZAKHSTAN STEPPES

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ABSTRACT

This study investigates the regulation of glycogenolysis in livestock adapted to high-altitude environments in the Kazakhstan steppes. Animals living in elevated terrains are exposed to hypoxia, cold stress, and energy-demanding physiological conditions, requiring efficient metabolic adaptation to maintain performance and survival. The research focused on key enzymatic and hormonal mechanisms controlling glycogen breakdown in muscle and liver tissues. The findings indicate that high-altitude adapted livestock display more efficient regulation of glycogenolysis, allowing better energy mobilization under stress. Differences in enzyme activity suggested enhanced metabolic flexibility and improved capacity to sustain glucose availability during periods of reduced oxygen supply. The study also showed that these adaptive traits were associated with improved tolerance to environmental stress and more stable physiological performance. Such mechanisms are important for maintaining growth, reproduction, and productivity in challenging pasture systems. The research contributes to understanding how livestock species adapt to extreme ecological conditions and may support selective breeding programs aimed at improving resilience. In addition, the findings have broader implications for animal physiology, metabolic regulation, and sustainable livestock production in mountain regions. Overall, the study highlights the importance of glycogen metabolism in supporting adaptation to high-altitude environments and provides a biochemical basis for future research on animal performance under climatic stress.

Keywords: glycogenolysis, high-altitude adaptation, livestock physiology, Kazakhstan steppes, metabolic regulation

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MICROBIAL ENZYME DISCOVERY FOR BIOFUEL PRODUCTION FROM KAZAKH WHEAT RESIDUES

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ABSTRACT

This study explores the discovery of microbial enzymes from Kazakhstan wheat residues and their potential application in biofuel production. Agricultural by-products such as wheat straw and husks represent an abundant lignocellulosic resource, but efficient conversion requires enzymatic systems capable of breaking down complex biomass. In this research, microbial isolates obtained from residue-rich environments were screened for cellulase, hemicellulase, and related hydrolytic enzyme activities. The results showed that several strains produced enzyme complexes with strong biomass-degrading potential. These enzymes demonstrated effective action under conditions relevant to industrial biofuel processing, suggesting their suitability for pretreatment and saccharification steps. The study further indicates that microbial adaptation to plant residue environments may contribute to the production of robust enzymes with high stability and catalytic efficiency. Such enzymes can improve the conversion of agricultural waste into fermentable sugars, thereby supporting sustainable bioethanol and bioproduct development. The findings underline the importance of regional microbial resources in advancing renewable energy technologies. By linking waste valorization with enzyme discovery, the research offers a practical pathway for reducing dependence on fossil fuels while adding value to agricultural residues. Overall, this work contributes to biotechnology, bioenergy, and circular economy research, and it supports future development of indigenous enzyme-based systems for biomass conversion in Kazakhstan.

Keywords: microbial enzymes, biofuel production, wheat residues, biomass conversion, biotechnology

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CRISPR-CAS9 MEDIATED GENE EDITING FOR DROUGHT RESISTANCE IN ROMANIAN WHEAT VARIETIES

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ABSTRACT

This study explores the potential of CRISPR-Cas9 gene editing to improve drought resistance in Romanian wheat varieties, with a focus on traits associated with water-use efficiency, root architecture, and stress-responsive gene regulation. As climate variability increasingly affects cereal production in Eastern Europe, breeding strategies that combine precision biotechnology with locally adapted germplasm have become essential. In this research, target genes associated with abscisic acid signaling, stomatal regulation, and osmotic adjustment were selected based on previous genomic and functional studies in wheat. Edited lines were generated using a tissue-culture-based transformation approach and subsequently evaluated under controlled drought conditions and comparative greenhouse trials. The results indicate that genome-edited lines exhibited improved physiological performance under water deficit, including delayed wilting, better chlorophyll retention, and enhanced root development. Molecular analyses confirmed stable editing in the selected loci without visible detrimental effects on plant morphology or grain quality. The study also emphasizes the importance of using indigenous wheat varieties as genetic backgrounds because their adaptation to local soils and climates may support more reliable field performance than exotic cultivars. In addition, the findings suggest that CRISPR-based breeding can complement conventional selection methods by shortening breeding cycles and increasing precision in trait improvement. The research contributes to the growing literature on sustainable crop innovation and demonstrates the value of integrating modern molecular tools into regional agricultural development strategies. It also highlights the need for future field-scale validation, regulatory consideration, and public engagement regarding the use of genome editing in food crops.

Key word: CRISPR-Cas9, drought resistance, wheat breeding, genome editing, Romania

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EPIGENETIC MODIFICATIONS ASSOCIATED WITH CANCER PROGRESSION IN ROMANIAN POPULATIONS

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ABSTRACT

This research investigates epigenetic alterations associated with cancer progression in Romanian populations, with particular attention to DNA methylation patterns, histone modification profiles, and their relationship to tumor aggressiveness and clinical outcomes. Epigenetic regulation has become a central area in oncology because it helps explain how gene expression changes occur without alterations in DNA sequence. In this study, tissue and blood samples were analyzed to identify molecular signatures linked to malignancy development across major cancer types observed in Romanian patients. The findings show that abnormal promoter methylation in tumor suppressor genes is strongly associated with disease advancement, reduced treatment response, and poorer survival indicators. In parallel, dysregulated histone marks appear to contribute to altered chromatin accessibility and oncogenic transcriptional activity. The study also indicates that these epigenetic patterns vary according to cancer stage and tissue type, suggesting the presence of disease-specific regulatory mechanisms. A further important outcome of the research is the demonstration that some epigenetic changes may serve as potential biomarkers for early detection, prognostic assessment, and therapeutic monitoring. The study discusses the relevance of integrating epigenomic profiling into clinical oncology in Romania, especially as personalized medicine becomes increasingly important in cancer care. It also highlights the promise of epigenetic therapies, including inhibitors targeting DNA methylation and chromatin remodeling pathways, as future treatment options. Overall, the research contributes to a better understanding of cancer biology in Romanian populations and supports the development of precision oncology approaches grounded in molecular evidence.

Key word: epigenetics, cancer progression, DNA methylation, biomarkers, Romania

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NANOPARTICLE DELIVERY SYSTEMS FOR SIRNA THERAPEUTICS IN EGYPTIAN LIVER CANCER MODELS

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ABSTRACT

This study examines the design and therapeutic potential of nanoparticle-based delivery systems for siRNA in Egyptian liver cancer models. RNA interference has emerged as a promising strategy for silencing oncogenic pathways, but clinical translation is limited by the instability of siRNA molecules and their inefficient cellular uptake. To address these barriers, the present research developed biocompatible nanoparticle carriers engineered to protect siRNA from degradation and promote selective delivery to hepatocellular carcinoma cells. The formulations were evaluated for particle size, surface charge, encapsulation efficiency, release behavior, and cellular internalization. Biological testing demonstrated that the nanoparticle systems improved siRNA stability in physiological conditions and enhanced uptake by cancer cells compared with free siRNA. Functional assays further showed suppression of genes implicated in tumor growth, proliferation, and survival, indicating that the delivery platform effectively supported therapeutic gene silencing. The study also observed reduced cancer cell viability and increased apoptotic activity following treatment, suggesting meaningful antitumor potential. The results are particularly relevant to liver cancer, which remains a major health concern in Egypt due to its high incidence and complex molecular profile. By combining nanotechnology with molecular therapeutics, this research contributes to the development of more targeted and less toxic treatment strategies. It also underlines the value of locally relevant preclinical models for assessing translational therapies in liver cancer. Overall, the findings support nanoparticle-mediated siRNA delivery as a promising approach for future precision oncology applications in hepatocellular carcinoma.

Key word: siRNA therapeutics, nanoparticles, liver cancer, gene silencing, Egypt

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MITOCHONDRIAL DNA MUTATIONS LINKED TO METABOLIC DISORDERS IN EGYPTIAN COHORTS

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ABSTRACT

This research investigates mitochondrial DNA mutations associated with metabolic disorders in Egyptian cohorts, focusing on the relationship between mitochondrial genome variation and clinical features such as insulin resistance, dyslipidemia, and impaired energy metabolism. Mitochondria play a central role in cellular bioenergetics, and mutations in mitochondrial DNA may disrupt oxidative phosphorylation, contributing to a range of metabolic diseases. In this study, genetic samples from affected individuals were analyzed to identify pathogenic and potentially pathogenic mitochondrial variants. The findings indicate that several mutations were recurrently observed in patients with metabolic dysfunction and were associated with altered clinical parameters relevant to disease severity. These variants appear to influence mitochondrial efficiency and may contribute to increased oxidative stress, reduced ATP production, and disturbance of lipid and glucose homeostasis. The study further suggests that mitochondrial mutations may interact with environmental and lifestyle factors, thereby shaping disease expression in the Egyptian population. A major implication of the research is that mitochondrial genetic screening could improve the early identification of individuals at risk for metabolic disorders. The results also support the use of mitochondrial DNA analysis as part of broader personalized medicine strategies in metabolic disease management. By emphasizing population-specific molecular data, the study contributes to a more nuanced understanding of metabolic disease etiology in Egypt and highlights the need for future functional investigations to clarify the biological consequences of the identified mutations.

Key word: mitochondrial DNA, metabolic disorders, genetic mutation, oxidative stress, Egypt

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TELOMERE LENGTH VARIATIONS AND AGING PROCESSES IN AZERBAIJANI ELDERLY POPULATIONS

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ABSTRACT

This study explores telomere length variations and their relationship to aging processes in Azerbaijani elderly populations. Telomeres serve as protective chromosome end structures, and their progressive shortening is widely considered a biomarker of biological aging and cellular senescence. In this research, peripheral blood samples from older adults were evaluated to assess telomere dynamics in relation to age, health status, lifestyle patterns, and chronic disease burden. The findings indicate that shorter telomeres are associated with advanced biological aging and are more frequently observed in individuals with cardiovascular risk factors, reduced physical activity, and chronic inflammatory conditions. The study also suggests that psychosocial stress and environmental exposures may contribute to accelerated telomere attrition. These results support the view that telomere length is not only a genetic marker but also a measurable indicator of cumulative life-course influences on health. The research further discusses the potential value of telomere assessment in aging research, preventive medicine, and geriatric risk profiling. In the Azerbaijani context, where demographic aging is becoming increasingly important, such findings may help guide future public health strategies aimed at promoting healthy longevity. Overall, the study contributes to molecular aging research by linking telomere biology to population-specific patterns of aging and disease vulnerability.

Key word: telomeres, aging, elderly populations, biomarkers, Azerbaijan

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NON-CODING RNA REGULATION OF GENE EXPRESSION IN AZERBAIJANI CARDIOVASCULAR PATIENTS

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ABSTRACT

This research investigates the role of non-coding RNA molecules in regulating gene expression among Azerbaijani cardiovascular patients, with emphasis on microRNAs and long non-coding RNAs implicated in vascular inflammation, myocardial remodeling, and atherosclerotic progression. Non-coding RNAs have emerged as critical regulators of post-transcriptional control, and their dysregulation is increasingly associated with cardiovascular disease development and severity. In this study, patient-derived biological samples were analyzed to identify expression patterns related to disease phenotypes. The results show that several non-coding RNAs are significantly altered in patients with hypertension, coronary artery disease, and heart failure, suggesting their involvement in disease-specific regulatory networks. These molecules appear to influence inflammatory signaling, endothelial function, and apoptotic pathways, thereby shaping cardiovascular pathology. The study also indicates that certain non-coding RNAs may have value as diagnostic or prognostic biomarkers due to their association with clinical indicators of disease progression. Beyond biomarker potential, the findings support the idea that non-coding RNA-based therapeutic strategies may become important in future cardiovascular medicine. The research contributes to a growing body of evidence showing that gene regulation in cardiovascular disease is mediated by complex non-coding mechanisms rather than protein-coding genes alone. By focusing on Azerbaijani patients, the study provides population-relevant molecular data that can inform future translational and precision-medicine approaches in cardiology.

Key word: non-coding RNA, cardiovascular disease, gene regulation, biomarkers, Azerbaijan

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GENOME-WIDE ASSOCIATION STUDIES FOR DIABETES SUSCEPTIBILITY IN PAKISTANI POPULATIONS

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ABSTRACT

This study applies genome-wide association approaches to investigate genetic susceptibility to diabetes in Pakistani populations, aiming to identify loci associated with disease risk and metabolic dysfunction. Diabetes represents a major public health challenge in South Asia, and population-specific genomic research is essential for understanding its genetic architecture. In this work, case-control cohorts were analyzed using genome-wide marker data to detect variants associated with type 2 diabetes and related metabolic traits. The results indicate that several loci show significant association with insulin resistance, glucose dysregulation, and impaired beta-cell function. These findings suggest that both common and population-enriched genetic variants may contribute to diabetes vulnerability in Pakistan. The study also demonstrates that genomic risk is shaped by interactions between hereditary background, obesity, diet, and sedentary lifestyle patterns. By identifying risk-associated loci, the research contributes to the development of more accurate genetic screening tools and supports future efforts in personalized prevention. The findings further highlight the importance of conducting large-scale genomic studies within underrepresented populations, as global reference data may not fully capture local disease mechanisms. Overall, this work strengthens the evidence base for precision medicine in diabetes research and offers new directions for functional validation, risk prediction, and public health intervention in Pakistani communities.

Key word: genome-wide association study, diabetes susceptibility, Pakistani population, genetic risk, precision medicine

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MICRORNA BIOMARKERS FOR BREAST CANCER EARLY DETECTION IN PAKISTANI WOMEN

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ABSTRACT

This study examines the diagnostic potential of microRNA biomarkers for the early detection of breast cancer in Pakistani women. Breast cancer remains one of the leading causes of cancer-related morbidity and mortality among women in Pakistan, and delayed diagnosis continues to limit treatment outcomes. In this research, circulating and tissue-derived microRNA profiles were analyzed to identify expression signatures associated with malignant transformation. The findings show that several microRNAs are differentially expressed in breast cancer patients compared with healthy controls, indicating their value as candidate biomarkers for early screening. These microRNAs are involved in pathways related to cell proliferation, apoptosis, epithelial-to-mesenchymal transition, and tumor invasion. Their altered expression suggests a regulatory role in tumor development and progression. The study also highlights that biomarker panels may be more informative than single markers because they better capture the molecular complexity of breast cancer. In addition, the results point to the potential clinical utility of non-invasive sampling methods, particularly blood-based assays, for early disease detection. Such approaches could be especially useful in Pakistan, where screening access remains uneven and many patients present at advanced stages. By focusing on a local patient population, the research contributes population-specific evidence for biomarker development and supports the broader goal of precision oncology. Overall, the study indicates that microRNA-based testing may improve early diagnosis, risk stratification, and future therapeutic planning for breast cancer in Pakistani women.

Key word: microRNA, breast cancer, early detection, biomarkers, Pakistan

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PROTEIN-PROTEIN INTERACTION NETWORKS IN PAKISTANI LUNG CANCER PATHOGENESIS

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ABSTRACT

This study investigates protein-protein interaction networks involved in lung cancer pathogenesis among Pakistani patients, with the aim of identifying key molecular hubs that may contribute to tumor initiation and progression. Lung cancer is a highly complex disease characterized by dysregulated signaling pathways, altered protein interactions, and multiple layers of molecular control. In this research, interaction network analysis was conducted to map the relationships among proteins associated with cell proliferation, apoptosis, DNA repair, angiogenesis, and metastatic behavior. The findings reveal that several proteins occupy central positions in the interaction network, suggesting that they may serve as regulatory hubs or potential therapeutic targets. The analysis also indicates that network topology can help clarify how oncogenic pathways become activated and maintained in lung cancer cells. By integrating computational and molecular perspectives, the study demonstrates the value of systems biology for understanding cancer mechanisms at a broader level than single-gene analysis allows. The results further suggest that network-based biomarkers may improve the identification of clinically relevant pathways and support the development of targeted therapies. In the Pakistani context, where lung cancer remains a major health burden and molecular profiling is still limited in many settings, such research is particularly important. Overall, the study contributes to the growing field of network oncology by providing a biologically informed framework for identifying critical interaction nodes in lung cancer pathogenesis and by supporting future translational research on diagnostic and therapeutic innovation.

Key word: protein-protein interaction, lung cancer, pathogenesis, network biology, Pakistan

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PHYTOREMEDIATION POTENTIAL OF ROMANIAN DANUBE DELTA PLANTS FOR HEAVY METAL POLLUTION

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ABSTRACT

This study examines the phytoremediation potential of native plant species from the Romanian Danube Delta as a sustainable strategy for mitigating heavy metal pollution in wetland ecosystems. The Delta represents one of Europe's most ecologically sensitive regions, yet it is increasingly exposed to contamination from industrial discharge, agricultural runoff, and river-borne pollutants. The research focuses on the ability of selected halophytic and hydrophytic plants to absorb, translocate, and stabilize metals such as cadmium, lead, zinc, and copper under natural field conditions. Through comparative ecological assessment and laboratory analysis, the study evaluates how plant morphology, root structure, and physiological tolerance contribute to remediation efficiency. Results indicate that several native species show strong accumulation capacity in their below-ground tissues, suggesting their potential for both phytoextraction and phytostabilization. The findings also highlight the importance of plant-microbe interactions in enhancing metal tolerance and rhizosphere activity. Beyond remediation performance, the study considers ecological compatibility, seasonal resilience, and habitat preservation, which are crucial in fragile wetland environments. The discussion emphasizes that phytoremediation offers a low-cost, environmentally friendly, and biodiversity-supporting alternative to conventional remediation methods. The research contributes to the growing understanding of how native wetland vegetation can be integrated into regional environmental restoration strategies while maintaining ecosystem integrity. It also provides a scientific basis for future monitoring and conservation planning in the Danube Delta.

Key word: phytoremediation, Danube Delta, heavy metals, wetland plants, environmental restoration

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MICROBIAL DIVERSITY IN CARPATHIAN CAVE ECOSYSTEMS AND THEIR BIOTECHNOLOGICAL APPLICATIONS

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ABSTRACT

This research explores the microbial diversity of Carpathian cave ecosystems and evaluates their potential for biotechnology and environmental applications. Cave environments are characterized by stable temperatures, low nutrient availability, and long-term isolation, which create unique selective pressures for microbial adaptation. The study investigates bacterial and fungal communities collected from subterranean rock surfaces, sediments, and drip waters, with attention to species composition, metabolic versatility, and survival strategies. Findings show that cave microbiomes contain organisms capable of producing enzymes, antimicrobial metabolites, and stress-resistant biomolecules with possible industrial value. Particular attention is given to microorganisms adapted to oligotrophic and mineral-rich conditions, as these traits may support applications in bioremediation, bioactive compound discovery, and novel enzyme production. The analysis also considers the ecological significance of microbial communities in nutrient cycling and cave mineral formation. In addition to taxonomic diversity, the research examines functional traits associated with biofilm formation, metal tolerance, and secondary metabolite synthesis. The study argues that cave microbiota represent an underexplored reservoir of biological innovation, especially for sectors seeking sustainable and nature-based solutions. At the same time, it emphasizes the need to protect cave ecosystems from uncontrolled bioprospecting and habitat disturbance. Overall, the findings highlight the Carpathian caves as important natural laboratories for understanding microbial adaptation and for developing future biotechnological resources.

Key word: cave microbiology, microbial diversity, biotechnology, Carpathian Mountains, bioprospecting

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CORAL BLEACHING RESISTANCE MECHANISMS IN INDONESIAN RAJA AMPAT MARINE PROTECTED AREAS

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ABSTRACT

This study investigates the mechanisms that support coral bleaching resistance in marine protected areas of Raja Ampat, Indonesia, one of the world's most biologically diverse reef systems. Rising sea temperatures and environmental stress have intensified coral bleaching events across tropical oceans, making it necessary to identify reefs with greater resilience and the ecological factors that sustain them. The research examines coral physiology, symbiotic relationships with zooxanthellae, water quality, habitat complexity, and local management practices that may contribute to bleaching tolerance. Results indicate that coral communities in well-protected sites show stronger recovery potential, greater symbiont stability, and healthier reef-associated biodiversity than more disturbed locations. The study also emphasizes the role of lower sedimentation, reduced fishing pressure, and effective conservation governance in maintaining reef resilience. Environmental monitoring suggests that structural reef complexity and high species diversity create microhabitats that buffer thermal stress. In addition, certain coral genotypes appear more capable of surviving temperature fluctuations, indicating the importance of genetic diversity in adaptation. The findings support the view that marine protected areas can function as climate refuges when they are managed effectively and supported by strong community involvement. This research contributes to coral reef conservation by identifying ecological and governance factors that improve resistance to bleaching, offering practical guidance for marine management in Indonesia and other tropical regions facing climate change.

Key word: coral bleaching, Raja Ampat, marine protected areas, reef resilience, climate adaptation

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GENETIC DIVERSITY ASSESSMENT OF SUMATRAN ORANGUTAN POPULATIONS USING NON-INVASIVE SAMPLING

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ABSTRACT

This research assesses the genetic diversity of Sumatran orangutan populations through non-invasive sampling methods, with the aim of supporting conservation planning for this endangered primate. Habitat fragmentation, poaching, and forest degradation have placed increasing pressure on orangutan populations in Sumatra, making genetic monitoring essential for understanding population structure and long-term viability. The study uses fecal and hair samples collected from different forest zones to examine levels of genetic variation, relatedness, and dispersal patterns without disturbing the animals. The findings reveal clear population differentiation among forest fragments, suggesting limited gene flow caused by habitat isolation. Such fragmentation may reduce adaptive potential and increase vulnerability to disease, environmental change, and inbreeding. The study also identifies the value of non-invasive sampling as a practical and ethical tool for monitoring elusive and endangered species in difficult terrain. By comparing genetic markers across regions, the research provides useful information for identifying conservation units and planning wildlife corridors. The results support habitat restoration, forest connectivity, and targeted protection of key populations as priorities for orangutan conservation. More broadly, the study demonstrates how molecular ecology can inform evidence-based management of threatened species in biodiversity-rich tropical landscapes.

Key word: orangutan conservation, genetic diversity, non-invasive sampling, Sumatra, molecular ecology

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PHYTOCHEMICAL ANALYSIS OF PERSIAN GULF MANGROVE SPECIES FOR ANTICANCER ACTIVITY

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ABSTRACT

This study investigates the phytochemical composition and anticancer potential of mangrove species from the Persian Gulf, focusing on their value as natural sources of bioactive compounds. Mangrove ecosystems are known for producing chemically diverse secondary metabolites that help plants survive in harsh coastal environments, and these compounds may also possess pharmacological properties. The research examines plant extracts for flavonoids, phenolics, terpenoids, and other metabolites with possible cytotoxic and antioxidant effects. The study evaluates their activity against selected cancer cell lines and explores the relationship between phytochemical richness and biological response. Results indicate that mangrove extracts contain compounds capable of inhibiting cancer cell proliferation and promoting oxidative stress regulation, suggesting promising therapeutic potential. The findings also emphasize the importance of species-specific chemical profiles, as different mangrove plants show varying levels of activity. In addition to laboratory evaluation, the study considers ecological sustainability and the need to protect mangrove habitats while exploring their biomedical use. The work contributes to natural product research by identifying coastal flora as an underexplored source of anticancer agents. It also highlights the broader significance of biodiversity conservation for future drug discovery efforts. The study supports further phytochemical isolation, mechanism-based testing, and preclinical research to assess the feasibility of developing plant-derived anticancer compounds from Persian Gulf mangrove species.

Key word: mangrove plants, phytochemical analysis, anticancer activity, Persian Gulf, natural products

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MICROPLASTIC INGESTION EFFECTS ON IRANIAN CASPIAN SEA FISH GUT MICROBIOMES

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ABSTRACT

This study examines the effects of microplastic ingestion on the gut microbiomes of fish from the Iranian sector of the Caspian Sea, addressing an emerging environmental and ecological concern. Microplastics have become widespread in marine and freshwater systems, and their presence in fish digestive tracts may alter microbial balance, nutrient absorption, and overall physiological health. The research evaluates the composition of gut microbial communities in fish exposed to contaminated habitats and compares them with individuals from less polluted areas. Findings suggest that microplastic ingestion is associated with shifts in microbial diversity, including changes in beneficial bacteria and a rise in stress-related microbial groups. These alterations may influence digestion, immune function, and susceptibility to disease. The study also considers the role of polymer type, particle size, and pollutant adsorption on the severity of microbiome disruption. Beyond gut ecology, the research highlights the broader implications for fish health, food safety, and aquatic ecosystem stability. It argues that microplastics function not only as physical contaminants but also as carriers of chemical substances and microbial assemblages that may further affect host organisms. The study underscores the need for continuous monitoring of plastic pollution in the Caspian Sea and for integrated management strategies to reduce coastal contamination. It contributes to environmental microbiology by linking plastic pollution to host-microbe interactions in an economically important fishery region.

Key word: microplastics, fish microbiome, Caspian Sea, aquatic pollution, gut health

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CUBAN BIO-DIVERSITY CONSERVATION STRATEGIES FOR ENDANGERED AMPHIBIAN SPECIES

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ABSTRACT

This study explores biodiversity conservation strategies for endangered amphibian species in Cuba, with a focus on habitat protection, population monitoring, and species recovery planning. Cuban amphibians are highly vulnerable to habitat loss, climate variability, invasive species, and restricted geographic distribution. The research examines conservation approaches that combine ecological surveys, habitat assessment, and species-specific management measures. Findings show that effective conservation depends on the protection of forested wetlands, the restoration of breeding sites, and the control of environmental pressures in fragile habitats. The study also emphasizes the importance of community involvement and national conservation policy in safeguarding species with limited range. Amphibians are presented as important indicators of ecosystem health, and their decline reflects broader environmental degradation. The research argues that conservation strategies must be based on local ecological conditions and supported by long-term monitoring. It also highlights the value of ex situ measures, such as captive breeding and genetic management, in reinforcing wild populations when habitat conditions are severely degraded. Overall, the study provides a framework for integrated amphibian conservation in Cuba that links field ecology, habitat restoration, and public policy. Its findings contribute to island biodiversity science and to the protection of one of the Caribbean's most distinctive amphibian faunas.

Key word: amphibian conservation, Cuba, biodiversity, habitat restoration, endangered species

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MOLECULAR EVOLUTION OF VIRUS-HOST INTERACTIONS IN CUBAN BAT RESERVOIRS

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ABSTRACT

This research examines the molecular evolution of virus-host interactions in Cuban bat reservoirs, focusing on the ecological and evolutionary dynamics that shape pathogen persistence. Bats are recognized as important reservoirs for a range of viruses, and their unique immune systems allow them to coexist with pathogens that may pose risks to other species. The study investigates viral genetic variation, host immune response, and the influence of ecological conditions on virus maintenance within bat populations. Results suggest that coevolutionary processes play a significant role in shaping virus-host relationships, with host adaptation and viral mutation occurring in parallel. The study also indicates that bat colonies in stable ecological settings may support long-term viral circulation without obvious signs of disease. This makes them important subjects for understanding spillover risk and disease ecology. The analysis highlights the value of molecular surveillance in identifying viral lineages and monitoring ecological change in reservoir systems. It also underscores the importance of protecting bat habitats, since environmental disturbance may alter host behavior and increase contact with other species. The findings contribute to the broader field of zoonotic disease research by clarifying how viral persistence is influenced by host biology, ecology, and evolutionary pressure. The study supports future interdisciplinary monitoring of bat-virus systems in the Caribbean and beyond.

Key word: bat reservoirs, virus-host interaction, molecular evolution, Cuba, zoonotic ecology

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THERMOTOLERANT YEAST STRAINS FROM CUBAN HOT SPRINGS FOR BIOETHANOL PRODUCTION

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ABSTRACT

This study investigates thermotolerant yeast strains isolated from Cuban hot springs for their potential use in bioethanol production. The search for efficient biofuel-producing microorganisms is increasingly important because high temperatures during fermentation can reduce productivity and increase production costs. The research evaluates yeast isolates for growth stability, sugar utilization, ethanol tolerance, and fermentation performance under elevated thermal conditions. Results show that certain strains maintain strong metabolic activity at temperatures that typically inhibit conventional industrial yeasts. These isolates are able to ferment a range of carbohydrate substrates efficiently, making them promising candidates for second-generation bioethanol processes. The study also considers the ecological significance of hot spring environments as reservoirs of extremophilic microorganisms with valuable industrial traits. In addition to laboratory evaluation, the research discusses the potential for using locally sourced microbial resources to support sustainable energy development in Cuba. Thermotolerant yeasts may reduce cooling requirements, improve process resilience, and enhance the economic feasibility of ethanol production in tropical climates. The findings suggest that bioprospecting in geothermal habitats can contribute to renewable energy innovation while expanding knowledge of microbial adaptation. The study recommends further strain optimization, pilot-scale fermentation, and genomic analysis to support future industrial application.

Key word: thermotolerant yeast, bioethanol, hot springs, Cuba, renewable energy