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September 7-9, 2022

5th International Conferences on Science and Technology

Budva, Montenegro

Natural Science and Technology

Abstracts & Proceedings Book

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International Conferences on Science and Technology

Natural Science and Technology

September 7-9, 2022 in Budva, MONTENEGRO

ABSTRACTS
&
PROCEEDINGS BOOK

ICONST NST 2022

International Conferences on Science and Technology

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Natural Science and Technology

September 7-9, 2022 in Budva, MONTENEGRO

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Dear Readers;

The fifth of ICONST organizations was held in Budva/Montenegro between 7-9 September 2022 with the theme of '***science for sustainable technology***' again. In recent years, weather changes due to climate change have reached a perceptible level for everyone and have become a major concern. For this reason, scientific studies that transform technological progress into a sustainable one is seen as the only solution for humanity's salvation. Here we ask ourselves "which branch of science is responsible for sustainability?". Sustainability science is an interdisciplinary field of study that covers all basic sciences with social, economic, ecological dimensions. If we consider technology as the practical application of scientific knowledge, the task of scientists under these conditions is to design products that consume less energy, require less raw materials, and last longer.

ICONST organizations organize congresses on sustainability issues of three main fields of study at the same time in order to present different perspectives to scientists. This year, 128 papers from 27 different countries presented by scientists in **ICONST Organizations**.

71 papers from 19 countries presented in our **International Conference on Engineering Science and Technology** organized under ICONST organizations. The total rate of countries excluding Türkiye is 53%. Türkiye is the country with the highest participation with 47%, followed by Poland 7%, Montenegro 5%, India 4%, Italy 4%, Kosovo 4%, Hungary 4%, Slovakia 4%, Afghanistan 2%, Czech Republic 2%, Iran 2%, Algeria 2%, Ethiopia 2%, Central African Republic 2%, Romania 2%, Russia 2%, Serbia 2%, North Macedonia 2% and Moldova %2.

31 papers from 9 countries presented in our **International Conference on Life Science and Technology** organized under ICONST organizations. The total rate of countries excluding Türkiye is 58%. Türkiye is the country with the highest participation with 42%, followed by Poland 16%, Bulgaria 12%, Slovakia 8%, Philippines 6%, Czech Republic 3%, Tunisia 3%, Algeria 3% and Switzerland 3%.

Finally, 26 papers from 10 countries presented in our **International Conference on Natural Science and Technology** organized under ICONST organizations. The total rate of countries excluding Türkiye is 65%. Türkiye is the country with the highest participation with 35%, followed by Serbia 19%, Slovakia 11.5%, Algeria 8%, India 8%, Croatia 3.8%, Ethiopia 3.8%, Hungary 3.8%, Kosovo 3.8%, South Africa 3.8%.

As ICONST organizations, we will continue to organize organizations with the value you deserve in order to exchange ideas against the greatest threat facing humanity, to inspire each other and to contribute to science. See you at your future events.

ICONST Organizing Committee

ICONST NST 2022

International Conferences on Science and Technology

Natural Science and Technology

September 7-9, 2022 in Budva, MONTENEGRO

Contents

International Conferences on Science and Technology
Natural Sciences and Technology
7-9 September 2022 in Budva, MONTENEGRO
ICONST NST 2022

Electrospun Innovative Tannic Acid/Chitosan Fiber: Preparation and Characterization

Serbülent Türk[1*], Mahmut Özacar[2]

Abstract: Electro-spining, which is a cost-effective and efficient technique of producing nanofibers from natural and synthetic polymers, is one of the methods to produce 3D structures. Electrospun fibers obtained thanks to their high porosity and large surface areas can find a place in a wide range of applications. Innovative fibers was prepared by electrospinning using tannic acid (TA) and chitosan (CHI) in this study. Concentrations of polymer solutions were chosen experimentally to ensure uniform spinning. First, for the preparation of 2% (w/v) CHI solution, CHI was dissolved in 2% (v/v) acetic acid aqueous solution at 50°C by mixing until a homogeneous mixture is obtained. To prepare the final composite solutions, TA in different proportions (0, 0.5, 1, and 2 wt% based on the weight of chitosan) was added to the mixture solution. Electrospinning was performed using a flow rate of 0.5 mL/h, a voltage of 15 kV and a distance of 18 cm between the electrodes. The fibers were collected on the plate collector covered with aluminum foil. A metallic needle with an outer diameter = 0.8 mm and an inner diameter = 0.5 mm attached to a plastic syringe was used in all experiments. The spinning solutions were analyzed by measuring the surface tension. It was confirmed by SEM analysis that the obtained electrospun mats were uniform and error-free.

Acknowledgments: This work was supported by the Scientific Research Projects Commission of Sakarya University (Project number: 2019-6-23-223).

Keywords: Electrospinning, Fiber, Composite, Polymer

[1] Sakarya University, Biomedical, Magnetic and Semiconductor Materials Application and Research Center, Sakarya, Turkey
[2] Sakarya University, Faculty of Science Department of Chemistry, Sakarya, Turkey
* Corresponding author: serbulentturk@sakarya.edu.tr

International Conferences on Science and Technology
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7-9 September 2022 in Budva, MONTENEGRO
ICONST NST 2022

Structural and Spectroscopic Analysis of Methyl 2-(pyridin-2-yl)acetate with Quantum Mechanical Calculations

Nuri Öztürk

Abstract: The structural and spectroscopic features of the methyl 2-pyridin-2-ylacetate (C8H9NO2) have been investigated by using 1H and 13C NMR, UV-Vis., FT-IR and Raman spectroscopies. The molecular geometry optimization parameters (bond lengths and angles), NMR chemical shifts, UV-Vis. parameters (wavelengths, excitation energies, oscillator strength), HOMO (the Highest Occupied Molecular Orbital), LUMO (the Lowest Unoccupied Molecular Orbital) energies, MEP (Molecular Electrostatic Potential) surface, and vibrational wavenumbers have been calculated using DFT/B3LYP quantum chemical method with 6-311++G(d,p) level of theory to compare with the experimental results. Assignments of the vibrational wavenumbers have been performed by PED (Potential Energy Distribution) analyses using VEDA 4 software. UV-Vis. parameters, HOMO-LUMO analyses, and MEP surface of the molecule have been investigated to report electronic transitions, intramolecular charge transfer, and nucleophilic and electrophilic reactive sites in the molecule. It can be said that the calculated molecular geometry parameters, proton and carbon NMR chemical shift values, vibrational wavenumbers, and UV-Vis. parameters have been in good agreement with the experimental data obtained from the literature.

Keywords: Methyl 2-pyridin-2-ylacetate, HOMO-LUMO, NMR chemical shifts, UV-Vis. parameters, Vibrational Spectroscopy

Giresun University, Property Protection and Security Division Occupational Health and Safety Program, Giresun, Turkey
* Corresponding author: nuri.ozturk@giresun.edu.tr

International Conferences on Science and Technology
Natural Sciences and Technology
7-9 September 2022 in Budva, MONTENEGRO
ICONST NST 2022

Theoretical Investigation of the Structural, Electronic and Spectroscopic Properties of the 4'-dimethylamino-3-nitrochalcone

Nuri Öztürk

Abstract: The structural, electronic, and spectroscopic properties of the trans-4'-dimethylamino-3- nitrochalcone (C17H16N2O3) have been researched theoretically by using nuclear magnetic resonance, ultraviolet-visible and vibrational spectroscopies. Theoretical molecular geometry optimization parameters, vibrational wavenumbers, 1H and 13C NMR chemical shifts, UV- Vis. parameters (wavelengths, excitation energies, oscillator strength) and HOMO (the highest occupied molecular orbital) and LUMO (the lowest unoccupied molecular orbital) energies have been calculated using Density Functional Theory (DFT/B3LYP) quantum chemical method with 6-311++G(d,p) basis set to compare with the experimental results. Assignments of the vibrational wavenumbers have been performed by PED (Potential Energy Distribution) analyses. UV-Vis. electronic absorption parameters, HOMO-LUMO analyses, MEP (Molecular Electrostatic Potential) surface of the compound have been studied to explain electronic transitions, intramolecular charge transfer, and interaction sites in the compound. The computed molecular geometry parameters, vibrational wavenumbers, NMR chemical shifts, and UV-Vis. parameters have been in good agreement with the experimental data in the literature.

Keywords: Chalcone, DFT/B3LYP, NMR chemical shifts, UV-Vis. parameters, Vibrational Spectroscopy

Giresun University, Property Protection and Security Division Occupational Health and Safety Program, Giresun, Turkey
* Corresponding author: nuri.ozturk@giresun.edu.tr

International Conferences on Science and Technology
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7-9 September 2022 in Budva, MONTENEGRO
ICONST NST 2022

Water Resources Management

Fathi Ahmednur

Abstract: Water Resources Management Policy Goals Overview Water Policy of Ethiopia Synopsis Water Policy of Ethiopia is often referred as the aquatic keep tower of East Africa for the reason that of its many rivers and water systems that drain neighbouring waterless countries. Estimates show that the surface water potential is about 111 billion m3, which represents a significant per capita. Major problem in developing this enormous resource is limited capacity and uneven distribution of the resource itself. As result of this, the country did not use its optimal irrigation potential and other uses that can be derived from the resource. Unfluctuating non a significant share of the probable is utilized for power generation. Therefore, the water policy aims at equitable, sustainable, and rational development of the water resources potential. In this policy, issues such as drought mitigation are addressed. Location Ethiopia is located between approximately 30 -150 N latitude and 330 -480 E longitude. The country covers a land area of about 1.12 million km2 occupying a significant portion of the Horn of Africa. It shares boundaries to the east and southeast with Djibouti and Somalia, to the north with Eritrea, to the south with Kenya, and to the west with the Sudan.and South Sudan Ethiopia also shows a high spatial and temporal variability and rainfall. The highest mean annual rainfall, which is more than 2700 mm, occurs in the Southwestern highlands of the country and gradually decreases in the North to less than 250 mm., Northeast to less than.

Keywords: Policy, water, resources, management

* Corresponding author: Fbarrex@gmail.com

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Natural Sciences and Technology
7-9 September 2022 in Budva, MONTENEGRO
ICONST NST 2022

Effect of Chemical Proprieties on Detection Efficiency in Cylindrical Containers: Application to Environmental Samples

Boukhalfa Salma*[1], Ould Mohamed Mounir[1], Khelifi Rachid[1]

Abstract: For environmental gamma spectrometry analysis, the detection efficiency curves required pre-knowledge of sample and detector characteristics. Via Monte Calo code, the goal of this study is to explore the effect of chemical proprieties e.g., density and elemental composition on detection efficiency curves. Firstly, Monte Carlo through the Geant4 toolkit was validated by 137Cs punctual radioactive source. The employed detector was modelled following the same manufacturer description. Good agreement between the experimental and the simulated gamma spectrum was obtained. Then, we select randomly ten different types of environmental samples. The investigated samples, biological and geological, were taken to the laboratory, they were air-dried for one week, homogenized by mortar, sieved at 100 µm, pressed in capsule format, and exposed to an X-ray beam. The validated detector geometry and the chemical proprieties of the samples were used to determine the detection efficiency curves in an energy range from 59 to 1408 keV corresponding to 241Am and 152Eu energies. The results revealed the high dependency on detection efficiency and the incident particle energy. Further, it appears from results that detection efficiency decrease, wherever chemical composition, as high particle energy. Contrary, the detection efficiency increase for high densities. Furthermore, there is no correlation between energy, density, and elemental composition. Besides, the obtained efficiency curves can be used for gamma spectrometry analysis, especially when e.g., sample activity is needed.

Keywords: Geant4, Detection efficiency, gamma spectrometry, chemical composition

[1]Université Blida, Buleyde, Algeria

* Corresponding author: boukhalfasalma@gmail.com

International Conferences on Science and Technology
Natural Sciences and Technology
7-9 September 2022 in Budva, MONTENEGRO
ICONST NST 2022

Environmental Radioactivity in Commercial Fertilizers: Levels, Origin, and Radiological Assessement

Boukhalfa Salma*[1], Khelifi Rachid[1], Ould Mohamed Mounir[1]

Abstract: At present, the environmental monitoring of natural and/or artificial radionuclides in agricultural soil has increased along with: the dose-radiation impact on the human body and information's lack of radionuclides distribution in the environment. Nitrogen fertilizers also called chemical fertilizers are characterised by their high impact on food toxicity, soil contamination, food quality etc. The present work aims to evaluate the radioactivity level in different types of commercial fertilizers employing a calibrated 2"×2"LaBr3:Ce scintillator detector. The collected samples were separately crushed, sieved, homogenized, catalogued, and stored for 4 weeks to ensure secular equilibrium between 226 Ra and 224Ra progenies. The specific activities (in Bq.kg- 1) of 40K, 238U, and 232Th were measured at gammas lines corresponding to 1.46, 1.76, and 2.6 MeV respectively. To correct the surface area corresponding to the selected peaks, the background radiation, counted during one week, was subtracted from all the spectrums. The gamma spectrometry analysis shows the presence of natural radionuclides where high concentrations of 40K, 238U, and 232Th with different concentrations. The radiological assessment shows: that the means values of radium equivalent activity, external and internal hazard index, absorbed and annual dose arelowerthanUNSCEAR limit. This work provides experimental results of natural radionuclides in commercial fertilizers and their impact on the environment.

Keywords: Natural radioactivity; fertilizers; labr3:ce detector; dosimetry.

[1]Université Blida, Buleyde, Algeria

* Corresponding author: boukhalfasalma@gmail.com

International Conferences on Science and Technology
Natural Sciences and Technology
7-9 September 2022 in Budva, MONTENEGRO
ICONST NST 2022

Photocatalytic Degradation of Quinmerac in Aqueous Medium in the Presence of Doped Carbon Quantum Dots as Photocatalysts

Vesna Despotović*[1], Jovana Prekodravac[1], Nataša Zec[1], Nina Finčur[1]
Daniela Šojić Merkulov[1]

Abstract: Heterogeneous photocatalysis using semiconductors has become an intensive field of research focused, among other things, on the development of materials able to decompose organic pollutants. Substituted quinolinecarboxylic acids are a relatively new class of highly selective auxin herbicides, of which quinmerac is quite effective in controlling dicotyledonous weeds in sugar beet, oilseed rape and wheat. In view of the fact that quinoline compounds are extremely toxic, there exists a need for developing new methods for their removal from water. Carbon quantum dots (CQD) consist of sp2 and sp3 hybridized carbon atoms functionalized with oxygen containing groups. CQD can act as electron donors and acceptors, therefore besides antioxidant activity, they can generate reactive oxygen species under illumination (UV or visible). This dual behavior of CQD allows them to exhibit photocatalytic, photodynamic and antioxidant application. Modification of CQD by doping with different heteroatoms, can increase their electronic density and therefore the antioxidant activity. Its photodegradation performance in degradation of herbicides quinmerac solution was investigated under UV and simulated sunlight. The CQD were synthesized by one step microwave-assisted method from glucose water solution and in the presence of ammonium hydroxide as nitrogen source. The efficiency of elimination the herbicide from water was monitored by UFLC–DAD technique.

ACKNOWLEDGEMENT: This work was supported by the Science Fund of the Republic of Serbia (Grant No 7747845, In situ pollutants removal from waters by sustainable green nanotechnologies-CleanNanoCatalyze).

Keywords: Herbicides, photocatalytic degradation, quinmerac, carbon quantum dots

[1] Novi Sad University, Department of Physical Chemistry, Serbia

* Corresponding author: vesna.despotovic@dh.uns.ac.rs

International Conferences on Science and Technology
Natural Sciences and Technology
7-9 September 2022 in Budva, MONTENEGRO
ICONST NST 2022

Sunlight-driven Photocatalytic Degradation of Antibiotic Ciprofloxacin Using Newly Synthesized ZnO Nanomaterials

Nina Finčur *[1], Dušica Jovanović [1], Nataša Zec[1], Vesna Despotović [1]
Tamara Ivetić [1], Daniela Šojić Merkulov[1]

Abstract: Nowadays, huge amounts of pharmaceuticals are being manufactured. Among them,antibiotics can be found in the environment due to improper disposal of unused or expired drugs and their low biodegradability in wastewater treatments. Ciprofloxacin belongs to fluoroquinolones class, representing one of the most important classes of synthetic antibiotics, which has been widely used for a broad spectrum of antimicrobial activities. In the last few years, environmental criteria have been tightened with the aim of reducing pollutant emissions. Therefore, it is necessary to find an efficient, safe and environmentally friendly method for the removal of pharmaceutically active compounds, which will be able to effectively eliminate them from the polluted aquatic environment. A possible solution would be using of photocatalytic degradation processes. These processes under mild experimental conditions can produce highly reactive hydroxyl radicals, which can oxidize and mineralize organic molecules to harmless products. In this work, photocatalytic degradation of ciprofloxacin was studied under simulated sunlight and using ZnO based nanomaterials (prepared by mechanochemical method in a molar ratio of 2:1). Namely, the effect of catalyst type (ZnO/ZrO2, ZnO/CeO2 and ZnO/MgO), catalysts loading, and pH on the efficiency of the ciprofloxacin removal were investigated. Ultrafast liquid chromatography with a diode array detector was applied to monitor the efficiency of ciprofloxacin removal from water.

ACKNOWLEDGEMENT: This work was supported by the Science Fund of the Republic of Serbia (Grant No 7747845, In situ pollutants removal from waters by sustainable green nanotechnologies-CleanNanoCatalyze).

Keywords: Photocatalytic degradation, antibiotic, ciprofloxacin, ZnO, sunlight

[1] Novi Sad University, Department of Analytical Chemistry, Serbia

* Corresponding author: nina.fincur@dh.uns.ac.rs

International Conferences on Science and Technology
Natural Sciences and Technology
7-9 September 2022 in Budva, MONTENEGRO
ICONST NST 2022

Enhanced Photocatalytic Activity of Rare Earth Doped Lanthanum Manganite Nanomaterials in the Degradation of Endocrine Disruptors

Daniela Šojić Merkulov *[1], Szabolcs Bognár [1], Nataša Zec[1], Paula Sfirloaga [1]
Vesna Despotović [1], Nina Finčur [1], Predrag Putnik[1]

Abstract: Environmental pollution is an emerging global issue, with severe impact on natural fields. The water contamination is the second most serious problem, right after the air pollution. 17α-Ethinylestradiol (EE2) is a synthetic steroid estrogen and commonly applied in controlling ovulation, as well as in treating alopecia, prostate and breast cancer in humans and reproductive disorders. Furthermore, it is also applied in contraceptives and in hormone replacement therapy. EE2 belongs to the group of endocrine disruptors and has been globally detected in surface water, sanitary sewage, drinking water and wastewater effluents from sewage treatment plants. Unfortunately, even at low concentrations EE2 can cause serious damages to the living organisms. Heterogeneous photocatalytic degradation is considered as competitive, effective, sustainable and green alternatives in the removal of persistent, non- biodegradable pollutants, since they can turn them into nontoxic CO2 and H2O. In this study, the efficiency of photocatalytic degradation of EE2 in the presence of undoped and doped (Eu, Tb, Ho) LaMnO3 nanomaterials (synthesized by sol-gel technique) was investigated under UV light and simulated sunlight irradiation. The best removal efficiency was reached in the presence of LMO:Ho, when 77% of endocrine disruptors EE2 was degraded after 30 min of UV irradiation.

ACKNOWLEDGEMENT: This work was supported by the Science Fund of the Republic of Serbia (Grant No 7747845, In situ pollutants removal from waters by sustainable green nanotechnologies-CleanNanoCatalyze) and by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No 451-03-68/2022- 14/200125.

Keywords: Photocatalytic degradation, green nanotechnology, 17α-ethynilestradiol, LaMnO3, rare earths

[1] Novi Sad University, Department of Chemistry, Biochemistry and Environmental Protection, Serbia

* Corresponding author: daniela.sojic@dh.uns.ac.rs

International Conferences on Science and Technology
Natural Sciences and Technology
7-9 September 2022 in Budva, MONTENEGRO
ICONST NST 2022

Laboratory Menu And Oviposition Preference In Drosophila Melanogaster

Jelena Trajković*[1], Milica Spasojević [1], Tatjana Savić[1], Sofija Pavković-Lučić [1]

Abstract: In nature, fruit flies use volatile compounds produced by rotting fruits and vegetables to locate feeding, breeding and oviposition sites. The choice of oviposition site is very important for Drosophila melanogaster offspring survival and fitness. Females make decisions about egg-laying sites by evaluating complex information obtained from multiple sensory pathways. When choosing a place for oviposition, they carefully evaluate specific nutrients and substrate strength. The aim of this study was to investigate the oviposition preference of three D. Melanogaster strains, reared for 22 years on different diets (standard cornmeal, tomato and carrot substrates). Virgin flies were kept on a neutral agarose substrate until the experiment started. Oviposition preference was tested in ten replicates, in transparent plastic boxes that contained five Petri dishes filled with cornmeal, tomato, banana, carrot and apple substrates. Twenty five individuals of each sex and strain, 4-5 days old, were placed in boxes overnight, after which the eggs were counted. Results pointed to significant differences among strains. Females from standard strain chose more often the standard substrate for laying eggs. On the other hand, females reared on tomato substrate chose the carrot substrate more often, whereas females from strain reared on carrot substrate significantly preferred tomato substrate for egg laying. The results of this study confirmed that the substrate on which the adults developed did not necessarily affect the preference for the egg-laying substrate. Further, substrates that contained carrot and tomato were previously determined as ones that enabled both the fastest.

Keywords: Food, fruit fly, egg-laying preference, behaviour

[1] Belgrad University, Department of Biology, Serbia

* Corresponding author: jelena.trajkovic@bio.bg.ac.rs

International Conferences on Science and Technology
Natural Sciences and Technology
7-9 September 2022 in Budva, MONTENEGRO
ICONST NST 2022

Traveling Waves for a Model of Thin Film Flows

Gökçen Çekiç

Abstract: Families of traveling wave solutions will be presented for a model of a falling viscous film on the upstanding solid. Each family contains a single solution with smaller film thickness than all others in the family. It was previously conjectured that at a given value of a film thickness separating two distinct flow regimes observed in analytical studies as well as two distinct types of behavior in transient solutions to the model which is on an inclined solid. These studies over a range of parameter values using a combination of numerical and analytical techniques will be compared with the spectrum of upstanding type. The linear stability of these solutions will also be discussed; both large- and small-amplitude solutions will be shown to be unstable, though the instability mechanisms are different for each wave type.

Keywords: Traveling waves, thin films, flows, linear stability, fluid flows

* Corresponding author: gokcen.cekic@gmail.com

International Conferences on Science and Technology
Natural Sciences and Technology
7-9 September 2022 in Budva, MONTENEGRO
ICONST NST 2022

Adsorption and Photodegradation of Methylene Blue by Using WO3/PVC Composite

Ivana Jagodić*[1], Imre Guth[1], Svetlana Lukić-Petrović[1], Nemanja Banić[1]

Abstract: With growing industry expansion and significant use of chemical pollutants, water pollution has been the inevitable consequence. Dyes are often found in wastewaters and are increasingly becoming an environmental problem. One of the promising strategies for degradation organic pollutants in water is advanced oxidation processes, which are breaking down pollutants into biodegradable products. WO3 is a potential semiconductor for photochemical processes with favorable properties: suitable bandgap (~2.6 eV), good chemical stability under intense solar exposure, oxygen-evolution capability, long minority carrier diffusion length (~500 nm – 6 μm). Due to the more economical use of catalysts in photocatalytic processes, we have synthesized a new catalyst in the form of tablets with a diameter of 5 mm and a thickness of 2 mm. The WO3 was immobilized on commercial polyvinyl chloride support. The efficiency of WO3/PVC composites with a different mass ratio of WO3 to PVC (1%, 1.75%, 2.5%, 3.75%, 5%, and 7.5%) for methylene blue (c0 = 2.45·10□5 mol dm□3) removal in the presence/absence of simulated solar radiation was investigated. The optimal mass ratio of WO3 towards PVC, in the presence of the simulated solar radiation, was 2.5%. The contribution of adsorption and photodegradation to the total removal efficiency of methylene blue was observed. The methylene blue removal kinetics were monitored by UV/Vis spectrophotometry. The photocatalysts were characterized by scanning electron microscopy, X-ray diffraction, UV/Vis diffuse reflectance, and Raman spectroscopy. The possible photodegradation mechanism of methylene blue dye with (WO3)/PVC was assumed using the scavenger test.

Keywords: Methylene blue, WO3/PVC composite, photocatalytic degradation, adsorption

[1] Novi Sad University, Department of Chemistry, Biochemistry and Environmental Protection, Serbia

* Corresponding author: ivana.jagodic@dh.uns.ac.rs

International Conferences on Science and Technology
Natural Sciences and Technology
7-9 September 2022 in Budva, MONTENEGRO
ICONST NST 2022

Drought Analysis and Forecasting in Odisha Using Machine Learning Techniques

Saroj Kumar Dash *[1], Kaibalya Pattnaik [1]

Abstract: Drought is a natural phenomenon that damages agricultural land severely. The severity of drought must be reduced in order to decrease its impact on the agricultural productivity. The study of drought was carried out for the state Odisha that experienced drought for 8 times during last 20 years due to failure of monsoon. The drought forecasting was carried out using machine learning techniques like Auto-regressive model (AR), Long Short-Term Memory (LSTM) and Auto regressive Integrated Moving Average (ARIMA) using daily rainfall data collected for 28 years (1993-2020). Further using this data each district was categorized into four different categories namely Flood (FL), No Drought (ND), Moderate Drought (MD), Sever Drought (SD). In order to classify the districts after forecasting, classification models were used like Support Vector Classifier (SVC) and Naïve Bayes. The results of the forecasting model as well as the classification model was compared. It becomes important to forecast drought for a proper planning and management of water resource system to decrease the damage due to such calamities. This study is valuable for the government, farmers, and other stakeholders to understand the pattern and reason behind severity of drought to take relevant precautionary measures and improve decisions and facilities to tackle with such natural calamities.

Keywords: Drought, long short-term memory (LSTM), auto-regressive model (AR), Auto-regressive integrated moving

[1] VIT University, Department of Mathematics, India

* Corresponding author: sarojkumar.dash@vit.ac.in

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Polyvinyl Chloride Matrices with Xerogel-Carbon Nanoparticles-AgCuS Composites as a Sensitive Element for Electrochemical

Zoran Grubač *[1], Marijo Buzuk [1], Slobodan Brinić [1]

Abstract: The most exploited and reported techniques, suitable for the low-level detection of hydrogen peroxide, are electrochemical techniques. In comparison with the above-mentioned techniques, electrochemical sensors have remarkable properties such as fast response, low cost, safe and easy handling, simplicity and the possibility of being constructed in a form of a portable system. The latter has importance in the development of a "point-of-care" system for biochemical, biomedical and clinical applications. In this paper, a development of composite sol-gel (as xerogel) material containing carbon-nanotubes impregnated with inorganic ternary sulfide-AgxCuyS for the determination of the low concentration of hydrogen peroxide is presented. The developed xerogel composites were used as a sensing element in polyvinyl chloride matrices. These composites were used for surface modification of glassy carbon electrode (GCE). The electrochemical behaviour of prepared electrodes reveals the possibility of direct electrochemical detection of hydrogen peroxide on + 0.3 V at pH 9. As a suitable electrochemical method for the determination of hydrogen peroxide, amperometry was chosen. Electroanalytical optimization of the developed method shows a possibility of detection of hydrogen peroxide in the concentration range between 5 and 90 μg cm-3 with the sensitivity of 1.47 nA cm3 μg-1 and the detection limit of 0.068 μg cm-3. Being a robust and simple electrode, with remarkable stability, repeatability and reproducibility, the optimized electrode was successfully tested for determination of the hydrogen peroxide in a commercially available contact lens solution

Keywords: Hydrogen peroxide, amperometry, sol-gel composites, ternary sulfides, modification

[1] Split University, Department of Chemistry and Technology, Croatia

* Corresponding author: brinic@ktf-split.hr

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Weighted Darna Distribution with Applications in Real Life Data

Aafaq Ahmad Rather *[1], C. Subramanian [1]

Abstract: This manuscript addresses a new model of Darna distribution known as Weighted Darna distribution by applying the weighted technique. Several statistical properties of the newly introduced distribution are derived and discussed in detail. This newly introduced weighted Darna distribution has three parameters. The parameters of the newly proposed distribution are estimated by using the maximum likelihood estimation method. Finally, the usefulness and applicability of the newly proposed model is investigated by using the two real-life data sets.

Keywords: Weighted distribution; darna distribution; reliability measures; order statistics; maximum likelihood estimation

[1] Annamalai University, India

* Corresponding author: aafaq7741@gmail.com

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Comparison of Methods for Testing Antioxidant Activity in Milk

Ilmije Vllasaku *[1], Julijana Tomovska [2]

Abstract: Milk is a rich source of all necessary nutritients, it is a colloidal solution of proteins, milk fat, milk sugar and mineral substances. Milk, also contains non-nutritive substances, antioxidants that act to prevent the oxidation and degradation of lipids, vitamins, carotenoids and other substances that are susceptible to auto-oxidation. The purpose of this paper is to examine the total antioxidant activity of milk using two methods, the phosphomolybdate method and the hydrogen peroxide cleaning method, and to compare the obtained results. Milk samples from several farms from different regions of our country taken as material for analysis. The milk first extracted with 3% trichloroacetic acid. The phosphomolybdate test used for antioxidant activity based on the reduction of Mo (VI) to Mo (V) from the sample and the subsequent formation of a green phosphate / Mo (V) complex at acid pH. Absorbance measurement is at 695nm on a Merck Spectrocuant Pharo 300. The hydrogen

Keywords: Antioxidant activity, antioxidants, milk, phosphomolybdate method, hydrogen peroxide scavenging

[1] 1UBT - Higher Education Institution, Kalabrija nn, 10000 Prishtina, Kosovo
[2]Faculty of Biotechnical Sciences, University “St. Kliment Ohridski” - Bitola, Partizanska bb, 7000 Bitola, Macedonia
* Corresponding author: ilmijevllasaku@hotmail.com

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Environmentalists of the Centuries, from the Poles to Tropics: Lichens

Fadime Eryılmaz Pehlivan

Abstract: Lichens, which mostly lives on trees and moist soils as epiphytes, are a symbiotic partnership formed by Fungi and Algae, and they have a wide spread as a result of their mutualistic life. They are found in all kinds of terrestrial habitats of the earth, even at the extremes where most macro-organisms reach the limit of life. they continue to exist by exhibiting an adaptation. With these features Lichens, which have been the subject of increasingly interesting and important studies in recent years, also known as air quality indicators with their bioindicator properties. Lichens are found in all kinds of terrestrial habitats of the earth, even at the extremes where most macro-organisms reach the limit of life. they continue to exist by exhibiting an adaptation. With these features Lichens, which have been the subject of increasingly interesting and important studies in recent years, also known as air quality indicators with their bioindicator properties. In this study lichen and climate change are discussed together with the habitats of vulnerable lichens, with special attention to antarctics– the most likely place for possible extinction of lichens as a result of global warming. The objective of this review was to identify the general responses to climate change in functional and ecological traits.

Keywords: Lichenes, global warming, bioindicator, antarctic, tropic

Faculty of Science, Department of Biology, Department of Botany, İstanbul, Turkey
* Corresponding author: eryilmazfadime6@gmail.com

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Challenges in Lecturing Sport and Exercise Technology using Online Platforms during the COVID-19 Pandemic

Chantell Gouws*[1], Gerrit Breukelman[1] , Lourens Millard [1]

Abstract: This presentation details the development of transitioning of in-person sport and exercise technology diploma lecturing to online lecturing through the course of the COVID-19 pandemic. Due to practical components of the course, specific challenges emerged involving course design and practical learning. This article considers the problem-solving process and strategies for remotely teaching, discussing, and experiencing the global exercise trend. Specifically, accessibility, adjustments and attitudes as important pedagogical tools for reimagining experiential learning and creating meaningful human connections.

Keywords: COVID-19, Sport and Exercise Technology, accessibility, adjustments, attitudes

* Corresponding author: Gouwsc@unizulu.ac.za

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Testing Various Numerical Methods for the Efficient Optimization of Detailed Chemical Reactio Mechanisms

Goitom Turanyi

Abstract: Features of several global and local optimization methods for effective parameter estimation are discussed. The methods are compared on complex analytical benchmark functions in terms of final objective function value, number of function evaluation, CPU time and success rate (number of times the method converges to the global optima).The best one are selected for optimization of large reaction mechanism models where its chemical kinetic and thermodynamic parameters are fitted within their uncertainty limits to better describe an experimental data. These are extreme optimization tasks due to the large number of fitted parameters (typically 50-100), large number of experimental data considered (up to 25000), and the slow calculation of the simulation results. The latter is based on the solution of several thousand systems of ordinary and partial differential equations and nonlinear algebraic systems. The selected local and global methods were compared with the FOCTOPUS (home made new global optimization method) on the H_2/O_2/NO_x reaction system. As a first trial, the comparison was carried out on optimizing of rate parameters of the H2/O2/NOx reaction system by fitting the parameters to 732 experimental and theoretical data points implemented in Matlab programming language.

Keywords: Local optimization methods, global optimization methods, FOCTOPUS method

* Corresponding author: simretab1222@gmail.com

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Comparison of Sheet Material and Polymer Materials with Stiffness Analysis

Remzi Varol[1], Omer Can Ersek[1*]

Abstract: In this study, it is aimed to perform linear, non-linear stiffness analyzes and modal analyzes of the automobile front hood model by creating a finite element model in Hypermesh or Ansys program and using Nastran Abaqus software as a solver. The contribution of this study to the automotive industry is that we will see in advance what the results will be if the front hoods are produced with the chosen polymer material instead of sheet metal. If, according to the results of the analysis, it is revealed that the plastic material is close to the sheet metal values used in the existing vehicles, examples will be given on what can be done to correct the inappropriate constraints in the next stage. The production of automobile front hoods, which are produced with sheet materials in the sector, with plastic material is also important in terms of cost, because cost is an important concept. If plastic material comes to the fore in terms of cost and amortization times, it would be logical to produce with plastic material. There will be pros and cons according to the sheet material, but the important thing here is to give examples of what can be done to correct the constraints that will arise for plastic. So the contribution of this analysis, As a result of the comparison of the sheet material used in the market and the selected polymer material, the sector will be illuminated, whether positive or negative.

Keywords: Automotive, front hood, polymer, sheet

* Corresponding author: omercanersek16@gmail.com

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Structural Features of Metamorphic Rocks Around Budağan Mountain (Emet/Kütahya)

Furkan Öztürk[1], Ali Kamil Yüksel[2*]

Abstract: Western Anatolia, comprises Menderes massif, Lycian nappes, Afyon and Tavşanlı zones, experienced regional high pressure/low temperature metamorphism by the contractional and extensional deformations of Alpine orogeny. The studied area, located 13 km NE of the Emet (Kütahya), is in Afyon Zone. Middle-Upper Triassic İkibaşlı Formation which consists of metaclastics and carbonate lenses, forms the lower part of the study area. Jurassic Budağan limestone, composed of dolomitic carbonates, conformably overlies the İkibaşlı Formation. These two formations are overlain tectonically and metamorphosed by an ophiolitic nappe of the Dağardı Melange. The direction of the nappe transportation which caused the metamorphism, can be determined with kinematic studies along this tectonic contact zone.

At the tectonic contact, Mesoscopic and microscobic structures which are used in kinematic studies, show that two different deformation phases, while the initial deformation phase (D1) is in ductile (Alpine), the last phase (D2) is brittle (Neotectonic). Neotectonic brittle high angle normal and oblique faulting (D2) overprint the ductile deformation phase (D1) in the study area. The measured linear structures in the metamorphic rocks of İkibaşlı Formation trend in NW−SE direction. Asymmetric sigmoids in the outcrops and rotated clasts in the oriented thin sections are used for determining the sense of movement of the tectonic slices and indicate top-to-the-NW sense of shear.

This research which was prepared within the scope of the master's thesis, has been supported by Balıkesir University Scientific Research Projects.

Keywords: Ophiolite emplacement, Kinematic indicators, Afyon Zone

1. Introduction

Western Turkey, separeted by the İzmir-Ankara suture, is commanly divided into the Pontides in the north and Anatolide–Tauride Block in the south (Ketin, 1966; Şengör and Yılmaz, 1981; Okay et al. 1996) (Figure 1). The northern edge of the Anatolide-Tauride continent experienced regional HP/LT (high pressure/low temperature) metamorphism during the Alpine orogeny (Candan et al. 2005). The HP/LT Anatolides are generally subdivided into Tavşanlı Zone and Afyon Zone in the north and Menderes Massif and Lycian Nappes further south by the type and age of the metamorphism (Okay and Tüysüz, 1999). Metamorphism of these tectonic units of the Anatolide-Tauride block in western Turkey is suggested by the

[1]Balıkesir University, Institute of Science and Technology, 10145, Çağış Campus, Balıkesir, TURKEY
[2]Balıkesir University, Faculty of Engineering, 10145, Çağış Campus, Balıkesir, TURKEY
*Corresponding author: akyuksel@balikesir.edu.tr

transportation of nappes during Alpine evolution times (Şengör and Yılmaz, 1981; Şengör et. al. 1984). The emplacement direction of this nappe packages are in debate. Despite the debate about direction and age of the emplacement of the nappes, there are many structural analysis about Menderes Massif and Lycian Nappes (Bozkurt and Park, 1999; Güngör and Erdoğan, 2001) but there has not sufficient kinematic study in the Afyon Zone.

The studied area, composed of metamorphic rocks, carbonates, ophiolitic rocks and Neogen aged sedimentary rocks, located in Afyon Zone (Figure 1). Afyon Zone is made up of pre-Mesozoic basement and unconformably overlying Mesozoic cover series. Metamorphic rocks of this zone, which are unconformably overlain by Upper Palaeocene–Lower Eocene sedimentary rocks, indicate a Paleocene age for the regional HP/LT metamorphism related to northward subduction of the Anatolide–Tauride platform beneath the Sakarya Zone (Candan et. al. 2005). The burial depth of this zone was approximately 35 km and it experienced HP/LT metamorphism under blueschist facies depending on mineral paragenesis including Fe-Mg carpholites and estimated P/T conditions for this zone rocks give 350 ^{0}C and 6–9 kbar (Candan et. al. 2005). In this paper the features and sense of movement of the Afyon Zone rocks are described.

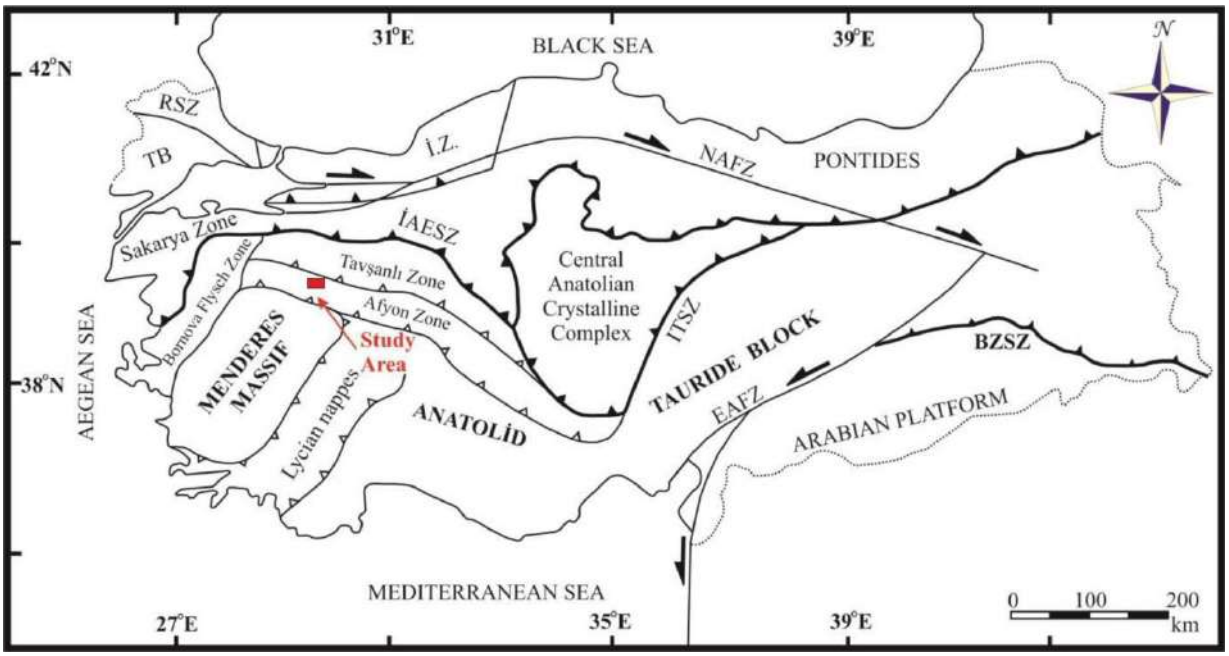

Figure 1: Simplified map shows the main tectonostratigraphic units and zones of Turkey and location of the study area (modified from Şengör and Yılmaz, 1981; Okay et al., 1996) (RSZ: Rhodope-Strandja Zone; TB: Thrace Basin; İ.Z.: İstanbul Zone; İAESZ: İzmir-Ankara Erzincan Suture Zone; BZSZ: Bitlis-Zagros Suture Zone; ITSZ: Inner Tauride Suture Zone; NAFZ: North Anatolian Fault Zone; EAFZ: East Anatolian Fault Zone)

2. Material and Method

2.1. Stratigraphy

Candan et. al. (2005) defined the stratigraphy of the Afyon Zone as a Pan-African basement and unconformably overlying Mesozoic cover rocks. In the Emet (Kütahya) region, Akay et. al. (2011) defined the only upper part of the Afyon Zone and distinguished the zone into

İkibaşlı Formation and Budağan limestone. In this study, in the Budağan mount region (Emet/Kütahya), only the Mesozoic upper part of the Afyon Zone exposes also. There are not any outcrops of the Pan-African basement of the Afyon Zone in the study area. The stratigraphy of the study area starts at the base with Middle-Upper Triassic İkibaşlı Formation (Figure 2). Jurasic Budağan limestone, comprises dolomitic carbonates, overlies the İkibaşlı Formation conformably. These two formations are overlain tectonically by an ophiolitic nappe with a low angle.

İkibaşlı Formation consists of metaconglomerates, schists and marble interclations. The metaconglomeratic rocks, extending between the exit of the Işıklar Village and Saraycık road, forms the lowermost of the formation. The upper part of the formation passes to light-dark gray and light brown sericite-quartz schist, chlorite-sericite schist, biotite-sericite-quartz schist and chloritoid schist. In the metaclastics, there are white-gray-light blue coloured, thin recrystallized limestones and rarely dolomitic limestone lenses. The metaclastic rocks of the formation pass upward conformably to the Budağan limestone. The fossil contents of the formation indicate Triassic-Liassic age for the transition between the İkibaşlı and Budağan limestone (Akay et. al. 2011). In the Emet region, metaclastics and platform carbonates of the Afyon Zone are tectonically overlain along a low angle fault by Dağardı Melange of the İzmir-Ankara Suture Zone. Ultramafics which are composed of generally peridotites and serpentinized peridotites, are the most dominant rock type of Dağardı Melange in the study area. And also sub-ophiolitic metamorphic rocks were defined in this melange by Yüksel et. al. (2014) at Mount Murat region. The hornblende from these sub-ophiolitic metamorphic rocks yielded 100.7±1.3 Ma (Albian) (Yüksel et. al. 2014). The Baklan Granite and Eğrigöz pluton intruded into Mesozoic rocks after the emplacement of the ophiolitic nappe. The Baklan Granite was dated at 17,8±0,7–19,4±0,9 Ma by K/Ar method (Aydoğan et. al. 2008). In the study area, all units are unconformably overlain by Neogene units which bears borate minerals.

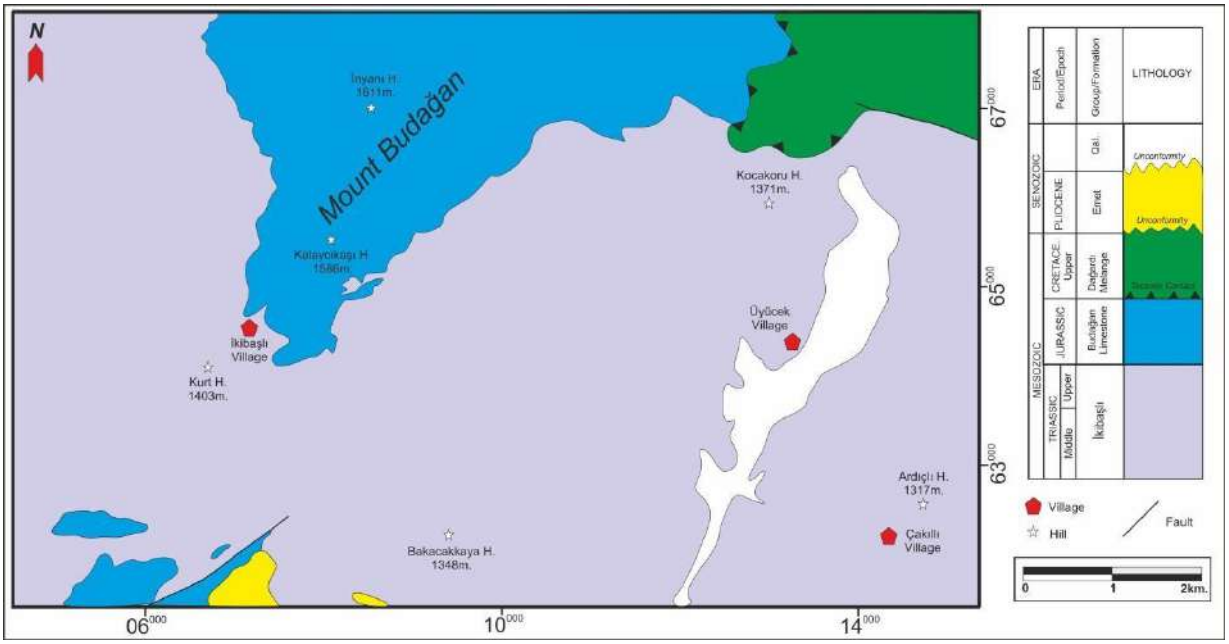

Figure 2: Geological map of the study area (modified from Akdeniz and Konak, 1979)

2.2. Kinematic studies

Kinematic studies from the contact zone between the Dağardı Melange and the underlying İkibaşlı Formation give information about the original position of these tectonic slices. Accordingly, we attached importance to mesoscopic and microscopic structures along the contact zone between these units. The methodology comprises systematic definitions and measurements of the mesoscopic shear criteria in outcrops oriented normal to the foliation and parallel to the associated stretching lineation and examinations of microscopic structures in oriented thin sections. Kinematic data gathered from mesoscopic and microscobic structures preserved in the metaclastics along the boundary between İkibaşlı Formation and Dağardı melange suggest two deformation phases (D1 and D2) and associated foliation and lineation (S1 and L1). The initial deformation phase (D1) is in ductile character and the last phase (D2) is in brittle character.

The L_1 mineral elongation/stretching lineation in the metaclastics is often contained within the S_1 foliation and is defined mostly by the preferred parallel alignment of mostly micas and quartz (Figure 3a). Trending of these lineations have been used to define the tectonic transport direction. Linear structures measured in the metaclastics are shown in the Schmidt diagram and generally lineations are NW−SE-trending (Figure 3b).

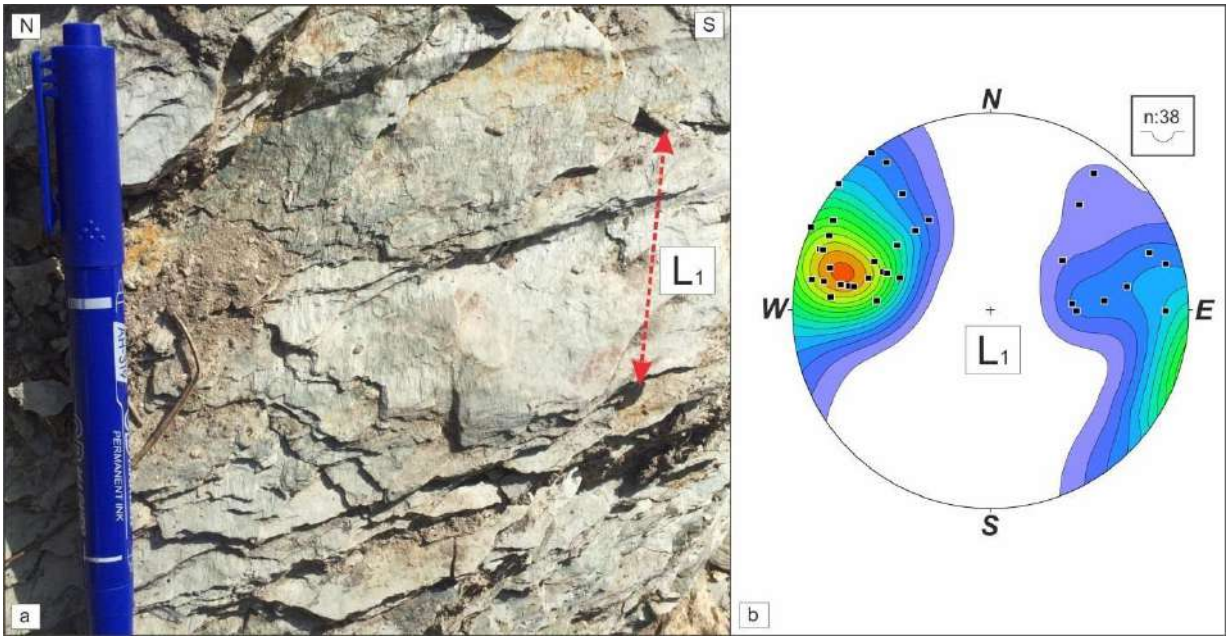

Figure 3: (a) The L1 mineral elongation/stretching lineation in the metaclastics of the İkibaşlı Formation (b) Contour diagram shows attitude of the linear fabric in the İkibaşlı Formation.

Asymmetric sigmoids in the outcrops and in the oriented thin sections and also rotated clasts are used for determining the sense of movement of the tectonic slices. Close to the tectonic contact, these sigmoids are asymmetric in sections normal to the foliation and parallel to the stretching lineation and indicate top-to-the-NW sense of shear (Figure 4a,b,c).

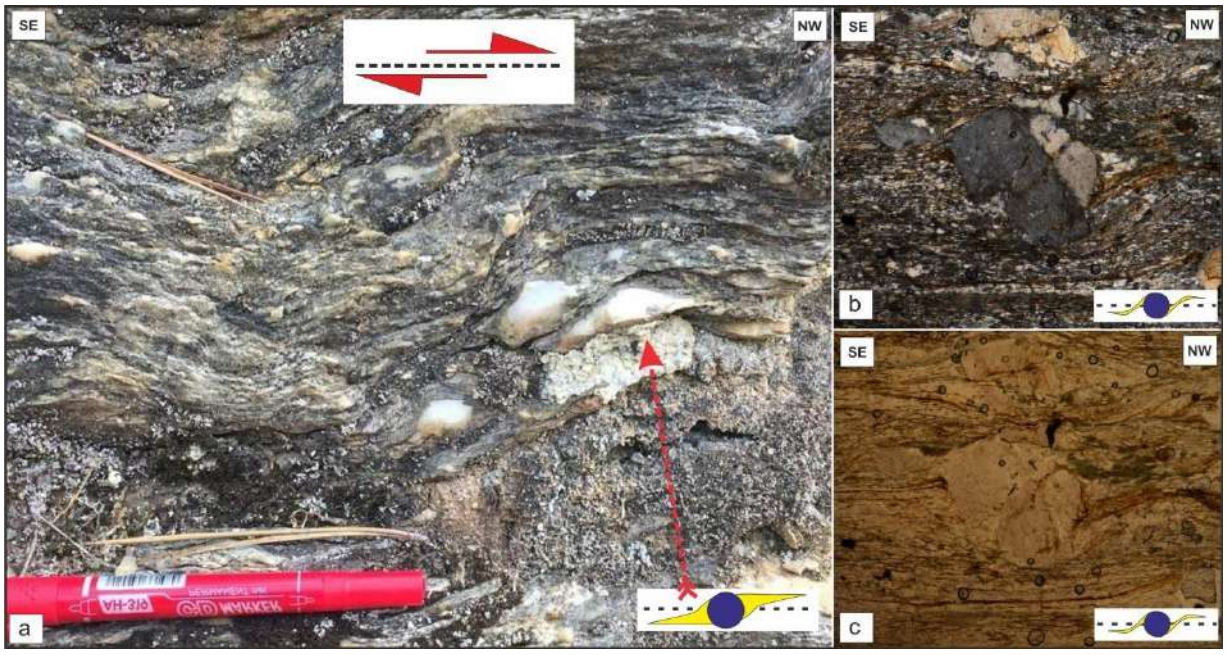

Figure 4: (a) Photograph shows asymmetrically deformed quartz pebble in the İkibaşlı Formation. (b) and (c) Rotated clast in the oriented thin section point to a top-to-the-northwest tectonic transport direction (Width of view is 2 mm)

The second deformation phase (D2) affected the study area and also the western Anatolia is the neotectonic brittle high angle normal and oblique faulting. These recent faults are the youngest structures in the study area and cut the well-developed S1 foliations in the İkibaşlı Formation and rocks of the ophiolitic nappe.

3. Results

Kinematic data gathered from mesoscopic and microscobic structures preserved in the metaclastics along the boundary between the İkibaşlı Formation and Dağardı melange, suggest two deformation phases (D1 and D2) and associated foliation and lineation (S1 and L1). The initial deformation phase (D1) is in ductile character and the last phase (D2) is in brittle character. A penetrative NW-trending strecthing lineation (L1) associated with thrusting was produced during the first deformation phase (D1). The kinematic indicators, the asymmetrical sigmoids, which observed both in the field and in the oriented thin sections, indicate top-to-the NW sense of shear at the tectonic contact between the İkibaşlı Formation and ophiolitic nappe. Neotectonic brittle high angle normal and oblique faulting (D2) overprint the ductile deformation phase (D1).

4. Discussion and Conclusions

Structural analysis in the study area (Afyon Zone) indicate top-to-the NW movement. This shear sense is inconsistent with previous studies of the metamorphism of the northern edge of the Anatolid-Tauride. Similarly, but in the Menderes Massif, Bozkurt and Park (1999) have adressed same incompatibility and proposed that top-to-the N-NNE sense of shear may have been related to the north-directed back-thrusting and internal imbrication of the Menderes Massif. Same top-to-the N-NNE fabrics of the Alpine contractional and extensional deformation phases have been determined in the submassives of the Menderes Massif (Verge, 1995; Işık and Tekeli, 2001; Lips et al. 2001; Hetzel et al. 1998). This study in the Afyon

zone was carried out in a narrow area within the scope of the master's thesis. More comprehensive studies are needed to better understand the tectonics of the region.

Acknowledgements

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New locality record of *Inosperma bongardii* with notes on its taxonomy, molecular phylogeny and ecology

Oğuzhan Kaygusuz[1*]

Abstract: Collections of *Inosperma bongardii*, which belongs to the Inocybaceae family, were collected from the Aegean Region of Turkey. The DNA sequences of the samples were isolated, and the results were compared with the sequences at the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST). According to the taxonomic and molecular results, the species was determined as *Inosperma bongardii*. In this study, detailed taxonomic data, a phylogenetic tree based on nrITS data, and habitat information for *I. bongardii* are presented.

Keywords: Agaricales, *Inocybe*, phylogeny, taxonomy, diversity.

1. Introduction

One of the most diverse families of Agaricales Underw., Inocybaceae Jülich has recently been revised by Matheny et al. (2020) using a six-gene phylogeny and seven separate genera (*Inocybe* (Fr.) Fr., *Inosperma* (Kühner) Matheny & Esteve-Rav., *Mallocybe* (Kuyper) Matheny, Vizzini & Esteve-Rav., *Nothocybe* Matheny & K.P.D. Latha, *Pseudosperma* Matheny & Esteve-Rav., *Auritella* Matheny & Bougher and *Tubariomyces* Esteve-Rav. & Matheny) have been recognized to constitute it. Members of this family occur worldwide, forming ectomycorrhizal (ECM) associations with numerous families of angiosperms and gymnosperms (Matheny et al. 2020).

Previously defined as a subgenus in the large *Inocybe* genus (Kühner 1980), *Inosperma* is comprised of gilled mushrooms distinguished morphologically from other genera species by longer stipe, the absence of pleurocystidia, and the shape of the spores. *Inosperma* is divided into two sections: *Cervicolores* (with a scaly cap) and *Rimosae* (with a radially fibrose or radially cracking ("rimose") pileus. *Inosperma bongardii* (Weinm.) Matheny & Esteve-Rav. and *I. calamistratum* (Fr.) Matheny & Esteve-Rav. are included in *Cervicolores* section (Knudsen and Vesterholt 2018).

Inosperma bongardii is an agaric fungus that was originally described as a species of Agaricus (Weinmann, 1836), then it was transferred to the genus *Inocybe* (Quélet 1872). *I. bongardii* was reported from the hemiboreal zone in Finland by von Schulmann (1960), later the species was reported from the Parainen region of this country (Vauras and Huhtinen 1986). It was rarely found in central Sweden, where it was occurred in a luxuriant stand of mountain birch

[1] Isparta University of Applied Sciences, Atabey Vocational School, Department of Plant and Animal Production, Isparta, Turkey
* Corresponding author: okaygusuz03@gmail.com

(Jacobsson 1984). Recently, *Inosperma bongardii* was found to be relative to the subgenus *Inosperma* (Matheny et al. 2020).

The purpose of this research is to present the new locality record of *Inosperma bongardii* for Turkey, to compare its characteristics with published data, and to provide a concise summary of the current understanding of its taxonomy, ecology, and distribution.

2. Materials and Methods

2.1. Morphological analyses

The samples were collected in the Aydın and Denizli Provinces of Turkey. By employing standard techniques, specimens were gathered, recorded, and conserved. Macroscopic characteristics were examined in fresh material. Microscopic structures were examined on the dry material. Dry samples were rehydrated and examined in Congo red and 3% potassium hydroxide (KOH). For each collection, 30 spores were measured.

2.2. Molecular analysis

2.2.1. DNA extraction, PCR, sequencing, and phylogenetic analysis

Genomic DNA isolation, PCR amplification, and sequencing methods were conducted based on the previous study by Kaygusuz et al. (2019). PCR amplifications were done with the primers ITS1F and ITS4 (White et al. 1990, Gardes and Bruns 1993) for the Internal Transcribed Spacer (ITS) region. The PCR products were cleaned with the CleanUp Kit and sequenced at Macrogen Inc. using the same primers. Newly generated sequences for this study were uploaded to GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Sequences obtained in this study were compared with data from the GenBank and UNITE (https://unite.ut.ee) databases by using the BLASTN algorithm (http://www.ncbi.nlm.nih.gov), and the relevant sequences were retrieved from GenBank and UNITE. nrITS sequences were aligned using MAFFT V7.464 (Katoh et al. 2019), and then manually adjusted using BioEdit v.7.0.5. (Hall 1999). Maximum Likelihood (ML) analysis was performed with the RAxML algorithm (Stamatakis 2014) with a GTR + G model under 1000 rapid bootstrap replicates.

3. Results

3.1. Phylogeny

In the phylogenetic analysis based on nrITS (Figure 1), three sequences of *Inosperma bongardii* from Turkey clustered together with eight sequences from Italy, Lebanon, Norway, and Sweden, forming a well-supported clade (100%, Figure 1). Within sect. *Cervicolores*, *Inosperma bongardii* formed a sister clade with undefined China species (OL850873), with strong support (100% ML bootstrap).

3.2. Taxonomy

Inosperma bongardii (Weinm.) Matheny & Esteve-Rav., in Matheny, Hobbs & Esteve-Raventós, Mycologia, 19 (2020).

Basionym: *Agaricus bongardii* Weinm., Hymeno et Gastero-Mycetes hucusque in imperio Rossico observatos:190. (1836).

Synonymy: *Inocybe bongardii* (Weinm.) Quél., Mém. Soc. Émul. Montbéliard, Sér. 2 5: 319 (1872) var. *bongardii*; *Inocybe bongardii* var. *pisciodora* (Donadini & Riousset) Kuyper, Persoonia, Suppl. 3: 41 (1986); *Inocybe pisciodora* Donadini & Riousset, Docums Mycol. 5(no. 20): 5 (1975).

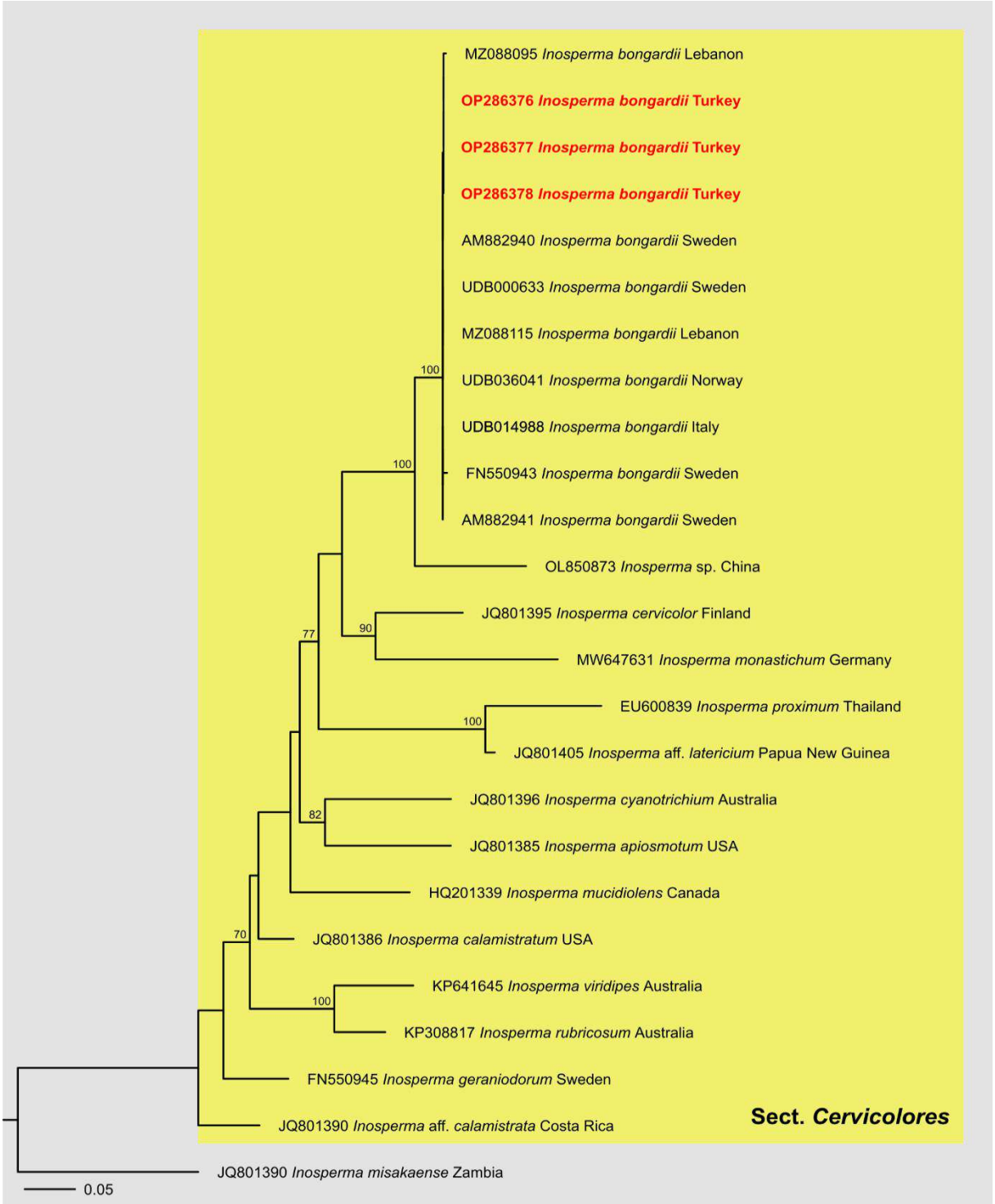

Figure 1. RAxML tree obtained from the nrITS sequence alignment showing the placement of *Inosperma bongardii*. *Inosperma misakaense* (Matheny & Watling) Matheny & Esteve-Rav. was used as outgroup taxon. Newly sequenced collections are shown in red. Maximum-likelihood bootstrap (MLB) ≥ 70% is given above branches.

Description. Pileus 35–65 mm in diam, hemispherical when young, later becoming conico-convex to convex to nearly plano-convex with a low and broadly umbo, dull, cracked or fibrillose-scaly surface, light brown to light ocher, darker brown at the disc and paler toward the margin, acute and involute margin. Lamellae broad, beige to grey-brown to light brown, adnexed to sinuate, and the edges uneven. Stipe 55–95 × 6–20 mm, cylindrical, somewhat wider at the apex, solid, white-fibrillose at first, later longitudinally reddish-brown fibrillose. Odor sweetish and fruity.

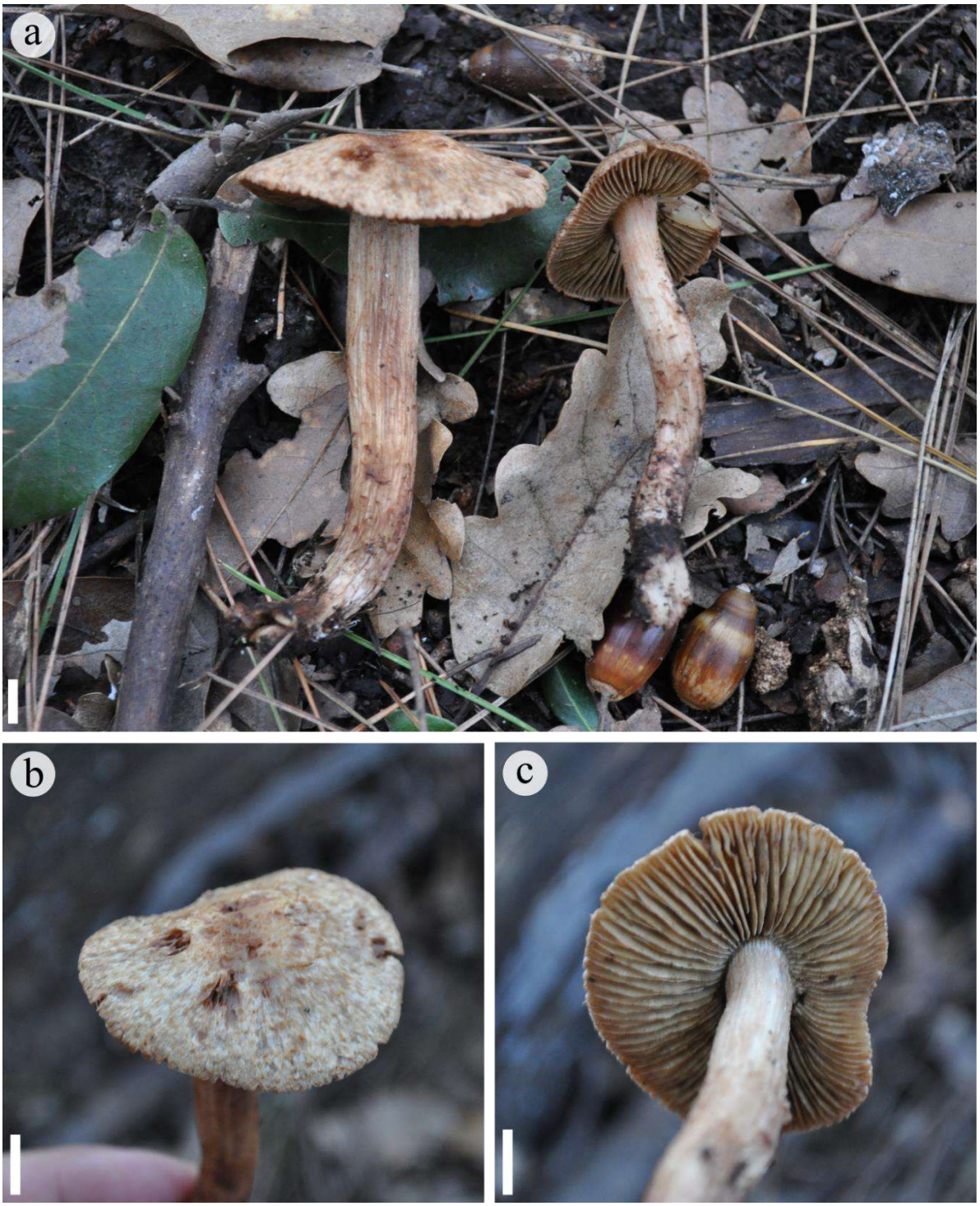

Figure 2. *Inosperma bongardii* collected in a *Quercus* forest in Aydın, Turkey. Scale bars: 10 mm.

Basidiospores (10.8–)11.5–15.0(–15.2) × (6.6–)6.7–8.6(–9.0) µm, Lm × Wm = 13.1 × 7.8 µm, Qm = 1.7, mostly oblong, sometimes ellipsoid, smooth, slightly thick-walled, brownish yellow in KOH. Basidia 45–60 × 8–11.5 µm, clavate, obtuse at the apex, tapering towards at the base, 4–spored, yellowish-brown content in KOH. Pleurocystidia absent. Cheilocystidia, abundant and crowded, 40–75(–94) × 10–13 µm, mostly clavate, broadly clavate to elongate-clavate or irregularly cylindrical, often flexuose, hyaline, thin-walled. Pileipellis a cutis consisting of cylindrical elements, 5–14 µm diam., with yellowish-brown intracellular pigment in KOH, thin-walled.

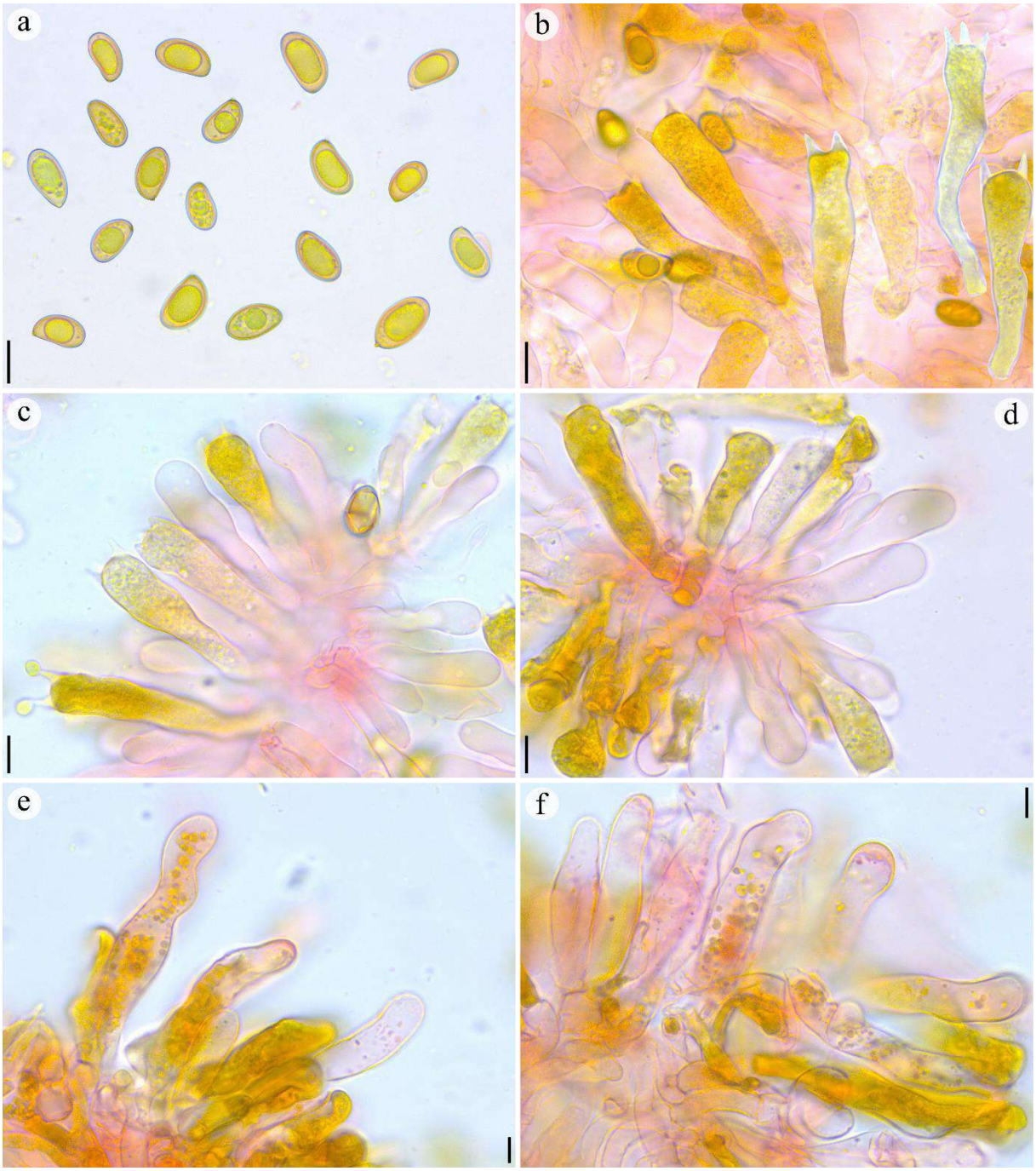

Figure 3. *Inosperma bongardii*. a. basidiospores, b-d. basidium and basidiole, e-f. cheilocystidia. Scale bars: 10 µm.

Habit and habitat: Scattered to gregarious in forests dominated by *Quercus pubescens* Willd.

Specimens examined: TURKEY, Aydın Province, Kuşadası district, around Davutlar, under *Quercus pubescens*, alt. 7 m, 09 April 2011, O. Kaygusuz, OKA-TR1857; GenBank: OP286376 for nrITS; *ibid.*, alt. 4 m, 14 March 2016, O. Kaygusuz, OKA-TR1859; GenBank: OP286378 for nrITS; Denizli Province, Buldan district, under *Q. pubescens*, alt. 630 m, 01 April 2013, O. Kaygusuz, OKA-TR1858; GenBank: OP286377 for nrITS.

4. Discussion and Conclusions

In the phylogenetic tree (Figure 1), *Inosperma bongardii* clusters together with the sequences of the species identified under the same name with high ML bootstrap support (100%). In addition, a sister *Inosperma* sp. (OL850873), an undescribed species from China takes place near this branch. Other closely related species are *Inosperma cervicolor* (Pers.) Matheny & Esteve-Rav. and *I. monastichum* Bandini & B. Oertel. *Inosperma cervicolor* differs from *I. bongardii* in that it has an earthy odor and an ochraceous brownish pileus with contrasting (very) dark squamules, and is associated with the Pinaceae (Kuyper 1986). *Inosperma monastichum* has smaller basidiospores (av. 10.6 × 6.3 μm) and mostly (sub)cylindrical cheilocystidia (Bandini et al. 2021). Furthermore, *Inosperma bongardii* (Weinm.) Matheny & Esteve-Rav. shares some morphological similarities with *I. geraniodora*. However, *I. geraniodora* differs from *I. bongardii* by the dark brown to chocolate-brown pileus, distinctly squamulose at the lower part of the stipe (Kuyper 1986).

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Antibacterial Activity Of The Vapor Phase Of *Thymus Mastichina* Essential Oil

Lucia Galovičová[1*], Natália Čmiková[1], Veronika Valková[1,2], Miroslava Kačániová[1]

Abstract: Essential oils have a diverse spectrum of biological activities, they are also low-toxic, and easily degradable in the human body. These properties make them suitable candidates for the protection and shelf-life extension of agricultural products. The aim of our study was to evaluate the efficacy of the vapor phase of *Thymus mastichina* essential oil against microorganisms on model fruit and vegetable crops. To compare the efficacy of vapor phase essential oil with contact application using the disc diffusion method against the tested microorganisms. Based on the methods we used, we concluded that *Thymus mastichina* essential has higher efficacy in a vapor application. For most of the tested microorganisms and on all the tested crops, the most significant inhibition was detected at the lowest tested concentration of 62.5 µL/L. Only moderate antimicrobial activity was detected in contact application and lower efficacy compared to antibiotics. These findings suggest that in the future *Thymus mastichina* essential oil could find application in crop storage to prevent crop deterioration due to microbial pathogens. Due to the need for low concentrations, it is assumed that the sensory properties of the crop for the consumer will not be affected. The replacement of synthetic fungicides and bactericides with natural alternatives could have a positive impact on the environment.

Keywords: *Thymus mastichina,* essential oil, vapor phase, disc diffusion method

1. Introduction

Thymus mastichina is also known as "Spanish Thyme" is native to the Iberian Peninsula. *Thymus mastichina* belongs to the Lamiaceae family and is a semi-woody shrub growing to a height of about 0.5 meters (Arantes et al. 2019). This plant is very resistant to frost, diseases, and pests. *Thymus mastichina* is mainly used for its antimicrobial, antioxidant, antirheumatic, and antitussive properties (Méndez-Tovar et al., 2015). For centuries, *Thymus mastichina* has been used as a spice to flavor food. From this mother plant, the essential oil of *Thymus mastichina* (TMEO) is extracted which is used in the pharmaceutical, food, cosmetic, and fragrance industries (Taghouti et al., 2019).

Essential oils (EOs) are volatile, aromatic compounds extracted from different parts of plants that possess diverse biological properties (Ishaq et al., 2017). EOs, due to their pronounced vapor-phase biological activities, low toxicity to humans, easy degradability, and antimicrobial effects, could find application in protecting and extending the shelf life of agricultural products during storage (Gutiérrez et al., 2009).

[1] Institute of Horticulture, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976 Nitra, Slovakia
[2] AgroBioTech Research Centre, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976 Nitra, Slovakia
* Corresponding author: l.galovicova95@gmail.com

Vegetables and fruits are among the perishable products with a short shelf life after harvesting. These commodities are at risk of mechanical damage, variation in physical parameters, and contamination during handling by various microbial pathogens (Ding and Lee, 2019). Control of postharvest microbial pathogens is largely provided by synthetic fungicides and bactericides (Palou, 2018). The use of these synthetic products can lead to the emergence of resistance of pathogens to the products used as well as high residues on agricultural products posing a high risk to consumer health and the environment (Hosseini et al., 2020).

Our study aimed to evaluate the efficacy of the vapor phase of *Thymus mastichina* essential oil against microorganisms on fruit and vegetable crops model. To compare the efficacy of vapor phase essential oil with contact application against the microorganisms tested.

2. Material and Method

2.1. Essential oil

Thymus mastichina essential oil was purchased from the Slovak company Hanus s.r.o. The essential oil was stored in the dark at 4 °C throughout the experiment. The composition of the essential oil stated by the manufacturer is cineol 45-65 %, linalool 8-30 %, 4-terpineol, β-pinene, α-pinene, limonene, α-terpineol.

2.2. Tested microorganisms

The tested microorganisms were obtained from the Czech collection of microorganisms Brno. Gram-negative bacteria *Yersinia enterocolitica* CCM 7204 and *Haemophilus influenzae* CCM 4454 were used. Gram-positive bacteria *Listeria monocytogenes* CCM 4699 and *Enterococcus faecalis* CCM 4224. Yeast *Candida tropicalis* CCM 8264. The biofilm-producing bacterium *Pseudomonas fluorescens* was obtained from a fish sample.

2.3. Antimicrobial activity of TMEO contact application

The antimicrobial activity of the contact application of TMEO was determined by the disk diffusion method. The bacterial inoculum was cultured for 24 hours in Tryptone soy agar (TSA, Oxoid, Basingstoke, UK) at 37 °C. The yeast inoculum was cultured for 24 h in Sabouraud dextrose agar (SDA, Oxoid, Basingstoke, UK) at 25 °C. The inoculum was adjusted to an optical density of 0.5 McFarland's standard (1.5 x 10^8 CFU/mL). The disc diffusion method was analyzed on Mueller Hinton agar (MHA, Oxoid, Basingstoke, UK). 100 µL of modified inoculum of the respective bacteria and yeast was applied to Petri dishes (PD) containing MHA. Sterile paper discs (Oxoid, Basingstoke, UK) with a diameter of 6 mm were placed on the PDs. The discs were saturated with 10 µL of TMEO. Samples were incubated for 24 h at 37 °C for bacteria and 25 °C for yeast. Two antibiotics (cefoxitin for gram-positive bacteria, gentamicin for gram-negative bacteria; Oxoid, Basingstoke, UK) and one antifungal (fluconazole; Oxoid, Basingstoke, UK) were used as positive controls for Gram-negative, Gram-positive bacteria and yeasts. An inhibition zone above 10 mm was determined as very strong antimicrobial activity, an inhibition zone of 5-10 mm was determined as moderate activity, and an inhibition zone below 5 mm was determined as weak activity. Antimicrobial activity was measured in triplicate.

2.4. Antimicrobial activity of vapor phase TMEO on model crops

The antimicrobial activity of vapor phase TMEO on model crops (apple, pear, carrot, white radish) was tested on gram-negative, gram-positive bacteria and yeast. SDA for yeast and MHA for bacteria was poured into 60 mm PD and capped. Sliced model crops (0.5 mm) were placed on agar. Using a microbial needle, three injections of inoculum were applied to the sliced model foods. TMEO was diluted in ethyl acetate to concentrations of 500, 250, 125, and 62.5 µL/L. A sterile filter paper was placed in the lid onto which 100 µL of the appropriate concentration was injected using a micropipette. The filter paper was dried for 1 min to evaporate the remaining ethyl acetate, then the plates were sealed and incubated at 37 °C for bacteria and 25 °C for yeast for 7 days.

Growth inhibition was assessed by stereological methods. Volume density (Vv) was estimated using ImageJ software. Colony (P) and substrate (p) stereological grid points were calculated. Growth density was calculated as % by the formula $Vv = P/p \times 100$. The antimicrobial activity of EO was expressed as growth inhibition $BGI = [(C - T)/C] \times 100$, where C was the growth density in the control group and T was the growth density in the treated group (Aman, 2015; Talibi et al., 2012). Negative results represented growth stimulation.

2.4. Statistical data processing

One-way analysis of variance (ANOVA) followed by Tukey's test at $p < 0.05$ was used to statistically process the data using Prism 8.0.1 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Antimicrobial activity of TMEO contact application

Based on the disc diffusion method, we found that TMEO achieved moderate inhibitory activity on how many inhibition zones in the range of 5-10 mm were observed for all tested microorganisms (Table 1). The highest zone of inhibition was detected against the yeast *C. tropicalis* (8.62 mm) and the gram-negative bacteria *H. infuenzae* (8.31 mm). Compared to the antibiotic control, the effect of TMEO was lower.

Table 1. Antimicrobial activity of TMEO contact application.

Microorganisms	Inhibition zones (mm)	ATB (mm)
Yersinia enterocolitica	5.62±0.58	29.62±0,58
Haemophylus influenzae	8.31±1.15	30.54±1,53
Lysteria monocytogenes	6.31±1.66	30.67±1.15
Enterococcus faecalis	7.54±0.58	28.83±0.33
Pseudomonas fluorescens-biofilm	5.31±1.15	25.62±0.58
Candida tropicalis	8.62±0.58	31.62±1.15

The inhibition zone above 10 mm was determined as very strong antimicrobial activity, the inhibition zone 5-10 mm was determined as moderate activity, and the inhibition zone below 5 mm was determined as weak activity. Antimicrobial activity was measured in triplicate.

2.4. Antimicrobial activity of vapor phase TMEO on model crops

In the analysis of the antimicrobial activity of vapor phase TMEO on the model apple crop (Table 2.), we detected inhibition in all tested microorganisms and at all tested concentrations, except for *E. faecalis. E. faecalis* with a concentration of 250 µL/L and for *P. fluorescens* biofilm with a concentration of 125 µL/L where we observed stimulation of growth. The most

pronounced inhibition was observed at the lowest tested concentration, 62.5 μL/L, for all tested microorganisms.

Table 2. Antimicrobial activity of TMEO vapor application on model food apple.

Apple	**BGI (%)**			
	Con. (μL/L)			
Microorganisms	**62.5**	**125**	**250**	**500**
Y. enterocolitica	54.40±0.08 [a]	44.23±0.99 [b]	36.39±2.12 [c]	26.02±2.09 [d]
H. influenzae	35.25±1.07 [a]	22.65±0.89 [b]	15.83±0.83 [c]	12.68±0.67 [d]
L. monocytogenes	44.74±0.95 [a]	34.35±0.98 [b]	13.32±1.05 [c]	8.32±0.52 [d]
E. faecalis	34.71±0.95 [a]	6.73±1.20 [b]	-9.28±0.55 [c]	2.76±0.42 [d]
C. tropicalis	53.40±1.15 [a]	-24.46±2.06 [b]	24.68±0.42 [c]	8.74±0.57 [d]
P. fluerescens-biofilm	36.55±2.03 [a]	21.06±0.63 [b]	12.03±1.60 [c]	2.99±0.59 [d]

Mean ± standard deviation. Values followed by different superscripts within the same line are significantly different (P < 0.05). Con.—concentration. BGI – bacterial growth inhibition.

In the analysis of the antimicrobial activity of vapor phase TMEO on the model crop pear (Table 3.), we detected inhibition in all tested microorganisms and at all tested concentrations. The most pronounced inhibition was observed at the lowest tested concentration, 62.5 μL/L, for all tested microorganisms.

Table 3. Antimicrobial activity of steam application of TMEO on model crop pear.

Pear	**BGI (%)**			
	Con. (μL/L)			
Microorganisms	**62.5**	**125**	**250**	**500**
Y. enterocolitica	36.63±2.04 [a]	33.72±5.34 [a]	12.36±0.84 [b]	6.84±0.95 [c]
H. influenzae	36.55±2.03 [a]	25.36±0.11 [b]	15.17±0.61 [c]	0.81±0.56 [d]
L. monocytogenes	44.09±2.28 [a]	23.85±2.03 [b]	12.56±0.48 [c]	5.37±0.44 [d]
E. faecalis	54.78±1.46 [a]	36.39±2.12 [b]	26.52±2.39 [c]	8.48±0.59 [d]
C. tropicalis	36.39±2.12 [a]	23.32±0.95 [b]	14.45±0.86 [c]	5.37±0.40 [d]
P. fluerescens-biofilm	64.89±1.00 [a]	36.46±2.72 [b]	17.43±0.06 [c]	6.98±0.97 [d]

Mean ± standard deviation. Values followed by different superscripts within the same line are significantly different (P < 0.05). Con.—concentration. BGI – bacterial growth inhibition.

In the analysis of the antimicrobial activity of vapor phase TMEO on a model carrot crop (Table 4.), we detected inhibition in all tested microorganisms and at all tested concentrations. The most pronounced inhibition was observed at the lowest concentration tested, 62.5 μL/L, for all microorganisms tested except *L. monocytogenes* where the most pronounced inhibition was at a concentration of 125 μL/L.

Table 4. Antimicrobial activity of steam application of TMEO on carrot model crop.

Carrot	**BGI (%)**			
	Con. (μL/L)			
Microorganisms	**62.5**	**125**	**250**	**500**
Y. enterocolitica	19.32±0.95 [a]	14.05±1.50 [b]	8.28±1.47 [c]	2.16±0.86 [d]
H. influenzae	56.18±1.17 [a]	43.66±1.02 [b]	27.47±2.13 [c]	8.27±0.52 [d]
L. monocytogenes	16.34±1.85 [a]	25.54±0.39 [b]	7.16±1.63 [c]	4.44±0.46 [d]
E. faecalis	44.21±0.48 [a]	28.17±5.90 [b]	13.24±0.55 [c]	3.43±0.83 [d]
C. tropicalis	56.05±0.95 [a]	33.72±1.06 [b]	12.47±0.45 [c]	4.96±0.34 [d]
P. fluerescens-biofilm	47.87±1.35 [a]	27.46±1.06 [b]	14.00±1.16 [c]	5.21±0.30 [d]

Mean ± standard deviation. Values followed by different superscripts within the same line are significantly different (P < 0.05). Con.—concentration. BGI – bacterial growth inhibition.

In the analysis of the antimicrobial activity of vapor phase TMEO on a model crop of white radish (Table 5.), we detected inhibition in all tested microorganisms and at all tested concentrations. The most pronounced inhibition was observed for *Y. enterocolitica, E. faecalis* and *C. tropicalis* at the lowest concentration tested, 62.5 µL/L. Against other microorganisms, efficacy was observed at higher concentrations for *H. influenzae* and *L. monocytogenes* at 250 µL/L and biofilm-producing *P. fluorescens* at 125 µL/L.

Table 5. Antimicrobial activity of steam application of TMEO on a model crop of white radish.

White radish	BGI (%)			
	Con. (µL/L)			
Microorganisms	**62.5**	**125**	**250**	**500**
Y. enterocolitica	43.62±0.56 ^{a}	23.65±1.53 ^{a}	17.67±0.48 ^{b}	8.87±0.59 ^{c}
H. influenzae	15.68±0.57 ^{a}	5.20±3.68 ^{b}	35.30±1.06 ^{c}	35.21±0.76 ^{c}
L. monocytogenes	25.93±1.80 ^{a}	23.49±18.65 ^{ac}	87.67±1.48 ^{b}	14.26±0.57 ^{c}
E. faecalis	86.97±1.34 ^{a}	4.26±1.08 ^{b}	16.52±2.02 ^{c}	37.10±1.85 ^{d}
C. tropicalis	55.85±1.14 ^{a}	32.80±1.00 ^{b}	23.65±1.53 ^{c}	16.37±0.79 ^{d}
P. fluerescens-biofilm	35.28±0.95 ^{a}	75.47±1.33 ^{b}	24.45±2.41 ^{c}	8.43±0.71 ^{d}

Mean ± standard deviation. Values followed by different superscripts within the same line are significantly different (P < 0.05). Con.—concentration. BGI – bacterial growth inhibition.

4. Discussion and Conclusions

Rodrigues et al. (2020) in their work found inhibition zones of TMEO against *L. monocytogenes* of 9.7-12.3 mm and for *P. fluorescens* of 9-10 mm, depending on the origin of the EO and the plant part used. The findings for *L. monocytogenes* are in agreement with our results for *P. fluorescens* we detected a lower zone of inhibition which could be because it is a biofilm-producing strain that is more resistant to inhibition. Faleiro et al. (2003) detected in their work the inhibitory activity of TMEO against *L. monocytogenes* with a zone of inhibition of 9.7 mm and also report that TMEO is most effective against the *Candida* genus with a zone of inhibition above 10 mm. These findings confirm our results. Ballester-Costa et al. (2013) in their work found a zone of inhibition against *P. fluorescens* of 9.0 mm. In our work, we detected a lower zone of inhibition which could be because it is a biofilm-producing strain which is more resistant to inhibition.

To our knowledge, TMEO has not yet been tested in the vapor phase on food models. In a previous study, we analyzed the vapor phase effect of *T. vulgaris* detecting a very good antifungal as well as antibacterial effect (Galovičová et al., 2021). Paris et al. (2020) in their work analyzed the antifungal and antimicrobial effect of EOs in washed and contact applications against fruit spoilage pathogens and concluded that the vapor phase is more effective than the contact phase as EOs are rich in volatile compounds which confirms our findings. In contrast, Ács et al. (2018) in their study reported that their EOs were stronger inhibitors in liquid form, which is probably due to direct contact with the pathogen. This finding contradicts our results which may be due to the different chemical compositions especially the content of volatile compounds which are more effective in the vapor phase.

The results of our study show that TMEO has higher efficacy in vapor application where we detected the most significant inhibition at the lowest tested concentration of 62.5 μL/L for most of the tested microorganisms and on all tested crops. In contact application, only moderate antimicrobial activity was detected, and lower efficacy compared to antibiotics. These findings suggest that in the future TMEO could find application in the storage of horticultural crops to prevent their deterioration due to microbial pathogens. In the future, it would be advisable to test even lower concentrations of TMEO as the trend of antimicrobial effect showed that in most cases the lowest concentration was the most effective. Due to the need to use very low concentrations, it is assumed that the sensory properties of the crop for the consumer will not be affected. The replacement of synthetic fungicides and bactericides with natural alternatives could have a positive impact on the environment.

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Antifungal Efficacy Of Mint Essential Oil Against *Penicillium* Spp.Inoculated On Carrots

Veronika Valková[1,2*], Hana Ďúranová[2], Lucia Galovičová[1], Nenad L. Vukovic[3], Milena Vukic[3], Miroslava Kačániová[1,4]

Abstract: Current research aimed to investigate the chemical composition, antioxidant, and in vitro and in situ antifungal activities of commercially available Mentha arvensis essential oil (mint, MEO). The identification of the volatile substances was done using Gas chromatography–Mass spectrometry (GC-MS) analysis. A total of 42 components representing 99.5% of the total oil were identified. The main compounds in the oil were menthol (37.3%), menthone (17.4%), neo-menthol (14.1%), and 1,8-cineole (4.9%). Antioxidant assay (1,1-diphenyl-2-picrylhydrazyl radical) revealed only a weak activity of the MEO with values of 195.00 ± 5.30 µg TEAC.mL^{-1}, indicating 22.8 ± 1.2% free radical-scavenging inhibition. Evaluation of in vitro and in situ antifungal activities of the MEO (in four concentrations: 62.5 µL/L, 125 µL/L, 250 µL/L, and 500 µL/L) against three strains of Penicillium (P.) spp. (P. expansum, P. citrinum, and P. crustosum) were assessed by disc diffusion and vapor contact methods on a carrot as a model food, respectively. The suitability of carrot samples as a substrate for analyzes was verified by determining their moisture content (MC) and water activity (a_w), which showed values of 82.80 ± 2.33% and 0.959 ± 0.001, respectively. The MEO exhibited promising antifungal activity against analyzed strains of test fungi manifested by diameter of inhibition zones (ranging from 2.88 ± 0.55 to 12.33 ± 1.14 mm), as well as by their inhibited growth on the carrot model (from -5.41 ± 7.35 to 100.00 ± 0.00%). Moreover, it can be concluded that the growth inhibition of fungal strains significantly depends ($P < 0.05$) on the concentration of the MEO used in both procedures. Our results suggest that MEO, as a promising natural antifungal agent, can be applied in the innovative packaging of food products including carrots.

Keywords: Mentha arvensis, DPPH assay, volatile compounds, antifungal activity, model food

1. Introduction

Currently, the cultivation of herbs and aromatic plants to derive essential oils (EOs) is greatly increasing primarily due to the expanding demand generated by the food, pharmaceutical, and cosmetics industries (Lubbe and Verpoorte, 2011). Among medicinal plants, the production of mint (*Mentha* species) is very agriculturally profitable because of a large number of practical applications (Souza et al., 2014).

[1] Institute of Horticulture, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976 Nitra, Slovakia
[2] AgroBioTech Research Centre, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976 Nitra, Slovakia
[3] Department of Chemistry, Faculty of Science, University of Kragujevac, 34000 Kragujevac, Serbia
[4] Department of Bioenergy, Food Technology and Microbiology, Institute of Food Technology and Nutrition, University of Rzeszow, 4 Zelwerowicza Str., 35-601 Rzeszow, Poland
* Corresponding author: veronika.valkova@uniag.sk

Mentha (M; Lamiaceae) is a well-known genus including approximately 30 reported species that grow across the world in temperate areas (Trucker and Naczi, 2007). The most common species of *Mentha* include *M. aquatica, M. arvensis, M. citrata*, *M. longifolia, M.piperita, M. pulegium*, *M. rotundifolia*, and *M. spicata* (Anwar et al., 2019) which are widely used in savory dishes, food, beverages, and confectionary products (Tafrihi et al., 2021). These plants exhibit a great chemical diversity with respect to their EOs and important biological activities (Trucker and Naczi, 2007) which are effectively used in the management of plant pathogens and insect pests, in traditional medicine, as well as culinary and cosmetics (Singh and Pandey, 2018). In general, EOs are a highly volatile lipophilic mixture produced in plant secondary metabolism responsible for plant protection and communication (Saeed et al., 2022). At the laboratory scale, steam distillation and hydrodistillation are the most commonly used methods for their extraction (Ribeiro-Santos et al., 2018).

Mentha arvensis, popularly known as corn mint, wild mint or field mint (Nazim et al., 2020), has unique importance among the mint family due to its high concentration of menthol (Thawkar, 2016) ranging up to 71.40% (Pandey et al., 2003). In effect, menthol has antiseptic, carminative, refrigerant, stimulative, and diuretic properties (Thawkar et al., 2016). Other primary compounds of *M. arvensis*, responsible for its typical aroma, are menthone and its isomers, menthyl esters, and piperitone (Trucker and Zarowin, 2006). Generally, EO obtained from *Mentha arvensis* (MEO) is yellow in color with a very strong and persistent odor of mint (Makkar et al., 2018). Its chemical profile is affected by many factors, such as environmental and cultivar conditions, soil nutrients, humidity, temperature, and biotic and abiotic circumstances (de Sousa Barros et al., 2015). Regarding its biological properties, MEO was found to be a potential candidate for antimicrobial (Bokhari et al., 2016; Bibi et al., 2021), antioxidant (Benabdallah et al., 2018), and fungicidal activities (Makkar et al., 2018).

In this report, the antifungal activity of the MEO and its possible application as a bio-preserver of carrots were evaluated. For a detailed description, chemical profile and antioxidant properties of the EO were also taken into consideration.

2. Material and Method

2.1. Essential Oil

Mint EO (MEO; *Mentha arvensis*) was extracted by steam distillation of flowering stems. This EO was obtained by a commercial producer Hanus Ltd. (Nitra, Slovakia), and was preserved at 4 °C in the laboratory refrigerator until its next application.

2.2. Chemical Analysis

The chemical composition of the MEO was analyzed using gas chromatography with mass spectrometry (GC-MS), as it was described by Valková et al. (2022a). In brief, the analysis was carried out by Agilent Technology 6890N (Agilent Technologies, Santa Clara, CA, USA) coupled to quadrupole mass spectrometer 5975B (Agilent Technologies, Santa Clara, CA, USA). Separation of compounds was carried out using HP-5MS capillary column (30 m × 0.25 mm × 0.25 m). The temperature program was as follows: 60 °C to 150 °C (increasing rate 3 °C/min) and 150 °C to 280 °C (increasing rate 5 °C/min), using helium 5.0 as the carrier gas with a flow rate of 1 mL/min. Samples of essential oils were dissolved in pentane, and injection volume was 1μL. The split/splitless injector temperature was set at 280 °C. The investigated samples were injected in the split mode with a split ratio at 40.8:1. Electron-impact mass spectrometric data (EI-MS; 70 eV) were acquired in scan mode over the m/z range 35–550. The mass spectrometry ion source temperature was 230 °C, while the temperature of MS quadrupole

was set at 150 °C. Solvent delay time of 3 min. After the separation, the components were identified based on the comparison of their relative retention index and compared with the library mass spectral database (Wiley and NIST databases). The percentage composition of compounds (relative quantity; amounts higher than 0.1%) was measured based on the peak area. The retention indices were experimentally determined by injection of standard n-alkanes (C6–C34) under the same chromatographic conditions.

2.3. Determination of MEO Antioxidant Activity

To measure the antioxidant activity (AA) of the MEO, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used, as previously described by Galovičová et al. (2021). The AA was expressed as the percentage of DPPH inhibition, which was calculated using the following equation: (A0 − A1)/A0 × 100; where A0 was the absorbance of DPPH and A1 was the absorbance of the sample. The power of AA was recognized as follows: weak (0–29%) < medium–strong (30–59%) < strong (60 and more %). Moreover, the value for total AA was expressed according to the calibration curve as 1 μg of the standard reference Trolox to 1 mL of the MEO sample (TEAC).

2.4. Evaluation of MEO Antifungal Potential

2.4.1. Fungal Strains and Culture Media

In the current study, three strains of genus *Penicillium* (*P. expansum*, *P. crustosum, P. citrinum*), isolated from berry samples of *Vitis vinifera* were employed. Consequently, the microscopic filamentous fungi were classified using a reference-based MALDI-TOF MS Biotyper, and validated by comparison with the taxonomic identification using 16S ribosomal RNA (16S rRNA) gene sequences analysis.

2.4.2. *In Vitro* Antifungal Activity of MEO

Evaluation of the *in vitro* antifungal activity of the EOs was performed using the agar disc diffusion method, according to Valková et al. (2022a). For this purpose, an aliquot of 100 μL of culture media was inoculated on SDA. To prepare culture media, the strains were inoculated in Sabouraud Dextrose Agar (SDA; Oxoid, Basingstoke, UK) and incubated for 5 days at 25 °C. Subsequently, small aliquots of the fungi were transferred to test tubes, each containing 3 mL of distilled water. The inoculum concentration was standardized by comparison with the 0.5 McFarland scale (1.5 × 108 CFU/mL). After that, the discs of filter paper (6 mm) were impregnated with 10 μL of MEO sample (in four concentrations: 62.5, 125, 250, and 500 μL/L), and applied on the SDA surfaces. Fungi were incubated aerobically at 25 ± 1 °C for 5 days. After the incubation, diameters of the inhibition zones in mm were measured. The values for inhibitory activity increased in the following manner: weak antifungal activity (5 - 10 mm) < moderate antifungal activity (10 - 15 mm) < very strong antifungal activity (zone > 15 mm).

2.4.3. *In Situ* Antifungal Activity of MEO

All three fungal strains (*P. expansum*, *P. crustosum*, and *P. citrinum*) were used to evaluate the antifungal activity of the EOs *in situ*.

2.4.4. Food Model

Carrot was applied as substrates for the growth of the fungi. This vegetable was purchased at the local market (Nitra, Slovakia).

2.4.5. Moisture Content and Water Activity of Food Model

To predict the suitability of substrates for fungal growth, moisture content (MC) and water activity (a_w) of the carrot were determined, as reported by Valková et al. (2022a).

2.4.6. Vapor Contact Method

The experiment itself was performed as reported by Valková et al. (2022a). Firstly, sliced carrot (5 mm) was placed on the bottom of Petri dishes (PDs), and the inoculum was applied by stabbing one time with an injection pin on the vegetable surface. Further, 10 µL of the MEO (in the same four concentrations) was applied on the sterile filter paper disc (60 mm), then, it was placed at the top of PD. Subsequently, PDs were hermetically closed using parafilm and cultivated at 25 °C for 14 days.

2.4.7. Determination of Fungal Growth Inhibition

In situ fungal growth was determined using stereological methods. In this concept, the volume density (Vv) of visible fungal colonies was firstly established using ImageJ software counting the points of the stereological grid hitting the colonies (P) and those (p) falling to the reference space (growth substrate used: carrot). The volume density of the strain colonies was consequently calculated as follows: Vv (%) = P/p. Finally, the antifungal potential of the EOs was expressed as the percentage of mycelial growth inhibition (MGI) according to the formula MGI = [(C − T)/C] × 100, where C and T is the growth of fungal strains (expressed as Vv) in the control and treatment group, respectively (Valková et al., 2022a).

2.5. Statistical Analysis

The data were submitted to one-way analysis of variance (ANOVA) and the means were compared by the Tukey test at 5% of probability using statistical software Prism 8.0.1 (GraphPad Software, San Diego, CA, USA). All analyses were performed in triplicate.

3. Results

3.1. Chemical Profile of MEO

GC-MS analysis revealed that a total of 42 substances, accounting for 99.5% of the whole constituents, were identified in the MEO chemical composition. The major compounds were shown to be menthol (37.3%), menthone (17.4%), neo-menthol (14.1%), and 1,8-cineole (4.9%), as presented in Table 1.

Table 1. Chemical composition of MEO

NO	RI [a]	Compound [b]	% [c]
1	926	α-thujene	0.1
2	938	α-pinene	2.3
3	948	camphene	0.3
4	977	sabinene	0.5

5	980	β-pinene	1.5
6	992	β-myrcene	0.4
7	993	3-octanol	0.6
8	1004	α-phellandrene	tr
9	1016	α-terpinene	0.1
10	1023	p-cymene	1.0
11	1028	α-limonene	3.6
12	1033	1,8-cineole	4.9
13	1047	(*E*)-β-ocimene	tr
14	1060	ɣ-terpinene	0.5
15	1088	α-terpinolene	0.4
16	1148	isopulegol	1.7
17	1151	menthone	17.4
18	1160	pinocarvone	1.2
19	1162	iso-menthone	1.0
20	1164	neo-menthol	14.1
21	1170	borneol	0.2
22	1173	menthol	37.3
23	1189	α-terpineol	0.6
24	1217	trans-carveol	0.1
25	1229	cis-carveol	tr
26	1239	pulegone	0.9
27	1241	carvone	0.2
28	1253	3-carvomenthenone	1.0
29	1254	(*Z*)-anethole	0.4
30	1276	*p*-penth-1-en-7-al	5.2
31	1289	(*2E*)-hexenyl valerate	0.3
32	1297	menthyl acetate	tr
33	1298	*p*-menth-1-en-9-ol	tr
34	1378	α-ylangene	tr
35	1379	α-copaene	tr
36	1385	β-bourbonene	0.2
37	1388	β-elemene	tr
38	1422	(*E*)-caryophyllene	0.8
39	1443	aromadendrene	tr
40	1483	germacrene D	0.2
41	1525	δ-cadinene	0.4
42	1583	caryophyllene oxide	0.1
Total			99.5

Note: [a] Values of retention indices on HP-5MS column; [b] Identified compounds; [c] tr - compounds identified in amounts less than 0.1%.

3.2. Antioxidant Activity of MEO

It was found that values for AA of the MEO were 195.00 ± 5.30 μg TEAC.mL^{-1}, with 22.8 ± 1.2% free radical-scavenging inhibition linked to a weak AA.

3.3. *In vitro* Antifungal Activity of MEO

The results from the antifungal effects of the MEO on the growth of *P. expansum*, *P. crustosum*, and *P. citrinu*m are shown in Table 2. It can be concluded that the growth inhibition of fungal strains significantly depended ($P < 0.05$) on the MEO concentration used. Concretely, moderate antifungal activities (12.13 ± 0.48 mm, 11.56 ± 0.86 mm, and 12.33 ± 1.14 mm) were observed in the highest concentration (500 µL/L) of the MEO against the growth of *P. expansum*, *P. crustosum*, and *P. citrinu*m, respectively. On the other hand, 125 µL/L and 250 µL/L concentrations of the EO showed weak antifungal activities against all evaluated strains; whereas the lowest concentration of the MEO resulted in only a very low inhibitory efficiency.

Table 2. *In vitro* antifungal activity of MEO in analyzed concentrations (inhibition zones in mm)

Fungi	MEO (µL/L)			
	62.5	125	250	500
P. expansum	2.88 ± 0.55 [a]	5.92 ± 0.39 [b]	8.13 ± 1.25 [c]	12.13 ± 0.48 [d]
P. crustosum	4.36 ± 0.78 [a]	6.23 ± 0.44 [b]	7.89 ± 0.96 [c]	11.56 ± 0.86 [d]
P. citrinum	3.71 ± 0.61 [a]	5.84 ± 1.06 [b]	8.56 ± 1.01 [c]	12.33 ± 1.14 [d]

Note: Mean ± standard deviation. MEO - Mint essential oil. Values in the same line with different small letters are significantly different (P < 0.05).

3.4. Moisture content and water activity of carrot

The results from the moisture content (MC) and water activity (a_w) measurements showed that the parameters of carrot in our study had values of 82.80 ± 2.33% and 0.959 ± 0.001, respectively.

3.5. *In situ* antifungal activity of MEO

The antifungal effectiveness of the MEO against the growth of the *Penicillium* spp. inoculated on carrots is demonstrated in Table 3. From the findings it is clearly evident that with an increasing concentration, the MEO exhibited an enhancing antifungal activities against all analyzed strains, with the strongest one revealed in the highest concentration (500 µL/L).

Table 3. *In situ* antifungal activity of MEO on carrot.

Fungi	MGI (%)			
	MEO (µL/L)			
	62.5	125	250	500
P. expansum	28.57 ± 4.31 [a]	88.89 ± 5.12 [b]	89.09 ± 6.13 [b]	98.08 ± 4.78 [c]
P. crustosum	-5.41 ± 7.35 [a]	3.68 ± 2.93 [a]	72.04 ± 6.71 [b]	98.43 ± 4.88 [c]
P. citrinum	-23.33 ± 6.09 [a]	71.81 ± 8.12 [b]	96.92 ± 5.13 [c]	100.00 ± 0.00 [c]

Note: Mean ± standard deviation. MEO - Mint essential oil. Values in the same line with different small letters are significantly different (P < 0.05). The negative values indicate a profungal activity against Penicillium strains.

4. Discussion and Conclusions

Generally, the biological potencies of plant EOs are attributable to their chemical composition and especially to their major substances (Kasrati et al., 2015). Therefore, the detection of

individual volatile components, which we implemented in our study, is an important tool for knowing the effect of EOs. In line with our findings, Pandey et al. (2008), Chagas et al. (2020), and Mahn and Tuyet (2020) detected the major substance in EO obtained from *M. arvensis* to be menthol (71.4%, 86.1%, 66.04%, respectively). However, this compound was in our analyzed MEO presented in lower concentration (37.3%). Similarly, lower concentration of this substance (21.35%) was also confirmed by Khan et al. (2019). The authors also find high content of menthone (29.42%) in the EO conception which also created a high proportion of our oil sample (17.4%). We assume that differences in the percentage of the chemical components in the MEO between mentioned studies may be related to different cultivars of mint or different growing stage of plants (Verma et al., 2010).

DPPH assay is a widely employed procedure to estimate the free radical scavenging ability of materials due to its simplicity and rapidity (Gudimella et al., 2021). This method is based on the reduction of the commercially available radical (DPPH) and shows a color change from deep purple to pale yellow upon reaction (Higgins et al., 2021). Due to its properties, this technique is often used to analyze the antioxidant characteristics of EOs (Valková et al., 2022a; 2022b; 2022c). In our study we found that despite the diverse chemical profile, the MEO showed weak values for AA (195.00 ± 5.30 μg TEAC.mL^{-1}, with 22.8 ± 1.2%). Accordingly, in our previous research works, the weak AAs of green mandarin EO (Valková et al., 2021a), rosalina EO, fir EO, and niaouli EO (Valková et al., 2022b) were reported. We propose that the weak AA of our MEO may be related to the high concentration of monoterpene and sesquiterpene hydrocarbons in its conception, which have low solubility in the assay medium and also do not have the ability to donate hydrogen atoms (Mata et al., 2007). These properties can be an essential limitation for the determination of the DPPH radical scavenging activity of some types of samples, including various EOs (Viuda-Martos et al, 2010). Therefore, the choice of methodology largely affects AA of the samples. In the future, we plan to carry out the determination of AA using several methods including the FOMO and the ABTS ones.

Essential oils from *Mentha* spp. were screened for their antifungal activities (Saba and Anwar, 2018). Confirming our findings, Hussain et al. (2010) also detected the antifungal effects of *M. arvensis* EO against seven fungal strains including *Aspergillus* (*A.*) *flavus*, *Alternaria* (*A.*) *solani*, *Fusarium* (*F.*) *solani*, *Rhizopus* (*R.*) *solani*, *A. alternata*, *A. niger*, and *Rhizopus* spp. Their results from the disc diffusion method showed strong antifungal activity of MEO with large inhibition zones varying from 16 to 30 mm against the fungal strains. The antifungal efficacy of MEO may be related to its high menthol content, which we also detected in our study (37.3%). It is known that menthol exhibits antifungal effects against various fungal strains including *Candida (C.) albicans* (Piran et al., 2017), *Aspergillus* (*A.*) *niger*, *A. fumigatus*, *A. flavus*, *A. ochraceus*, *A. alternata*, *Botrytis* (*B.*) *cirenea*, *Cladosporium* spp., *P. citrinum*, *P. chrysogenum*, *F. oxysporum* and *Rhizopus oryzae* (Abbaszadeh et al., 2014). Although the exact mechanism of menthol action is not fully understood, its antifungal effect can result from a perturbation of the lipid fraction of fungi plasma membrane, leading to alterations in membrane permeability, and to leakage of intracellular materials (Trombetta et al., 2005). Moreover, Samber et al. (2015) demonstrated the efficacy of menthol due to its integration with PM-H+ ATPase enzyme which participates in an electrochemical proton gradient across the cell membrane necessary for nutrient uptake. However, we assume that individual compounds of MEO and their interaction are crucial for their final inhibitory effects on mycelial growth. Therefore, the antifungal efficacy of our EO may be related to other volatile components present in its conception, as well.

MC and a_w largely influence the ability of microorganisms to grow on food products (Qiu et al., 2019). The presence of water in the form of MC is the major factor aiding the growth and activities of the microorganisms because it increases their metabolic activities. Without water or in the presence of a limited volume of water, agricultural products will become inhospitable

to the microorganism and inhibit their growth (Rajeev et al., 2012). In this context, many fresh foods including vegetables are perishable due to their high MC (> 40%; Akdogan, 1999; Dagnas et al., 2017). Further a_w is defined as the ratio of the vapor pressure of water over a substrate compared to that over pure water at the same temperature and pressure (Cazier and Gekas, 2001).Concerning a substantial impact on the growth of microorganisms it was found out that a_w above 0.7 supports the microbial spoilage (Syamaladevi et al., 2016). In line with our findings, similar values for MC (82.80 ± 2.33%) and a_w (0.959 ± 0.001) of carrot we also detected in our last research (86.83 ± 0.42%; 0.945 ± 0.002; Valková et al., 2022c). Anyway, our results indicate the suitability of carrot as food model for *in situ* antifungal analysis of the MEO investigated.

Some studies have reported that vapor generated by EOs has a greater antifungal effect compared with EOs in liquid form applied by direct contact (Tullio et al. 2007; Fisher and Phillips 2008). Moreover, the vapor phase allows for free attachment of EOs to microorganisms, unlike lipophilic molecules in the liquid phase are associated with formation of micelles which restrain the attachment of EOs to microorganisms (Boukhatem et al., 2014). In this way, the vapor phase of EOs has a specific impact on fungi due to their superficial growth reflecting more susceptibility to EO volatile compounds (Edris and Farrag 2003). Furthermore, the composition of the food system impacts the antifungal effectiveness of EOs, and this activity is typically decreased in *in situ* conditions compared to *in vitro* ones. However, the low-fat content of vegetables can participate in the successful antifungal effects also on the food model (Burt, 2004). Therefore, in the current study, this effect has been investigated for carrots as a food substrate. In accordance with our results, the antifungal effect of MEO obtained from *M. piperita* against the same fungal strains (*P. expansum, P. citrinum,* and *P. crustosum*) inoculated on bread was also reported (Valková et al., 2021b). In both cases, the effect of EOs on food models was dependent on their concentration, with the highest efficiency recorded in the highest concentrations (500 μL/L), except for *P. citrinum* inoculated on bread (Valková et al., 2021b); in this case, the highest efficiency was recorded in concentrations of 125 μL/L. Moreover, our findings are in accordance with our previous studies, in which the antifungal efficacies of various types of EOs, such as mandarin EO (Valková et al., 2021a), coriander EO (Kačániová et al., 2020), fir EO, rosalina EO, and niaouli EO (Valková et al., 2022c), against the same fungal species analyzed were confirmed.

From the results of all our analyses, it can be concluded that MEO may be a promising agent with potential use for extending the shelf-life of vegetables including carrots on the commercial scale of the food industry.

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Antifungal Activities of Essential Oil Obtained from *Mentha spicata* var. *crispa* Against Selected *Penicillium* Species

Hana Ďúranová[1*], Veronika Valková[1,2], Lucia Galovičová[2], Nenad L. Vukovic[3], Milena Vukic[3], Miroslava Kačániová[2,4]

Abstract: Attention of the scientific community has still focused on application of essential oils (EOs) as natural antifungal agents in the food industry to prolong the shelf-life of food products. In this regard, the current study was designed to evaluate chemical composition, antioxidant capacity, and antifungal (in vitro, in situ) activities of spearmint (Mentha spicata var. crispa) essential oil (SEO) commercially obtained from Slovak company against selected Penicillium species. The EO was used in four concentrations (62.5, 125, 250, and 500 µL/L) chosen, and gas chromatography–mass spectrometry, DPPH, agar disc diffusion and vapor phase methods were employed for such analyses. Our results revealed carvone (57.5%) and α-limonene (17.6%) to be the principal constituents in the EO chemical composition. Although only a weak antioxidant capacity (20.40 ± 0.80% free radical-scavenging inhibition) was displayed by the SEO, the highest EO concentration (500 µL/L) was shown to be a moderate growth inhibitor of P. expansum (inhibition zone of 11.46 ± 0.63 mm) and P. crustosum (inhibition zones of 12.93 ± 0.46 mm). The growth of P. citrinum was only weakly inhibited by the SEO (≥ 250 µL/L). Most importantly, the ability of the SEO to inhibit the mycelial growth of three Penicillium spp. tested was pronounced ($P < 0.05$) for all applied concentrations. Accordingly, the results from the current study complement our previous ones dealing with the possibility of utilizing diverse EOs commercially achieved from the same company in the food sector.

Keywords: Mentha spicata, essential oil, volatile substances, DPPH assay, antifungal activity, vegetable

1. Introduction

Due to microbial contamination of food products, food sector is facing a great challenge to find a promising alternative to synthetic preservatives reducing food spoilage but being bio-incompatibile, non-biodegradable, and environmentally unsustainable (Maurya et al., 2021). One of the major emerging technologies to extend the shelf-life of foods seems to be an extraction of essential oils (EOs) from various plant organs (particularly leaves and flowers) and their application to food systems (Fernández-López and Viuda-Martos, 2018; Ribeiro-Santos et al., 2018; LaLonde et al., 2019; Rao et al., 2019; Santos et al., 2022). Indeed, as a valuable source of diverse biologically active compounds, EOs possess antioxidant and antimicrobial properties which participate in food shelf-life enhancement, thus making them an

[1] AgroBioTech Research Centre, Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia
[2] Institute of Horticulture, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia
[3] Department of Chemistry, Faculty of Science, University of Kragujevac, 34000 Kragujevac, Serbia
4 Department of Bioenergy, Food Technology and Microbiology, Institute of Food Technology and Nutrition, University of Rzeszow, 4 Zelwerowicza Str., 35-601 Rzeszow, Poland
* Corresponding author: hana.duranova@uniag.sk

ideal natural, eco-friendly, renewable, and cost-effective replacement of synthetic food additives (Fernández-López and Viuda-Martos, 2018; Basavegowda and Baek, 2021; Saeed et al., 2022).

Spearmint [*Mentha* (*M.*) *spicata*, equivalent to *M. viridis*] is a perennial herbaceous medicinal plant of the Lamiaceae family with a pungent smell, commercially cultivated in many regions of the world (Ounoki et al., 2021; El Menyiy et al., 2022). Leaves of the plant are traditionally used as tea (Dhifi et al., 2013) and an ingredient in a variety of mixed drinks including the mojito and the mint julep (Ounoki et al., 2021). Spearmint EO (SEO) belonging among the 10 most commercialized EOs (Delfine et al., 2022) is produced and stored in the glandular trichomes of the leaves. The EO is characterized by high presence of monoterpenes (Chrysargyris et al., 2017), mainly of carvone as the major component responsible for its aroma (Dionísio et al., 2012). Hence, it is especially applied in the flavoring of chewing gums, toothpastes, and other oral products (Kokkini et al., 2003). There are many *in vitro* and *in vivo* experimental reports demonstrating *M. spicata* extracts and EOs as agents with remarkable antimicrobial, antiparasitic, antidiabetic, anti-inflammatory, and anticancer biological activities (El Menyiy et al., 2022). Additionally, SEO is reputed for its carminative, antispasmodic, and diuretic properties (Dhifi et al., 2013). Regarding its antimicrobial efficacy, common food-borne pathogenic bacteria, such as *Staphylococcus aureus*, *Bacillus* (*B.*) *subtilis*, *B. cereus*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Pseudomonas aeruginosa*, *Shigella flexneri* (Ullah et al., 2012; Shahbazi, 2015; Horváth and Koščová, 2017) were shown to be sensitive to SEO actions. Also, antifungal activity of the EO is extensively tested (Şarer et al., 2011; Houicher et al., 2016; Bardaweel et al., 2018). However, it is well-known that chemical profile and relative proportions of organic compounds of any EO extracted from a selected single plant species depend on a plethora factors such as agricultural aspect (e.g., environment, climate, soil conditions, time of harvesting and postharvest handling prior to isolation) (Sankarikutty and Narayanan, 2003), extraction methods used (Berka-Zougali et al., 2012; Pintatum et al., 2020; Messaoudi et al., 2021), plant parts being used for extraction (Pintatum et al., 2020), and many others, which are principal causes of serious discrepancies identified among the studies concerning this field of research area. Taking into account this fact along with adding another piece to our comprehensive view creation of various antifungal actions exerted by diverse commercial EOs achieved from the same company, the current study evaluated *in vitro* and *in situ* antifungal activities of the EO obtained from *M. spicata* var. *crispa* against selected *Penicillium* spp. inoculated on potato slices as a model of food substrate. In such a way, application of the SEO in active food packaging to prolong shelf-life of foods can be considered.

2. Material and Method

2.1. Essential oil

Spearmint (*M. spicata* var. *crispa*) essential oil (SEO) was purchased from Hanus s.r.o Company (Nitra, Slovakia) to complement our previous studies (Galovičová et al., 2021a; Galovičová et al., 2021b; Kačániová et al., 2021a; Valková et al., 2021a; Valková et al., 2021b; Galovičová et al., 2022; Kačániová et al., 2022a; Valková et al., 2022a; Valková et al., 2022b). The EO was prepared by steam distillation of flowering stems.

2.2 Fungal strains

To assess *in vitro* and *in situ* antifungal activities of the SEO, three *Penicillium* (*P.*) strains (*P. crustosum*, *P. citrinum*, and *P. expansum*) isolated from *Vitis vinifera* berries were employed.

The strains were classified using a reference-based MALDI-TOF MS Biotyper followed by comparison with the taxonomic identification obtained by 16S rDNA sequences analysis.

2.3 Chemical characterization of SEO

Gas chromatography/mass spectrometry (GC/MS) analysis of the SEO was performed using an Agilent 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to a quadrupole mass spectrometer 5975B (Agilent Technologies, Santa Clara, CA, USA) according to the methodology described by Valková et al. (2021a). The individual volatile constituents of the injected EO samples were identified based on their retention indices (Adams, 2007), and a comparison with reference spectra (Wiley and NIST databases). The retention indices were experimentally determined using the standard method which included retention times of n-alkanes (C6–C34), injected under the same chromatographic conditions. The percentages of the identified compounds (amounts higher than 0.1%) were derived from their GC peak areas.

2.4 Antioxidant activity of SEO

Antioxidant activity (AA) of the SEO was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay as it was carried out in our previous experiments (Galovičová et al., 2021a; Valková et al., 2021a; Valková et al., 2021b; Kačániová et al., 2021b; Kačániová et al., 2022b). The AA was expressed as the percentage of DPPH inhibition, and calculated according to the formula: (A0 – A1)/A0 × 100; where A0 and A1 were absorbances of the DPPH and the samples, respectively. The power of AA was assessed based on the following scheme: weak (0 – 29%) < medium-strong (30 – 59%) < strong (60% and more). Moreover, the value for total AA was expressed as Trolox equivalent antioxidant capacity (TEAC) according to the calibration curve as 1 μg of standard reference Trolox to 1 mL of the SEO sample.

2.5 *In vitro* antifungal activity of SEO

In vitro antifungal activity of the SEO was performed using the agar disc diffusion method (Valková et al., 2021a; Valková et al., 2021b; Valková et al., 2022b). To prepare fungal media, the strains were inoculated in Sabouraud Dextrose Agar (SDA; Oxoid, Basingstoke, UK) and incubated for 5 days at 25 °C. Subsequently, small aliquots of the fungi were transferred to test tubes, each containing 3 mL of distilled water, and the inoculum concentration was standardized by comparison with the 0.5 McFarland scale (1.5×10^8 CFU/mL). For the analysis, an aliquot of 100 μL of the culture media was firstly inoculated on the SDA, and the discs of filter paper (6 mm) impregnated with 10 μL of the SEO sample (in four concentrations: 62.5, 125, 250, and 500 μL/L; diluted in 0.1% dimethyl sulfoxide, DMSO) were applied on the SDA surfaces. Fungi were consequently incubated aerobically at 25 ± 1 °C for 5 days. After the incubation, diameters of the inhibition zones were measured in mm. The values for inhibitory activity were expressed in the following manner: weak antifungal activity (5 - 10 mm) < moderate antifungal activity (10 - 15 mm) < very strong antifungal activity (zone > 15 mm).

2.6 Moisture content and water activity of potato food model

Potato slices as a substrate for fungal growth were applied. The vegetable was purchased at the local market (Nitra, Slovakia). Its moisture content (MC) and water activity (a_w) were measured using the Lab Master a_w Standard (Novasina AG; Lachen, Switzerland) and the Kern DBS 60-3 moisture analyzer (Kern and Sohn GmbH, Balingen, Germany), respectively (Kačániová et al., 2020a; 2020b).

2.7 *In situ* antifungal analysis

To assess *in situ* antifungal activity of the SEO, the vapor phase (contact) method was employed (Valková et al., 2022a; 2022b). Firstly, sliced potato (5 mm) was placed on the bottom of Petri dishes, and the inoculum was applied by stabbing one time with an injection pin on the vegetable surface. Next, 10 μL of the SEO in the same four concentrations was applied on the sterile filter paper disc (60 mm) which was consequently placed at the top of Petri dishes. The dishes were hermetically closed by parafilm and cultivated at 25 °C for 14 days.
After the cultivation, the size of the fungal colonies with visible mycelial growth and visible sporulation (Kačániová et al., 2020a; 2020b) was evaluated using stereological methods. In this concept, the volume density of the colonies was firstly assessed using ImageJ software (National Institutes of Health, Bethesda, MD, USA), counting the points of the stereological grid hitting the colonies and those falling to the reference space (growth substrate used, potato). The antifungal activity of the SEO was expressed as the percentage of mycelial growth inhibition (MGI), which was calculated using the formula: $MGI = [(C - T)/C] \times 100$ (Sempere-Ferre et al., 2021), where C and T were volume fractions of fungal colonies in the control (untreated) and treated samples, respectively.

2.8 Statistical analysis

Statistical analysis of obtained data was performed using Prism 8.0.1 (GraphPad Software, San Diego, California, USA). To assess significant differences between the analyzed groups of samples, one-way analysis of variance (ANOVA) followed by Tukey's test were employed. A value for $P < 0.05$ denoted the level of statistical significance. All analyses were performed in triplicate.

3. Results

Using GC/MS analysis, a total of 35 organic components were identified in our SEO, completely accounting for 99.7% of the EO chemical composition (Table 1). Among them, carvone (57.5%) and α-limonene (17.6%) were the most prominent, followed by dihydrocarveol (4.3%), cis-dihydrocarvone (4.1%), and dihydrocarveol acetate (3.8%). Hence, the SEO investigated can be ascribed to carvone/limonene chemotype.

Table 1. Chemical composition of essential oil obtained from *Mentha spicata* var. *crispa*

No	RI [a]	Compound [b]	% [c]
1	938	α-pinene	0.6
2	977	sabinene	0.1
3	980	β-pinene	1.0
4	992	β-myrcene	0.4
5	993	3-octanol	0.2
6	1023	*p*-cimene	0.9
7	1028	α-limonene	17.6
8	1033	1,8-cineole	0.8
9	1060	γ-terpinene	tr
10	1068	cis-sabinene hydrate	tr
11	1148	isopulegol	tr
12	1151	menthone	1.1
13	1162	iso-menthone	0.4
14	1164	neo-menthol	0.3
15	1173	menthol	1.8

16	1178	4-terpinenol	tr
17	1192	cis-dihydrocarvone	4.1
18	1199	dihydrocarveol	4.3
19	1200	trans-dihydrocarvone	0.7
20	1217	trans-carveol	0.7
21	1229	cis-carveol	1.0
22	1241	carvone	57.5
26	1253	3-carvomenthenone	tr
27	1254	(*Z*)-anethole	tr
28	1297	menthyl acetate	0.9
29	1306	iso-menthyl acetate	0.5
30	1311	dihydrocarveol acetate	3.8
31	1379	α-copaene	tr
32	1385	β-bourbonene	0.2
33	1388	β-elemene	tr
34	1422	(*E*)-caryophyllene	0.6
35	1583	caryophyllene oxide	0.2
total		**99.7**	

[a] Values of retention indices on HP-5MS column; [b] Identified compounds; [c] tr - compounds identified in amounts less than 0.1 %

In spite of only a weak (20.40 ± 0.80% free radical-scavenging inhibition) antioxidant capacity with a value of 107.66 ± 3.0 μg TEAC.mL^{-1} displayed by the SEO, an *in vitro* antifungal activity of the EO against the growth of *Penicillium* spp. selected (Table 2) was observed. In effect, the disc diffusion method revealed a moderate inhibitory efficiency of the highest SEO concentration (500 μL/L) against the growth of *P. expansum* and *P. crustosum*, with inhibition zones of 11.46 ± 0.63 mm and 12.93 ± 0.46 mm, respectively. Against *P. citrinum* mycelial growth, the highest concentration of the EO induced only a weak inhibitory action, which was also noticed for 250 μL/L of the EO against all three fungal species.

Table 2. *In vitro* antifungal activity of spearmint essential oil expressed as the diameter of the inhibition zone (in mm)

Fungi	SEO (μL/L)			
	62.5	125	250	500
P. expansum	1.73 ± 0.55 [a]	4.55 ± 1.03 [b]	9.05 ± 0.83 [c]	11.46 ± 0.63 [d]
P. crustosum	2.76 ± 0.57 [a]	5.14 ± 0.74 [b]	8.36 ± 1.09 [c]	12.93 ± 0.46 [d]
P. citrinum	2.88 ± 0.36 [a]	4.86 ± 0.77 [b]	6.56 ± 0.34 [c]	9.47 ± 0.59 [d]

Note: Mean ± standard deviation. SEO: spearmint essential oil. Values in the same line with different superscripts are significantly different ($P < 0.05$).

In addition, all concentration of the SEO was able to considerably ($P < 0.05$) inhibit the mycelial growth of *P. expansum*, *P. crustosum*, and *P. citrinum* inoculated on potato slices as a food model substrate. The data from *in situ* analysis is summarized in Table 3. Moreover, there was a more pronounced inhibitory action of higher concentrations of SEO (≥ 125 μL/L; ≥ 250 μL/L) on the growth of *P. expansum* and *P. citrinum*, respectively, as compared to the lower concentrations. On the other hand, *P. crustosum* was equally sensitive to all the EO concentrations. Moisture content and a_w of the potato substrate were estimated to be 75.18 ±

1.23% and 0.969 ± 0.002, respectively; the data of the physical parameters demonstrates the suitability of the food model for microbial (fungal) spoilage.

Table 3. *In situ* antifungal activity of spearmint essential oil expressed as mycelial growth inhibition

Fungi	MGI (%)			
	SEO (μL/L)			
	62.5	125	250	500
P. expansum	55.07 ± 5.15 [a]	91.35 ± 9.39 [b]	100.00 ± 0.00 [b]	91.67 ± 9.24 [b]
P. crustosum	100.00 ± 0.00 [a]	93.30 ± 10.17 [a]	97.03 ± 5.84 [a]	95.12 ± 5.99 [a]
P. citrinum	78.43 ± 3.69 [a]	92.80 ± 12.92 [ab]	95.96 ± 7.58 [b]	96.74 ± 9.15 [b]

Note: Mean ± standard deviation. MGI: mycelial growth inhibition; SEO: spearmint essential oil. Values in the same line with different superscripts are significantly different ($P < 0.05$).

4. Discussion and Conclusions

In general, biological activity of EOs being interested in the food and cosmetic industries, as well as in the field of human health is strongly dependent on their chemical composition (Dhifi et al., 2016). Similarly to our results, carvone (65.33%), limonene (18.19%), and dihydrocarvone (2.97%) as the major compounds of Chinese *M. spicata* EO were reported by Liu et al. (2012). Additionally, the carvone/limonene chemotype of SEO has also been identified in the research by Bardaweel et al. (2018) and Snoussi et al. (2015). However, while first authors determined values for carvone (49.5%) and limonene (16.1%) to be close to our ones, the latter authors showed that both compounds participate in different proportions (40.8 ± 1.23% of carvone, 20.8 ± 1.12% of limonene) in the EO chemical profile in comparison with our study. Carvone (56.6%; 41.1%; 78.76%; 62.9%; 62–65%; 41.1%) and limonene (27.3%; 20.1%; 11.50%; 8.5%; 11–13%; 14.1%) as the major constituents of *M. spicata* EO have also been detected by other researchers (Aggarwal et al., 2002; Martins et al., 2012; Shahbazi, 2015; Ounoki et al., 2021; Piras et al., 2021; Giménez-Santamarina et al., 2022), respectively. Generally, the concentrations of monoterpenes (such as carvone) being extracted from the same species is strongly influenced by the plant parts and the method itself selected for such procedure (Bouyahya et al., 2021). Also, already above-mentioned (in the part "Introduction") other factors must be kept in mind, all of them contributing to different findings identified between studies employed for data comparison as it was shown in our report.

The DPPH free radical-scavenging activity is commonly used for measurement of AA of diverse EOs (Bag and Chattopadhyay, 2015; Inaam et al., 2015; Anggraeni et al., 2018; Olmedo et al., 2018; Chambre et al., 2020; Galovičová et al., 2021a; Valková et al., 2021a; Valková et al., 2021b; Kačániová et al., 2022a). According to many authors (Amiri, 2012; Bag and Chattopadhyay, 2015), the DPPH has been largely used as an easy, quick, reliable, and reproducible assay for screening *in vitro* antioxidant activity of EOs or plant extracts. Using the method, we have found that the SEO was able to scavenge the radical; however, only to a lesser extent indicating its weak antioxidant capacity. The same finding was also displayed by EO from *M. spicata* growing in Portugal (Martins et al., 2012) and Poland (Grzeszczuk and Jadczak, 2009). By contrast, moderate inhibition of DPPH radicals (54.68%) of *M. spicata* EO from Oman have been observed by Alsaraf et al. (2021). Furthermore, Ahmad et al. (2012) have detected even higher values for AA (61-71%) of *M. spicata* methanolic extract. As compared to our previous study, the EO from *M. spicata* exhibited lower AA than that from *M. piperita* (36.85 ± 0.49%) recognizing menthol, menthone, and menthyl acetate to be the major

constituents (Valková et al., 2021a). The same fact was also reported by other researchers (Dorman et al., 2003; Nikavar et al., 2008).
The characterization of our EO from *M. spicata* from both *in vitro* and *in situ* antifungal activities has shown that the SEO is an effective inhibitor of the *Penicillium* spp. growth. Similarly to our study, low to moderate antimicrobial activity of SEO against pathogenic microorganisms including Gram positive, Gram negative bacteria, and fungi was also reported by Bardaweel et al. (2018). Spearmint EO has also been found to be active against *P. citrinum* (Liu et al., 2012), *Candida albicans*, *Aspergillus niger*, *Fusarium oxysporum* (Martins et al., 2012), and *Vibrio* spp. strains (Snoussi et al., 2015). Aggarwal et al. (2002) have tested the effect of SEO, as well as their main isolated components, carvone and limonene, on a wide spectrum of human pathogenic fungi and bacteria revealing their high *in vitro* bioactivity. The use of carvone as an antifungal agent against various fungal strains is suggested by many reports (Morcia et al., 2012; Boni et al., 2016; Hassan et al., 2017; Moro et al., 2017). From this aspect it can be assumed that the low to moderate antifungal activity of our SEO demonstrated by the disc diffusion method can be attributed mainly to the carvone abundance in its chemical composition.
Food spoilage is a very common phenomena in which genus *Penicillium* plays an important concern because of its ubiquity and mycotoxin production (Pitt, 2014). In the current study, potato slices as a food model substrate for *Penicillium* spp. growth was employed. In effect, its values for MC and a_w being around 80% and higher than 0.60, respectively, indicate its suitability for the *in situ* antifungal activity analysis of diverse EOs (Valková et al., 2022b). Moreover, our data of both physical parameters have been found to be in accordance with the study of the mentioned authors (Valková et al., 2022b). A strong antifungal potential of our SEO against the mycelial growth of all three *Penicillium* species could be explained by the presence of oxygenated monoterpenes, such as carvone that has emerged as a promising antifungal compound (Bouyahya et al., 2021) due to its disruptive impact on the cell membrane and fungal mitochondria (Zhang et al., 2022). Also, limonene (another major component of SEO) is able to inhibit the growth of *C. albicans* by generation of oxidative stress in the cell envelope and induction of oxidative DNA damage, leading to cell-cycle modulation and apoptosis (Sales et al., 2022). Thus, the *in situ* antifungal activity of SEO demonstrated in our study can be associated mainly with the two major compounds and their mutual interactions. However, the biological activities of EOs as a multicomponent mixture cannot be easily ascribed to only one or two specific components but they are rather a result of additive, synergistic or antagonistic actions of different constituents present in their chemical profile (Bardaweel et al., 2018).
Anyway, the findings from all our analyses suggest the SEO to be a promising natural agent for extending the shelf life of vegetables (including potato) which can be a very helpful aspect for the food sector in terms of active food packaging.

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Spectroscopic Investigations (IR and NMR) by DFT method of 3,3'-(2,5- Dimethoxybenzylidene)-bis-[4-hydroxycoumarin]

Hacer Gümüş*

Abstract: In this study, in order to better understand the molecular definition of the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule, the physicochemical properties were examined in detail by theoretical methods. The ground level geometries of all molecules were obtained by using the 6-311G(d,p) basis set with the help of DFT (B3LYP) methods in Gaussian 09 program and were compared with the data of this molecule, which were illuminated by the X-ray diffraction method in the literature. As a result of the calculations, FT-IR spectra were recorded and vibration properties were examined. In addition, the ^{1}H and ^{13}C nuclear magnetic resonance (NMR) chemical shifts values, Mulliken charge analysis, potential energy surface, electronic properties, molecular orbital energies were investigated and electronegativity, molecular hardness and softness parameters were obtained from these energies.

Keywords: DFT, IR, NMR and MEP

1. Introduction

Experimental studies prove that synthetic and natural coumarins derivatives are endowed with good chemical reactivity and different bioactivity. Therefore, natural coumarins and their derivatives play an important role in plant physiology and biochemistry. The natural coumarins and their derivatives behave as antioxydants, enzyme inhibitors and precursors of toxic substances. They are also involved in the actions of plant growth hormones and growth regulators, the control over the respiration and photosynthesis, as well as in the defense against various infections (Borges, F., (2005)).

Synthetic coumarin derivatives have been obtained by chemical modification of the coumarin ring. As a substitution can conceptually occur at any of the six available sites of the basic molecule, these compounds are widely variable in structure and activity. The biological activities of coumarin derivatives, in particular their therapeutic application as anticoagulant and antibacterial agents (Chiang, C.C., (2008)), has stimulated further interest for the synthesis of this class of compounds (Ćavar, S., (2009)).

A variety of synthesized coumarin derivatives have been experimentally shown to exert pharmacological activities including anti-proliferative (Jung, J.C., (2009)), antifungal, (Kostova, (2007)) anti-psoriasis, antiinflammatory, as well as antiviral activities (Symeonidis, T., (2009)).

Kocaeli University, Golcuk Vocational School, Automotive Technology Program, Kocaeli, Turkey
* Corresponding author: hacer.gumus@kocaeli.edu.tr

In previous publication, of 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule was synthesized and characterized with X-Ray diffraction method and its structure was elucidated with ^{1}H and ^{13}C and NMR spectra (in the DMSO-d6 solution), and IR spectra by Davorka Završnik et al. (Završnik, D., (2011)). To the best of our knowledge, the theoretical calculations of molecular geometry, vibrational modes, molecular frontier orbital energy and electronic properties of the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule have been not investigated yet. The aim of the present work is to describe and characterize the molecular structure, vibrational frequencies, ^{1}H and ^{13}C NMR chemical shifts, the total energy, molecular frontier orbital energies (HOMO and LUMO), electronegativity (χ), hardness (η), softness (S), electronic properties, Mulliken charges analysis, molecular electrostatic potential (MEP) maps of the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule.

2. Material and Method

2.1. Computational Details

The theoretical modeling was calculated for the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule by Gaussian 09 software in the ground state. The molecular structure of the optimized molecule was drawn by Gaussian View 5 program. All theoretical calculations of the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule performed with B3LYP/6-311G(d,p).

3. Results

3.1 Geometric structure

The experimental (Završnik, D., 2011)) and obtimized structure of the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule was shown in Figure 1.

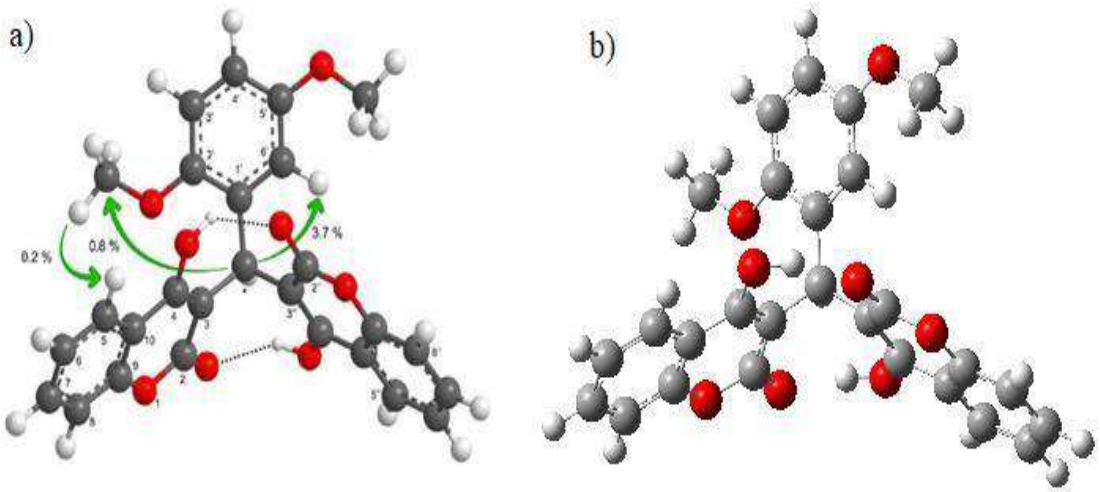

Figure 1. The a) experimental b) optimized structure of the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule.

The optimized (with B3LYP/6-311G(d,p)) geometrical parameters (bond distances and bond angles) were presented in Table 1 comparing with the experimental X-ray diffraction.

Table 1 Selected theoretical and experimental geometrical parameters

Parameters	X-Ray	DFT/B3LYP
Bond Lenght (Å)		
C_4-C_{10}	1.47082	1.45154
C_3-C_4	1.33508	1.37381
C_5-C_{10}	1.37807	1.40556
O_1-C_2	1.34557	1.37033
C_2-C_3	1.45125	1.44282
C_8-C_7	1.35041	1.38773
C_{10}-C_9	1.36542	1.39907
C_7-C_6	1.40660	1.40160
C_9-C_8	1.37677	1.39353
C_6-C_5	1.40437	1.38527
C_2''- C_3''	1.42824	1.44074
C_1'- C_2'	1.38504	1.40866
C_4'- C_5'	1.35959	1.39185
C_2'- C_3'	1.38100	1.39616
C_5'- C_6'	1.36939	1.39752
C_3'- C_4'	1.37617	1.38958
C_6'- C_1'	1.38859	1.39659
Bond Angles ( )°		
C- C_3''- C_2''	117.54075	119.97285
O_1-C_2-C_3	119.31544	119.20880
C_4-C_{10}-C_5	123.64224	123.28118
C_2-C_3-C_4	119.45233	119.50747
C_3-C_4-C_{10}	120.30707	119.81613
C_5'-C_6'- C_1'	120.97280	121.51073
C_3-C-C_3''	112.52285	112.18003
C_4-C_{10}-C_9	117.46040	118.08555
C_8-C_7-C_6	121.11161	120.57683
C_{10}-C_9-O_1	121.58466	121.18839
C_7-C_6-C_5	118.82608	120.10435
C_9-O_1-C_2	121.07733	121.69766
C_3'-C_4'- C_5'	119.30764	119.83840
C_6-C_5-C_{10}	119.66419	120.24086
C_{10}-C_9-C_8	123.04578	121.56124
C_9-C_8-C_7	118.44013	118.88180
C_1'-C_2'- C_3'	121.34067	120.28304
C_4'-C_5'- C_6'	121.13338	119.55118
C_6'-C_1'- C_2'	117.33317	118.16557
C_2'-C_3'- C_4'	119.88476	120.59934

Obtained geometric data listed in Table 1. In the study, Theoretical bond lengths (for C-C) were found in the range 1.36610-1.45154 Å at B3LYP/6-311G(d,p). Experimental bond lengths (C-C) are seen in the range of 1.33508 and 1.45125 Å. Experimental O_1-C_2 bond lengths are 1.34557 Å. Calculated bond lengths were observed as 1.37033 Å for the B3LYP/6-311G(d,p). The experimental C_9-O_1-C_2 bond angle is 121.07733˚, and this angle has been seen at 121.69766˚ for the B3LYP/6-311G(d,p).

3.2 Vibration (IR) analyses

The vibrational frequencies were calculated and fundamental vibrations were assigned based on the scaled theoretical wavenumbers. The bands calculated in the measured region 4000-400 cm^{-1} arise from the vibrations of O-H, C-H stretching, the internal vibrations and the vibrations of the lattice, etc. of the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule and listed in Table 2.

Table 2. Comparison of the observed and calculated vibrational wavenumbers and assignments for the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule.

Assigments	Exp. (cm^{-1})	Calculated
CH	2960	2999
C=C–C=O	1656	1688
C=C	1602	1625
C=C	1568	1589
C=C	1500	1534
C=C	1452	1473
OCH3	1416	1446
OH	1186	1204
CO	1154	1171

Vibrational modes: ν, stretching; a, assymmetric; s, symmetric; ρ, rocking; δ, scissoring; w, wagging; t, twisting; τ, torsion.

In the present study, in FT-IR spectrum symmetric vibration C-H stretching was assigned at 2960 cm^{-1}. The OCH3 deformation modes of the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule observed at 1416 cm^{-1} in FT-IR.

3.3 NMR spectra analyses

In this study, ^{1}H and ^{13}C NMR chemical shifts are calculated within the gage-including atomic orbital (GIAO) B3LYP method with 6-311G(d,p) basis set. A comparison of the experimental and theoretical spectra can be very useful in making correct assignments and understanding the basic chemical shift molecular structure relationship (as can see Table 3).

Table 3. The calculated and experimental ^{13}C and ^{1}H isotropic NMR chemical shifts (with respect to TMS, all values in ppm) for the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule.

^{1}H			**^{13}C**		
$H_{C5''}$	7.90	8.22	$C_{4''}$	164.03	171.98
H_{C5}	7.90	8.04	C_{4}	164.03	171.10
$H_{C7''}$	7.57	7.60	$C_{2''}$	163.96	170.84
H_{C7}	7.57	7.53	C_{2}	163.96	167.55
$H_{C8''}$	7.34	7.42	$C_{5'}$	152.79	158.92
H_{C8}	7.30	7.38	$C_{9''}$	152.03	158.59
$H_{C6''}$	7.31	7.37	C_{9}	152.03	158.31
H_{C6}	7.31	7.28	$C_{2'}$	151.63	156.59
$H_{C3'}$	6.81	6.85	$C_{7''}$	131.57	130.75
$H_{C4'}$	6.73	6.71	C_{7}	131.57	129.34
$H_{C6'}$	6.71	6.43	$C_{1'}$	130.23	128.78
H_{C*}	6.22	5.65	$C_{6''}$	123.63	127.37
$H_{C5'-OCH3}$	3.62	3.81	C_{6}	123.63	127.03
$H_{C5'-OCH3}$	3.62	3.65	$C_{5''}$	123.61	122.12
$H_{C5'-OCH3}$	3.62	3.60	C_{5}	123.61	121.37
$H_{C2'-OCH3}$	3.51	3.29	$C_{10''}$	117.77	119.64
$H_{C2'-OCH3}$	3.51	3.29	C_{10}	117.77	119.58
$H_{C2'-OCH3}$	3.51	3.17	$C_{8''}$	115.96	119.54
			C_{8}	115.90	113.23
			$C_{3'}$	111.84	113.23
			$C_{4'}$	110.03	111.79
			C_{3}	104.63	109.68
			$C_{5'-OCH3}$	56.13	54.07
			$C_{2'-OCH3}$	55.13	53.84
			C_{*}	33.02	37.64

3.4 Electronic properties

The electronic absorption basically means the transition from the ground to the first excited state and is mainly described by one electron transitions from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). In other words, HOMO is the orbital that acts as an electron donor, whereas LUMO is an orbital that acts as electron acceptor. The molecular orbital energies of 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule were calculated at the B3LYP/6-311G(d,p) level and are presented in Figure 2.

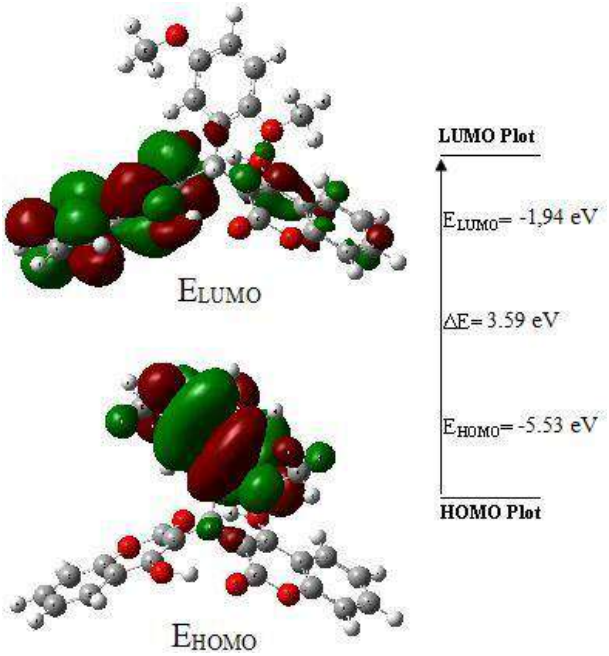

Figure 2. Molecular frontier orbital pictures and energies obtained at B3LYP/6-311G(d,p) level for the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule.

The total energy, HOMO (π donor) and LUMO (π acceptor) energies, the energy gap (ΔE), the ionization potential (I), the electron affinity (A), the absolute electronegativity (χ), the absolute hardness (η) and softness (S) for the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule has been calculated at B3LYP/6-311G(d,p) and levels and the results are given in Table 5.

Table 4. The calculated electronic parameters of the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule.

Parameters	**B3LYP /6-311G(d,p)**
E_{HOMO} (eV)	-5.53239
E_{LUMO} (eV)	-1.94264
$\Delta E = E_{LUMO}-E_{HOMO}$ (eV)	3.58975
I (eV)	5.53239
A (eV)	1.94264
χ (eV)	3.737515
η (eV)	1.794875
S (eV^{-1})	0.090377
E_{TOTAL} (Hartree)	-1643.1722

The calculated HOMO and LUMO energies shows that charge transfer occurs within molecule. HOMO and LUMO energies were calculated as -5.53 and -1.94 eV at B3LYP/6-311G(d,p) respectively. The energy gap between the HOMO and LUMO orbital was predicted as 3.59 eV at the B3LYP/6-311G(d,p) level, and this energy gap showed that the intramolecular charge transfer occur in 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule. It is well known that the more HOMO-LUMO energy gap is low, the more charge transportation is prospective.

3.5 Analysis of molecular electrostatic potential (MEP) surfaces

The molecular electrostatic potential (MEP) has been used as a useful method in research of molecular structure with its physicochemical property relationship. The calculated 3D MEP of was calculated from optimized molecular structure by using B3LYP/6-311G(d,p) level and are presented in Figure 3. According to the results, the color scheme for the MEP surface is red electron rich or partially negative charge; blue-electron deficient or partially positive charge; light blue-slightly electron deficient region; yellow slightly electron rich region, respectively. Areas of low potential, red, are characterized by an abundance of electrons. Areas of high potential, blue are characterized by a relative absence of electrons.

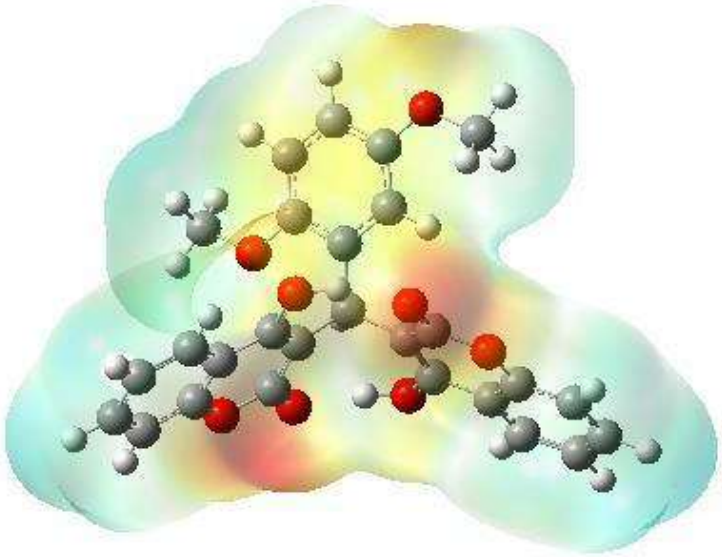

Figure 3. Molecular surfaces obtained using B3LYP/6-311G(d,p) level of the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule.

3.6 Mulliken and APT charge analysis

Mulliken population analysis method and the density matrix based normal population analysis. The Mulliken population analysis is one of the oldest and simplest, with the electrons being divided up amongst the atoms according to the degree to which different atomic AO basis functions contribute to the overall wave function. The Mulliken charge on each atom of the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule is presented in the graphical representation is shown in Figure 4.

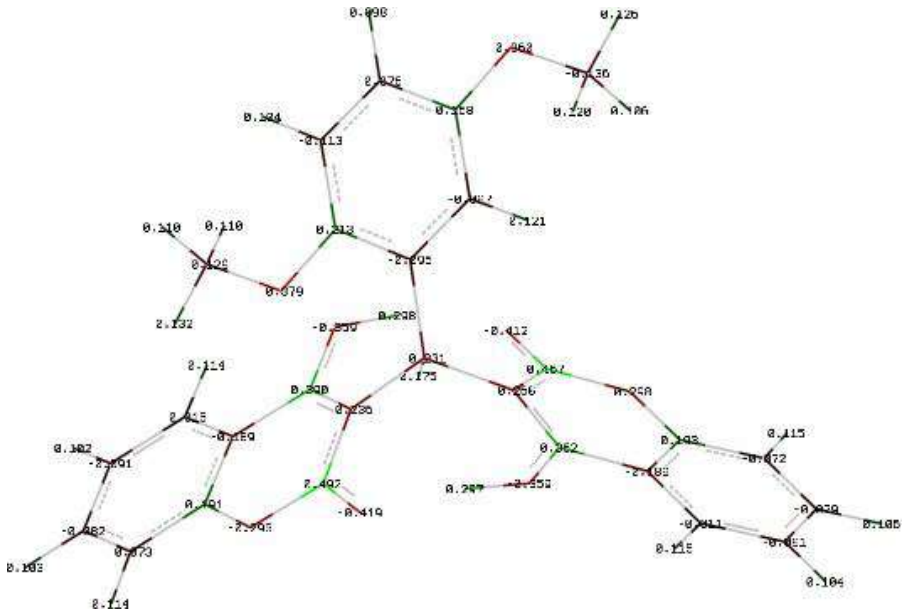

Figure 4. Mulliken charge of the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule

Calculated Mulliken and APT atomic charges analysis results are shown in Figure 5.

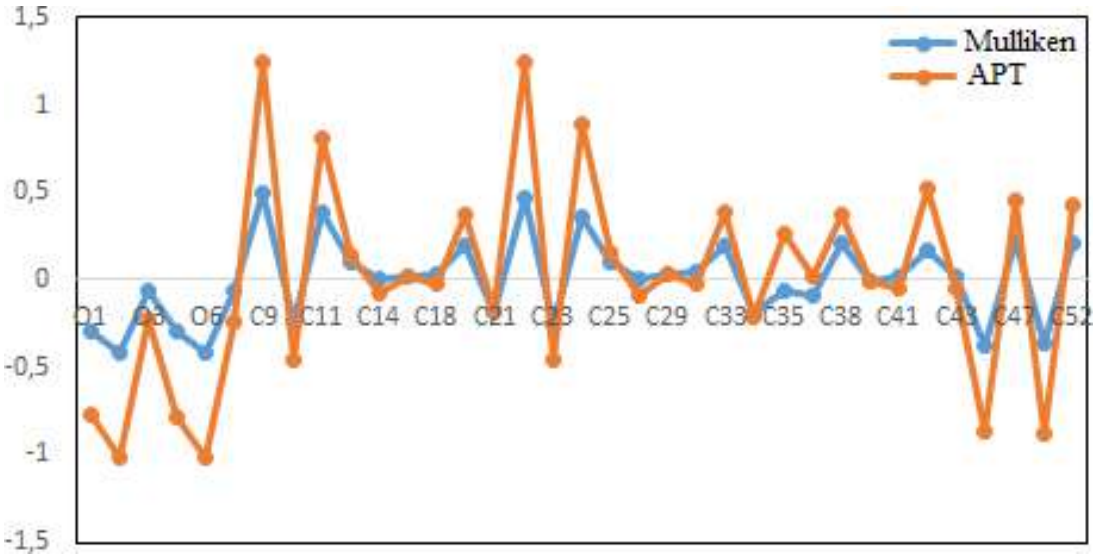

Figure 5. Comparative of Mulliken and APT plots of the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule.

The atomic charges of the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule acquired by Mulliken analysis with B3LYP method with 6-311G(d,p) basis set are listed in Table 5. As can be seen from the Table 5, the magnitudes of the carbon Mulliken charges, found to be either positive or negative, were noted to change from 0.49 to -0.42 at B3LYP/6-311G(d,p) levels. The whole protons have a positive charge while the oxygen atoms have negative charges. The charges of the carbon atoms were found to be either positive or negative.

4. Discussion and Conclusions

In this study, we tried to clarify the characterization of the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule by means of computational methods. The calculated structural parameters (bond distances, bond angles and dihedral angles) were optimized in different methods. All compared data are shown to have in a good agreement with each other. This good agreement is well within the accuracy of computational results. The IR and NMR analysis were calculated and the assignments made were compared with the experimental values. Electronic and electric properties of the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule were investigated and interpreted. Molecular electrostatic potential surface (MEPs) and atomic charges were also determined for the identification of the 3,3'-(2,5-Dimethoxybenzylidene)-bis-[4-hydroxycoumarin] molecule. In conclusion, all the calculated data and simulations not only show the way to the characterization of the molecule but also help for the fundamental researches in chemistry and biology in the future.

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