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This issue includes the abstracts of the 6th edition of The Young Researchers in Bio-Sciences (YRBS) International Symposium, organized by: Students for Nature Organization (OSpN), The Institute for Science and Innovation in Society (ISIS), and The Faculty of Biology and Geology, Babeş-Bolyai University, Cluj-Napoca, Romania, between 22 and 25 July, 2021, in Cluj-Napoca, Romania.

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YEAR **Volume 66 (LXVI) 2021**
MONTH **JUNE**
ISSUE **1**

PUBLISHED ONLINE: 2021-06-30
PUBLISHED PRINT: 2021-06-30
ISSUE DOI:10.24193/subbbiol.2021.1

STUDIA UNIVERSITATIS BABEŞ-BOLYAI

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Original picture on front cover: The Young Researchers in Bio-Sciences (YRBS) International Symposium logo

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 9-10)

Influence of PDMS microtopographies on cells morphology

Simona Stroescu (Nistorescu)[1,2], Cristina Staicu[1,2], Anca Bonciu[2], Felix Sima[2], Anişoara Cîmpean[1], Valentina Dincă[2]✉

[1]University of Bucharest, Faculty of Biology, Bucharest, Romania;
[2]National Institute of Laser, Plasma and Radiation Physics, Măgurele, Ilfov, Romania;
✉***Corresponding author, E-mail: valentina.dinca@inflpr.ro.***

Abstract

The cellular adhesion and morphology influence direct cell development in the tissue engineering mechanisms (Hashemzadeh *et al*., 2020; Yeh *et al*., 2017). Our work aims at knowing the impact of substrate properties by manufacturing biocompatible microstructured in polydimethylsiloxane (PDMS). Using various roughness and topography (lines, points, or unpatterned), we systematically analyze in vitro response of the cells. Initially, we characterized the physical characteristics of the PDMS samples such as contact angle, surface free energy, roughness, and SEM images. Besides, we show how plasma can turn the PDMS surface to hydrophilic after only 5 minutes exposure. Furthermore, bioassays were performed to investigate the viability, adhesion, and morphology of the cells. In vitro biocompatibility was evaluated by visualizing the actin filaments that provide mechanical support, determine cell shape, and allow movement of the cell surface, thereby enabling cells to migrate. The wettability property has been determined to be improved for plasma-treated PDMS. Morphological observations revealed good biocompatibility with the PDMS samples, cell cytoskeleton is not affected by the substrates. Finally, we showed a correlation between roughness, surface free energy, and cell adhesion. Our results suggest that PDMS microtopographies have a very high potential for cell behavior studies and future therapies.

Keywords: cells morphology, microtopographies, PDMS, wettability.

Acknowledgements. This work was supported by PN-III-P4-ID-PCE2020-2375.

References

Hashemzadeh, H., Allahverdi, A., Sedghi M., Vaezi, Z., Tohidi, Moghadam, T., Rothbauer, M., Fischer, M.B., Eartl, P., & Naderi Manesh, H. (2020). PDMS nano-modified scaffolds for improvement of stem cells proliferation and differentiation in microfluidic platform. *Nanomaterials* (Basel, Switzerland), **10**(4). https://doi.org/10.3390/nano10040668

Yeh, Y.-C., Corbin, E.A., Caliari, S.R., Ouyang L., Vega S.L., Truitt, R., Han, L., Margulies, K.B., & Burdick, J.A. (2017). Mechanically dynamic PDMS substrates to investigate changing cell environments. *Biomaterials* 145, 23-32. https://doi.org/10.1016/j.biomaterials.2017.08.033

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 11-12)

Low concentration of Zearalenone affects the biochemical and immunological parameters in swine

Valeria Cristina Bulgaru[1]✉, Gina Cecilia Pistol[1], Daniela Eliza Marin[1] and Ionelia Taranu[1]

[1]*INCDBNA-IBNA, Baloteşti, Romania;*
✉***Corresponding author, E-mail:** cristina.bulgaru@ibna.ro.*

Abstract

Zearalenone (ZEA) is a mycotoxin produced by fungi of the genus *Fusarium,* that frequently contaminate wheat, barley and rye crops and affects both human and animal health. Swine is one of the most susceptible species to ZEA intoxication, this fact being due to the high intake of cereals in the diet, but also to a high sensitivity to mycotoxins. The aim of this study was to investigate the effects produced by exposure of weaned piglets to a low dose of ZEA. Some immune parameters as immunoglobulins (IgG, IgA, IgM), nitric oxyde (NO) and also biochemical parameters related to energetic (glucose, cholesterol, triglyceride), proteic (total protein, albumin, ureea, creatinine), mineral (Ca, Fe, Mg, P) profile as well as the activity of some hepatic enzymes (aspartat amino-transferase, alanil-amino-transferase, gamma-glutamil transferase and alcaline phosphatase) were assesed in piglets serum. The *in vivo* experiment was performed on 12 crossbred weaned piglets (TOPIGS-40), randomly assigned to two groups (6 animals/group): control group (C) fed uncontaminated feed and experimental group (E) fed a ZEA contaminated diet (75µg/kg feed) for 21 days. The main immunological change induced by ZEA was the decrease in IgG (P=0.0428) and NO concentration (P=0.0176). The exposure to ZEA significatly decreased the bilirubin concentration (P=0.0077) and tended to decrease the serum glycemia (P=0.07). By contrast ZEA increased the triglycerides concentration (P=0.0004) and gammaGT activity (P=0.0003). In conclusion, ZEA can affect immunological and biochemical parameters in weaned piglets, even in concentrations lower than recommended by European Comission for swine feed.

Keywords: biochemistry, immunology, swine, zearalenone.

Acknowledgements. This work was supported by ADER 9.2.1 financed by Ministry of Agriculture and Rural Development.

References

Bulgaru, C. V., Marin, D. E., Pistol, G. C., & Taranu, I. (2021). Zearalenone and the Immune Response. *Toxins*, *13*(4), 248.

Marin, D. E., Pistol, G. C., Neagoe, I. V., Calin, L., & Taranu, I. (2013). Effects of zearalenone on oxidative stress and inflammation in weanling piglets. *Food and Chemical Toxicology*, *58*, 408-415.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 13-14)

Novel combined therapy based on IL-13-PEG-LCL-SIM and PEG-EV-DOX to reduce murine melanoma aggresiveness *in vivo*

Giorgiana Negrea[1]✉, Valentin-Florian Rauca[1], Laura Pătraş[1], Lavinia Luput[1], Emilia Licărete[1], Vlad-Alexandru Toma[1,2], Bogdan Dume[1], Alina Porfire[3], Manuela Banciu[1] and Alina Sesărman[1]

[1] *Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babeş-Bolyai University, Cluj-Napoca, Romania;* [2] *Department of Experimental Biology and Biochemistry, Institute of Biological Research, branch of NIRDBS Bucharest, Cluj-Napoca, Romania;* [3]*Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania;*
✉***Corresponding author, E-mail:*** ***giorgiana.negrea@ubbcluj.ro.***

Abstract

Melanoma is one of the most aggressive type of cancer worldwide, which rapidly develops resistance to conventional treatments, resulting in metastasis and recurrence. This study aims to test the potential of IL-13-PEG-LCL-SIM and PEG-EVs-DOX to decrease the aggressiveness of B16.F10 murine melanoma by applying an innovative therapy that targets both tumor-associated-macrophages and cancer cells. Melanoma-bearing mice intravenously received the combined therapy, or the individually formulations of SIM or DOX. In order to detect the level of expression of HIF-1α, a key promoter of hypoxia and of Bcl-xL and Bax, apoptotic proteins, western blot analysis was performed. The concentration of malondialdehyde (MDA) in tumor lysates, a marker of oxidative damage, was assessed by HPLC. Our data showed a strong inhibition of tumor development for the group treated with the combined therapy. Also, there was a substantial decrease of expression of HIF-1α due to PEG-EVs-DOX therapy, which was not noticed in the case of combined therapy. However, the concentration of MDA was highly increased, indicating a disruption of intratumor ROS levels, that alters the balance needed for HIF-1α proper activity. Thus, our findings suggest that the combined active targeted therapy which was tested, strongly inhibits tumor growth. Additional studies must be performed to understand the actions of this new active therapy on other processes which maintain the tumor development, such as angiogenesis and inflammation.

Keywords: melanoma**,** targeted therapy, tumor microenvironment

Acknowledgements. This work was supported by UEFISCDI national project, PN-III-P4-ID-PCE-2016-0342, contract no: 91/2017, Project manager: Dr. Manuela Banciu, Babeş-Bolyai University, Cluj-Napoca, Romania.

References

Alupei, M. C., Licarete, E., Banciu, M., *et al*. (2015). Liposomal simvastatin inhibits tumor growth via targeting tumor-associated macrophages-mediated oxidative stress. *Can. Lett*., 356(2), 946–952.

Buranrat B., *et al*. (2017). Simvastatin potentiates doxorubicin activity against MCF7 breast cancer cells, *Onco. Let*. 14: 6243-6250.

Doublier, S., Belisario, D.C., *et al.* (2012). HIF-1 activation induces doxorubicin resistance in MCF7 3-D spheroids, *BMC Can*., 12(1).

Licărete, E., Rauca, V. F., Luput, L., Banciu, M., *et al*. (2020). Overcoming Intrinsic Doxorubicin Resistance in Melanoma by Anti-Angiogenic and Anti-Metastatic Effects of Liposomal Prednisolone Phosphate on Tumor Microenvironment. *International Journal of Molecular Sciences*, 21(8), 2968.

Liu, J., & Wang, Z. *et al.* (2015). Increased Oxidative Stress as a Selective Anticancer Therapy. *Oxid. Med. and Cell. Long*., 2015, 1–12, 2015.

Spampanato, C., De Maria, S., Sarnataro M., *et al.* (2012). Simvastatin inhibits cancer cell growth by inducing apoptosis correlated to activation of Bax and down-regulation of Bcl-2 gene expression. *Int. J. Oncol*. 40:935–941.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 15)

Identifying heavy metal multi-resistant bacteria isolated from the rhizosphere of potential metallophytes

Mihai-Cristian Grimm[1,2]✉, Lucian Barbu-Tudoran[3] and Dorina Podar[1,2]

[1]Department of Molecular Biology and Biotechnology, Babeş-Bolyai University, Cluj-Napoca, Romania; [2]Centre for Systems Biology, Biodiversity and Bioresources (3B), Babeş-Bolyai University, Cluj-Napoca, Romania; [3]National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania;
✉***Corresponding author, E-mail: mihai.grimm@ubbcluj.ro.***

Abstract

Soil heavy metal pollution has attracted considerable attention due to the negative effects on human health and on ecosystems. Decontamination of heavy metal polluted soils by engineering-based remediation technologies is -expensive and invasive as they affect the topsoil and thus the micro- and macrobiota on the site. Bioremediation technologies based on using microbiota able to survive the metal toxicity has been proposed.

The aim of the study was to identify and describe the multi-resistant bacteria present in the rhizosphere of some potential metallophytes from a heavy metal polluted site in Romania. Concentration of heavy metal in soil samples was determined via Vanta pXRF analyzer - (Olympus, - USA). Culturable soil bacteria were isolated by the plate culture method using metal supplemented media. Isolates displaying metal resistance were further identified based on 16S rDNA sequence and assessed for the presence of metal resistance molecular determinants such as merA, merB, czcA, nccA, copA. Cell morphology and heavy metal accumulation was investigated by SEM-EDX.

In this study, 309 bacterial isolates were obtained based on their resistance to different metals. More than 75% of the isolates displayed multi metal resistance. The majority (93%) of the isolates were resistant to Hg^{2+} that was found in very high concentrations in soil. Most of the isolates were identified as part of the genus *Pseudomonas.* These data support the idea that the natural rhizobiota of some plants is already developed to withstand toxic concentrations of heavy metals, making them suitable candidates for new bioremediation technologies.

Keywords: Bioremediation, heavy metals, rhizosphere.

Acknowledgements. This work was supported by CNCS - UEFISCDI PN-III-P2-2.1-PED-2019-5254, contract no. 390PED/2020.

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Knowledge, habits and beliefs regarding usage of antibiotics - comparative study of medical and non-medical students from Nis University, Serbia

Nemanja R. Kutlesic[1]✉ and Aleksandra Jovanovic[2]

[1]University of Camerino, Department for Biosciences and Veterinary Medicine, Camerino MC, Italy; [2]University of Nis, Faculty of Medicine, Nis, Serbia;
✉***Corresponding author, E-mail: nemanja.kutlesic@studenti.unicam.it.***

Abstract

Introduction. Many studies in the developing world have reported a high level of ignorance among youth towards proper usage of antibiotics. This study wanted to examine the knowledge, beliefs and habits of Nis University students regarding this important topic and discover whether an anticipated difference in knowledge, beliefs and habits existed between medical and non-medical students.

Materials and methods. The data was acquired through an online questionnaire which addressed knowledge, beliefs and habits regarding antibiotics. Obtained data was classified into two groups and tested for statistical significance using the Chi-squared test. The study took into account the margin of error for the sample of 5%. The study adhered to principles of the Helsinki declaration.

Results. The research showed that the majority of students were able to correctly identify bacteria as the main target of antibiotics. More students from non-medical faculties thought viral infections can be treated with antibiotics (37.35% vs. 7.45% of medical, SE_D*=0.042, $p<0.05$), and identified incorrectly *Paracetamol* as an antibiotic (42.17% vs. 8.51% of medical, SE_D*=0.043, $p<0.05$).

However, a similar percentage in both groups claimed they interrupted their regimen before the prescribed time and admitted to alcohol usage.

Conclusion. While students of the medical faculty demonstrated much better knowledge and beliefs on antibiotics, their habits were not found to be significantly different. Overall, a large percentage of students from both

* SE_D = Standard error of the difference (between the two groups).

groups uses the medicines as they please. These results are similar to available studies from the developing world. Campaigns are necessary to inform students better on the subject.

Keywords: antibiotics, antibiotic resistance, appropriate use of antibiotics, public health, university students

References:

Grigoryan, L., Burgerhof, J. G. M., Degener, J. E., Deschepper, R., Lundborg, C. S., Monnet, D. L., … & Haaijer-Ruskamp, F. M. (2007). Attitudes, beliefs and knowledge concerning antibiotic use and self-medication: a comparative European study. *Pharmacoepidemiology and Drug Safety*, *16*(11), 1234–1243. doi: 10.1002/pds.1479

Afzal Kahn, A. K., Banu, G., & Reshma, K. K. (2013). Antibiotic Resistance and Usage—A Survey on the Knowledge, Attitude, Perceptions and Practices among the Medical Students of a Southern Indian Teaching Hospital. *Journal Of Clinical And Diagnostic Research, 7*(8), 1613-1616. doi: 10.7860/jcdr/2013/6290.3230

Jairoun, A., Hassan, N., Ali, A., Jairoun, O., Shahwan, M., & Hassali, M. (2019). University students' knowledge, attitudes, and practice regarding antibiotic use and associated factors: a cross-sectional study in the United Arab Emirates. *International Journal of General Medicine*, *Volume 12*, 235–246. doi: 10.2147/ijgm.s200641

Scaioli, G., Gualano, M. R., Gili, R., Masucci, S., Bert, F., & Siliquini, R. (2015). Antibiotic Use: A Cross-Sectional Survey Assessing the Knowledge, Attitudes and Practices amongst Students of a School of Medicine in Italy. *Plos One*, *10*(4). doi: 10.1371/journal.pone.0122476

Wang, X., Peng, D., Wang, W., Xu, Y., Xudong, Z., & Hesketh, T. (2017). Massive misuse of antibiotics by university students in all regions of China: implications for a national policy, *International Journal of Antimicrobial Agents, 50* (3), 441-446. doi: 10.1016/j.ijantimicag.2017.04.009

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Production of polyhydroxybutyrate using renewable carbon sources by the extremely halotolerant bacterium *Halomonas elongata* DSM 2581^T

Andreea-Melisa Tripon[1], Adorján Cristea[1]✉,
Timea Kristea[1], Călin Gabriel Floare[2] and Horia Leonard Banciu[1]

[1] *Babeş-Bolyai University, Faculty of Biology and Geology, Department of Molecular Biology and Biotechnology, Cluj-Napoca, Romania;* [2] *National Institute for Research and Development of Isotopic and Molecular Technologies, Department of Biomolecular and Molecular Physics, Cluj-Napoca, Romania;* ✉***Corresponding author: E-mail:*** ***adorjan.cristea@ubbcluj.ro***.

Abstract

Polyhydroxybutyrate (PHB) is a natural polyester which is produced under nitrogen and/or phosphorous limitation and excess of carbon (C) source. Under these conditions, the C source is converted to PHB and stored as intracellular C and energy reserves. PHB derivatives might be used as alternatives to synthetic plastics due to their biodegradability and biocompatibility. The aim of this study was to assess the ability of the extremely halotolerant bacterium *Halomonas elongata* DSM 2581^T to synthesise PHB from two renewable C sources namely industrial (IM) and commercial molasses (CM). To induce PHB production, a liquid mineral medium with high salinity (8% w/v NaCl) was used. Three C sources where used separately in the experiments (final concentration of C-source 1% w/v) (D-glucose – as control, IM and CM) and 0.1% w/v yeast extract. Batch cultures were incubated at 37^{o}C and 180 rpm. For cell count and PHB visualisation, DAPI and Nile Red staining where employed. ^{1}H-NMR spectroscopy and crotonic acid assay were used for the chemical analysis of the PHB (Cristea *et al*., 2018). The highest PHB yield was obtained after 96 h of growth on D-glucose (2.61 g/L), followed by CM (2.63 g/L after 90 h) and IM (1.07 g/L after 48 h). The weight percentage (wt%) of PHB fraction was 85% when cultivation occurred on CM, 86% on D-glucose and 35% on IM. Our preliminary results have showed that this strain could use renewable C sources (like molasses) to produce PHB that may be further applied in sustainable circular economy strategy.

Keywords: *Halomonas elongata*, high salinity, molasses, polyhydroxybutyrate, renewable source.

Acknowledgements. The current work was supported by grants of the Romanian National Authority for Scientific Research, CNCS–UEFISCDI, project numbers PN-III-P4-ID-PCCF-2016-0016 and PN-III-P4-ID-PCE-2020-1559.

References

Cristea, A., Baricz, A., Leopold, N., Floare, C., Borodi, G., Kacso, I., Tripon, S., Bulzu, P., Andrei, A. -Ș., Cadar, O., Levei, E. A., & Banciu, H. L. (2018). Polyhydroxybutyrate production by an extremely halotolerant *Halomonas elongata* strain isolated from the hypersaline meromictic Fără Fund Lake (Transylvanian Basin, Romania). *J. Appl. Microbiol.* 125**(5)**: 1343-1357.

Evaluation of selenium content in biofortified *Allium* species by chromatographic means

Bogdan M. Boşca[1]✉ and Augustin C. Moţ[1]

[1]*Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University, Cluj-Napoca, Romania;*
✉***Corresponding author, E-mail:*** ***bogdan.bosca@stud.ubbcluj.ro.***

Abstract

Selenium is an essential trace element in animals and humans. It has chemical properties similar to sulfur. In plants, selenium is involved in several important biochemical routes, including antioxidative processes (Germ *et al*., 2007). In animals and humans, compared to other micronutrients, there is a much thinner line between the normal concentration and the toxic amount of selenium. The inorganic forms of selenium (selenate, selenite) present a much higher toxicity than its organic combinations (e. g. selenocysteine, selenomethionine) (Herrero Latorre *et al*., 2013). Thus, a sensitive method is required for detection of selenium species in biological samples. A distinct method is represented by the formation of piazselenol. This kind of complexes represent the product of reaction between an aromatic *o*-diamine and Se(IV) species, in acidic conditions. This study describes an HPLC-based optimized, sensitive and selective method for determination of selenium in biological samples. The analyzed *Allium* species were grown in our laboratory in a phytochamber. Before the analysis procedures, the biofortified *Allium* samples have been mineralized and pre-concentrated using solid-phase microextraction technique. This analytical step affords an excellent precision and accuracy as it removes the interferences form a typical biological sample, thus allowing a limit of detection as low as 0.5 ng/mL Se.

Keywords: *Allium,* biofortification, piazselenol, selenium determination

Acknowledgements. This work has been financially supported by PN-III-P1-1.1-TE-2019-1396.

References

Germ, M., Stibilj, V., & Kreft, I. (2007). Metabolic importance of Selenium for plants. *Eur J Plant Sci Biotechnol* **1** (1):91-97.

Herrero Latorre, C., Barciela García, J., García Martín, S., & Peña Crecente, R.M. (2013). Solid phase extraction for the speciation and preconcentration of inorganic selenium in water samples: A review. *Anal Chim Acta* **804**:37-49.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 21-22)

The influence of abiotic factors on phosphate solubilizing bacteria

Loredana-Elena Mantea[1]✉ and Marius Ştefan[1]

[1]*Alexandru Ioan Cuza University, Faculty of Biology, Biology Department, Iaşi, Romania;*
✉***Corresponding author, E-mail:*** ***mantealoredana9@gmail.com.***

Abstract

Phosphorus is one of the most important macronutrients needed for optimal plant growth and development. Although P compounds are relatively abundant in agricultural soils, the concentration of soluble phosphorus accessible to plants is very low. The use of phosphate solubilizing bacteria as biofertilizers is a sustainable alternative for improving agricultural productivity globally, but the efficiency of these bacteria can be affected by some abiotic factors, such as: pH, temperature and salinity. In this context, the purpose of this study was to identify bacterial strains that have the ability to solubilize tricalcium phosphate ($Ca_3(PO_4)_2$) under abiotic stress. Bacterial strains were isolated from the rhizosphere of maize using the serial dilution method. The bacterial isolates were further analyzed for their ability to solubilize tricalcium phosphate in Pikovskaya liquid medium. Ten out of fifteen isolated bacterial strains solubilize $Ca_3(PO_4)_2$ in quantitative assay and the amount of phosphorus varied between 9.82 and 17.06 µg P/ml. The strain that solubilized the highest amount of $Ca_3(PO_4)_2$ was further subjected to abiotic stress (pH and temperature). When the bacterial strain P2.1S grew in medium with an acidic pH (4.9) it solubilized a lower amount of $Ca_3(PO_4)_2$ compared to that solubilized at pH 7.2, respectively pH 9. The highest amount of phosphorus solubilized by the P2.1S strain was recorded at 28°C. In conclusion, the bacterial strain P2.1S solubilizes $Ca_3(PO_4)_2$ regardless of pH and temperature values tested, but more studies are needed before this bacterial strain can be used in agriculture.

Keywords: bacteria, pH, temperature, tricalcium phosphate.

Acknowledgements. This work was supported by Microbiology Laboratory, Faculty of Biology, Alexandru Ioan Cuza University of Iaşi.

References

Alori, E., Glick, B., & Babalola, O. (2017). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in Microbiology* **8**(971):1-8.

Dandessa, C., & Bacha, K. (2018). Review on Role of Phosphate Solubilizing Microorganisms in Sustainable Agriculture. *International Journal of Current Research and Academic Review*, **6**(11):48-55.

Mihalache, G., Mihasan, M., Zamfirache, M., Stefan, M., & Raus, L. (2018). Phosphate solubilizing bacteria from runner bean rhizosphere and their mechanism of action. *Romanian Biotechnological Letters*, **23**(4):13853-13861.

Santoyo, G., Hernández-Pacheco, C., Hernández-Salmerón, J., & Hernández-León, R. (2017). The role of abiotic factors modulating the plant-microbe-soil toward sustainable agriculture. A review. *Spanish Journal of Agricultural Research*, **15**(1):1-15.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 23-24)

Simultaneous determination of torulene, torularhodin and β-carotene in *Rhodotorula mucilaginosa* using UV-Vis spectroscopy and chemometric approaches

Anca-Elena Anghel[1]✉, Ioana Grosu[2] and Augustin Cătălin Moţ[3]

[1]Babeş-Bolyai University, Faculty of Chemistry and Chemical Engineering, Department of Chemistry, Cluj-Napoca, Romania; [2] National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania; [3] Babeş-Bolyai University, Faculty of Chemistry and Chemical Engineering, Chemometrics and Bioanalytical Chemistry Laboratory, Analytica Research Center, Cluj-Napoca, Romania; ✉***Corresponding author**, **E-mail:** **ancu.anghel@gmail.com**.*

Abstract

Carotenoids are pigments of biotechnological importance, with antioxidant and radical-scavenging properties, confered by their extensive system of conjugated double bonds. They are synthesized via the mevalonate pathway in red yeasts, such as those belonging to the genus *Rhodotorula,* which have been explored as natural pigment-producing living factories, a safer and more consumer-friendly alternative than chemically synthesized carotenoids.

The aim of this work was to develop a method for the simultaneous, non-destructive determination of the main carotenoids produced by *Rhodotorula mucilaginosa* (β-carotene, torulene and torularhodin), using Principal Component Regression (PCR), a multivariate chemometric technique.

The fungal pigments were purified after chemical cell wall disruption via normal-phase column chromatography. PCR was used to obtain multiple regression equations for the determination of each carotenoid, using the UV-Vis spectra of carotenoid mixtures as input data. The method was validated on samples of known pigment concentration and tested on real samples, prepared by subjecting yeast cells to sodium selenate stress, to investigate the antifungal activity of selenocompounds.

An inverse correlation between total carotenoid content and seleno-compound concentration was observed. Intermediary selenate concentrations appear to shift the carbon flux in the biosynthetic pathway of carotenoids, favoring carotene synthesis in the detriment of xanthophylls.

To our knowledge, no previous method for the simultaneous determination of *Rhodotorula mucilaginosa* carotenoids using PCR has been developed.

The method could be used in industrial settings, when optimization of the culture media for the overproduction of a specific carotenoid is desired. It could also facilitate future studies regarding selenium-mediated fungal toxicity.

Keywords: carotenoids*, R. mucilaginosa*, simultaneous determination, selenocompounds.

Acknowledgements. This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI – UEFISCDI, project number TE-2019-1396, within PNCDI III.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 25)

Danube water physico–chemical parameters in relation with ichthyofauna diversity

Cristian-Emilian Pop[1]✉, Abdulhusein Jawdhari[2] and Nicolae Crăciun[2]

[1]University of Bucharest, Faculty of Chemistry, Department of Organic Chemistry, Biochemistry and Catalysis, Bucharest, Romania; [2]University of Bucharest, Faculty of Biology, Bucharest, Romania; ✉***Corresponding author, E-mail:*** ***cristian.pop@s.unibuc.ro.***

Abstract

Aquatic habitats are sharply deteriorating all over the world due to increasing human impact. The Danube River not an exception especially due to its high economically importance and therefore intense exploatation. Biodiversity inventories are needed to understand the impact of human activities on the Danube ecosystem and to detect potential early warning signals for catastrophic ecological changes in the species communities. In this work we used known physico–chemical indicators (Ilie *et al*., 2017) and fish diversity to assess the ecotoxicological status of the Danube River from eleven locations. Electrofishing was performed in linear 500 m transects, upstream and downstream over shore, as well as over the main water body. Most of the high levels of inorganic pollutants found could be explained by point and diffuse sources scattered along the sector such as discharged household and farm wastewater, as well as nearby agricultural areas where fertilizers have been used and then leaked in the water stream during rainfalls (Ivan *et al*., 2021). Although the Danube water analysis was found to be in what are considered normal ranges, heavy metals concentrations shown a slight correlation with the current status of fish communities which we surveyed, therefore requiring a more detailed investigation for future prospects.

Keywords: ecotoxicology; ichthyofauna; pollution.

References

Ilie, M., Marinescu, F., Ghita, G., Anghel, A. M., Tociu, C., Popescu, I., ... & Bogdan, U. (2017). Spatial distribution of heavy metal contamination and ecological risk assessment in water from the Danube River. *International Journal of Education and Learning Systems*, *2*.

Ivan, M. A., Curuţiu, C., Crăciun, N., Jujea, V., & Pop, C. E. (2021). Microbiological and Physico-Chemical Parameters of the Danube River (Romanian–Bulgarian Sector) with Ichthyofaunal Diversity. Preprints 2021, 2021010051 (doi: 10.20944/ preprints202101.0051.v1).

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 26-27)

Preliminary results of a new approach for *in-vitro* culture conservation of the sand bindweed (*Convolvulus persicus*): seeds

Valentin Adrian Kiss[1]✉ and Liliana Jarda[2]

[1]*Romanian Institute of Science and Technology, Cluj-Napoca, Romania;*
[2]*Alexandru Borza Botanical Garden, Babeş-Bolyai University, Cluj-Napoca, Romania;*
✉***Corresponding author, E-mail: kissvalentinadrian@gmail.com.***

Abstract

Convolvulus persicus is a critically endangered species, endemic to the embryonic shifting dunes of the Caspian Sea and the Black Sea. The risk factors for the populations of *C. persicus* in Romania have an anthropic origin (Kiss and Szatmari, 2020). At the same time, natural risks are also present, such as the solidification of the sands. The efficient approach of plant conservation involves the combination of *ex-situ* and *in-situ* strategies, having as the main objective the maintenance of genetic diversity. In our case, *ex-situ* conservation presents a viable and even indicated alternative. *In-vitro* conservation has been conducted before by Holobiuc on this species using fragments of the stem or root (Holobiuc *et al*., 2015) but not seeds. The study aims to obtain an optimal protocol for *ex-situ* conservation using seeds, as well as the multiplication of plant *in-vitro* culture. Seeds from 20 individuals were used and kept over the winter at 18-20 °C, as well as a month at 4 °C. To initiate the *in-vitro* culture, the seeds were disinfected and inoculated into culture vessels containing 50 ml of MS solid medium supplemented with 20% sucrose. The infection rate after 30 days from inoculation is 8%. The germination rate is 1%. Only one of the seeds germinated, and after 20 days from germination, it was passed on a medium supplemented with phytohormones. In conclusion, we can say that germination of *C. persicus* is quite difficult and it is easier to induce *in-vitro* cultures from fragments of the stem or root as was done in Holobiuc's study.

Keywords: critically endangered, *in-vitro*, seeds, endemic, *ex-situ* conservation.

Acknowledgements. This work was supported by Alexandru Borza Botanical Garden.

References

Kiss, V. A., & Szatmari, P. M. (2020). Distribution and characteristic habitat of *Convolvulus persicus L.* in South-East Romania: threats and protection solutions*. Studia Universitatis Babeş-Bolyai Biologia,* 65, 1, 29-36.

Holobiuc, I., Voichita, C., & Catana, R. (2015). Critically endangered taxon *Convolvulus persicus L. in vitro* conservation and regenerants evaluation. *Studii şi comunicări. Ştiinţele Naturii. Muzeul Olteniei Craiova. Oltenia.* 31, 2, 51-59.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 28-29)

Spread status of *Corythucha arcuata* (Heteroptera, Tingidae) in Romania

Virgil Ionel Oltean[1]✉, Alexandru Sabin Bădărău[1] and Olivia Elena Pistrui[1]

[1]*Babeş-Bolyai University, Faculty of Environmental Science and Engineering, Cluj-Napoca, România;* ✉***Corresponding author, E-mail: olteanvirgil@gmail.com.***

Abstract

Corythucha arcuata (Say, 1832), commonly named the Oak lace bug (OLB), is an insect that belongs to the order Heteroptera, family Tingidae, native to North America. The allogeneic species is known to be invasive and its presence was first reported on the European continent in May 2000, in the region of Lombardy, Italy (Bernardinelli and Zandigiacomo, 2000), then reached Turkey (Mutun, 2003) and spread further in most southern European countries: Bulgaria (Dobreva et al., 2013), Croatia (Hrašovec et al., 2013), Hungary (Csóka *et al.*, 2013), Slovenia (Jurc and Jurc, 2017), Slovakia (Zubrik *et al.,* 2019), progressing very rapidly and affecting very large areas of *Quercus* forests. Also, the species was detected for the first time in the southern Romania in 2016 (Chireceanu *et al.,* 2017), and after a relatively short period, the presence of the insect was reported in several areas of Romania.

The aim of the research was to evaluate the current spread status and level of infestation of the species. We used direct observation method and evaluation of leaves in situ. We also used drone photography as a technique for large areas evaluation The field work was carried out in the vegetative periods of 2020 and 2021. We made over 50 observations in *Querqus* forests and our results shows that the species was detected in over 50% of the survey points, some of the areas being highly infested.

Keywords: biodiversity, conservation, invasive species

References

Bernardinelli, I., & Zandigiacomo, P. (2000) First record of the oak lace bug *Corythucha arcuata* (Say) (Heteroptera, Tingidae) in Europe. *Informatore fitopatologico*, no. 12, 47-49.

Chireceanu, C., Teodoru, A., & Chiriloaie, A. (2017). First record of oak lace bug *Corythucha arcuata* (Tingidae: Heteroptera) in Romania. In 7th ESENIAS Workshop with Scientific Conference (pp. 28-30).

Csóka, G., Hirka, A., & Somlyai. M. (2013). A tölgy csipkéspoloska (*Corythuca arcuata* Say, 1832 – Hemiptera, Tingidae) első észlelése Magyarországon. Növényvédelem. 49. 293-296.

Dobreva, M., Simov, N., Georgiev, G., Mirchev, P., & Georgieva, M. (2013). First record of *Corythucha arcuata* (Say) (Heteroptera: Tingidae) on Balkan Peninsula. *Acta Zoologica Bulgarica*. 65. 409-412.

Hrasovec, B., Posarić, D., Lukić, I., & Pernek, M. (2013). First record of oak lace bug (*Corythucha arcuata*) in Croatia. *Sumarski List*. 137. 499-503.

Jurc, M., & Jurc, D. (2017). The first record and the beginning the spread of oak lace bug, *Corythucha arcuata* (Say, 1832) (Heteroptera: Tingidae), in Slovenia. *Sumarski List*. 9–10, CXXXXI. 485–488.

Mutun, S. (2003). First report of the oak lace bug, *Corythucha arcuata* (Say, 1832) (Heteroptera: Tingidae) from Bolu, Turkey. *Israel journal of zoology*, 46, 323–324.

Zúbrik, M., Gubka, A., Rell, S., Kunca, A., Vakula, J., Galko, J., Nikolov, C., & Leonotvyč, R. (2018). First record of *Corythucha arcuata* in Slovakia – Short Communication. *Plant Protection Science*. 55. 10.17221/124/2018-PPS.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 30-31)

Global warming promotes adaptive changes in the freshwater cyanobacterium *Microcystis aeruginosa*

Maria Nicoară[1]✉ and Bogdan Drugă[1]

[1]National Institute of Research and Development for Biological Sciences (NIRDBS), Institute of Biological Research, Cluj-Napoca, Romania; ✉***Corresponding author,***
E-mail: maria.nicoara@icbcluj.ro.

Abstract

Global warming has a substantial impact on aquatic ecosystems, especially on microalgae, influencing their growth and physiology (Padfield *et al.,* 2015; Sandrini *et al.,* 2015; Schaum and Collins, 2014). In this study, three strains of freshwater cyanobacterium Microcystis aeruginosa have been grown in two different conditions (22°C: A - ambient temperature and 26°C: H - the estimated temperature for the end of the century) for a period of 12 months. In order to observe their response to global warming, thermal reaction norms were calculated in a temperature range of 20-40°C. The results showed that after 100 generations, the H lineage gained remarkable competitive skills, being able to grow even at 38°C, whereas the A lineage did not survive. Moreover, after being re-incubated in the ambient temperature, the development of H strains was reduced, representing an irreversible change suggesting adaptive evolution. This study highlights the necessity of adaptive evolution experiments during a long period of time and with multiple strains, in order to understand the effects of climate change upon aquatic ecosystems.

Keywords: adaptive evolution, cyanobacteria, freshwater, global warming, *Microcystis*.

Acknowledgements. The authors express their gratitude to the Faculty of Biology and Geology from Cluj-Napoca for support.

References

Padfield, D., Yvon-Durocher, G., Buckling, A., Jennings, S., & Yvon-Durocher, G. (2015). Rapid evolution of metabolic traits explains thermal adaptation in phytoplankton. *Ecology Letters* **19**: 133-142. https://doi.org/10.1111/ele.12545

Sandrini, G., Cunsolo, S., Schuurmans, J.M., Matthijs, H.C.P., & Huisman J. (2015). Changes in gene expression cell physiology and toxicity of the harmful cyanobacterium *Microcystis aeruginosa* at elevated CO_2. *Frontiers in Microbiology* **6**: 401. https://doi.org/10.3389/fmicb.2015.00401

Schaum, C.E., & Collins, S. (2014). Plasticity predicts evolution in a marine alga. *Proceedings of Royal Society B* **281**: 20141486. https://doi.org/10.1098/rspb.2014.1486

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 32-33)

Diversity of cultivable heterotrophic bacteria from three Romanian karst caves

Nicoleta-Patricia Vaniga[1]✉, Diana Felicia Bogdan[1,]✉, Oana Teodora Moldovan[2] and Horia-Leonard Banciu[1]

[1]Babeş-Bolyai University, Faculty of Biology and Geology, Department of Molecular Biology and Biotechnology, Cluj-Napoca, Romania; [2]Emil Racoviță Institute of Speleology, Cluj-Napoca Department, Cluj-Napoca, Romania; ✉***Corresponding authors, E-mails:*** ***nicoleta.vaniga@stud.ubbcluj.ro; diana.bogdan@ubbcluj.ro.***

Abstract

Karst caves are peculiar ecosystems accommodating specific biota that readily include microbial communities with key roles in the biogeochemical cycling of main elements and thus, in the functioning of trophic webs (Barton and Northup, 2007). In caves, macroscopic colonies of microorganisms are frequently encountered on the walls, ceilings, in aquatic sediments and on the surface of speleothems. To assess the cultivable microbial diversity in timely, accurately and cost-effective manner, the molecular (marker gene-based) identification of isolates is presently preferred as preceding detailed genotypic and phenotypic characterization. The revealing of microbial diversity in the cave ecosystem is crucial to the understanding of their ecological roles and, in addition, to the discovery of novel taxa and metabolic capabilities. The aim of this study was to isolate and evaluate the taxonomic diversity of bacterial strains from three Romanian karst caves, namely Cloşani, Ferice and Muierilor, located in different geographic areas. To achieve this goal, sample plating and isolation on non-selective, oligotrophic solid culture media and subsequent molecular identification of bacterial isolates have been employed. In total, 41 strains were isolated from soil sediments of the above-mentioned caves. These underwent genomic DNA extraction, 16S rRNA gene amplification, and Sanger sequencing. The retrieved 16S rRNA gene sequences were analyzed and a phylogenetic tree was constructed by bioinformatic tools. The isolates were assigned to *Proteobacteria* (37% of total number of isolates), followed by *Firmicutes* (36%), *Actinobacteria* (26%), and *Bacteroidetes* (2%). *Pseudomonas glareae* (Cloşani), *Aeromonas* sp. and *Polaromonas jejuensis* (Ferice) and *Streptomyces sp.* (Muierilor) were the most frequently recovered isolates. *Bacillus sp.* and *Paenibacillus sp* were retrieved in all caves, while *Flavobacterium sp*. was found only in Cloşani samples. Well-known as bioactive compound-producers, *Actinobacteria* members isolated from the floor sediments of the

explored cave soils were assigned to *Arthrobacter* sp. (Cloșani and Ferice), *Paeniglutamicibacter kerguelensis* (Ferice), and *Streptomyces* sp. (Muierilor). Our findings indicated that the diversity of cultivable bacteria varied among the studied caves, probably due to the geographic distances and slightly different physicochemical setting of each particular cave. However, further detailed investigations are needed to reveal the full picture of bacterial diversity and its roles in the tested cave ecosystems.

Keywords: karst cave; microbiota; phylogeny; 16S rRNA gene; cultivable diversity

Acknowledgements. The present work has received financial support through the project: Entrepreneurship for innovation through doctoral and postdoctoral research, POCU/380/6/13/123886 co-financed by the European Social Fund, through the Operational Program for Human Capital 2014- 2020. HLB was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI – UEFISCDI, project number 2/2019 (DARKFOOD), within PNCDI III.

References

Barton, H., A., & Northup, D. E. (2007). Geomicrobiology in cave environments: past, current and future perspectives. *J. Cave Karst Stud.,* 69(1), 163-178.

Ghosh, S., Kuisiene, N., & Cheeptham, N. (2017). The cave microbiome as a source for drug discovery: Reality or pipe dream?, *J. Biochem. Pharmacol*., 134, 18-20.

Howarth, F., G., & Moldovan, O., T. (2018). Where cave animals live, In: Cave Ecology, Ed. Springer, 235, 28-29.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 34-35)

Downregulated microRNAs as mediators of retinoic acid-dependent transcriptome homeostatic mechanisms in mouse embryos

Silvia Alda[1], Andreea-Daniela Andreescu[1] and
Ioan Ovidiu Sîrbu[1]✉

[1]Victor Babeş University of Medicine and Pharmacy from Timisoara, Biochemistry Department, Timişoara, Romania; ✉***Corresponding author, E-mail: ovidiu.sirbu@umft.ro.***

Abstract

Retinoic acid (RA) is an important transcriptional gene expression regulator which functions as a ligand for retinoic acid receptors (RAR), which bind retinoic acid response elements (RARE) within the regulatory regions of target genes. During antenatal development, RA signaling starts at late gastrulation stages and is required for cell differentiation, cell migration, axial elongation and organogenesis.

In mouse embryos, the most important source of RA is RALDH2, expressed in the paraxial mesoderm starting with E7.5. RA has been shown to travel over long distances and to activate gene expression and orchestrate morphogenetic events in axial and paraxial tissues of neuroectodermal and mesodermal origin. Several *ex vivo* and *in vivo* studies have described the impact of RA on transcriptome homeostasis; however, little is known about the post-transcriptional mechanisms altered by RA in the context of vertebrate embryo development.

The aim of this study was to provide a better understanding of these mechanisms. Here we used Exiqon qRT-PCR arrays to analyze the small RNA profile in E8.5 *raldh2-/-* embryos and identified a set of 26 downregulated microRNAs. MicroRNAs are small non-coding RNAs that regulate gene expression at post-transcriptional level. In order to identify the signaling pathways putatively modulated by RA-microRNAs interactions during early organogenesis stages, we combined mirWalk3.0 target prediction algorithms (for 3'UTR, 5'UTR, and CDS regions), complex network analysis, and DAVID gene ontology analysis.

Further, more complex investigations are needed in order to understand the transcriptomic impact certain microRNAs have on RA signaling during mouse embryogenesis.

Keywords: mouse embryo, gene regulation, miRNA, retinoic acid

References

Huang, D.W., Sherman, B.T., & Lempicki, R.A. (2009). Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. *Nature Protoc.* 4(1):44-57.

Paschaki, M., Schneider, C., Rhinn, M., Thibault-Carpentier, C., Dembélé, D., *et al.* (2013) Transcriptomic Analysis of Murine Embryos Lacking Endogenous Retinoic Acid Signaling. *Plos One* 8(4): e62274.

Sticht, C., De La Torre, C., Parveen A., Gretz, N. (2018). miRWalk: An online resource for prediction of microRNA binding sites. *PLoS One*. Oct 18;13(10).

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 36-37)

Upregulated microRNAs as mediators of retinoic acid-dependent transcriptome homeostatic mechanisms in mouse embryos

Alexandru Alexandru[1], Cristiana-Smaranda Ivan[1] and Ovidiu Sîrbu[1]✉

[1]*Victor Babeş University of Medicine and Pharmacy from Timisoara, Biochemistry Department, Timişoara, Romania;* ✉***Corresponding author**, **E-mail:** ovidiu.sirbu@umft.ro.*

Abstract

A major transcriptional gene activator, retinoic acid (RA), exerts its function by binding to retinoic acid receptors (RAR), which recognize retinoic acid response elements (RARE) within the regulatory regions of target genes. During embryo-fetal development, RA signaling orchestrates axial elongation, organogenesis, cell differentiation, and cell migration, starting with late gastrulation stages.

In mouse embryos, RA is synthesized starting with E7.5 by RALDH2 expressed in the paraxial mesoderm and diffuses over long distances to activate gene expression and initiate morphogenetic events in the adjacent tissues. The impact of RA on mouse embryo transcriptome homeostasis has already been described; however, little is known about the RA-dependent post-transcriptional mechanisms of gene expression regulation in the context of vertebrate embryo development.

MicroRNAs are small non-coding RNAs that operate as endogenous post-transcriptional gene expression regulators. Here, we used Exiqon qRT-PCR arrays to identify the set of 11 upregulated microRNAs in *raldh2-/-* mouse embryos at E8.5. By combining mirWalk3.0 target prediction algorithms (for 3'UTR, 5'UTR, and CDS regions), complex network analysis, and DAVID gene ontology analysis, we identified and described the signaling pathways putatively modulated by RA-microRNAs interactions during early organogenesis stages of mouse embryo development.

Our work provides a conceptual framework for future, more complex investigations of microRNAs' role as mediators of RA signaling during mouse embryogenesis.

Keywords: mouse embryo, gene regulation, miRNA, retinoic acid

References

Huang, D.W., Sherman, B.T., & Lempicki, R.A. (2009). Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. *Nature Protoc.* 4(1): 44-57.

Paschaki, M., Schneider, C., Rhinn, M., Thibault-Carpentier, C., Dembélé, D., *et al.* (2013) Transcriptomic Analysis of Murine Embryos Lacking Endogenous Retinoic Acid Signaling. *Plos One* 8(4): e62274.

Sticht, C., De La Torre, C., Parveen A., Gretz, N. (2018). miRWalk: An online resource for prediction of microRNA binding sites. *PLoS One*. Oct 18;13(10).

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 38-39)

Photooxidation of skin cells with titanium oxide systems excited with visible light

Iulia Moigrădan[1]✉, Ştefania M. Milin[1] and Maria Suciu[1,2]

[1]Babeş-Bolyai University, Faculty of Biology and Geology, Cluj-Napoca, Romania; [2]National Research and Development Institute for Isotopic and Molecular Technologies, Cluj-Napoca, Romania; ✉***Corresponding author**; **E-mail:** iuliamoigradan@gmail.com.*

Abstract

The interest for nanoparticles increased in the last few years thanks to their unique proprieties and wide use. Although, there are some concerns about the exposure to these nanoparticles because, in certain quantities, can be toxic. In the current study, the effects of graphene nanoparticles doped with titanium dioxide (TiO_2) and silver (Ag) or TiO_2 and copper (Cu) were identified on two human cell lines, A375 and HaCaT, in the absence and presence of visible light. Cytotoxicity and oxidative stress were investigated in the two cell lines with the help of three assays: testing cell viability, measuring the quantity of lactate dehydrogenase (LDH) released and the level of nitric oxide (NO). More precise, the two cell lines were treated with TiO_2/Ag/TRGO and TiO_2/ Cu/TRGO at five different concentration (0.01-1 mg/mL) and incubated for 24 hours in the absence or presence of visible light; after the treatment, three specific assays were made. The findings of this research proved that the toxicity induced by the nanoparticles, in the absence or presence of visible light, damaged the A375 cell line much more compared to the HaCaT cell line. In addition, the results also showed that the treatment TiO_2/Ag/TRGO at high concentrations had the most aggressive effect. In conclusion, this study proved the antitumoral effect of nanoparticles doped with TiO_2/Ag or TiO_2/Cu and opened an opportunity for potential treatments of various diseases using photooxidation. However, due to the fact that the nanoparticles used in this study are known to be present in many everyday products that could come in contact with the human skin, further studies are recommended to be made regarding the toxicity of these nanoparticles on the HaCaT cell line.

Keywords: A375; cytotoxicity; graphene; HaCaT; nanoparticles.

Acknowledgements: The research leading to these results has received funding from the Norwegian Financial Mechanism 2014 -2021, under Project RO-NO-0616, contract no. 29/2020.

References

Gurunathan, S., & Kim, J. H. (2016). Synthesis, toxicity, biocompatibility, and biomedical applications of graphene and graphene-related materials. *International journal of nanomedicine*, *11*, 1927.

Lalwani, G., D'Agati, M., Khan, A. M., & Sitharaman, B. (2016). Toxicology of graphene-based nanomaterials. *Advanced drug delivery reviews*, *105*, 109-144.

Li, Y., Chang, Y., Lian, X., Zhou, L., Yu, Z., Wang, H., & An, F. (2018). Silver nanoparticles for enhanced cancer theranostics: in vitro and in vivo perspectives. *Journal of biomedical nanotechnology*, *14*(9), 1515-1542.

Rabek, J. F., & Rånby, B. (1974). Studies on the photooxidation mechanism of polymers. I. Photolysis and photooxidation of polystyrene. *Journal of Polymer Science: Polymer Chemistry Edition*, *12*(2), 273-294.

Siddiqi, K. S., Husen, A., & Rao, R. A. (2018). A review on biosynthesis of silver nanoparticles and their biocidal properties. *Journal of nanobiotechnology*, *16*(1), 1-28.

Wang, H., Wang, G., & Zhang, Y. (2019). Preparation of RGO/TiO_2/Ag Aerogel and Its Photodegradation Performance in Gas Phase Formaldehyde. *Scientific Reports 9(1)*. doi:10.1038/s41598-019-52541-7.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 40-41)

CRISPR-Cpf1 system and its utility in editing the *Paenarthrobacter nicotinovorans* genome

Iustin-Tiberius Munteanu[1], Fakhri Kallabi[1] and Marius Mihăşan[1]✉

[1] *Alexandru Ioan Cuza University of Iaşi, Faculty of Biology, BioActive Research Group, Iaşi, Romania;* ✉***Corresponding author, E-mail: marius.mihasan@uaic.ro.***

Abstract

Paenarthrobacter nicotinovorans is a Gram-positive bacterium that is best known for its ability to metabolize nicotine. The stain has proven its potential for converting nicotine containing waste into useful chemicals (Hritcu and Mihasan, 2019; Yu *et al.,* 2017). Its applications in biotechnology are hampered by the lack of reliable gene editing systems that would permit rational engineering of the nicotine degradation pathway.

CRISPR systems have been extensively used for genomic editing of eukaryotic cells and proved to be reliable and accurate. The applicability of CRISPR system for genomic editing of *Paenarthrobacter* strains remains elusive. The main goal of this work is to evaluate the applicability and functionality of the CRISPR-Cpf1 system in *P. nicotinovorans.* CRISPR-Cpf1 is a class 2 type V CRISPR system known to work in the closely related *Corynebacterium glutamicum* strains (Jiang *et al.,* 2017). The draft genome of *P. nicotinovorans* was used to screen for the presence of incompatible CRISPR systems using CRISPRs web server (https://crispr.i2bc.paris-saclay.fr/). A number of 4 CRISPRs candidates were found on different contigs, but none were related to CRISPR-Cpf1. Hence, we concluded that the system might work in this strain and the pJYS3_ΔcrtYf plasmid containing a functional a CRISPR-Cpf1 system was electroporated into *P. nicotinovorans*. No transformants were obtained upon selection with kanamycin, indicating that the pJYS3 replicating origin might not be functional in *P. nicotinovorans*. Next, the CRISPR-Cpf1 genes from pJYS3_ΔcrtYf were amplified by PCR and ongoing work aims to clone these genes into a plasmid known to work in *P. nicotinovorans* – pART2 (Sandu *et al.,* 2005).

Keywords: CRISPR, genetic engineering, *P. nicotinovorans*

Acknowledgements. This work was suported by PN-III-P4-ID-PCE-2020-0656, Sequencing the genome of a useful bacteria: *Paenarthrobacter nicotinovorans* next step in extending its biotechnological applications.

References

Hritcu, L., & Mihasan, M. (2019). 6-Hydroxy-l-Nicotine and Memory Impairment. In: *Neuroscience of Nicotine*; Victor R. Preedy, Ed. Elsevier; pp 165–172.

Jiang, Y., Qian, F., Yang, J., Liu, Y., Dong, F., Xu, C., Sun, B., Chen, B., Xu, X., Li, Y., Wang, R., Yang, S. (2017). CRISPR-Cpf1 Assisted Genome Editing of Corynebacterium Glutamicum. *Nat. Commun.*, *8* (1), 15179.

Sandu, C., Chiribau, C.-B., Sachelaru, P., Brandsch, R. (2005). Plasmids for Nicotine-Dependent and - Independent Gene Expression in Arthrobacter Nicotinovorans and Other Arthrobacter Species. *Appl. Environ. Microbiol*, *71* (12), 8920–8924.

Yu, W., Wang, R., Li, H., Liang, J., Wang, Y., Huang, H., Xie, H., & Wang, S. (2017). Green Route to Synthesis of Valuable Chemical 6-Hydroxynicotine from Nicotine in Tobacco Wastes Using Genetically Engineered Agrobacterium Tumefaciens S33. *Biotechnol. Biofuels*, *10* (1), 288.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 42-43)

Effects of graphene materials on A375 and HaCaT cell lines due to exposure to visible light

Ştefania Milin[1]✉, Iulia Moigrădan[1], Crina Socaci[2], Sergiu Gabriel Macavei[2] and Maria Suciu[1,2]

[1]Babeş-Bolyai University, Faculty of Biology and Geology, Departament of Molecular Biology and Biotehnology, Cluj-Napoca, Romania; [2] National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania;
✉ ***Corresponding author, E-mail: stefania.milin@stud.ubbcluj.ro.***

Abstract

The wide scale use of nanoparticles (NPs) due to their unique properties and important applications in sensor devices, clothing, alimentation and cosmetics makes human being more prone to the exposure of NPs and its potential to adverse effects. Exposure is mainly through skin. Therefore, the aim of the present study was to investigate the effects of graphene oxide nanoparticles complexed with titanium dioxide and copper or copper oxide (TiO_2/CuO/GO and TiO_2/Cu/TRGO) on A375 and HaCaT cell lines exposed to visible light. We explored the cytotoxicity and oxidative stress induced by nanoparticles. Cell viability, nitric oxide (NO) levels and extracellular release of lactate dehydrogenase (LDH) were assayed in A375 and HaCaT cells after 24 hours incubation with 0.01-1 mg/ml TiO_2/CuO/GO and TiO_2/Cu/TRGO nanoparticles. The results showed that nanoparticles under light iradiation reduced cell viability, induced nitric oxide generation and impaired cell membrane integrity of A375 and HaCaT in a dose dependent manner. It is valuable to inform that HaCaT cells appeared to be slighty more susceptible to TiO_2/CuO/GO treatment than A375 cells, TiO_2/Cu/TRGO nanocomposite has the potential for antitumor treatment by photooxidation, as green and blue lights intensify the toxicity. These results provide a basic comparative toxic effect of TiO_2/CuO/GO and TiO_2/Cu/TRGO nanoparticles on normal keratinocytes and cancerous epitelial cells. Considering the diverse results, further studies using different conditions are recommended.

Keywords: A375, cytotoxicity, graphene, HaCaT, nanoparticles;

Acknowledgements. This work was supported by a grant of Romanian Ministry of Research and Innovation, PCCCDI – UEFISCDI, Project number PN-III-P1-1.2-PCCDI-2017 0743/44PCCDI/2018, within PNCDI III.

References

Dröge, W. (2002). Free radicals in the physiological control of cell function. *Physiological reviews*.

Love, S. A., Maurer-Jones, M. A., Thompson, J. W., Lin, Y. S., & Haynes, C. L. (2012). Assessing nanoparticle toxicity. *Annual review of analytical chemistry*, *5*, 181-205.

Lowenstein, C. J. (2007). Nitric oxide regulation of protein trafficking in the cardiovascular system. *Cardiovascular research*, *75*(2), 240-246.

Novo, E., & Parola, M. (2008). Redox mechanisms in hepatic chronic wound healing and fibrogenesis. *Fibrogenesis & tissue repair*, *1*(1), 1-58.

Saud Alarifi, D. A., Verma, A., Alakhtani, S., & Ali, B. A. (2013). Cytotoxicity and Genotoxicity of Copper Oxide Nanoparticles in Human Skin Keratinocytes Cells.

Urbańska, K., & Orzechowski, A. (2019). Unappreciated role of LDHA and LDHB to control apoptosis and autophagy in tumor cells. *International journal of molecular sciences*, *20*(9), 2085.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 44-45)

Epigenetic changes induced by commonly used metal and metal oxide nanoparticles

Bogdan Andrei Miu[1]✉ and Anca Dinischiotu[1]

[1]*University of Bucharest, Faculty of Biology, Department of Biochemistry and Molecular Biology, Bucharest, Romania;* ✉***Corresponding author, E-mail: miu.bogdan-andrei@s.bio.unibuc.ro.***

Abstract

Nanotechnology is one of the fastest developing fields in science and engineering. Based on market-estimated size and online repositories listing nanoproducts (e.g., Nanodatabase), it is obvious that silver, titanium dioxide and silicon oxide nanoparticles (NPs) are widely used in day-to-day items such as cosmetic and skincare products or clothing materials. However, consumer products containing NPs may become a source of air pollution, raising concerns regarding human health. Toxic effects of NPs are relatively well documented, although experimental data regarding epigenetic alterations is limited. Our review study aims to provide a general description of the epigenetic changes induced by these three commonly used NP types. We considered recent *in vivo* and *in vitro* studies and discussed which molecular pathways associated with DNA methylation and histone post-translational modifications were impaired by NPs exposure. Knowledge gaps related to the subject were also highlighted. Our work could contribute to the improvement of knowledge about NPs toxicity by compiling the related data available so far and clearly illustrating general NPs effects observed on key molecules from epigenetic signalling pathways. Epigenetic changes play a crucial role in triggering different human disorders. Considering the widespread of NPs, all their toxic effects, including epigenetic impairment, need to be documented to completely assess their safety. We concluded exposure to NPs affects genes involved in establishing and maintaining the normal epigenetic pattern. It remains unknown whether epigenetic changes occur as an indirect consequence of other NPs toxic effects such as oxidative stress and inflammation. These data may be considered in developing appropriate public policies for nanomaterials market.

Keywords: DNA methylation, histone modification, epigenetic, nanoparticle.

References

Inshakova, E., & Inshakov, O. (2017). World market for nanomaterials: structure and trends. MATEC Web Conf. 129: 02013.

Jayaram, D. T., & Payne, C. K. (2020). Intracellular Generation of Superoxide by TiO2 Nanoparticles Decreases Histone Deacetylase 9 (HDAC9), an Epigenetic Modifier. *Bioconjugate Chem* 31(5): 1354-1361.

Liu, L., Li, Y., & Tollefsbol T. O., (2008). Gene-Environment Interactions and Epigenetic Basis of Human Diseases. *Curr Issues Mol Biol* 10(1-2): 25-36.

Maki, A., Lin, Y., Aoyama, M., Sato, K., Gao, J. Q., Tsujino, H., Nagano, K., Higashisaka, K., & Tsutsumi, Y. (2020). Silver Nanoparticles Induce DNA Hypomethylation through Proteasome Mediated Degradation of DNA Methyltransferase 1. *Biol Pharm Bull* 43(12): 1924-1930.

Pogribna, M., Koonce, N. A., Mathew, A., Word, B., Patri, A. K., Lyn-Cook, B., & Hammons, G. (2020). Effect of titanium dioxide nanoparticles on DNA methylation in multiple human cell lines. *Nanotoxicology* 14(2): 1-20.

Rossnerova, A., Honkova, K., Pelclova, D., Zdimal, V., Hubacek, J. A., Chvojkova, I., Vrbova, K., Rossner, P., Topinka, J., Vlckova, S., Fenclova, Z., Lischkova, L., Klusackova, P., Schwarz, J., Ondracek, J., Ondrackova, L., Kostejn, M., Klema, J., & Dvorackova, S. (2020). DNA Methylation Profiles in a Group of Workers Occupationally Exposed to Nanoparticles. *Int J Mol Sci* 21: 2420.

Seidel, C., Kirsch, A., Fontana, C., Visvikis, A., Remy, A., Gaté, L., Darne, C., & Guichard, Y. (2017). Epigenetic changes in the early stage of silica-induced cell transformation. *Nanotoxicology* 11: 923-935.

Toyooka, T., Amano, T., Ibuki, Y., (2012). Titanium dioxide particles phosphorylate histone H2AX independent of ROS production. *Mutat Res* 742: 84-91.

Tulve, N. S., Stefaniak, A. B., Vance, M. E., Rogers, K., Mwilu, S., LeBouf, R. F., Schwegler-Berry, D., Willis, R., Thomas, T. A., & Marr, L. C. (2015). Characterization of silver nanoparticles in selected consumer products and its relevance for predicting children's potential exposures. *Int J Hyg Environ Health* 218: 345-357.

Vance, M. E., & Marr, L. C. (2015). Exposure to airborne engineered nanoparticles in the indoor environment. *Atmos Environ* 106: 503-509.

Zhao, X., Rao, Y., Liang, J., Lin, S., Wang, X., Li, Z., Huang, J. (2019). Silver Nanoparticle-Induced Phosphorylation of Histone H3 at Serine 10 Involves MAPK Pathways. *Biomolecules* 9(2): 78.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 46-47)

Subcloning and expression of recombinant glucose-dehydrogenase from *Bacillus subtilis*

Andrea Popa[1]✉, Sebastian Emanuel Gherman[1] and Iulia Lupan[2]

[1]Babeş-Bolyai University, Cluj-Napoca, Romania; [2]Interdisciplinary Research Institute on Bio-Nano-Sciences, Cluj-Napoca, Romania; ✉***Corresponding author, E-mail:*** ***andreapopa3101@gmail.com.***

Abstract

Glucose-dehydrogenase (GDH) is an enzyme that catalyzes the oxidation of glucose to gluconate, reducing NAD(P)+ to NAD(P)H + H+. This enzyme is of particular interest due to the fact that it can be used to regenerate the NAD cofactor using a cheap substrate such as glucose. The aim of this study was the fusion of recombinant GDH gene with the His-Tag at the N-terminus. For this purpose, the recombinant GDH gene was subcloned into the pET28a expression vector and expressed in *E. coli* BL21(DE3) cells. The recombinant protein was expressed both in soluble form (10% of total proteins) and in inclusion bodies. The recombinant GDH was purified by Ni-agarose affinity chromatography and tested for enzymatic activity (glucose and NAD). In conclusion, after subcloning the ORF did not change, the protein was fused with His-tag, and this fusion did not affect the activity or solubility of the enzyme. The results obtained in this study may be used to optimize glucose-dehydrogenase production so that it would increase its biotechnological importance.

Keywords: cofactor regeneration; glucose-dehydrogenase; recombination; subcloning; vector

Acknowledgements. We thank Maria Ciobanu for her kind technical help.

References

Chen, X., Haitao, D., Du, Y., Lin, H., Li, Z., & Zhao, Y. (2011). Cloning, expression and characterization of a glucose dehydrogenase from Bacillus sp. G3 in Escherichia coli, *Afr. J. Microbiol. Res*. 5(32): 5882-5888.

Chiriac, M., Lupan, I., Bucurenci, N., & Popescu O. (2007). Palibroda N., Stereoselective synthesis of L-[15N] amino acids with glucose dehydrogenase and galactose mutarotase as NADH regenerating system. *J Labelled Comp Radiopharm*. 51(4): 171-174.

Howe, C. (2007). Gene Cloning and Manipulation, 2nd edition, ed. Cambridge Press University, pp. 1-73.

Pongtharangkul, T., Chuekitkumchorn, P., Suwanampa, N., Payongsri, P., Honda, K., Panbangreg, W. (2015). Kinetic properties and stability of glucose dehydrogenase from Bacillus amyloliquefaciens SB5 and its potential for cofactor regeneration, *AMB Express*., 5: 68.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 48-49)

CRISPR/Cas9 – mediated gene silencing of OXCT1 in HeLa cells favors cell proliferation

Marta Biesiekierska[1]✉, Maciej Studzian[1], Paweł Hikisz[1], Souad Nasser[2], Luciano Pirola[2] and Aneta Balcerczyk[1]

[1]University of Lodz, Faculty of Biology and Environmental Protection, Department of Molecular Biophysics, Lodz, Poland; [2]INSERM Unit 1060, CarMeN Laboratory, Lyon 1 University, France;
✉***Corresponding author, E-mail:*** ***marta.biesiekierska@edu.uni.lodz.pl.***

Abstract

Extensive evidence suggests that the utilization of ketone bodies as an alternative source of energy to carbohydrates may have a beneficial effect on cancer treatment due to the fact that the major ketone body – β-hydroxybutyrate – directly affects inflammatory processes. The rate-limiting enzyme in ketone bodies catabolism is succinyl-CoA:3-ketoacid-coenzyme A transferase 1 (SCOT1), encoded by the OXCT1 gene. Our previous studies showed an elevated OXCT1 expression in various cancer cell lines, including cervical cancer cell line HeLa. The main aim of the research was to knockout the OXCT1 gene from HeLa cells using the CRISPR/Cas9 technique in order to analyze the proliferation rate and possible new functional characteristics of this model.

The knocking-out procedure of OXCT1 was performed by CRISPR/Cas9 and gDNA transient transfection, followed by a Surveyor test to scan for the occurrence of the DNA mutation. The SCOT1 protein ablation in multiple clones was determined by Western blotting and immunofluorescent microscopy. Moreover, we performed a cytometric analysis of the cell cycle phase distribution and analyzed the gene expression of selected cell cycle determinants, including p21 and cyclins.

We observed that OXCT1 knockout increases the proliferation rate of HeLa cells, suggesting that OXCT1 gene may be non-essential for cell proliferation or even involved in attenuation of cell proliferation, suggesting that a switch to an alternative energy source to glucose/carbohydrates in cancer cells may render them less susceptible to proliferation.

Keywords: cancer cells, ketone bodies, metabolism

Acknowledgements. This work was supported by HARMONIA project 2019/30/M/NZ3/00682, funded by Polish National Science Centre.

References:

Dąbek, A., Wojtala, M., Pirola, L., & Balcerczyk, A. (2020). Modulation of cellular biochemistry, epigenetics and metabolomics by ketone bodies. Implications of the ketogenic diet in the physiology of the organism and pathological states. *Nutrients*, *12*(3), 788.

Chriett, S., Zerzaihi, O., Vidal, H., & Pirola, L. (2017). The histone deacetylase inhibitor sodium butyrate improves insulin signalling in palmitate-induced insulin resistance in L6 rat muscle cells through epigenetically-mediated up-regulation of Irs1. *Molecular and cellular endocrinology*, *439*, 224-232.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 51-72)
doi:10.24193/subbbiol.2021.1.01

Fatty acid composition and biological activity of four olive oils from Kabylia (Algeria) against *Rhyzopertha dominica* (Coleoptera: Bostrychidae) infesting wheat seeds

Safia Kerbel[1], Ines Abdelli[1], Hakim Azzi[2], Jean-Francois Debras[3] and Abdellah Kellouche[1]✉

[1]Laboratoire de production et de sauvegarde des espèces menacées et des récoltes. Influence des variations climatiques. Faculté des sciences biologiques et des sciences agronomiques, Université Mouloud Mammeri, Algeria; [2]Laboratoire des Ressources Naturelles, Faculté des Sciences Biologiques et des Sciences Agronomiques, UMMTO, Algeria; [3]Unité Plantes et Systèmes de Cultures Horticoles, INRA, Domaine Saint-Paul, Avignon, France;
***Corresponding author, E-mail:** kader_kellouche@yahoo.fr.*

Article history: Received 6 February 2021; Revised 7 May 2021; Accepted 18 May 2021; Available online 30 June 2021

Abstract. The use of conventional insecticides is one of the most widely used methods of controlling pests of stored grains. But the presence of toxic residues in treated commodities and the emergence of insect resistant strains are becoming a growing concern. Olive oil is well known throughout the world for its benefits to human health, but little known for its biological activity against insect pests.

The aim of this work is to study the fatty acid composition and the insecticidal activity of oils according to origin of plantation, against one of the main insect pests of stored grain *Rhyzopertha dominica* (Coleoptera: Bostrychidae). The olive oils were obtained using an oleodoser from olives of the 'Chemlal' variety harvested in 4 olive groves in Kabylia (Algeria) and the analysis of the fatty acid composition was carried out by gas chromatography. The main fatty acids found are oleic, palmitic and linoleic acids. Biological tests conducted under laboratory conditions, at a temperature of 30±1°C and a relative humidity of 70 ± 5 %, revealed that the 4 olive oils, applied on soft wheat grains, showed a contact toxicity against *R. dominica*. The toxicity of the oils varied as a function of the dose and the duration of treatments. After 24 h of exposure, all oils tested at the highest dose (0.4 mL/25 g) were found to be highly toxic to adults of *R. dominica,* with mortality rates ranging from 72.5 to 95 %. The toxicity of the 4 oils based on the LD_{50} (mL/25 g) values for 24 h mortality is established as follows: Maatkas (213), Bachloul (232), Tadmait (234) and M'Chedellah (263).

The number of the F1 offspring decreases as the dose of oil is increased to reach zero with the highest dose, for all treatments. All oils tested completely preserve soft wheat seeds from *R. dominica* attacks using the same highest dose. On the other hand, results also revealed that treatments with olive oil do not affect the germination capacity of soft wheat seeds.

Keywords: wheat grains, insect pest, *Rhyzopertha dominica*, olive oil, fatty acids, toxicity, damage.

Introduction

Cereals occupy a fundamental place in Algerian agriculture, with a surface area of 2.7 million hectares, i.e. nearly 40% of the country's overall agricultural area, and 3.3 million tons in 2014 (FAO, 2015).They also constitute a major part of the human diet worldwide (Chen and Dubcovsky, 2012; Merouche *et al.*, 2014).

Soft wheat (*Triticum aestivum* L.) is an important food crop worldwide due to its nutritional value (FAO, 2019; Valenzuela-Aragon *et al.*, 2019). In Algeria, it ranks third in terms of production after durum wheat (*Triticum durum* Desf.) and barley (*Hordeum vulgare* L.), with an annual cultivated area of 0.8 million hectares, representing 24.2 % of the area devoted to cereals country-wide (Bellatreche *et al.*, 2019; Kara *et al.*, 2020; Meziani *et al.*, 2020).

The conservation of these products is the only way to ensure the link between the year's harvest and continuous consumption. During storage, various biotic and abiotic factors depreciate it qualitatively and quantitatively, the most important being insect pests (Arve *et al.*, 2014; Hamdi *et al.*, 2015; Abdelli *et al.*, 2016; Aoues *et al.*, 2017; Brahmi*et al.*, 2017; Djidel *et al.*, 2018). According to the assessment of Algerian Inter-Professional Office of Cereals (AIOC), losses that can exceed 35 % have been recorded in recent years (Ahmed, 2016).

Among the pests of wheat grains during storage, *Rhyzopertha dominica*, is a serious pest of stored grain worldwide, due to the quantitative and qualitative losses that it causes (Bashir, 2002; Hagstrum and Flinn, 2014; Filomeno *et al.*, 2020). It is considered a highly destructive pest of wheat, sarrasin rice, maize, sorghum, barley, rye, millet, etc. (Aitken, 1975; Mason and McDonough, 2012; Eydozehi and Ravan, 2013; Kakde *et al.*, 2014; Ridley *et al.*, 2016). As a primary colonizer, *R. dominica* larvae and adults can infest sound kernels (Hill, 2002; Batta *et al.*, 2007); they spend most of their life inside the kernel, feeding on both the germ and endosperm, directly causing damage and changes the physical and

chemicalproperties of the grain. The adult is responsible for losses that are estimated to be eight times greater than those caused by the larvae (Toews *et al.*, 2000; Huchet, 2017). The infestation of stocks by *R. dominica* causes weight loss (Park *et al.*, 2008; Hendrival *et al.*, 2019; Arthur *et al.*, 2020), a decrease in essential amino acids (Jood *et al.*, 1995; Edde, 2012 ; Boukouvala *et al.*, 2020), a decrease in the germination capacity of grains used as seed and a reduction in plant vigor at emergence (Limonta *et al.*, 2011; Saad *et al.*, 2018; Waongo *et al.*, 2018). Grains infested by *R. dominica* are then vulnerable to attack by secondary pests and moulds (Srivastava and Subramanian, 2016; Win and Rolania, 2020).

Control measures are currently based on the application of chemicals because of their effectiveness and low cost (Boyer *et al.*, 2012; Kumar and Kalita, 2017). Nevertheless, the use of conventional insecticides has caused adverse effects on the agro-ecosystem such as the development of insect resistance, resurgence of secondary pests and environmental contamination, affecting target pests, domestic animals and human health (Mau *et al.*, 2012; Kim *et al.*, 2017; Collins and Schlipalius, 2018; Daglish and Nayak, 2018; Morrison *et al.*, 2019; Nayak *et al.*, 2020).

These drawbacks have highlighted the need for sustainable alternatives that are available, affordable, less toxic to mammals and less detrimental to the environment and plants such as botanicals (Kellouche and Soltani, 2004; Kellouche *et al.*, 2010; Hamdi *et al.*, 2015; García-Lara and Serna-Saldivar, 2016; Lougraimzi *et al.*, 2018; Righi *et al.*, 2018; Ekoja and Ogah, 2020; Nia *et al.*, 2020).

Recently, there has been an increasing interest in the use of natural oils, including vegetable, essential and mineral oils (Obeng-Ofori and Amiteye, 2005; Lal and Raj, 2012; Rayhan *et al.*, 2014;Rolania and Bhargava, 2015; Singh *et al.*, 2016; Baccari *et al.*, 2020; Chenni *et al.*, 2020; Ebrahimifar *et al.*, 2020; Haouel-Hamdi *et al.*, 2020; Yakhlef *et al.*, 2020).

Among vegetable oils, olive oil, due to its high oleic acid content, detected as an insecticidal component, could be used as a biopesticide in insect pest management (Abdallah *et al.*, 2001; Kellouche *et al.*, 2004; Uddin and Sanusi, 2013; Ekoja and Ogah, 2020; Zohry *et al.*, 2020).

The aim of the present work was to study the efficacy of four olive oils extracted from olives collected from four localities in Kabylia (Algeria), against *R. dominica.* The importance of this research resides in the development of a natural method of sustainable conservation that can be used locally to protect cereal seeds, which are strategic products in Algeria.

Materials and methods

Plant material and oil extraction

The extra virgin olive oils used in this work came from Chemlal, the main Algerian olive variety, at the same stage of maturity (maturity index = 3), in the 2016/2017 crop season (November). Four representative regions of olive oil production in Algeria were selected to obtain the EVOO samples Tadmaït (3. 98778 36° 36′ 44″ North, 3° 59′ 16″ East), Maatkas (3.90186 36° 44′ 34″ North, 3° 54′ 7″ East), M'Chedellah (4.24858 36° 23′ 50″ North, 4° 14′ 55″ East) and Bechloul (4.06667 36° 19′ 0″ North, 4° 4′ 0″ East). The olive fruits were randomly and manually picked from all parts of the selected fully grown olive trees (COI, 2011). The olive maturity index (MI) was determined according to the method developed by the agricultural station in Jaén (Uceda and Hermoso, 1998), on the basis of the evaluation of the olive skin and pulp colors. MI values range from 0 (100% intense green skin) to 7 (100% purple flesh and black skin).

Olive oil samples were obtained using a laboratory-scale oil mill (S.I.O.L. 20240 GHISONACCIA, France), consisting of three basic elements: a hammer crusher, a thermo-beater (mixer) and a pulp centrifuge. Olives were first crushed and the resulting paste was slowly mixed for 30 min at 25 °C. Olive oil was obtained after the centrifugation of the paste at 3000 rpm for 3 min (Bengana *et al.*, 2013).

The oil was separated by decanting, classified according to the origin of the olives; OO1 (Tadmaït), OO2 (Maatkas), OO3 (M'Chedellah), OO4 (Bechloul), and stored at 4 °C in darkness using amber glass bottles without headspace prior to use.

Fatty acid analysis

The analytical methods for the determination of fatty acid composition were described in regulation EEC 2568/91 (EEC, 1991). Fatty acids were converted to fatty acid methyl esters before analysis by vigorous shaking of a solution of oil in hexane (0.2 g in 3 mL) with 0.4 mL of 2 N methanolic potassium hydroxide and analyzed by a GC Chrompack CP 9002 (Les Ulis, France) equipped with a FID detector, an split–splitless injector and a DB23 (50% cyanopropyl) capillary column (30 m x 0.32 mL, 0.25 μm film; Agilent Technologies, Palo Alto, California, USA). The carrier gas was nitrogen (Linear velocity, 0.5 cm/min; split ratio of 1:30, v/v). The temperatures of the injector, detector and the oven were set at 250 °C, 280 °C and 200 °C, respectively. The injection volume was 0.8 μL (EEC, 1991). One replicate was prepared and analyzed per sample.

Insects rearing

The mass breeding of *R. dominica* was conducted in the laboratory, in a dark oven at 30±1°C and 70±5% relative humidity, on soft wheat (*Triticum aestivum*). The strain of *R. dominica* comes from the storage commodities of CCLS Tizi-Ouzou (Cooperative of Cereals and Dried vegetables). The same conditions of temperature and humidity were chosen to perform our experiments.

Bioassays

Soft wheat seeds of local origin, free of infestation and pesticides, were used for the biotests. Each olive oil extracted was mixed with soft wheat seeds in glass Petri dishes (13 cm diameter and 3 cm height), at three doses: 0.1, 0.2 and 0.4 mL/25 g. All trials were repeated four times for each dose and control. These Petri dishes were shaken manually for 15 min to achieve an equal distribution of the oils in the entire grain mass. Then, 20 unsexed adults <1 weeks old were introduced into each dish, which was immediately closed. Mortality was assessed 24, 48, 72 and 96 h after treatment application. Dead adults were removed and counted during each assessment. Dead insects from oil-treated grain showed signs of rapid immobilization, with their legs flexed and clinging to either the grain or the container surface. Since mean mortality in untreated control was less than 5 %, mortality data were not corrected for natural mortality (Abbott, 1925). LD_{50} values were determined after 24 h of exposure by Probit analysis (Finney, 1971).

At the end of the tests, all the adults (dead and alive) were removed and the Petri dishes were kept in the oven under the same conditions for an additional period of 45 days to assess emergence of F1 progenies. On day 45, samples of treated or control grains were taken to evaluate weight loss and germination capacity.

The weight loss was obtained using the formula described by Tefera *et al.* (2011): **Percent weight loss = (Initial weight - Final weight) × 100.**

In order to assess the viability of seeds, seed germination was tested using 50 randomly picked seeds from each Petri dish. The seeds were placed on moistened cotton in glass Petri dishes (13 cm in diameter and 3 cm in height) and incubated at room temperature (28-32°C) for 5 days (Kellouche *et al.*, 2004; Kumawat and Naga, 2013). The germination percentage was calculated as follows: **Germination (%) = (number of germinated seeds / total number of seeds) × 100.**

Statistical analysis

Data were expressed as mean values and standard deviations (SD) were calculated**.** All data were subjected to analysis of variance, using Stat Box Pro

(version 6.40). Significant differences between means were determined using Newman-Keuls test at 5% probability.

Results

Fatty acid composition

The results obtained (Tab. 1) show that the fatty acid composition of the olive oils analyzed meets the standards set by Commission Regulation EEC/2568/91 of July 11, 1991 for the EVOO category. The major fatty acids present in Chemlal olive oil were oleic (C18:1), linoleic (C18:2), palmitic (C16:0) acids (Tab.1). Oleic acid (C18:1) is the main monounsaturated fatty acid and is present in higher concentrations (59.08–63.59 %). Palmitic acid content, the major saturated fatty acid in olive oil, varied between 17.66 and 18.81% according to the plantation zones. Concerning linoleic acid (C18:2), the highest percentage was observed in EVO2 (15.06%), whereas the lowest was found in EVO1 (12.51%). Chemlal olives also contained low amounts of linolenic acid (C18:3), arachidic acid (C20:0) and traces of palmitoleic acid (C16:1) (Tab.1). Our results are in agreement with those of previous research (Bengana *et al.*, 2013; Bakhouche *et al.*, 2015; Laincer *et al.*, 2016; Medjkouh *et al.*, 2016; Boudour-Benrachou *et al.*, 2017; Guissous *et al.*, 2018).

Table 1. Fatty acid composition (%) of the four oils (C16:0, palmitic acid; C16:1, palmitoleic acid; C17:0, heptadecanoic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, alpha linolenic acid; C20:0, Arachidic acid; C20:1, eicosenoic acid;C22:0, behenic acid; MUFA/PUFA, monounsaturated fatty acids/ polyunsaturated fatty acids; UFA/SFA, unsaturated fatty acids/saturated fatty acids).

Fatty acid composition (%)					**Legal limits EEC/2568/91**
	EVO1	**EVO2**	**EVO3**	**EVO4**	
C16:0	17.8	18.81	17.97	17.66	7.5-20.0
C16 :1	2.32	3.13	2.26	2.26	0.3-3.5
C17:0	0.1	0.1	0.27	TR	
C18:0	1.88	2.19	2.12	2.58	0.5-5
C18 :1	63.59	59.08	62.28	60.39	55-83
C18 :2	12.51	15.06	13.68	14.81	3.5-21.0
C18 :3	0.68	0.38	0.52	0.51	≤1.0
C20:0	0.48	0.64	0.59	0.73	≤0.6
C20:1	0.44	0.27	0.27	0.27	≤0.4
C22:0	0.14	0.12	TR	TR	≤0.2
MUFA/PUFA	5.03	0.71	0.52	0.53	-
Oleic acid/ linoleic acid	5.08	3.92	4.55	4.08	-
UFA/SFA	3.89	2.25	/	/	-

Contact toxicity of olive oils against R. dominica

The results indicated that the four olive oils tested revealed contact toxicity as a function of the tested dose and the time of exposure. Variance analysis using 2 classification criteria shows that dose rate (F= 9599.58, *P*= 0,000) and origin of oil (F= 11.68, *P*= 0,000), as well as their interaction, act with a high degree of significance on the percentage of adult mortality.

In contact mortality assay after 24 h, 48, 72 and 96 h, the ascending concentrations of the four oils caused increased mortality of the beetles. After 24 h of exposure, all oils, at the highest dose (0.4 mL/25 g), are very toxic to *R. dominica*, the mortality rate ranging from 72.5 to 95%.

With the lowest dose (0.1 mL/25 g grains), all oils tested caused a low mortality rate ranging from 18.75 to 48.75%, even after 96 h of exposure (Fig.1A, B, C, D).The order of toxicity of the four oils, taking into account the LD_{50} (mL/25 g) calculated after 24 h of exposure, is as follows: OO2 (LD_{50} = 213), OO3 (LD_{50} = 232), OO1 (LD_{50} = 234) and OO4 (LD_{50} =263).

F1 progeny emergence

The four oils significantly reduced the emergence of adults, compared to the control. Variance analysis shows a highly significant effect of the factor dose (F= 1010.706, *P*= 0,000) on the number of emerged F1 progeny. However, the oil origin and the interaction (dose x oil origin) are not significant (F= 0.517, *P*= 0.676; F=0.424, *P*=0.915, respectively).

The highest numbers of emergence are observed in the control lots, with an average number of 92.5 adults. Moreover, the number of offspring is inversely proportional to the dose used. In fact, this number decreases as the dose is increased and becomes zero at the highest dose (0.4 mL/25 g grains), in all treatments (Fig.2).

Grain weight loss

All of the oils tested very significantly reduced the weight loss percentage, compared to the control. Variance analysis revealed a significant influence, at the 5% level, of the treatment dose on grain weight loss (F=749.465, *P*=0.000); however, the factor oil origin and the interaction between the two factors are not significant (F= 0.98, *P*= 0,411; F=0.681, *P*=0.723, respectively).

According to the results obtained (Fig.3), we observe that *R. dominica* caused higher weight loss in the control lots (9.62 %).On the other hand, in lots treated with oils from different regions, a considerable reduction in grain weight loss rates is observed as the treatment dose increases. For all oils tested, treatment with the highest dose 0.4 mL/25 g completely preserves the soft wheat seeds from pest attack.

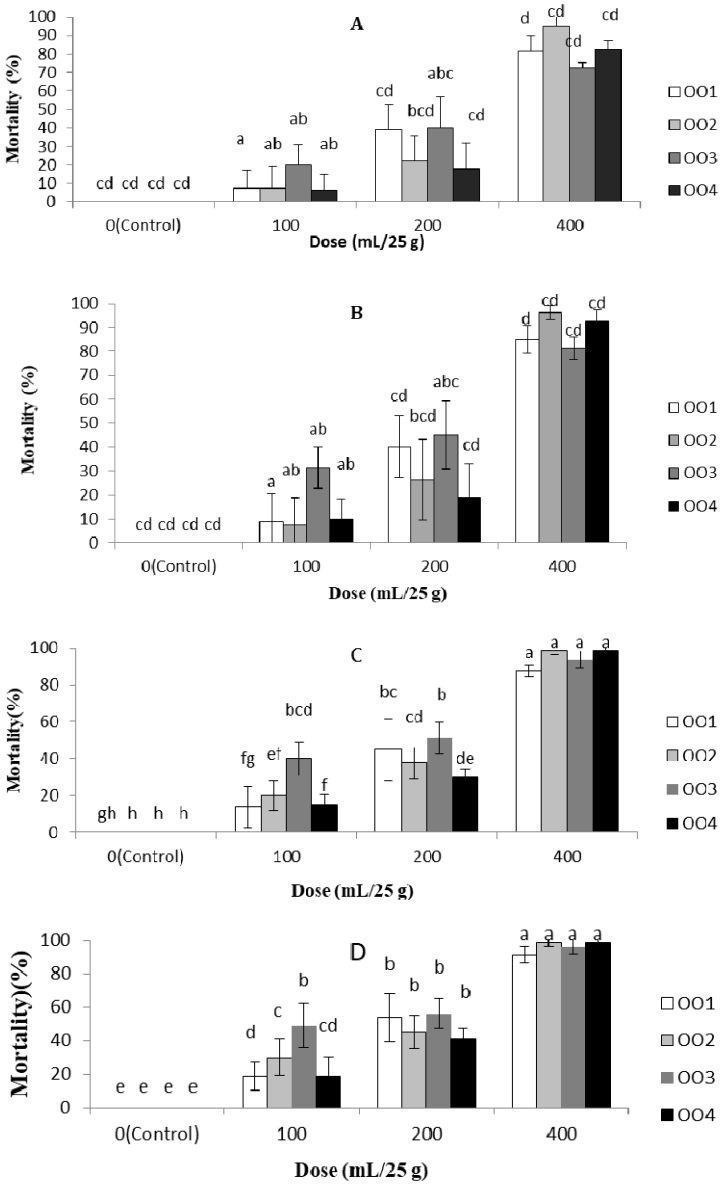

Figure1. Mortality of *R. dominica* in contact toxicity assay with four extra-virgin olive oils. (A)24h of treatment, (B) 48h, (C) 72h, (D) 96h. N = 80 (4 × 20 insects) for each treatment. Bars with the same letter are not significantly different.24h of treatment. Kruskal Wallis test with multiple comparisons, $P< 0.05$.

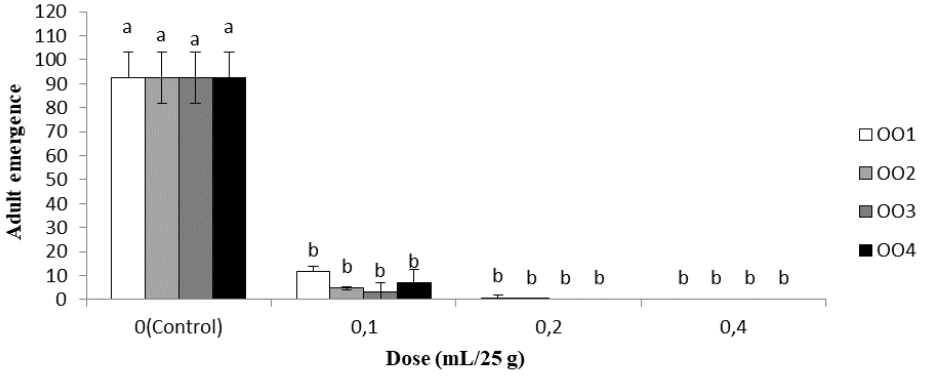

Figure 2. Mean progeny production (number of individuals/dish ±SE) on soft wheat treated with the four oils at three doses. Bars with the same letter are not significantly different. Kruskal Wallis test with multiple comparisons, *P*< 0.05.

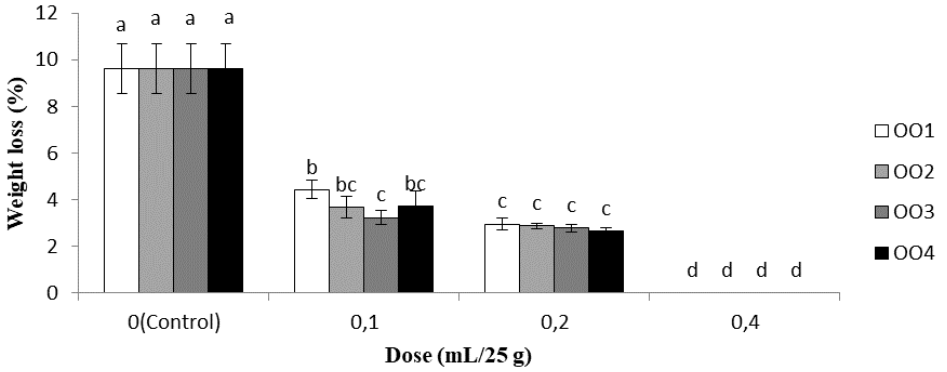

Figure 3. Percentage weight loss (%±SD) on soft wheat seeds treated with the four oils at three doses. Bars with the same letter are not significantly different. Kruskal Wallis test with multiple comparisons, *P*< 0.05.

Seed germination

According to the ANOVA, seed germination was significantly adversely affected as olive oil concentration increased (F=29.952, *P*=0.000). As the olive oil dose levels increased, the soft wheat seed germination rate decreased. But the oil origin factor and the dose-origin interaction do not influence germination, F=0.367, *P*=0.780 and F=1.153, *P*=0.345, respectively.

The tests carried out show that the germination capacity of wheat seeds from untreated and infected control lots reaches 92.50%, while this rate decreases as the dose increases in seeds treated with the various oils, ranging from 79.5% (0.1 mL/25g) to 45% (0.4 mL/25g) (Fig.4).

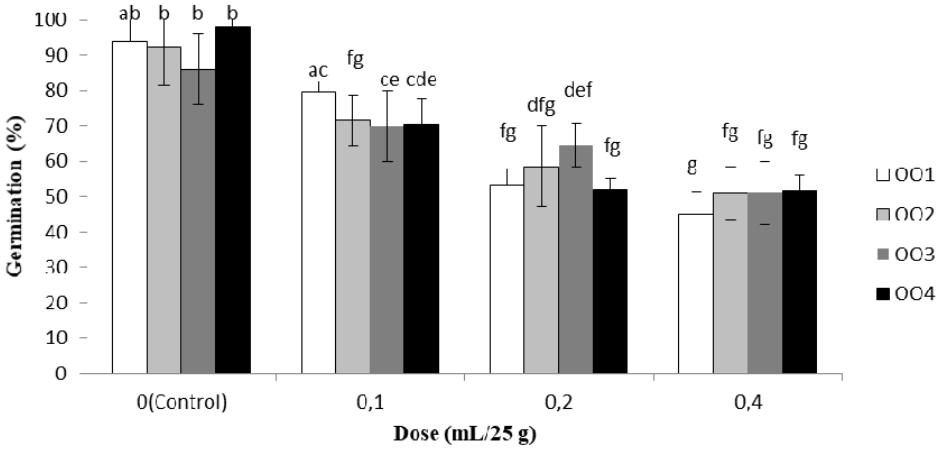

Figure 4. Percentage germination (%±SD) of soft wheat seeds treated with the four oils at different rates and infested by *R. dominica*. Bars with the same letter are not significantly different. Kruskal Wallis test with multiple comparisons, $P< 0.05$.

Discussion

This study showed the bioactivity of olive oils from different localities on one of the main insect pests of stored cereals, *R. dominica*. These oils showed contact toxicity against adults of this pest, a significant reduction of its offspring and consequently a reduction in weight loss caused to grains. Moreover, the effectiveness of treatments varies according to the dose of these natural substances. These results confirm those of previous studies that have highlighted the efficacy of different vegetable oils to protect cereal grains against damage caused by different species of insects in stored products (Saxena and Singh 1994; Abdallah *et al.*, 2001; Chander, 2003; Rahman *et al.*, 2003; Yadav *et al.*, 2008; Fogang *et al.*, 2012; Hossain *et al.*, 2014; Rayhan *et al.*, 2014; Gumaa and Elamin, 2015; Wahedi *et al.*, 2015; Chakravarty *et al.*, 2020).

The results obtained reveal that after treatment of soft wheat seeds, at the dose of 0.4 mL/25g, the adults of *R. dominica* live less than 24 h, thus preventing the females from laying eggs and, consequently, the emergence of new offspring.

Similar observations have been reported by other authors (Uvah and Ishaya, 1992; Ibrahim, 2012; Uddin and Sanusi, 2013; Parmar and Patel, 2015; Ekoja and Ogah, 2020) on the toxicity of olive oil and other vegetable oils against *Callosobruchus maculatus* (Coleoptera: Bruchidae) infesting cowpea seeds.

Other authors such as Wale and Assegie (2015) found that castor bean oil (*Ricinus communis*L.), applied at a dose of 4mL, against *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) caused 85% mortality after 1 h of exposure.In addition, groundnut, rape seed and sunflower vegetable oils, at 10 mL/kg of grain, caused considerable mortality in adults of *S. granarius* L. (Coleoptera: Curculionidae) (60-80%) in 14 days (Tembo and Murfitt, 1995). On the other hand, treatment of maize kernels with fixed oils from *Jatropha curcas* seeds (Euphorbiaceae) pretreated with different methods (cooking, roasting and raw) (1.50 µl / cm^2, for 3 h) induced47.52, 46.96 and 47.36% mortality for oil obtained from roasted, cooked and raw seeds, respectively, in *S. zeamais* (Babarinde *et al.*, 2019). Zohry *et al.* (2020) showed that black seed oil (*Nigella sativa*), sesame oil (*Sesamum indicum*) and olive oil (*Olea europaea*) at 1mL/100g are toxic to adults of *S. granarius* (83.33-100%) in 72h. The research of Kellouche *et al.* (2004) also reveals that olive oil causes total mortality of adults of *C. maculatus* after 2 h of treatment with 0.8 mL/50g cowpea seeds. The same is true for the work carried out by Gemechu *et al.* (2013) who demonstrated the efficacy of mustard and cottonseed oils (0.2 to 0.5 mL/250 g wheat), which cause mortality rates ranging from 25 to 100 % and reduce egg-laying in *S. zeamais*, without affecting the germination capacity of the seeds. According to the aforementioned authors, these treatments with olive oil induce the formation of a film that causes asphyxiation of the insect pest, as also demonstrated by Ait-Aider *et al.* (2016) in *C. maculatus*. In addition, adult mortality may have been caused by the action of saturated and unsaturated fatty acids that compose the chemical profile of olive oils (Hil and Schoonhoven, 1981). Moreover, some authors (Don-Pedro, 1990; Ait-Aider *et al.*, 2016) have highlighted the insecticidal effect of oleic acid and linoleic acid against *C. maculatus.* Regnault-Roger *et al.* (2002) also revealed the insecticidal activity of certain fatty acids such as oleic acid and undecylenic acid. These compounds cause the rupture of cell membranes, oxidative phosphorylation and insect cuticles (Weinzierl, 2000).

More recently, the insecticidal properties of certain volatile fatty acids (formic, acetic, propiopic, butyric and valeric acid) (Krzyżowski *et al.*, 2020) and a mixture of three free fatty acids, octanoic, nonanoic and decanoic acids (C8910) (Ramadan *et al.*, 2020) have been demonstrated against *C. maculatus, Lasioderma serricorne* (Coleoptera: Ptinidae) and *R. dominica*, respectively.

Variation in oleic and linoleic acid content observed in olive oil samples obtained from the Chemlal variety are probably related to both genetic factors and environmental conditions during fruit development and maturity (Arslan *et al.*, 2013; Essiari *et al.*, 2014; Rondanini *et al.*, 2014; Piscopo *et al.*, 2016; Borges *et al.*, 2017; García-Inza *et al.*, 2018).

Concerning the effect of treatments on the emergence of *R. dominica*, we observed a significant reduction in the number of first generation offspring when the dose is increased, regardless of the geographical origin of the oil tested. This may be a consequence of the reduction in oviposition and the ovicidal and larvicidal effects of the products tested (Shaaya *et al.*, 1997; Rolania and Bhargava, 2015).

This toxicity has also been observed by many authors such as Singh and Mall (1991) in *S. oryzae* exposed to soft wheat seeds treated with castor, neem, mustard and linseed oils (dose= 0.1%, v/w), Kellouche *et al.* (2004) with *C. maculatus* (reduction of emergence greater than 90%) in treatments carried out with 1st and 2nd pressing olive oils, and Singh *et al.* (2016) who demonstrated the biocidal activity of neem and castor oils and recorded a significant reduction in progeny in *R. dominica*.

In lots treated with olive oils, we observed a very significant reduction in weight losses of wheat seeds infested by *R. dominica*, which is most probably the consequence of the reduction in emergence of the insect pest as observed by Khinchi *et al.* (2017) in *Callosobruchus chinensis* (Coleoptera: Bruchidae), infesting chickpea grains treated with neem, groundnut, coconut and sesamum (4, 8 and 12 mL/kg grains), or Dey and Sarup (1993) with *S. oryzae* on maize seeds treated with mustard, soya bean, coconut, neem, groundnut, cotton seed, sesame and castor oils. The same observations were also reported by Kumawat and Naga (2013), Akter *et al.* (2019) and Chakravarty *et al.* (2020) on pests such as *R. dominica*, with wheat grains, and *C. chinensis*, infesting green mung pulse (*Vigna radiata*) or chickpeas, in treatments with several vegetable oils such as black seed (*Nigella sativa*), neem, castor, Karanj, coconut, sesame, soybean, and mustard, for example.

Regarding germination tests, the results obtained reveal that the treatments with the different oils affect the germination capacity of the wheat grains as olive oil concentrations increased, as reported by Tembo and Murfitt (1995) testing high doses of groundnut, rape seed and sunflower (10 mL/kg) against *S. granarius*, as also reported by Yun-tai and Burkholder (1981) with oils of cotton seed, soybean, maize and peanut (5 or 10mL/kg), and Ivbijaro (1984) obtaining a maize kernel viability ranging from 6 to 13% after treatment with groundnut oil (10 to 20 mL/kg), compared to the untreated control (100%). However, several authors have reported the safety of vegetable oils

as noted by Singh *et al.* (2016) testing neem and castor oils (0.1 to 0.20% v/w) on *R. dominica* infesting wheat grains, and Hassan (2001)testing sesame, sunflower and castor oils (0.1 to 1.25 % v/w) on *Trogoderma granarium* (Coleoptera: Dermestidae).

Conclusion

The results of our tests show that the four olive oils tested are highly toxic to *R. dominica.* In fact, his toxicity is increased as the dose increases. Comparison of LD_{50} indicates that olive oil extracted from Maatkas olives is more effective than oils from other olive groves (Bachloul, Tadmait and M'Chedellah).

This study confirmed the agro-phytosanitary potential of Algerian olive oil, which can be used locally as a bio-pesticide for the protection of wheat seeds whose preservation is a major challenge for Algeria.

Since Algeria has a diversified olive-growing heritage, the valorization of these plant extracts as part of an integrated control program in the eco-chemical fight against insect pests of stored grains, in order to reduce the significant economic loss recorded each year in storage warehouses, is an interesting option.

Acknowledgements. We are grateful to the Laboratory of Entomology, University of Tizi-Ouzou (Algeria). We would like also to thank M. Paul for checking the English translation.

References

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol*. 18, 265-267.

Abdallah, S.A., Badawy, H.M.A., Barakat, A.A., & El-Sabaay, T.N. (2001). Efficacy of certain vegetable oils as wheat grain protectants against the lesser grains borer, *Rhyzopertha dominica* (Fabricius). *Bull Facult Agric Cairo Univ,*52, 167-82.

Abdelli, M., Moghrani, H., Aboun, A., & Maachi, R. (2016). Algerian *Mentha pulegium* L. leaves essential oil: chemical composition, antimicrobial, insecticidal and antioxidant activities. *Ind. Crop. Prod*, 94, 197-205.

Ahmed, M. (2016). Faunal diversity in a cereal medium of Chlef region (Algeria). *J Entomol Zool Stud*, 4(5), 1102-1106.

Ait-Aider, F., Kellouche, A., Fellag, H., & Debras, JF. (2016). Evaluation of the bio-insecticidal effects of the main fatty acids of olive oil on *Callosobruchus maculatus* F. (Coleoptera-Bruchidae) in cowpea (*Vigna unguiculata* (L.). *J. Plant Dis. Prot*,123(5), 235-245.

Aitken, A.D. (1975). Insect Travelers, I; Coleoptera. Techn. Bull.31. *H.M.S.O*, London, pp.190.

Akter, S., Sultana, S., Akter, T., & Begum, S. (2019). Oviposition deterrent and ovicidal activity of three edible oils (black seed, sesame and soybean oils) against pulse beetle, *Callosobruchus chinensis* (lin.) (Coleoptera: Bruchidae) on green gram, *Vigna radiata. Bangladesh j. zool*, 47(1), 59-65.

Aoues, K., Boutoumi, H., & Benrima, A. (2017). Phytosanitary status of local durum wheat stored in Algeria. *J. Agrobiol*, 7 (1), 286–296.

Arslan, D., Karabelkir, Y., & Schreiner, M. (2013). Variation of phenolic compounds fatty acids and some qualitative characteristics of Sarıulak olive oi as induced by growing area. *Food Res. Int*, 54, 1897-1906.

Arthur, F.H., Bean, S.R., Smolensky, D., Cox, S., Lin, H.H., Peiris, K.H.S., &Peterson, J. (2020). Development of *Rhyzopertha dominica* (Coleoptera: Bostrychidae) on sorghum: Quality characteristics and varietal susceptibility. *J Stored Prod Res*, 87, 101-569.

Arve, S.S., Chavan, S.M., & Patel, M.B. (2014). Effect of rice weevil (*Sitophilus oryzae* Linnaeus) infestation on the biochemical properties of six wheat varieties. *Tr Biosci,* 7, 925-934.

Babarinde, G.O., Babarinde, S.A., Ojediran, T.K., Odewole, A.F., Odetunde, D.A., & Bamido, T.S. (2019). Chemical composition and toxicity of *Jatropha curcas* seed oil against *Sitophilus zeamais* Motschulsky as affected by pre-extraction treatment of seeds. *Biocatal Agric Biotechnol*, 21, 101-333.

Baccari, W., Znati, M., Zardi Bergaoui, A., Chaieb, I., Flamini, G., Ascrizzi, R., & Jannet, H. B. (2020). Composition and insecticide potential against *Tribolium castaneum* of the fractionated essential oil from the flowers of the Tunisian endemic plant *Ferula tunetana* Pomel ex Batt. *Ind Crops Prod*, 111888.

Bakhouche, A., Lozano-Sanchez, J., Bengana, M., Guttièrrez, A. F., & Carretera, A. S. (2015). Time course of algerian Azeradj extra virgin olive oil quality during olive ripening. *Eur J Lipid Sci Technol*, 117(3), 389-397.

Bashir, T. (2002). Reproduction of *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) on different host grains.*Pakist J Biol Sci,*5, 91-93.

Batta, Y., Saleh, A., & Salameh, A. (2007). Evaluation of the susceptibility of wheat cultivars to lesser grain borer *Rhyzopertha dominica* (Fab.), (Coleoptera: Bostrychidae). *Arab J Pl Prot*, 25(2),159–162.

Bellatreche, A., Mnasri, S., Ben Naceur, M., & Gaouar, S.S.B. (2019). Study of the Molecular Biodiversity of the Saharan Bread Wheat in Algeria. *Cereal Res Comm*, 47, 724-739.

Bengana, M., Bakhouche, A., Lozano-Sánchez, J., Amir, Y., Youyou, A., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2013). Influence of olive ripeness on chemical properties and phenolic composition of Chemlal extra-virgin olive oil. *Int. Food Res. J*, 54, 1868 – 1875.

Borges, T.H., Pereira, J.A., Cabrera Vique, C., Lara, L., Oliveira, A.F., & Seiquer, I. (2017). Characterization of Arbequina virgin olive oils produced in different regions of Brazil and Spain: physicochemical properties, oxidative stability and fatty acid profile. Food Chem, 215, 454-462.

Boudour-Benrachou, N., Plard, J., Pinatel, C., Artaud, J., & Dupuy, N. (2017). Fatty acid compositions of olive oils from six cultivars from east and south-western Algeria. *Adv Food Technol Nutr Sci*, 3(1), 1-5.

Boukouvala, M.C., Romano, D., Kavallieratos, N.G., Athanassiou, C.G., Stefanini, C., Conte, G., Canale, A., & Benelli, G. (2020). Does geographical origin affect lateralization and male mating success in *Rhyzoperha dominica* beetles. *J. Stored Prod Res*., 88, 101630.

Boyer, S., Zhang, H., & Lempérière, G. (2012). A review of control methods and resistance mechanisms in stored-product insects. *Bull. Entomol. Res*, 102, 213–229.

Brahmi, F., Khodir, M., Mohamed, C., &Pierre, D. (2017). Chemical Composition and Biological Activities of *Mentha* Species, In Tech: *In Aromatic and Medicinal Plants-Back to Nature*, pp.47-80.

Chakravarty, M.K., Prasanthi, S.J., & Kumar, S.K.J.R. (2020). Evaluation of eight plant oils against pulse beetle, *Callosobruchus chinensis* Linnaeus (Bruchidae: Coleoptera) in chickpea under storage conditions. *Entomol Zool Stud*, 8(5), 1262-1267.

Chander, R. (2003). Host preference and bio-control studies of *Rhyzopertha dominica* (Fabricius) on barley and its management. Ph.D (Agri.), Rajasthan Agricultural University, Bikaner, pp. 243.

Chen, A., & Dubcovsky, J. (2012). Wheat TILLING mutants show that the vernalization gene *VRN1* down-regulates the flowering repressor *VRN2* in leaves but is not essential for flowering. *PLOS Genet*, 8, e1003134.

Chenni, M., El Abed, D., Neggaz, S., Rakotomanomana, N., Fernandez, X., & Chemat, F. (2020). Solvent free microwave extraction followed by encapsulation of *O. basilicum* L. essential oil for insecticide purpose. J. *Stored Prod. Res*., 86, 101575.

Collins, P.J., & Schlipalius, D.I. (2018). Insecticide resistance, In:*Recent Advances in Stored Product Protection*, Athanassiou, C.G., Arthur, F.H. (Eds.), Springer Verlag, *GmbH*, Germany, pp. 169-182.

Daglish, G.J., & Nayak, M.K. (2018). Prevalence of resistance to deltamethrin in *Rhyzopertha dominica* (F.) in eastern Australia. *J. Stored Prod. Res*, 78, 45e49.

Dey, D., & Sarup, P. (1993). Feasibility of protecting maize varieties with vegetable oils to save losses in storage due to *Sitophilus oryzae* (Linnaeus). *J Ent Res,* 17, 1-15.

Djidel, A., Daghbouche, S., Benrima, A., & Djazouli, Z. (2018). Evaluation of the insecticidal activity of the raw aquatic extract of the fabacae *Cytisus triflorusl'* Her. regarding *Tribolium castaneum* (herbst, 1797) (Coleoptera: Tenebrionidae). *J. Agrobiol*, 8 (2), 1093–1102.

Don-Pedro, K.N. (1990). Insecticidal activity of fatty acid constituents of fixed vegetable oils against *Callosobruchus maculatus* on cowpea. *Pestic. Sci,* 30, 295-302.

Ebrahimifar, J., Jamshidnia, A., Sadeghi, R., & Ebadollahi, A. (2020). Repellency of *Ferulago angulata* (Schlecht.) Boiss essential oil on two major stored-product insect pests without effect on wheat germination. *Int. J. Trop. Insect Sci*, 1-7.

Edde, P.A. (2012). A review of the biology and control of *Rhyzopertha dominica* (F.) the lesser grain borer. *J. Stored Prod. Res*, 48, 1e18.

Ekoja, E.E., & Ogah, B.E. (2020). Efficacy of oils from nine plant species as protectants against infestation by *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae). *WJARR*, 07(03), 007–015.

Essiari, M., Zouhair, R., & Chimi, H. (2014). Contribution to the study of the typical characteristics of the virgin olive oils produced in the region of Sais (Morocco), *OLIVÆ*, 119, 8-21.

European Union (2001). Commission Regulation (EEC) No. 1513/2001 of 23 July 2001 amending Regulations No. 136/66/EEC and (EC) No. 1638/98 as regards the extension of the period of validity of the aid scheme and the quality strategy for olive oil. *Official Journal of the European Communities*, L201, 4–7.

Eydozehi, K., & Ravan, S. (2013). Evaluation of wheat cultivars resistant to insect priest or fine grain beetle, *Rhyzopertha dominica* (Fabricius). *Ann Biol Res,* 4, 228-233.

FAO (2015). The State of Food Insecurity in the World,FAO, Rome, Italy, pp.1e56.

FAO (2019). World food and agriculture, Statistical Pocketbook, FAO, Rome, Italy,pp. 254.

Filomeno, C.A., Almeida Barbosa, L.C., Teixeira, R.R., Pinheiro, A.L., de Sá Farias, E., Ferreira, J.S., & Picanço, M.C. (2020). *C*hemical diversity of essential oils of *Myrtaceae* species and their insecticidal activity against *Rhyzopertha dominica*. *Crop Prot, 105309.*

Finney, D.J. (1971). Probit analysis, 3rd Ed, *Cambridge University Press*, London, U.K, pp.333.

Fogang, H.P.D., Womeni, H.M., Piombo, G., Barouh, N., & Tapondjou, L.A. (2012). Bioefficacy of essential and vegetable oils of *Zanthoxylum xanthoxyloides* seeds against *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). *J. Food Prot*, 75, 547–555.

García-Inza, G.P., Hall, A.J. & Rousseaux, M.C. (2018). Proportion of oleic acid in olive oil as influenced by the dimensions of the daily temperature oscillation. *Sci Hort,* 227, 305–312.

Garcia-Lara, S., & Serna-Saldivar, S.O. (2016). Insect pests, In: *Encyclopedia of food and health*. *Academic Press*, Caballero, B., Finglas, PM., (eds), London, pp. 432-436.

Gemechu, F., Santiago, D.R., & Sori, W. (2013). Laboratory evaluation of cotton *(Gossypium hirsutum)* and Ethiopian mustard *(Brassica cariata*) seed oils as grain protectants against maize weevil, *Sitophilus zeamais* M. (Coleoptera: Curculionidae). *African J. of Agric. Res*, 8(32), 4374-4379.

Guissous, M., Le Dréau, Y., Boulkhroune, H., Madani, T., & Artaud, J. (2018). Chemometric characterization of eight monovarietal Algerian Virgin Olive Oils. *J Am Oil Chem Soc*, 95, 267-281.

Gumaa, A.G.A., & Elamin, A.E.H. (2015). Efficacy of groundnut oil, wood ash, sand and some plant powders against *Callosobruchus chinensis* in cowpea seeds*. J. Agr. Sci. Technol, B*, 5, 647- 652.

Hagstrum, D.W., & Flinn, P.W. (2014). Modern stored- product insect pest management. *J Plant Prot Res*, 54, 205-210.

Hamdi, S.H., Hedjal-Chebheb, M., Kellouche, A., Khouja, M.L., Boudabous, A., & Jemaa, J.M.B. (2015). Management of three pests' population strains from Tunisia and Algeria using Eucalyptus essential oils. *Ind. Crops Prod*, 74, 551-556.

Haouel-Hamdi, S., Ben Hamedou, M., Bachrouch, O., Boushih, E., Zarroug, Y., Sriti, J., Messaoud, C., Hammami, M., Abderraba, M., Limam, F., & Mediouni-Ben Jemâa, J. (2020). Susceptibility of *Tribolium castaneum* to *Laurus nobilis* essential oil and assessment on semolina quality. *Int J Trop Insect Sci*, 40(3), 667–675.

Hassan, T.A. (2001). Effect of three plant oils (Sesame, sunflower and castor) against stored grain insects (*Trogoderma granarium* and *Sitophilus granarius*). *Uni of Aden, J. of Nat and Appl Scie*, 5, 103-110.

Hendrival, H., Afriani, D., & Aryani, D.S. (2019). Susceptibility and damage cereals to infestation *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) in storage*. Journal, Agro*, 6(1), 57-65.

Hill, D.S. (2002). Pests of Stored Foodstuffs and Their Control. *Kluwer Academic Publishers*, Boston, pp. 855.

Hill, J. & Schoonhoven, A.V. (1981). Effectiveness of vegetable oil fractions in controlling the Mexican bean weevil on stored beans. *J. Econ. Entomol*, 74, 478–479.

Hossain, M.A., Alim, M. A., Ahmed, K.S., & Haque, M.A. (2014). Insecticidal potentials of plant oils against *Callosobruchus chinensis* (Coleoptera: Bruchidae) in stored chickpea. *JESI*, 34(3), 47-56.

Huchet, J.B. (2017). Le Coléoptère, la Graine et l'Archéologue: approche archeoentomologique des principaux ravageurs des denrées stockées ; In: *Plantes, produits végétaux et ravageurs*, Diestch Sellami, M.F., *et al.* (Eds.), Aquitania, Burdeos, pp. 17–42.

Ibrahim, M.Y. (2012). Efficacy of some plant oils against stored-product pest cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Bruchidae) on chickpea seeds. *Persian Gulf crop* protect*,* 1(1), 4-11.

International Olive Council (IOC) (2011). Guide for the determination of the characteristics of oil-olives. COI/OH/Doc. No: 1. COI/OH/ Doc. n°1, November 2011. IOC, Madrid, Spain, pp.34.

Ivbijaro, M.F. (1984). Toxic effect of groundnut oil on the rice weevil, *Sitophilus oryzae* (L.). *Insect Sci. Appl*, 5, 251-252.

Jood, S, Kapoor, A.C., & Singh, R. (1995). Amino acid composition and chemical evaluation of protein quality of cereals as affected by insect infestation. *Plant Foods Hum. Nutr,* 48(2), 159–167.

Kakde, S.P., Dhonde, S.V., Sarda, A.L., Khillare, P.W., & Deshwal, H.L. (2014). Screening of wheat varieties and eco-friendly management of *Rhyzopertha dominica* (Fabricius) on wheat. *Pl Archs,* 14, 431-437.

Kara, K., Rached-Kanouni, M., MnasrI, S., Khammar, H., & Ben Naceur, M. (2020). Genetic variability assessment in bread wheat (*Triticum aestivum*) grown in Algeria using microsatellites SSR markers. *Biodiversitas*, 21, 2638-2644.

Kellouche, A., Ait-Aider, F., Labdaoui, K., Moula, D., Ouendi, K., Hamadi, N., Ouramdane, A., Frerot, B., & Mellouk, M., 2010. Biological activity of ten essential oils against cowpea beetle, *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae). *Int. J. Integr. Biol*, 10, 86–89.

Kellouche, A., & Soltani, N. (2004). Activité biologique des poudres de cinq plantes et de l'huile essentielle d'une d'entre elles sur *Callosobruchus maculatus* (F.). *Int. J. Trop. Insect Sci*, 24, 184–191.

Kellouche, A., Soltani, N., Kreiter, S., Auger, J., Arnold, I., & Kreiter, P. (2004). Biological activity of four vegetable oils on *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). *Redia.* LXXXVII, 39-47.

Khinchi, S.K., Sharma, M.M., Khinchi, M.K., Bairwa, D.K., Dinesh, A., Naga, B.L., & Naga, R.P. (2017). Studies on efficacy of certain vegetable oils against pulse beetle, *Callosobruchus chinensis* Linn. on chickpea, *Cicer arietinum* (L.). Int. J. *Chem. Stud*, 5(3), 255-259.

Kim, K.H., Kabir, E., & Jahan, S.A. (2017). Exposure to pesticides and the associated human health effects. *Sci Total Environ*, 575, 525-535.

Krzyżowski, M., Francikowski, J., Baran, B., & Babczyńska, A. (2020). *The short-chain fatty acids as potential protective agents against Callosobruchus maculatus infestation*. J. Stored Prod. Res*, 86,* 101570.

Kumar, D., & Kalita, P. (2017). Reducing postharvest Losses during Storage of Grain Crops to Strengthen Food Security in Developing Countries. *Foods*, 6(1), 8.

Kumawat, K.C., & Naga, B.L. 2013. Effect of plant oils on the infestation of *Rhyzopertha dominica* (Fabricius) in wheat, *Triticum aestivum* (Linnaeus). *J. Plant Prot. Res*, 53, 301-04.

Laincer, F., Iaccarino, N., Amato, J., Pagano, B., Pagano, A., Tenore, G., Tamendjari, A., Rovellini, P., Venturini, S., Bellan, G., Ritieni, L.M., Novellino, E., & Randazzo, A. (2016). Characterization of monovarietal extra virgin olive oils from the province of Béjaïa (Algeria). *Int. Food Res. J*, 89, 1123–1133.

Lal, D., & Raj, D.V. (2012). Efficacy of application of four vegetable oils as grain protectant against the growth and development of *Callosobruchus maculatus* and on its damage. *Adv Biores*, 3, 55-59.

Limonta, L., Morosini, M.C., & Locatelli, D.P. (2011). Development of *Rhyzopertha dominica* (Fabricius) (Coleoptera Bostrychidae) on durum wheat kernels and semolina. *J Ent Acarol Res Ser II*, 4, 33-38.

Lougraimzi, H., El Iraqui, S., Bouaichi, A., Gouit, S., Achbani, E.H., & Fadli, M. (2018). Insecticidal effect of essential oil and powder of *Mentha pulegium* L. leaves

against *Sitophilus oryzae* (Linnaeus, 1763) and *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Curculionidae, Tenebrionidae), the main pests of stored wheat in Morocco. *POL J ENTOMOL*, 87, 263– 278.

Mason, L.J., & McDonough, M., (2012). Biology, behavior, and ecology of stored grain and legume insects. In: *Stored Product Protection*, Hagstrum, D.W., Phillips, T.W., Cuperus, G. (Eds.), Kansas State University, Manhattan, KS, pp. 7e20.

Mau, Y.S., Collins, P.J., Daglish, G.J., Nayak, M.K., & Elbert, P.R. (2012). The rph2 gene is responsible for high level resistance to phosphine in independent field strains of *Rhyzopertha dominica. PloS One* 7: e34027.

Medjkouh, L., Tamendjari, A., Keciri, S., Santos, J., Nunes, M.A., & Oliveira, M.B. (2016). The effect of the olive fruit fly (*Bactrocera oleae*) on quality parameters, and antioxidant and antibacterial activities of olive oil. *Food Funct*, 7(6), 2780–2788.

Merouche, A., Debaeke, P., Messahel, M., & Kelkouli, M. (2014). Response of durum wheat varieties to water in semi-arid Algeria. *Afr. J. Agric. Res*, 9 (38) ,2880-2893.

Meziani, S., Saidani, S., Menadi, N., Mehida, H., Chenni, F.Z., Zairi, M., Labga, L., Benguella, R., & Benali, M. (2020). Comparative assessment of bioactive compounds and antioxidant activity of soft wheat bran on the Algeria market. *Not. Sci. Biol*, 12(3), 741-751.

Morrison, W.R., Bruce, A., Wilkins, R.V., Albin, C.E., & Arthur, F.H. (2019). Sanitation improves stored product insect pest management. *Insects*, 10, 1e20.

Nayak, M., Daglish, G., Phillips, T.W., & Ebert, P.R. (2020). Resistance to the fumigant phosphine and its management in insect pests of stored products: a global perspective. *Ann Rev Entomol*, 65,333–350.

Nia, B., Lekbir, A., & Ben Salah, M.K. (2020). Insecticidal and antioxidant activities of aqueous extracts of two Algerian medicinal plants. *Acta Entomol. Serbica*, 25(1), 67-75.

Obeng-Ofori, D., & Amiteye, S. (2005). Efficacy of mixing vegetable oils mixed with pirimiphosmethyl against the maize weevil, *Sitophilus zeamais* Motschulsky in stored maize.J. *Stored Prod. Res*, 41, 56- 57.

Park, S.H., Arthur, F.H., Bean, S.R., & Schober, T.J. (2008). Impact of differing population levels of *Rhyzopertha dominica* (F.) on milling and physicochemical properties of sorghum kernel and flour. *J. Stored Prod. Res,* 44, 322-327.

Parmar, V.R., & Patel, B.H. (2015). Evaluation of Plant Oils as Grain Protectants against Pulse Beetle, *Callosobruchus chinensis* on Mung Bean. *Trends in Biosci*, 8(15), 3855-3864.

Piscopo, A., De Bruno, A., Zappia, A., Ventre, C., & Poiana, M. (2016). Characterization of monovarietal olive oils obtained from mills of Calabria region (Southern Italy). *Food Chem*, 213, 313-318.

Rahman, M.A., Taleb, M.A., & Biswas, M.M. (2003). Evaluation of botanical product as grain protectant against grain weevil, *Sitophilus granarius* (Linnaeus) on wheat. *Asian J Pl Sci,* 2, 501-504.

Ramadan, G.R.M., Abdelgaleil, S.A.M., Shawir, M.S., El-bakary, A.S., Zhu, K.Y., & Phillips, T.W. (2020). Terpenoids, DEET and short chain fatty acids as toxicants and repellents for Rhyzopertha dominica (Coleoptera: Bostrychidae) and *Lasioderma serricorne* (Coleoptera: Ptinidae). *J. Stored Prod. Res*, 87, 101610.

Rayhan, M.Z., Das, S., Sarkar, R., Adhikary, S.K., Tania, S.N., Islam, M.M., & Rabbani, M.G. (2014). Bioefficacy of neem, mahogoni and their mixture to protect seed damage and seed weight loss by rice weevil in storage. J. Biodivers. Environ. Sci, 5(1), 582-589.

Regnault-Roger, C., Philogene, J.R.B., & VIncent, C. (2002). Bio- pesticides d'origine végétale. *2* Ed*. Tec et Doc* Paris ; Lavoisier, pp, 319.

Ridley, A.W., Hereward, J.P., Daglish, G.J., Raghu, S., McCulloch, G.A., & Walter, G.H. (2016). Flight of *Rhyzopertha dominica* (Coleoptera: Bostrychidae) a spatiotemporal analysis with pheromone trapping and population genetics. *J. Econ. Entomol*, 109, 2561e2571.

Righi, K., Assia Righi, F., Boubkeur, A., Boungab, K., Elouissi, A., & Djendara, A.C. (2018). Toxicity and repellency of three Algerian medicinal plants against pests of stored product: *Ryzopertha dominica* (Fabricius) (Coleoptera: Bostrychidae). *Banats J* Biotechnol, IX(17), 50–59.

Rolania, K., & Bhargava, M.C. (2015). Evaluation of different plant oils against *Lasioderma serricorne* Fab. on fennel. *J. Nat. Appl*, 7 (1), 83 – 87.

Rondanini, D.P., Castro, D.N., Searles, P., & Rousseaux, M.C. (2014). Contrasting patterns of fatty acid composition and oil accumulation during fruit growth in several olive varieties and locations in a non-Mediterranean region. *Eur. J. Agron*, 52, 237–246.

Saad, A.S.A., Tayeb, E.H.M., El-Shazli, M.M., & Baheeg, S.A. (2018). Susceptibility of certain Egyptian and imported wheat cultivars to infestation by *Sitophilus oryzae* and *Rhyzopertha dominica*. *Arch. Phytopathol. Plant Prot*, 51, 14-29.

Saxena, A., & Singh, Y.P. (1994). Effect of some plant products as protectants against *Rhyzopertha dominica* Fabricius on wheat grains. *Bull Grain Tech,* 32, 117-121.

Shaaya, E., Kostjukovski, M., Eilberg, J., & Sukprakarn, C. (1997). Plant oils as fumigants and contact insecticides for the control of stored-product insects. *J Stored Prod Res,* 33, 7-15.

Singh, S., Sharma, D.K., & Gill, R.S. (2016). Evaluation of three plant oils for the control of lesser grain borer, *Rhyzopertha dominica* (Fabricius) in stored wheat. *J. Insect Sci*, 29,162-169.

Singh, Y.P., & Mall, N.P. (1991). Effect of various grain protectants on germination and damage of wheat grain by *Sitophilus oryzae* (Linnaeus). *Bull Grain Tech,* 29, 50-54.

Srivastava, C., & Subramanian, S. (2016). Storage insect pests and their damage symptoms: an overview. *Indian J Entomol* , 78, 53-58.

Tefera, T., Kanampiu, F., De Groote, H., Hellin, J., Mugo, S., Kimenju, S., Beyene, Y., Boddupalli, P.M., Shiferaw, B., & Banziger, M. (2011). The metal silo: an effective grain storage technology for reducing post-harvest insect and pathogen losses in maize while improving smallholder farmers' food security in developing countries. *J. Crop Prot*, 30, 240e245.

Tembo, E., & Murfitt, R.F.A. (1995). Effect of combining vegetable oil with pirimiphos-methyl for protection of stored wheat against *Sitophilus granarius* (L.). J. *Stored Prod. Res*, 31(1), 77-81.

Toews, M.D., Cuperus, G., & Phillips, T.W. (2000). Susceptibility of Eight U.S. Wheat Cultivars to Infestation by *Rhyzopertha dominica* (Coleoptera: Bostrychidae). *Environ Ent,* 29, 250-255.

Uceda, M., & Hermoso, M. (1998). La calidad del aceite de oliva en el cultivo del olivo. AAVV. 2ª Ed. *Coedición Junta de Andalucía- Mundiprensa*, Spain, pp.116-125.

Uddin, II R.O., & Sanusi, S.A. (2013). Efficacy of olive oil, groundnut oil, soybean oil and palm kernel oil in the control of *Callosobruchus maculatus* in stored cowpea (*Vigna unguiculata*),*Agrosearch,* 13,67-72.

Uvah, I.I., & Ishaya, A.T. (1992). Effect of some vegetable oils on emergence, oviposition and longevity of the bean weevil, *Callosobruchus maculatus* (F.). *Trop. Pest Manag*, 38, 257-260.

Valenzuela-Aragon, B., Parra-Cota F.I., Santoyo G., Arellano Wattenbarger G.L., & de Los Santos-Villalobos, S. (2019). Plant-assisted selection: a promising alternative for in vivo identification of wheat (*Triticum turgidum* L. subsp. Durum) growth promoting bacteria. *Plant Soil*, 435, 367–384.

Wahedi J.A., Zakariya, R., Danba, E.P., David, D.L., Mshelmbula,B.P., Buba, U., Barau, B.W., Dauda, U.D., & Bello, H. (2015). Ethnobotanical studies of the efficacy of five oils at graded levels on adult *Callosobruchus maculatus* reared on cowpea. *IJRR,* 2, 481-486.

Wale, M., & Assegie, H. (2015). Efficacy of castor bean oil (*Ricinus communis* L.) against maize weevils (*Sitophilus zeamais* Mots.) in northwestern Ethiopia. *J. Stored Prod. Res, 63*, 38–41.

Waongo, A., Traoré, F., Sankara, F., Dabiré-Binso, C., & Sanon, A. (2018). Evaluation du potentiel de développement de *Rhyzopertha dominica* F. (Coleoptera: Bostrychidae) sur deux variétés locales de sorgho (*Sorghum bicolor* [L.] Moench) du Burkina Faso*. Int. J. Biol. Chem. Sci,* 12**,** 5, 2143-2151

Weinzierl, R.A. (2000). Botanical insecticides, soaps and oils, In: *Biological and Biotechnological Control of Insect Pests*, Jack, E.R. (Ed.). Lewis Publishers, Boca Raton, pp. 101–121.

Win, N., & Rolania, K. (2020). Influence of seasonal variation on the biology of lesser grain borer, *Rhyzopertha dominica,* (Fabricius) on wheat. *J. Entomol. Zool*, 8(5), 285-290

Yadav, J.P., Bhargava, M.C., & Yadav, S.R. (2008). Effect of various plant oils on rice weevil, *Sitophilus oryzae* (Linnaeus) in wheat. *Indian J Pl Prot.,* 36, 35-39.

Yakhlef, G., Hambaba, L., Pinto, D.C.G.A., & Silva, A.M.S. (2020). Chemical composition and insecticidal, repellent and antifungal activities of essential oil of *Mentha rotundifolia* (L.) from Algeria. *Ind Crops Prod*, 158, 112988.

Yun-tai, Q.I., & Burkholder, W.E. (1981). Protection of stored wheat from the granary weevil by vegetable oils. *J. Econ. Entomol*., 74, 502-505.

Zohry, N.M.H., Ali, S.A., & Ibrahim, A.A. (2020). Toxicity of Ten Native Edible and Essential Plant Oils Against the Granary Weevil, *Sitophilus granarius* L. (Coleoptera: Curculionidae). *Egypt. Acad. J. Biolog. Sci*, 12(2), 219-227.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 73-84)
doi:10.24193/subbbiol.2021.1.02

Mycorestoration of crude oil polluted soil using *Pleurotus tuberregium*

Beckley Ikhajiagbe[1,3], Kingsley Erhons Enerijiofi[2,3]✉ and Paul O. Umendu[1]

[1]Environmental Biotechnology and Sustainability Research Group, Department of Plant Biology and Biotechnology, University of Benin, Nigeria; [2]Department of Biological Sciences, Samuel Adegboyega University, Ogwa Edo State, Nigeria; [3]Applied Environmental Bioscience and Public Health Research Group, Department of Microbiology, University of Benin, Nigeria; ✉***Corresponding author, E-mail: kingsmore1luv@gmail.com; kenerijiofi@sau.edu.ng****.*

Article history: Received: 9 March 2021; Revised 22 May 2021; Accepted 29 May 2021; Available online 30 June 2021.

Abstract. Crude oil contamination is known to cause unwholesome damage to man, his environment comprising of soil, air and water bodies as well as other forms of life. This study determined the effect of crude oil polluted soils on the composition of different microorganisms and plants and the growth of *Pleurotus tuberregium*. Oil polluted soils in bowls were amended with sawdust from *Brachystegia nigerica* as substrate. Fruiting bodies and the diameter of the mushroom cap were found to increase with increasing weeks of exposure to oil as against the control which had no fruiting bodies throughout the experiment. *Pepperomia pellucida* was found to be the predominant weed (n = 20), followed by *Asystasia gangetica* (n = 11). The bacterial and fungal counts were higher at the oil polluted soil attached to sclerotia than the control. The growth of *Pleurotus tuberregium* in the polluted soil samples showed its ability to degrade and utilize hydrocarbon as the source of carbon and energy, thereby remediating the contaminated soil environment. This work has shown that the fungus has bioremediation and pollution control capacity.

Keywords: crude oil, environmental sustainability, *Pleurotus tuberregium,* sawdust

Introduction

The rapid and progressive development of the oil industry and allied companies have led to the ever increasing release of different environmental pollutants, such as halogenated and polycyclic aromatic hydrocarbons (Prakash,

2017). This has posed a lot of challenges to the environment owing to the high level of contaminants in the environment (Deshmukh *et al*., 2016). This has brought untold hardships to individuals in the affected regions including health issues plus breathing problems and skin lesions; many have been deprived of basic human amenities of life such as food and potable water (Enerijiofi and Ikhajiagbe, 2021).

Bioremediation, which is a cheap and effective process uses several agents such as microorganisms and higher plants as main tools in treating oil spills currently in the environment (Enerijiofi, 2020). However, it is still regarded as an evolving technology because despite the extensive variety of mechanisms by microorganisms, some are still not known (Adenipekun *et al.,* 2013). Mycoremediation which involves the use of fungi mycelia to decontaminate toxic wastes from contaminated areas is gradually gaining ground. Fungi thrive in soils of diverse climatic circumstances as well as the life-threatening ones and proliferate through spores' dispersal in the air as well as assist in sustaining ecosystem equilibrium (Khatoon *et al*., 2021). The variety of habitats and the capability to release lots of enzymes avail fungi as viable nominees for bioremediation at different sites (Zhang *et al*., 2013). The central issue in mycoremediation is to establish the correct species of fungi to aim at the precise pollutant. Fungi such as *Phanaerochaete chrysosporium* and *Polyporus* sp. are capable applicants for bioremediation, since they possess the ability to metabolise greatly different types of recalcitrant environmental pollutants. One main benefit that fungi have over bacterial is that they do not need to get acclimatize to the pollutant of interest (Adenipekun and Lawal, 2012).

The possibility of debasing pollutants that do not require a specific method just as the potential for in situ and ex situ are thoroughly examined as the reward of mycoremediation innovation which utilizes mushroom. The genus *Pleurotus* which belongs to the order *Agaricales* is considered as one of the commercially vital edible mushrooms throughout the world. *Pleurotus* species popularly known as Oyster mushrooms have been generally utilized in the degradation of different organic pollutants due to their applications in biotechnology and environment (Thapa *et al.*, 2012; Mohammadi-Sichani *et al.,* 2017). Crude oil / its derivatives contaminated soils are generally of no value to the farmer due to the attendants' negative effects on soil, soil microorganisms and plants. The study was aimed at degrading crude oil polluted soils with the aid of a very economical and environmentally responsive substrate, *Pleurotus tuberregium* so as to guarantee environmental sustainability.

Materials and methods

Collection of materials

Twenty seven (27) buckets were purchased and washed properly. Sclerotia of *P. tuber-regium* were also bought from Ikpoba Hill market in Benin City. Garden soil was collected from an area beside the Botanical garden, University of Benin, Ugbowo Campus. Waste engine oil was collected from Total Filling Station at Mission Road in Benin City. Sawdust, used as substrate was collected from Ugbowo sawmills in Benin City and the sawdust was wood shavings of *Brachystegia nigerica*. Ten (10kg) of the garden soil was polluted with 1kg of the waste engine oil at 10% w/w. The soils were divided into two batches; Experiment 1 and Experiment 2.

Experiment 1:

Oil polluted soil (at 10%w/w of soil) that is substrate amended with sclerotia (at 6%w/w of soil) and divided into sub Experiments A1-D1.

Experiment 1:

A1: Oil polluted soil + Cubed Sclerotia (all mixed)
B1: Oil polluted soil + Powdered Sclerotia (all mixed)
C1: Oil polluted soil + Powdered Sclerotia as mulch
D1: Oil polluted soil + no Sclerotia.

Experiment 2:

Only oil polluted soil 10%w/w and has no sawdust at all.

A2: Oil polluted soil + Cubed Sclerotia (all mixed)
B2: Oil polluted soil + Powdered Sclerotia (all mixed)
C2: Oil polluted soil + Powdered Sclerotia as mulch
In all there were 7 expts and 2 controls totaling 9x3rep =27bowls.

A total of 10kg of oil polluted soil were obtained and each combination was inoculated with 6% w/w of sclerotia of *P. tuberregium* (0.6kg) both cubed and powdered and then divided into 2 Expts (Expt1 and Expt2).

The Expts 1 were subsequently amended with 2kg of sawdust (i.e. 20% w/w); whereas the Expt 2 was not amended with 2kg of sawdust. The entire set up was left in a screen house for observation.

Determination of mushroom emergence

The emergence of fruiting bodies was carefully observed 3 times a week. After emergence parameters were taken weekly (once a week). The different parameters were checked.

Height of stipe

This was done for the buckets treated with sclerotia. It was carefully observed for increase in height. This was done by using a metre rule to measure from the base of the shoot to the terminated bud and records of the length taken.

Diameter of cap

This was done using a tape to carefully measure the cap of the fruiting bodies for all the buckets respectively.

Number of fruiting bodies

The number of newly emerging fruiting bodies was carefully observed and recorded. Each treatment (in 3 replicates) was counted individually and added together to obtain a mean. The same procedure was also used to determine the number of fruiting bodies with caps.

Number of observable weeds

The number of weeds was determined by counting each weed that appeared in the buckets (> 3cm).

Bacteria and fungi analyses

Soil samples from each replicate were collected and mixed for each concentration. Thereafter the soil was air-dried, sieved and 1g was weighed from each of the different concentrations into the test tubes. Then 9ml of normal saline was added using a syringe and stirred for 30 seconds with the Vortex Genie mixer after which it was covered with foil paper and then allowed to stay for 24 hours (that was the aliquot). Three serial fold dilution was carried out in test tubes containing 9ml of normal saline. Thereafter, aliquot 0.1ml was inoculated into Petri dishes containing already prepared Nutrient agar and 1 tablet of dissolved ketoconazole to inhibit the growth of fungi. This was incubated at 37^{o}C for 24hrs for bacterial growth. Also, 0.1ml aliquot was inoculated into already prepared potato dextrose agar containing

chloramphenicol, which inhibits bacterial growth. The plates were incubated at room temperature of 25^{o}C for 72hrs for fungal growth. The colonies observed for bacterial and fungal were counted thereafter and recorded in CFU/g (Enerijiofi *et al*., 2020).

Identification of bacteria and fungi species

The bacteria were identified according to the method of Holt *et al*. (1994) while the fungi were identified following the protocol of Fawole and Oso, 2001.

Determination of total hydrocarbon content

The total hydrocarbon content of the soil and fruiting bodies were analysed by adding 5ml of n-hexane to 1gram of the different treatments of the soil sample and the fruiting bodies respectively after two months of planting. These samples were placed in a cuvette and passed through a visible spectrophotometer with the wavelength of 460nm where the absorbance was recorded and the total hydrocarbon content was calculated (Mohammadi-Sichani *et al.,* 2017).

Statistical analysis

Data obtained from the analysis were subjected to statistical analysis under descriptive statistics, in a Mean of 3 replicates. Single factor analyses of variance was used to evaluate the data obtained since the soil used in the experiment was homogenized and homogeneity of the entire plot was also assumed to evaluate the data obtained. Means of 3 determiantions were presented in Tables. Mean separation was acheived by using Least significant difference (LSD) where neccessary.

Results

In Table 1, oil polluted with Sclerotia and sawdust, A1 had the highest fruiting bodies with a cap at week 6 of 11.3 while B1, C1, and D1 had no fruiting bodies at weeks 3, 4, 5 and 6. In contaminated soils with Sclerotia, A2 had the highest number of fruiting bodies with a cap at weeks 4 and 6 of 8.3 while B2 had the lowest, 1.3 at week 6. However, the control had no fruiting bodies with caps throughout the experiment. All experimental soil samples did not support mushroom cap at weeks 3 and 4. In week 6, A2 had the highest of 4.5cm while C2 had the least of 2.2cm. It was also noted that no record was

observed for the control throughout the period. For the height of the stipe A1 and A2 had the highest of 8cm at week 6 while C2 had the least of 4.5cm. The weeds that grew in the setup are recorded in Table 2. *Pepperomia pellucida* was the most predominant weed with a count of 16 at control F. *Pepperomia pellucida* was found to be the predominant weed in all the treatments having a count of 20. This is followed by *Asystasia gangetica* with a count of 11. No weed was found in control E as well as treatments A1 and B1.

Table 3 recorded the bacterial and fungal count in contaminated soil after two months of planting *P. tuberregium.* B1 had the highest bacteria count of 3.58 $x10^6$ cfu/g while A1 had the highest fungi count of $7.4x10^5$cfu/g in the sawdust amended soil. However, in soils without sawdust, B2 had the highest bacteria count of $7.0x10^5$ cfu/g while A2 had the highest fungal count of $7.3 x10^5$ cfu/g.

Table 4 above shows the bacterial and fungal identified in different treatments after two months of planting. It was observed that *Bacillus* sp., *Micrococcus* sp. and *Staphylococci* sp. were among the bacterial isolates that were identified in all soil samples while *Aspergillus niger, Penicillium* sp., *Rhizopus* sp. were the predominant fungal isolates. However, *Proteus* sp. was identified in only B1, *Trichoderma* sp. only in C2, *Proteus* sp. in only B1 while *Microsporium* sp. was present in A1 and C2. Table 5 shows the total hydrocarbon present in different soil treatment and fruiting bodies after two months of planting. It was observed that C1 had the highest THC of 1.625ppm while control, F had the least of 0.29ppm. In the fruiting bodies, A2 had the highest THC of 0.955ppm while A1 had the least of 0.395ppm.

Table 1. Growth parameters of mushroom growing in oil polluted soil

Treatments	**Week 3**			**Week 4**			**Week 5**			**Week 6**		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Control E&F	0	0	0	0	0	0	0	0	0	0	0	0
A1 (O+X+S)	3.3	0	0	7.3	0	0	9.7	0	3	11.3	4.5	8
B1 (O+X+S)	0	0	0	0	0	0	0	0	0	0	0	0
C1 (O+X+S)	0	0	0	0	0	0	0	0	0	0	0	0
D1 (O+S)	0	0	0	0	0	0	0	0	0	0	0	0
A2 (O+X)	4	0	0	8.3	0	0	8	1.5	2.5	8.3	4.5	8
B2 (O+X)	0	0	0	1	0	0	1	0	0	1.3	2.7	6
C2 (O+X)	4.7	0	0	6.7	0	0	8.3	1.9	2.0	7.3	2.2	4.5

Legend: A= Mean number of mushrooms fruiting bodies with cap, growing in Oil polluted soil
B= Diameter of Cap of the mushrooms growing in Oil polluted soil (cm)
C= Height of mushrooms stipe growing in Oil polluted soil.
O= Oil-polluted soil, X= sclerotia, S sawdust, PS= pure soil

Table 2. Weeds grown from soil seed bank in the Oil polluted soil, with number per species of weed number

Treatments	Weeds Observed	Total
Control E (PS)	No weed	0
Control F (O)	*Pepperomia pellucida* (16) , *Asystasia gangética* (3)	19
A1 (O+X+S)	No weed	0
B1 (O+X+S)	No weed	0
C1 (O+X+S)	*Commelina erecta* (1)	1
D1 (O+S)	*Pepperomia pellucida* (1), *Cyperusspecies* (1)	2
A2 (O+X)	*Asystasia gangetica* (3)	3
B2 (O+X)	*Pepperomia pellucida* (3), *Asystasia gangetica* (2) *Eleucine indica* (2)	7
C2 (O+X)	*Asystasia gangetica* (3), *Cyperusspecies* (3)	6

Legend: O oil-polluted soil, X sclerotia, S sawdust, PS pure soil.
Values presented are means of 3 determinations and rounded off to the nearest integer

Table 3. Microbial load for bacterial and fungi count in oil polluted soil after two months of planting

Treatments	Oil polluted soil		Oil polluted soil attached to sclerotia	
	Bacterial counts ($x10^6$ cfu/g)	Fungal counts ($x10^5$ cfu/g)	Bacterial counts ($x10^7$ cfu/g)	Fungi counts ($x10^6$ cfu/g)
Control E (PS)	1.2	3.6	-	-
Control F (O)	0.77	4.9*	-	-
A1 (O+X+S)	2.34*	7.4*	2.19	4.45
B1 (O+X+S)	3.58*	5.3*	1.79	2.97
C1 (O+X+S)	2.34*	2.8	1.26	3.92
D1 (O+S)	0.66	3.8	-	-
A2 (O+X)	1.51	7.3*	2.46	2.77
B2 (O+X)	0.7	4.5	1.44	5.16
C2 (O+X)	1.15	2.7	1.4	3.57
LSD (0.05)	0.97	1.2	NA	NA
p-value	<0.001	0.004	NA	NA

Legend: O oil-polluted soil, X sclerotia, S sawdust, PS pure soil
*Means are significantly different from the control (PS), $p<0.05$

Table 4. Microorganisms present in the different oil polluted treated soil after two months of planting

Treatments	Oil polluted soil		Oil polluted soil attached to sclerotia	
	Bacterial isolates	**Fungal isolates**	**Bacterial isolates**	**Fungal isolates**
Control E (PS)	*Bacillus sp., Micrococcus letus,S. aureus*	*Penicillium* sp., *A. flavus., Rhizopus oryzea, A. niger, Mucor sp.*	-	-
Control F (O)	*Bacillus sp., Micrococcus sp, S. epidymis*	*Trichoderma* sp., *A. niger, Mucor* sp., *A. flavus, Penicillium* sp., *Rhizopus oryzea.*	-	-
A1 (O+X+S)	*Bacillus sp., Micrococcus sp.*	*Penicillium* sp., *Rhizopus oryzea, Trichoderma* sp., *A. niger.*	*Bacillus sp. Micrococcus* sp	*Rhizopus* sp., *A. niger, Microsporum* sp., *Mucor* sp., *Penicillium* sp.
B1 (O+X+S)	*Bacillus sp., Proteus sp., S. aureus, Micrococcus sp.*	*Candida sp., Penicillium sp., A. niger, Rhizopus* sp., *A. flavus.*	*Bacillus* sp, *Proteus* sp, *S. aureus, Micrococcus* sp.	*A. niger, Rhizopus oryzea, A. flavus, Penicillium* sp.,
C1 (O+X+S)	*Bacillus sp, Staph sp, Micrococcus*	*Mucor* sp., *Rhizopus* sp., *Penicillium* sp., *Tricodermal* sp., *A. niger.*	*Bacillus* sp, *Staph* sp, *Micrococcus*	*Fusarium sp., A. niger, Rhizopus* sp., *Mucor* sp., *Trichoderma* sp., *A. flavus.*
D1 (O+S)	*S. epidymis, Micrococcus*	*A. flavus, Fusarium* sp., *Rhizopus oryzea, Mucor* sp., *Penicillium* sp., *Aspergillus*	-	-
A2 (O+X)	*Bacillus sp, S. aureus, S. epidermidis*	*Rhizopus* sp., *Penicillium* sp., *A. flavis.*	*Bacillus* sp, *S. aureus, S. epidermidis*	*Rhizopus oryzea, A. niger, Fusarium* sp., *Penicillium* sp., *A. flavus*
B2 (O+X)	*Bacillus sp, S. epidermidis*	*A. niger, Trichoderma* sp., *Rhizopus oryzea, A. flavus, Penicillium* sp.	*Bacillus* sp, *S. epidermidis*	*A. flavus, Penicillium* sp., *Rhizopus* sp., *Fusarium* sp., *Mucor* sp., *A. niger.*
C2 (O+X)	*Bacillus sp, Micrococcus sp, S. aureus,*	*Penicillium* sp., *Aspergillus flavus, Trichoderma sp., Rhizopus oryzea, Mucor* sp.	*Bacillus* sp, *Micrococcus* sp, *S. aureus,*	*A. niger, A. flavus, Rhizopus* sp., *Microsporium* sp., *Mucor* sp., *Trichoderma* sp., *Penicillium* sp.

Legend: O oil-polluted soil, X sclerotia, S sawdust, PS pure soil

Table 5. Total Hydrocarbon Content of the soil sample and fruiting bodies after two months of planting

Treatments	Total Hydrocarbon Content (ppm)	
	Soil	Fruiting bodies
1st control (E) PS	1.375	-
2nd control (F) O	0.29*	-
A1 O+X+S	1.55	0.395
B1 O+X+S	1.42	-
C1 O+X+S	1.63	-
D1 O+S	0.65*	-
A2 O+S	0.77*	0.955
B2 O+S	2.53*	0.640
C2 O+S	1.42	0.590
LSD (0.05)	0.59	NA
p-value	0.024	NA

Legend: O oil-polluted soil, X sclerotia, S sawdust, PS pure soil
*Means different from the control (O), $p<0.05$

Discussion

The research was carried out to demonstrate the ability to utilize fungi for remediation purposes despite its importance as food to man. Three parameters: fruiting bodies, cap diameter and height of stipe were used to monitor the ability of the mushroom to grow in crude oil amended soil environment. The result as stated in Table 1 revealed that the mushroom was able to utilize crude oil in the presence of sawdust as a substrate over time for growth and proliferation. This could be responsible for the higher fruiting bodies recorded (Thenmozhi *et al.,* 2013). The increasing concern of the human population on the indiscriminate deposition of agricultural wastes into the environment has made focus on assessing the biodegradation ability of the white-rot fungi (mushrooms) on these waste (Isikhuemhen *et al*., 2010). Bioremediation of polluted soils by white-rot fungi is another area, using both local and exotic species to remediate different types of soils polluted with materials such as crude oil and its products of fractional distillation (Jonathan *et al.,* 2010). The usefulness of the treated soil was attested to by (Isikhuemhen *et al.,* 2011) where they reported its application on the cultivation of vegetables.

The ability of fungi to utilize crude oil profusely in the presence of sawdust is as also shown in Table 1. The isolates were found to proliferate with an increasing period of exposure. Similar work by Lawal *et al.* (2011), reported the cultivation of white-rot fungi which assessed the ability of white-rot fungi to degrade

agricultural wastes which are a nuisance to the environment. Also, Isikhuemhen *et al.* (2011) reported that white-rot fungi, *Pleurotus* species which are know to break down Polyaromatics (PAHs) and Polychlorinated biphenyls (PCB) into different fractions due to their ability to secrete lignocellulolytic enzymes are important in oxidizing persistent pollutants. Oluwafemi *et al*. (2011) reported that the use of Gas Chromatography-Mass Spetrophotometry and cation-exchange on sample analysis would enable one to characterize the genes that are regulated during growth and give a better understanding of the gene fractions involved by white-rot fungi during bioremediation.

The height of the stipe as recorded same Table 1 shows increased growth with prolonged exposure to the contaminated soil, which means that the mushrooms are able to utilize crude oil. The weeds that grew in the setup are recorded in Table 2. *Pepperomia pellucida* was the most predominant weed with the count at the oil polluted soil. This shows that the soil supported the growth of *Pepperomia pellucida* more than other experimental soils particularly the pure soil and oil polluted soil, and soil with soils with sclerotia and sawdust. This shows that the crude oil amended soil contained toxic substances that do not support the growth of weeds, hence no growth was recorded. Table 3 recorded the bacterial and fungal count in contaminated soil after two months of planting. The results of the bacterial and fungal counts revealed the ability of the isolates to grow profusely in the oil polluted soil particularly in the presence of sclerotia and sawdust. However, in general, the fungal isolates were observed to be more in numbers. This point to the obvious that fungi are better able to utilize and crude oil than bacterial species.

Table 4 above shows the bacterial and fungal identified in different treatments after two months of planting. It was observed that *Bacillus* sp., *Micrococcus* sp. and *Staphylococci* sp. were among the bacterial isolates that were identified in all soil samples while *Aspergillus niger, Penicillium* sp., *Rhizopus* sp. were the predominant fungal isolates. However, *Proteus* sp. was identified in only B1, *Trichoderma* sp. only in C2, *Proteus* sp. in only B1 while *Microsporium* sp. was present in A1 and C2. The bacterial and fungal isolates reported were not surprising as previous reports have documented them to be indigenous isolates in soil especially *Bacillus*, *Micrococcus, Staphylococci Aspergillus*, *Penicillium* and *Rhizopus* species. (Prakash, 2017; Thenmozhi *et al*., 2013). The fungal species reported were more than the bacterial isolates which also point to their robust morphology and diverse metabolic capacity. This makes fungal better agents of bioremediation and pollution control. Table 5 shows the total hydrocarbon present in different soil treatment and fruiting bodies after two months of planting. It was observed that C1 had the highest THC of 1.625ppm while control, F had the least of 0.29ppm. In the fruiting bodies, A2 had the highest THC of 0.955ppm while A1 had the least of 0.395ppm. It was observed that the crude oil polluted soil containing sclerotia and amended

with sawdust contained more hydrocarbons than the oil polluted soil with sawdust. This shows that fungi (sclerotia) are better degraders of hydrocarbons. In corroboration, Davis and Wilson (2005) reported that for efficient bioremediation, soil amendments are added to increase microbial activities. Also, *Pleurotus tuberregium* has been used to improve bioremediation of soils contaminated with crude oil (Adedokun and Ataga, 2007). The technology of bioremediation employed was simple, cheap, effective and environmentally friendly, whose biostimulant is pleasant and mainly of organic origin, i.e., sawdust, which is mostly referred to as waste and is of no economic value to the ordinary man.

Conclusions

This work has showed the potential of *Pleurotus tuberregium* in the degradation of crude oil polluted sites and confirmed the significant ecological role of fungi in petroleum-polluted environments.

References

Adedokun, O. M., & Ataga, A. E. (2007). Effects of amendments and bioaugmentation of oil-polluted with crude oil, automotive gasoline oil and spent engine oil on the growth of cowpea (*Vignaunguiculata* L. Walp). *Science Research Essay,* 2: 147-149.

Adenipekun, C. O., & Lawal, R. (2012). Uses of mushrooms in bioremediation: A review *Biotechnology and Molecular Biology Review*, 7(3): 62-68.

Adenipekun, C. O., Ayanleye, O. O., & Oyetunji, O. J. (2013). Bioremediation of soil contaminated by spent diesel oil using *Pleurotus pulmonarius* Fries (Quelet) and its effect on the growth of *Corchorus olitorius* (L). *Journal of Applied Biosciences*, 66: 5366–5373.

Davis, J. G., & Wilson, C. R. (2005). Choosing a Soil Amendment. Colorado State University Cooperative Extension Horticulture **7**: 235.

Deshmukh, R., Khardenavis, A. A., & Purohit, H. J. (2016). Diverse Metabolic Capacities of Fungi for Bioremediation, *Indian J Microbiology*, 56(3):247–264 DOI 10.1007/s12088-016-0584-6.

Enerijiofi, K. E. (2020). Bioremediation of environmental contaminants: a sustainable alternative to environmental management. In: Bioremediation for Environmental Sustainability: toxicity, mechanisms of contaminants degradation, detoxification and challenges. Saxena, G., Kumar, V, and Shah, M. P. (eds.), Elsevier, United States of America, 2**:** pp. 461–480.

Enerijiofi, K. E., Ahonsi, C. O., & Ajao, E. K. (2020). Biodegradation Potentials of Waste Engine Oil by three Bacterial Isolates. *Journal of Applied Sciences and Environmental Management*, 24 (3): 483–487. https://dx.doi.org/10.4314/jasem.v24i3.14.

Enerijiofi, K. E., & Ikhajiagbe, B. (2021). Plant-Microbe Interaction in Attenuation of Toxic Waste in Ecosystem. In: Rhizobiont in Bioremediation of Hazardous Waste.

Kumar, V., Prasad R. and Kumar, M. (eds). Springer, Singapore. https://doi.org/10.1007/978-981-16-0602-1_13.

Fawole, M. O., & Oso, B. A. (2001). Laboratory manual of Microbiology: Revised edition Spectrum books Ltd. Ibadan, 127 pp.

Holt, G. T., Krieg, R. N., Sneath, P. H. A., Staley, T. J., & Williams, T. S. (1994). Manual of Determinative Bacteriology. 9th edition. Williams and Wilkins Baltimore Md, USA. pp176-211.

Isikhuemhen, O. S., Adenipekun, C. O., & Ohimain, E. (2010). Preliminary studies on mating and improvement strain selection in the tropical culinary medicinalmushroom, *Lentinussquarrosulus* Mont. *(Agaricomycetales)*. *Internatio nal Journal of Medicinal Mushrooms*, 12 (2): 177 – 183.

Isikhuemhen, O. S., Mikiashvilli, N. A., Adenipekun, C. O., Ohimain, E. I., & Shahbazi, G. (2011). The tropical white rot fungus, *Lentinussquarrosulus.* Mont. Lignocellulolytic enzyme activities and sugar release from corn stalk under solid state fermentation. *World Journal of Microbiology and Biotechnology*, 28(5): 1961 – 1966.

Jonathan, S. G., Akinfemi, A., & Adenipekun, C. O. (2010). Biodegradation and *in-vitro* digestibility of maize husks treated with edible fungi (*Pleurotus tuber-regium* and *Lentinussubnudus*) from Nigeria. *Journal Electronic Environmental, Agricultural and Food Chemistry*, 9(4): 742 – 750.

Khatoon, H., Rai, J. P. N., & Jillani, A. (2021). Role of Fungi in bioremediation of contaminated soil, In: Fungi bioprospects in sustainable Agriculture, Environment and Nanotechnology. Sharma, V. K., Shah, M. P., Parmer, S. And Kumar, A. (eds). Elsevier, https://doi.org/10.1016/B978-0-12-821925-6.00007-1.

Lawal, R., Adenipekun, C. O., & Isikhuemhen, O. S. (2011). Effect of additives on the cultivation of *Auriculariaauricula* (St. Amans) on *Mansoniaaltissima* (A. chev) A. chev. Sawdust. *Advances in Food Sciences*, 33 (4): 199 – 204.

Mohammadi-Sichani, M. M., Mazaheri Assadi, M., Farazmand, A., Kianirad, M., Ahadi, A. M., & Ghahderijani, H. H. (2017). Bioremediation of soil contaminated crude oil by Agaricomycetes. *Journal of Environmental Health Science & Engineering*, 15 (8): 1 – 6. DOI 10.1186/s40201-016-0263-x.

Oluwafemi, F., Fasidi, I. O., & Adenipekun, C. O. (2011). Cultivation and fruit body production of *Lentinussquarrosulus* Mont. Singer on bark and leaves of fruit trees supplemented with agricultural wastes. *African Journal of Biotechnology*, 10 (22): 4608 – 4611.

Prakash, V. (2017). Mycoremediation of Environmental Pollutants *International Journal of ChemTech Research*, 10 (3): 149-155.

Thapa, B., Kumar, K. C., & Ghimire A. (2012). A review on bioremediation of petroleum hydrocarbon contaminants in soil. *Journal of Science, Engineering and Technology*, 8(1):164–70.

Thenmozhi, R., Arumugam, K., Nagasathya, A., Thajuddin, N., & Paneerselvam, A. (2013). Studies on Mycoremediation of used engine oil contaminated soil samples *Advances in Applied Science Research*, 4(2):110-118

Zhang, Y., Xie, J., Liu, M., Tian, Z., He, Z., van Nostrand, J. D., Ren, L., Zhou, J., & Yang, M. (2013). Microbial community functional structure in response to antibiotics in pharmaceutical wastewater treatment systems. *Water Res,* 47:6298–6308. doi:10.1016/j.watres.2013.08.003.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 85-92)
doi:10.24193/subbbiol.2021.1.03

=== SHORT COMMUNICATION ===

Preliminary data on terrestrial isopods from some railways in Dobruja, eastern Romania

Daniel-Răzvan Pop[1], Alexandra-Roxana-Maria Maier[2], Achim-Mircea Cadar[2] and Sára Ferenţi[2]✉

[1]University of Oradea, Doctoral School of Biomedical Sciences, Domain: Biology, Oradea, Romania;
[2]University of Oradea, Faculty of Informatics and Sciences, Department of Biology, Oradea, Romania; ✉ ***Corresponding author, E-mail: ferenti.sara@gmail.com.***

Article history: Received 6 December 2020; Revised 19 April 2021; Accepted 1 June 2021; Available online 30 June 2021.

Abstract. In July 2020 we analyzed the terrestrial isopod assemblages from different (mostly abandoned) railway constructions in Dobruja, eastern Romania. We identified 10 terrestrial isopod species, of which the most abundant and frequent were *Porcellionides pruinosus* (Brandt, 1833) and *Armadillidium vulgare* Latreille, 1804. We identified species that, in Romania, are present only in Dobruja: *Leptotrichus pilosus* Dollfus, 1905 and *Trachelipus squamuliger* (Verhoeff, 1907). Most of the species are common, generalist or synanthropic, connected to dry and open habitats. In abandoned railway-stations the number of species/samples was low (at most two), but at the base of a stone railway bridge in Babadag forest six species were found.

Key words: transportation network, habitats, artificial structures.

Introduction

Dobruja is a region from eastern Romania with a warmer and drier climate than other areas in the country (e.g. Mândruț, 2006; Croitoru *et al.,* 2013; Prăvălie and Bandoc, 2015). However, in Dobruja, there are numerous isopod species (Radu, 1983; 1985; Giurginca and Ćurčić, 2003; Tomescu and Teodor, 2018) compared with other regions in the country (Radu, 1983; 1985), even if their distribution is determined by humidity (Hornung, 2011).

Nevertheless, previous studies focused mainly on natural habitats (Giurginca and Ćurčić, 2003; Tomescu and Teodor, 2018), including caves (Tăbăcaru and Boghean, 1989; Gruia *et al.,* 1994; Gruia and Giurginca, 1998). However, it is known that isopods can use disturbed habitats, as they are present in large number in urban areas (Vilisics and Hornung, 2009; Ferenţi *et al.,* 2015; Giurginca *et al.,* 2017; Laza *et al.,* 2017; Pop *et al.,* 2019), but also on highways edges (Vona-Túri *et al.,* 2016; 2017; 2018; 2019). Thus, considering the rich terrestrial isopod fauna of Dobruja (Radu, 1983; 1985; Giurginca and Ćurčić, 2003; Tomescu and Teodor, 2018), we supposed that some of these species are present in artificial habitats too, like transportation network infrastructure. For this, we chose the railways, which have a dual relation with the fauna: on one hand they favor certain animals, like herpetofauna, isopods, spiders or pollinators (e.g. Covaciu-Marcov *et al.,* 2006; 2017; Wrzesień *et al.,* 2016; Graitson *et al.,* 2020), but on the other hand they cause the mortality of some animals, both vertebrates (e.g. Budzik and Budzik, 2014; Heske, 2015; Dornas *et al.,* 2019; Joshi and Puri, 2019) and invertebrates (Pop *et al.,* 2020). In Dobruja many railway stations were abandoned in the last years (C.F.R., 1993; C.F.R., 2019). Thus, our objective was to collect preliminary data about the terrestrial isopod fauna from Dobruja which populates different railway constructions.

Material and methods

The field work was realized at the end of July 2020. We investigated two secondary railway lines, which cross Dobruja on the north-south direction, diverging from the main line at Medgidia, namely Medgidia - Tulcea line and Medgidia - Negru Vodă line (C.F.R., 2019). The Tulcea line has a low number of passenger trains (maximum of six trains / day (C.F.R., 2019) and freight trains. On the Negru Vodă line passenger trains were suspended (C.F.R., 2019). We searched for terrestrial isopods around various constructions along these lines, especially in railway stations abandoned in the last years (C.F.R., 1993; C.F.R., 2019). We collected terrestrial isopods from five disused railway stations and from abandoned constructions belonging to two functional stations. Also, we collected isopods at the base of a stone railway bridge in Babadag forest. Under the bridge there is a tunnel with a road that leads to a stone quarry. The isopods were collected under the debris from the abandoned stations, or under the stones that fell at the base of the bridge. In each locality, the sampling took approximately 20 minutes, as in other cases (Ferenţi and Covaciu-Marcov, 2016; Pop *et al.,* 2019). The isopods were determined in the laboratory.

On the railways from Dobruja we collected 113 terrestrial isopod individuals, who belonged to 10 species (*Hyloniscus riparius* (Koch, 1838), *Haplophthalmus danicus* Budde-Lundt 1879, *Porcellium collicola* (Verhoeff, 1907),

Leptotrichus pilosus Dollfus, 1905, *Trachelipus nodulosus* (Koch, 1838), *Trachelipus squamuliger* (Verhoeff, 1907), *Porcellionides pruinosus* (Brandt, 1833), *Cylisticus convexus* (De Geer 1778), *Armadillidium vulgare* Latreille, 1804, and one *Armadillidium* species that could not be determined, as we collected only one juvenile). The species number / locality was reduced; in the case of the abandoned stations, we registered one, at most two species / locality. In the case of the stone bridge from Babadag forest, we identified six terrestrial isopod species. The percentage abundance differed between localities. The highest percentage abundance was registered by *P. pruinosus*, followed by *A. vulgare* (Tab. 1). *A. vulgare* also registered the highest frequency of occurrence.

Table 1. Percentage abundance and frequency of occurrence of terrestrial isopods in the studied railway constructions in Dobruja (1. – Istria, 2. Ciocârlia, 3. – Nazarcea, 4. – Târgușor Dobrogea, 5. – Ceamurlia de Jos, 6. – Zebil, 7. – Mihail Kogălniceanu, 8. – Babadag forests: S – station, B – Bridge, P% – Total percentage abundance, f% – Total frequency of occurrence)

Locality	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**	**P%**	**f%**
Type	S	S	S	S	S	S	S	B		
H. riparius	-	-	-	-	-	-	-	4.42	4.42	12.5
H. danicus	-	-	-	-	-	-	-	0.88	0.88	12.5
P. collicola	-	-	-	-	-	-	-	0.88	0.88	12.5
L. pilosus	-	-	-	6.19	-	-	-	-	6.19	12.5
T. nodulosus	-	-	-	-	-	-	-	2.65	2.65	12.5
T. squamuliger	-	-	-	-	-	-	-	8.84	8.84	12.5
P. pruinosus	8.84	-	-	3.53	21.2	3.53	-	-	37.1	50
C. convexus	-	-	-	-	-	-	0.88	2.65	3.53	25
A. vulgare	3.53	-	0.88	-	1.76	12.3	15.9	-	34.5	62.5
A. sp.	-	0.88	-	-	-	-	-	-	0.88	12.5
Species number	2	1	1	2	2	2	2	6		
P%	12.3	0.88	0.88	9.73	23	15.9	16.8	20.3		

Discussions

The number of terrestrial isopod species identified on the railways from Dobruja is reduced compared with the one recorded in the region's natural habitats, where more than 40 species are present (Giurginca and Ćurčić, 2003, Tomescu and Teodor, 2018). Nevertheless, the species number identified on the railways in Dobruja is close to the one registered in some towns in Romania (Ferenţi *et al.,* 2015; Laza *et al.,* 2017). Thus, as railways are artificial habitats, the species richness is close to the one registered in other artificial areas with more diverse habitats, like towns (Ferenţi *et al.,* 2015; Laza *et al.,* 2017). This could be a consequence of the high isopod diversity in Dobruja

(Giurginca and Ćurčić, 2003), some of the local species colonizing railways. Compared to abandoned railway tunnels (Covaciu-Marcov *et al.,* 2017) the number of isopods species identified on the railways in Dobruja was smaller, but the number of tunnels was higher compared with the number of railway constructions from Dobruja. At the same time, tunnels were situated in different (usually mountain) areas and the study was made in different periods (Covaciu-Marcov *et al.,* 2017).

The low number of isopod species registered on the railways does not necessary reflect the assemblages' poverty, but could be a consequence of the study period, as temperature and humidity also influences isopods near highways (Vona-Túri *et al.,* 2019). The study was made in summer, in the warmest and driest region of Romania (Mândruț, 2006; Croitoru *et al.,* 2013; Prăvălie and Bandoc, 2015), and terrestrial isopods are related to humidity (e.g. Warburg *et al.,* 1984; Hornung *et al.,* 2011). Previously in one of the investigated railway-stations the feeding of two frog species was studied in the spring (Covaciu-Marcov *et al.,* 2012) and those frogs consumed more isopod species than we identified now. Probably in the spring more species are active, but in the middle of the summer only the most drought-resistant ones were present. Probably in other seasons, the number of isopod species would have been higher than in many towns.

Compared with the isopods identified near highways (Vona-Túri *et al.,* 2017; 2019), the number of species registered near the railways from Dobruja was smaller. However, it is difficult to make such comparison, because we have fewer samples, from a smaller area, while near highways more road-areas were investigated, from a larger area, and at different distances from the roads (Vona-Túri *et al.,* 2017). Because the isopods near highways were sampled with pitfall traps, the individual number was huge (Vona-Túri *et al.,* 2017; 2019), and this reduced the species diversity compared with the railways from Dobruja, in the case of roads, the lowest species richness was near forests (Vona-Túri *et al.,* 2017), but in Dobruja in such habitats it was the highest. This could be a consequence of the species different ecological demands. Also, it could be caused by the differences between roads and railways, as roads generally have a more intense traffic, are wider and more polluted than railways (see in: Borda-de-Água *et al.,* 2017). In the same time, on roads the maximum species richness was registered at 40 meters from the roads (Vona-Túri *et al.,* 2017), but in Dobruja the isopods were collected at only few meters from the line.

Most of the terrestrial isopod species from the railways in Dobruja are common, generalist species, a fact which was also mentioned in the case of roads (Vona-Túri *et al.,* 2016; 2017; 2018). The most common species (*P. pruinosus* and *A. vulgare*) are synanthropic species, frequently mentioned in artificial, or at least partially modified habitats (e.g. Vilisics and Hornung, 2009; Laza *et al.,* 2017; Bodog *et al.,* 2018) even on the roads' edges (Vona-Túri *et al.,* 2018).

Both are considered species with Mediterranean affinities (e.g. Cochard *et al.,* 2010), thus their presence and high abundance in Dobruja and on the railways, should not be surprising. However, we also registered rare species with a limited distribution in Romania. This is the case of *T. squamuliger*, a species recently recorded for the first time in Romania, only in a few localities in Dobruja (Tomescu *et al.,* 2015, Tomescu and Teodor, 2018). *T. squamuliger* is rare also on railways, as it was encountered only on the bridge from Babadag forest. This is the fifth distribution record of this species in Romania, representing a connection between its previous distribution localities in northern and southern Dobruja (Tomescu *et al.,* 2015). At the same time, is seems to clarify at least partially its ecology (Tomescu *et al.,* 2015), indicating that in Dobruja it is probably related with forests. At Babadag it was encountered in an oak and hornbeam forest, at the base of a stone bridge, in a relatively humid habitat with humid soil and fallen leaves. Another rare species, present in Romania only in Dobruja is *L. pilosus* (Radu, 1985; Giurginca and Ćurčić, 2003; Schmalfuss, 2003). It was recorded only in central Dobruja, in the ruins of the abandoned water tower from Târguşor Dobrogea station, in an open and arid region, which seems to be characteristic for this species (Radu, 1985). *L. pilosus* is rare also in Dobruja, as it seams missing in the Danube Delta (Tomescu and Teodor, 2018). Also, in the case of this species the new locality seems to be a connection between the previously known ones (Radu, 1985; Giurginca and Ćurčić, 2003).

The terrestrial isopod fauna from the abandoned railway stations is poor, comprising numerous individuals from few species. As an exception, the stone bridge from Babadag forest shelters numerous species. Those species have different ecological demands compared with the ones from the railway stations, as species like *H. riparius*, *P. collicola* or *H. danicus*, are considered related to wet areas (e.g. Radu, 1983; 1985). This different fauna is a consequence of different neighboring areas, as the habitats from Babadag is the only one surrounded by forests. This fact confirms the importance of forests for the native terrestrial isopods in Romania (e.g. Ferenţi *et al.,* 2013; Ferenţi and Covaciu-Marcov, 2016), as the species identified in this habitat are generally native, many related with humid habitats. At the same time, it seems to indicate that also in the case of railways the neighboring areas are important for isopods, fact already mentioned in the case of towns (e.g. Herle *et al.,* 2016; Bodog *et al.,* 2018). Thus, a railway structure from natural areas will have a richer and more diverse terrestrial isopod fauna compared with an affected dry and open area. Terrestrial isopod assemblages vary according to the neighboring habitats characteristics on roads too (Vona-Túri *et al.,* 2017). As it is situated in a forest, the stone bridge from Babadag was colonized by the richer isopod fauna from the forest. Probably, in this way, the stone bridge is similar with the entrance of a tunnel (see in: Covaciu-Marcov *et al.,* 2017). Nevertheless, in this habitat we also recorded

species like *T. nodulosus*, which is related to dry and grassy areas (Farkas, 2010; Tomescu *et al.,* 2015). Moreover, *T. nodulosus* missed from the other localities, although it was common on the highway edges in Hungary (Vona-Túri *et al.,* 2019). Even if it is common in other regions from Romania, *T. nodulosus* seems rare in Dobruja (Tomescu *et al.,* 2015). This seems surprising since nowadays most of this region is covered with open grassy habitats, as forests were not a majority in the region even in the past (see in: Feurdean *et al.,* 2021). Probably, the climate in Dobruja, with hot and dry summers is too much for this species, which at least in summers needs more humidity, thus it remains active only in forests.

References

Bodog, E.-D., Popovici, P.-V., Molnár, K., Sas-Kovács, I., & Ferenți, S. (2018). Terrestrial isopods (Isopoda, Oniscidea) in Sebiș Town, Arad county (Romania). *Oltenia. Studii și Comunicări, Științele Naturii*, 34(2), 97-102.

Borda-de-Água, L. Barientos, R., Beja, P., & Pereira, H.M. (2017). Railway Ecology. In: *Railway Ecology*, Borda-de-Água, L., Barientos, R., Beja, P., Pereira, H.M. (eds.), Springer, pp: 3-9.

Budzik, K.A., & Budzik, K.M. (2014). A preliminary report of amphibian mortality patterns on railways. *Acta Herpetol*, 9(1), 103-107.

C.F.R. (1993). Mersul trenurilor de călători 23.05.1993 – 28.05.1994. Societatea Națională a Căilor Ferate Române, Serviciul Mersuri de Tren, *Societatea Tipografică Filaret* SA, București.

C.F.R. (2019). Mersul trenurilor de călători 15.12.2019 – 12.12.2020. Compania Națională de Căi Ferate "CFR" SA, Serviciul Mersuri de Tren, *Societatea Tipografică Filaret* SA, București.

Cochard, P.-O., Vilisics, F., & Sechet, E. (2010). Alien terrestrial crustaceans (Isopods and Amphipoda). *BioRisk*, 4(1), 81-96.

Covaciu-Marcov, S.-D., Bogdan, H.V., & Ferenți, S. (2006). Notes regarding the presence of some *Podarcis muralis* (Laurenti 1768) populations on the railroads of western Romania. *North-West J Zool*, 2(2), 126-130.

Covaciu-Marcov, S.-D., Ferenți, S., Cicort-Lucaciu, A-Ș., & Sas-Kovács, I. (2012). Terrestrial isopods in the diet of two amphibian species (*Epidalea viridis* and *Pelobates syriacus*) from Dobruja, Romania. *Entomologica romanica*, 17, 5-11.

Covaciu-Marcov, S.-D., Ferenți, S., Urák, I., Sas-Kovács, E.-H., Cicort-Lucaciu, A.-Ș., & Sas-Kovács, I. (2017). After the last train passes: data on the fauna from abandoned railway tunnels in Romania. *Ann Zool Fenn*, 54, 335-346.

Croitoru, A.-E., Piticar, A., Imbroane, A.M., & Burada, D.C. (2013). Spatiotemporal distribution of aridity indices based on temperature and precipitation in the extra-Carpathian regions of Romania. *Theor Appl Climatol*, 112, 597-607.

Dornas, R.A.P., Teixeira, F.Z., Gonsioroski, G., & Nóbrega, R.A.A. (2019). Strain by the train: Patterns of the toad fatalities on a Brazilian Amazonian railroad. *Science of the Total Environment*, 660, 493-500.

Farkas, S. (2010). Magyarország szárazföldi ászkarákfaunája (Isopoda: Oniscidea): *Trachelipus nodulosus* (C. L. Koch, 1938). *Natura Somogyiensis*, 17, 123-132.

Ferenţi, S., & Covaciu-Marcov, S.-D. (2016). Do terrestrial isopods from Vâlsan River protected area reflect the region's peculiarities? Zoogeographic and conservative implications of a possible answer. *Eco.mont – Journal on Protected Mountain Areas Research and Management*, 8(1), 5-11.

Ferenţi, S., Cupşa, D., Sas-Kovács, E.H., Sas-Kovács, I., & Covaciu-Marcov, S.-D. (2013). The importance of forests and wetlands from the Tur River natural protected area in conservation of native terrestrial isopod fauna. *North-West J Zool*, 9(1), 139-144.

Ferenţi, S., Lucaciu, M., & Mihuţ, A. (2015). Terrestrial isopods from Salonta town, western Romania. *South-west J Hortic Biol Environ*, 6(1), 21-31.

Feurdean, A., Grindean, R., Florescu, G., Tanţău. I., Niedermeyer, E., Diaconu, A.-C., Hutchinson, S.M., Nielsen, A.B., Sava, T., Panait, A., Braun, M., & Hickler, T. (2021). The transformation of the forest steppe in the lower Danube Plain of south-eastern Europe: 6000 years of vegetation and land use dynamic. *Biogeosciences*, 18: 1081-1103.

Giurginca A., & Ćurčić, S.B. (2003). A check-list of Oniscidea (Isopoda, Crustacea) from Dobruja (Romania). *Arch Biol Sci, Belgrade*, 55(1-2), 39-44.

Giurginca A., Baba Ş.-C., & Munteanu C.-M. (2017). New data on the Oniscidea, Diplopoda and Chilopoda from urban parks of Bucharest. *North-West J Zool*, 13(2), 234-243.

Graitson, E., Ursenbacher, S., & Lourdais, O. (2020). Snake conservation in anthropized landscapes: considering artificial habitats and questioning management of semi-natural habitats. *Eur J Wildl Res*, 66: 39. https://doi.org/10.1007/s10344-020-01373-2.

Gruia, M., Iavorschi, V., & Sarbu, S.M. (1994). *Armadillidium tabacarui* (Isopoda: Oniscidea: Armadillidiidae) a new troglobitic species from a sulfurous cave in Romania. *Proc Biol Soc Wash*, 107, 699-706.

Gruia, M., & Giurginca, A. (1998). *Haplophthalmus movilae* (Isopoda, Trichoniscidae), a new troglobitic species from Movile Cave, Dobrogea, Romania. *Mitt Hamb zool Mus Inst*, 95 S, 133-142.

Herle, A.I., Covaciu-Marcov, S.-D., & Ferenţi, S. (2016). Past industry vs. nature: which one influences more the terrestrial isopod assemblages from a town in western Romania? *Oltenia. Studii şi Comunicări, Ştiinţele Naturii,* 32(1), 55-60.

Heske, E.J. (2015). Blood on the Tracks: Track Mortality and Scavenging Rate in urban Nature Preserves. *Urban Naturalist*, 4, 1-13.

Hornung, E. (2011). Evolutionary adaptation of oniscidean isopods to terrestrial life: Structure, physiology and behavior. *Terr Arthropod Rev*, 4, 95-130.

Joshi, R., & Puri, K. (2019). Train-elephant collisions in a biodiversity-rich landscape: a case study from Rajaji National Park, north India. *Human – Wildlife Interactions*, 13(3), 370-381.

Laza D., Popovici P.V., Bodog D.E., Molnár K., & Ferenţi S. (2017). Terrestrial isopods in a small town in western Romania (Pâncota, Arad County): witnesses of the past human impact of the region? *Oltenia. Studii şi Comunicări, Ştiinţele Naturii*, 33(2), 53-60.

Mândruț, O. (2006): Mic Atlas de Geografie a României. *Editura Corint*, Bucharest, pp. 48.
Pop, D.-R., Dordea, D.-N., Cicort-Lucaciu, A.-Ș., Covaciu-Marcov, S.-D., & Ferenți, S. (2019). A hot-spot of native terrestrial isopods in an urban area in the Carpathians, Herculane Spa: an emerging of the past into the present. *Spixiana*, 42(2), 219-228.
Pop, D.-R., Maier, A.-R.-M., Cadar, A.-M., Cicort-Lucaciu, A.-Ș., Ferenţi, S., & Cupșa, D. (2020). Slower than the trains: Railway mortality impacts especially snails on a railway in the Apuseni Mountains, Romania. *Ann Zool Fenn*, 57, 225-235.
Prăvălie, R., & Bandoc, G. (2015). Aridity Variability in the Last Five Decades in the Dobrogea Region, Romania. *Arid Land Res Manag*, 29, 265-287.
Schmalfuss, H. (2003). World catalogue of terrestrial isopods (Isopoda: Oniscidea). *Stuttg Beitr Naturkd*, Serie A 654, 1-341.
Radu, V.G. (1983). Fauna R. S. R. Crustacea. vol. IV, Fascicola 13 Ordinul Isopoda, Subordinul Oniscoidea, Oniscoidee inferioare. *Editura Academiei R. S. R*., Bucharest, 168 pp.
Radu, V.G. (1985). Fauna R. S. R.. Crustacea. vol. IV, Fascicola 14 Ordinul Isopoda, Subordinul Oniscoidea, Crinochaeta. *Editura Academiei R. S. R.,* Bucharest, 158 pp.
Tăbăcaru, I., & Boghean, V. (1989). Decouverte, en Dobrogea (Roumanie), d'une espece troglobie du genre *Trachelipus* (Isopoda, Oniscoidea, Trachelipidae). *Misc Speol Rom*, 1, 53-76.
Tomescu, N., Teodor, L.A., Ferenţi, S., & Covaciu-Marcov, S.-D. (2015). *Trachelipus* species (Crustacea, Isopoda, Oniscidea) in Romanian fauna: morphology, ecology, and geographic distribution. *North-West J Zool*, 11(Supplement1), S1-S106.
Tomescu, N., & Teodor L. (2018). Terrestrial isopods (Isopoda, Crustacea) from the "Danube Delta" Biosphere Reserve. *Studia UBB Biologia*, 63, 15-23.
Vilisics, F., & Hornung, E. (2009). Urban areas as hot-spots for introduced and shelters for native isopod species. *Urban Ecosyst*, 12, 333-345.
Vona-Túri, D., Szmatona- Túri, T., Kádár, F., Kiss, B., Weiperth, A., & Gál, B. (2016). Ground-dwelling arthropod (Araneae, Coleoptera: Carabidae, Isopoda, Oniscidea) assemblages on Hungarian main road verges. *Acta Univ Sapientiae, Agric Environ*, 8, 96-111.
Vona-Túri, D., Szmatona-Túri, T., & Kiss, B. (2017). Effects of roads and adjacent areas on diversity of terrestrial isopods of Hungarian highway verges. *Biologia* 72(12), 1486-1493.
Vona-Túri, D., Szmatona-Túri, T., Gál, B., Weiperth, A., & Kiss, B. (2018). Invasive occurrence and abundance changes of Armadillidium vulgare (Latreille, 1804) in Hungarian roadside verges. *Period Biol*, 120(2-3), 91-103.
Vona-Túri, D., Szmatona-Túri, T., Weiperth, A., & Kiss, B. (2019). Diversity and Abundance of Isopods (Isopoda: Oniscidea) on Hungarian Highway Verges. *Acta Zool Bulgar*, 71(3), 385-398.
Warburg, M.R., Linsenmair, K.E., & Berkovitz, K. (1984). The Effect of Climate on the Distribution and Abundance of Isopods. *Symp zool Soc Lond*, 53, 339-367.
Wrzesień, M., Jachuła, J., & Denisow, B. (2016). Railway embankments - refuge areas for food flora, and pollinators in agricultural landscape. *J Apic Sci*, 60(1), 97-110.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 93-100)
doi:10.24193/subbbiol.2021.1.04

===SHORT COMMUNICATION===

The morphometric analysis of Eurasian coot eggs (*Fulica atra*) under the local conditions from Câmpeneşti, North-Western Romania

Valentin Adrian Kiss[1,2]✉

[1]*Babeş-Bolyai University, Faculty of Biology and Geology; Cluj-Napoca, Romania;*
[2] *Romanian Institute of Science and Technology, Cluj-Napoca, România;*
✉***Corresponding author, E-mail:*** ***kissvalentinadrian@gmail.com.***

Article history: Received 17 April 2020; Revised 27 November 2020; Accepted 29 April 2021; Available online 30 June 2021.

Abstract. The aim of the present paper was to analyze the measurements of the Eurasian Coot (*Fulica atra*) eggs in order to evaluate if the local conditions, presented here, differ in some way from older data found in references dating to 1955 in Romania. The data were collected from the Eurasian Coot nests identified (N=8) at Câmpeneşti fishponds, located in North-Western Romania, in May 2018. The clutch size was 7.5 ±1.6, ranging from 5 to 10 eggs. The mean egg length was 50.81 mm, and the mean egg breadth was 34.5 mm with higher variability in case of the first measurement. The mean egg volume was 31.3 cm^3 which is much smaller than reported in the Romanian references (36.13 cm^3). Regarding intra-clutch variance, we found that some clutches manifest a higher length, breadth and volume variance than others which can be a result of the intraspecific nest parasitism or environmental variances. The results brought some extensions of egg length and egg breadth limits and also may reflect a decrease in egg size over time.

Keywords: egg length, egg breadth, egg volume, intraspecific variation

Introduction

The present paper aims to analyze the Eurasian coot (*Fulica atra*) egg morphometry and to compare our data with similar findings from the literature dating to 1955 from Romania and also from Poland (Polak, 2010). In birds, the

genotype, laying date, laying order, and ambient temperature just before laying may influence the egg size variation (Custer and Frederick, 1990; Polak 2010). Clutch size and egg characteristics also vary among and within bird species (Figuerola and Green, 2005). Intraspecific and intraclutch variation may give a hint about the nestling hatching and its survival (Blackburn, 1991; Profus *et al*., 2004). It is known that larger eggs contain more nutrients, thus hatchlings from larger eggs are more sizeable, grow faster, and also show a higher survival rate than those from smaller eggs (Mitrus and Rogala, 2001). The egg size variation can also provide information about intraspecific brood parasitism (Cheng *et al*., 2016). Regarding the clutch size, the larger the clutch the lower the survival rate of the offspring due to an increased possibility of being detected by predators (David *et al*., 2018). The Eurasian coot is a sexually monomorphic, monogamous water bird of the family Rallidae (Cramp and Simmons, 1980; Samraoui and Samraoui, 2007). It breeds in inland wetlands where emergent vegetation is present. In this species, a pair's breeding success each season is positively related to the abundance of submerged vegetation (Nieoczym and Kloskowski, 2018) because emergent vegetation presumably provides nesting habitats and protection against aerial predators (Salathé, 1986). Coots are generally nesting on wet vegetation stands with rich food (plant or animal) resources (Glutz *et al*., 1981).

Egg size varies in relation to a lot of factors such as the age of the females, some anatomical and physiological features of the females or the mass of the oviduct and endogen proteins (Christians, 2002). The size of the clutch is influenced by the distance from shore of the nest emplacement and the weight of the egg is also dependent on this parameter (Uzun *et al*., 2010). The Carp population may also influence the clutch and egg measurements (Nieoczym and Kloskowski, 2018). In this context, our samples may seem insufficient (N=60 measured eggs can be interpreted as insufficient) but this number can reflect the situation found at Câmpeneşti and can reflect the general values that can be identified in the local conditions of those wetlands. The interpretation of data does not involve correlation in order to insist and isolate one parameter. Moreover, our results obtained through measurement, not calculation or statistical interpretation, bring novelty in relation to the existing data in the bibliography.

Materials and methods

Câmpeneşti fishing complex is in the North-Western Transylvania, Romania (46°50'01.60"N, 23°43'12.92"E). Seven fishponds are found at Câmpeneşti fishing complex, each of them having different ecological particularities (Kiss and Pripon, 2019a, b). In this study, we chose two of those ponds based on observations

indicating they the highest number of breeding pairs of coots. The distance between the two ponds where the nests were identified was 1.36 km. The data was collected between 8 and 9 May 2018. For the nest searching, we used the line transect method (Gregory *et al*., 2004). We measured the length and the breadth of the egg with a digital caliper. The volume was determined after Hoyt's formula: $V = k\ x\ L\ x\ B^2/1000$ where k = 0.51 (Hoyt, 1979), L = length and B = breadth. Standard deviation was calculated using standard statistical equations, as square root of the variance.

Results and discussion

At Câmpenești fishing ponds, the Eurasian coot is one of the most abundant species of waterbird (Kiss and Pripon, 2019a). A total of 60 eggs from 8 complete nests were identified and measured (Table 1 and Table 2) at Câmpenești. Regarding the clutch size, the mean value was 7.5 eggs/clutch with a variation similar to that found in eastern Poland in a similar habitat type in 2005 – 2008 (Polak, 2010). Regarding the minimum number of eggs per clutch, this was identical to the one mentioned by Polak (Polak, 2010) and lower than the one mentioned by Linția in 1955 (Linția, 1955). On the other hand, the maximum number of eggs per clutch was lower than the maximum number mentioned by Polak and by Linția (Table 1). We observed a negative trend regarding clutch size based on the minimum number and the maximum number of eggs mentioned 60 years ago in Romania. This trend should be studied more comprehensively for a longer period and museum collections should be also analyzed. In comparison with other studied Rallidae species (David *et al*., 2018), we found that the mean number of eggs in coot nests (7.5) is lower than in Water rails (*Rallus aquaticus*) – 8.36 and higher than in Little crakes (*Porzana parva*) – 6,22 (David *et al*., 2018).

Table 1. Clutch size characteristics in Eurasian Coot nests identified at Câmpenești in 2018 in comparison with other studies from Romania (Linția, 1955) and Poland (Polak, 2010)

	Linția (1955)	**Polak (2010)**	**This study**
Total number of clutches	-	106	8
Minimum number of eggs/clutch	7	5	5
Maximum number of eggs/clutch	12	14	10
Mean of eggs/clutch ±SD	-	7.52 ± 1.59	7.5 ±1.60

Table 2. Egg measurements from Eurasian Coot clutches identified at Câmpeneşti in 2018 in comparison with other studies from Romania (Linţia, 1955) and Poland (Polak, 2010)

		Linţia (1955)	**Polak (2010)**	**This study**
N (samples)		**100**	**797**	**60**
Length (mm)	Minimum	48.9		43.09
	Maximum	59.4		55.69
	Mean ±SD	**57.41**	**52.70 ±2.33**	**50.81 ±2.54**
Breadth (mm)	Minimum	32.1		32.22
	Maximum	39		43.38
	Mean ±SD	**35.13**	**36.52 ±1.22**	**34.55 ±1.48**
Volume (cm^3)	Minimum			24.73
	Maximum			50.3
	Mean ±SD	**36.13**	**35.72 ±3.44**	**31.3 ±3.74**

We obtained almost the same values for the egg dimensions as Polak's study (Polak, 2010 - Table 2) but in all cases, we obtained a slightly lower value and a slightly pronounced variation (Table 2). We can notice that a high difference is present between the data obtained from Câmpeneşti and those from 1955 obtained by Linţia (Linţia, 1955). Comparing the differences between the mean values, we saw an 11% decrease in length and a 13% decrease in volume. Regarding egg breadth, the difference is insignificant (1%) which shows that this dimension presents higher stability. On the other hand, we can observe that the minimum and maximum values for egg length at Câmpeneşti are considerably lower than those mentioned by Linţia (Linţia, 1955). Regarding egg breadth, we see that the minimum value is approximately identical to the one from 1955, but the maximum value is larger, meaning some eggs at Câmpeneşti are wider. If we compare our results with ones obtained for other species from the Rallidae family (David *et al*., 2018), we ascertain that the minimum egg length in the Eurasian coot is 3.5 mm larger than the maximum length of a Water rails' egg. The difference between the maximum and minimum egg length values for the Eurasian coot it is 12.6 mm, which makes the interspecific difference approximatively three times smaller than the intraspecific difference. Regarding the breadth of the little crake's eggs, some of the eggs have a bigger breadth (max: 36.93 mm (David *et al*., 2018)) than the Eurasian Coot's eggs (min: 32.22 mm – Table 2).

Concerning the egg volume, the minimum value was considerably lower compared with both Polak (Polak, 2010) and Linţia's studies (Linţia, 1955), showing a difference of 4.4 cm^3 in the first case and 4.83 cm^3 in the second

case (Table 2). The minimum value of the volume in Eurasian coot's egg at Câmpeneşti (24.73 cm^3 – Table 2) it is approximatively equal with the maximum value of the Water Rail's egg – 24.14 cm3 (David *et al*., 2018) which reflects a reduced interspecific difference regarding this parameter. Until now the discussions were focused on the intraspecific and interspecific differences regarding the clutch and the egg dimensions of the Eurasian Coot. Some interesting aspects can be observed from the variation of those parameters between clutches.

Inter-clutch analysis of the length, breadth and volume

In every clutch, the egg length varies to some degree (Fig. 1). Clutch number 8 contains 7 eggs and is the most variable clutch, while clutch numbers 1 (10 eggs) and 5 (6 eggs) are the least variables (Fig. 1). The rest of the clutches vary approximately equivalently. The egg breadth does not vary as much as egg length (Fig. 1).

For egg breadth, the most stable clutches are number 1 and number 7 and the most variable clutches are number 8 and 2 (Fig. 1). We can observe that in the case of breadth and length, clutch 8 varies considerably, reflecting that the nest may be intraspecific parasitized. The most pronounced variation regarding the egg breadth was found in clutch 2 where the egg length has an intermediary variation as shown in Figure 1.

If we compare clutch number 2 to the rest of the clutches which were influenced by the same environmental factors, we can also question the status of the clutch (if it was intraspecific parasitized or not). Regarding the volume of the eggs, we notice a similar spectrum of variance between the clutches (Fig. 1), where the eggs from clutches 2 and 8 have more variable eggs than clutches 1, 6, 5, and 7 where the egg volume is relatively constant even though clutch 1 has more eggs than the others.

Conclusions

In conclusion, we can say that the measured values for egg length, egg breadth and egg volume are similar to the eastern Poland study, but they are different from the ones in Romania in 1955, showing an evident decrease in egg length and egg volume in the latter case. A negative trend is also present in both egg dimensions, with the length of the egg being more variable and the breadth more stable. In both cases, we found high variation which goes over the limits stipulated by Linţia in 1955, stretching the limits for the maximum value of breadth by + 4.38 mm and the minimum value for length by -5.81 mm.

Regarding Eurasian coot and Water rail egg volumes, the interspecific differences are very small even though the body size difference between those species is significant. We can also say that the length, breadth and volume vary considerably between some clutches which can be a sign of intraspecific parasitism but also a result of the environmental influences during the laying period.

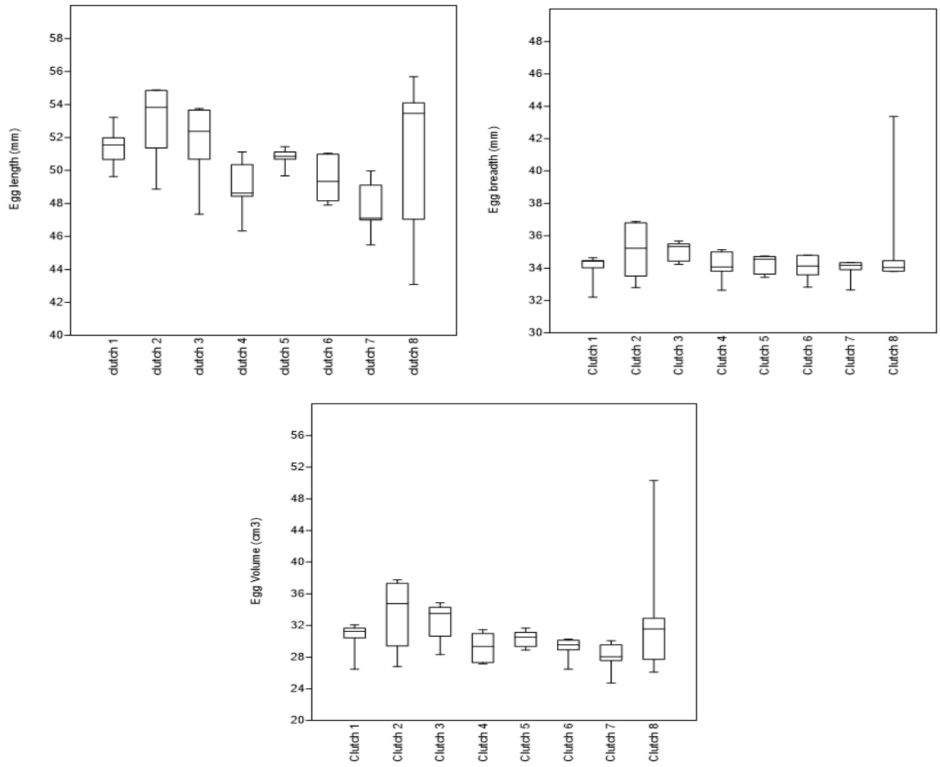

Figure 1. The variation of length (top), breadth (middle) and volume (down) of Coot (*Fulica atra*) eggs in 8 clutches identified at Câmpeneşti.

Acknowledgements: I want to thank Kurt Gielow for the text corrections and scientific content feedback and Liviu Pripon for his help searching for nests and measuring the eggs. This research was founded by Babeş-Bolyai University trough the special research scholarship.

References

Blackburn, T. M. (1991). An intraspecific relationship between egg size and clutch size in birds. *Auk*, 108: 209-211.

Cheng, K., Cheng, Z., Cai, T., & Ha., L. (2016). The effectiveness of egg morphology for detecting parasitized nests and eggs for common coots *(Fulica atra)* in Heilongjiang, China. *Waterbirds*, 39(3):306-311.

Christians, J., K. (2002). Avian egg size: variation within species and inflexibility within individuals Biol. Rev 77: 1–26

Cramp, S., & Simmons, K. E. L. (1980). The Birds of the Western Palearctic, Vol. 2. *Oxford University Press*, Oxford.

Custer, T. W., & Frederick, P. C. (1990). Egg size and laying order of Snowy Egrets, Great Egrets and Black-crowned Night-herons*, The Condor*, 92: 772-775.

David, A., Stermin, A. N., & Sevianu, E. (2018). Clutch size and egg repetability in three elusive bird species: Little Bittern (*Ixobrychus minutus*), Little Crake (*Zapornia parva*) and Water Rail (*Rallus aquaticus*) from north-west Romanian populations, Studia Universitatis Babeș-Bolyai Biologia, 63, 1, 81-88.

Figuerola, J., & Green A. (2005). A comparative study of egg mass and clutch size in the Anseriformes. *J. Oenithol.* 147: 57–68.

Glutz U. N., Bauer K., & Bezzel E. (1981). Handbuch der Vögel Mitteleuropas. Wiesbaden B.5: 688.

Gregory, R. D., Gibbons, D. W., & Donald, P. F. (2004). Bird census and survey techniques 1: 38-40 DOI: 10.1093/acprof:oso/9780198520863.003.0002

Hoyt, F. D. (1978). Practical methods of estimating volume and fresh weight of bird eggs. *The Auk* 96:73-77.

Kiss, V. A., & Pripon L. R. (2019a). Differences regarding habitat preferences of the Eurasian Coot (*Fulica atra* Linnaeus 1758) in the breeding and non-breeding season in local conditions of Câmpenești (Cluj, Transylvania), Conference paper: 12th congress of the European Ornithologists' Union - Cluj-Napoca.

Kiss, V. A., & Pripon, L. R. (2019b). A reevaluation of the avifauna from Câmpenești fishing complex (Cluj County – Romania) in the context of high human impact, *Brukenthal Acta Musei* XIV 3, 657-672.

Linția, D. (1955). Păsările din R.P.R. vol. III. *Editura Academiei Republicii Populare Române*, București.

Mitrus, C., & Rogala, B. (2001). Egg size variation in the Collared Flycatcher Ficedula albicollis in the Bialowieza Forest (NE Poland). *Acta Ornithologica* 36: 7-12.

Nieoczym, M., & Kloskowski, J. (2018). Habitat selection and reproductive succes of coot *Fulica atra* on ponds under different fish size and density conditions. *Hydrobiologia* 820: 267–279

Polak, M. (2010). Clutch and egg size variation in the coot *Fulica atra* breeding on fishponds in eastern Poland – test of the optimal egg dimensions hypothesis, *Acta zoologica cracoviensia,* 53A (1-2): 35-40.

Profus, P., Tryjanowski, P., Tworek, S., & Zduniak, P. (2004). Intrapopulation variation of egg size in the White Stork *(Ciconia ciconia)* in southern Poland, Polish *Journal of Ecology*, 52: 75-78.

Salathé, T. (1986). Habitat Use by Coots Nesting in a Mediterranean Wetland. Wildfowl 37: 136–171.

Samraoui, F., & Samraoui, B. (2007). The reproductive ecology of the Common Coot *(Fulica atra)* in the Hauts Plateux, Northeast Algeria, *Waterbirds* 30(1): 133-139.

Uzun, A., Uzun B., & Kopij G. (2010). The Effect of Clutch Size on Egg and Hatchling Mass and Measurements in the Common Coot *Fulica atra*, Ekoloji 19, 74:160-163.