BIOCELL, Vol.49, Suppl. 3, 2025
ISSN 0327-9545
ISSN 1667-5746 (online version)

Abstracts of the XLII Annual Meeting Sociedad de Biología de Cuyo

December 12th and 13th 2024

San Luis - Argentina

BIOCELL, Vol.49, Suppl. 3, 2025
ISSN 0327-9545
ISSN 1667-5746 (online version)

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ISSN 0327-9545
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LECTURES

A1
COMMON ERRORS IN THE DESIGN AND EXECUTION OF PRECLINICAL TRIALS: OBSTACLES IN CROSSING THE "VALLEY OF DEATH"

Ortega, Hugo H

Profesor Titular Cátedra de Biología Celular - Facultad de Ciencias Veterinarias del Litoral, Universidad Nacional del Litoral (UNL). Director del Centro de Medicina Comparada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral (UNL) /Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Esperanza, Santa Fe, Argentina. hhortega@fcv.unl.edu.ar

The "valley of death" in biomedical research refers to the challenging transition from laboratory discoveries to effective treatments or the transfer of results to specific sectors. This process often fails, largely due to errors in the design and execution of biological trials. Identifying these errors is crucial for improving knowledge transfer and reducing failure rates, particularly in the clinical phases of new drug studies. The discovery of a new active compound involves studying synthesis processes, isolating it from a natural source or obtaining it biotechnologically, characterizing its activity, and completing all preclinical phases, including toxicology, to confirm that the compound is acceptable in terms of efficacy and safety for human trials. However, translating knowledge from basic biomedical sciences to clinical applications that reach patients has been compared to crossing a "valley of death" due to numerous issues that separate the two ends, constantly threatening to halt progress. This gap between the lab benches that discover new molecules and the clinical sectors that need new treatments, diagnostics, and preventive measures has widened over time. Despite attempts made in both scientific and pharmaceutical industry settings to mitigate this problem, drug development failure rates remain high. One important factor is the lack of rigor in many aspects of basic research, which makes preclinical development require highly complex structures to meet the increasingly stringent regulatory agency requirements for authorizing clinical trials and allowing the translation of new molecules to advance. Intellectual property issues, weak hypotheses, irreproducible data, ambiguous experimental models, experimental and statistical design errors, and even the academic pressure to publish results prematurely all compromise many developments and hinder the transfer of new knowledge. Avoiding this requires the coordinated effort of an interdisciplinary team of professionals, along with comprehensive technological platforms that meet and certify the highest regulatory and quality standards to avoid compromising subsequent stages of result translation. A common error is the lack of a well-defined hypothesis and specific objectives that align the preclinical study with clinical needs. This can lead to redundant research or models that do not provide valuable information for future studies. The choice of animal models or cellular systems that are not representative of human pathology is a recurrent issue. Inadequate models generate data that do not translate well to clinical settings, leading to high failure rates in later clinical trials. The lack of randomization in the assignment of animals to groups and the absence of blindness in outcome evaluation are serious methodological errors. These factors introduce bias in data interpretation and can artificially inflate the effectiveness of an intervention. Many preclinical studies lack sufficient methodological detail, making replication by other researchers difficult. Reproducibility is essential for validating findings before moving to the clinical phase. There is a tendency to publish only positive results, distorting the perception of a treatment's effectiveness. This limits a complete view of an intervention's impact and promotes decision-making based on incomplete data. The use of inadequate statistical methods or selective analysis of results are common errors that can lead to incorrect interpretations and unreliable results. These errors not only hinder the translation of discoveries but also increase costs and time invested in developing new treatments. The high failure rates in clinical trials are a manifestation of these previous failures, leading to significant resource losses for both industry and research institutions. To overcome the "valley of death" in biomedical research, rigorous design and execution of trials are essential. Implementing practices such as randomization, blinding, using biologically relevant models, and ensuring transparency in documentation can significantly reduce failure rates in the clinical phase and improve the effectiveness of translational research.

A2
IMPORTANCE OF PLANT PRODUCTS FOR PLANT HEALTH IN CROPS OF AGRICULTURAL INTEREST

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The most common and destructive fungal diseases in oranges, strawberries, and peaches are caused by fungi of the genera *Penicillium*, *Botrytis*, *Colletotrichum*, *Rhizopus*, and *Monilinia*, respectively. Synthetic fungicides control these pathogens, but market demands regarding their residue limits have led to minimizing their use and seeking replacement alternatives. The general objective of this work was to evaluate products extracted from native plants of the Litoral region for their fungicidal capacity against fruit pathogens. This study summarizes the evaluation of 120 extracts/essential oils/pure molecules obtained from 52 plant species that were fungicidal or fungistatic against some of the mentioned pathogens, using *in vitro* and *ex vivo* assays. At least one type of extract from each plant species and all evaluated essential oils showed fungistatic or fungicidal activity against some of the fungi under study. The most active plant extracts were selected for bio-guided fractionation and isolation of the compounds responsible for their activity. The following compounds were isolated: Polygodial (1) from *Persicaria acuminata*; Pinostrobin (2) and Flavokawin B (3) from *Polygonum stelligerum*; and Solidagenone (4) from *Solidago chilensis*. A phytochemically standardized extract of *P. acuminata* was subjected to *ex vivo* assays on oranges, strawberries, and peaches inoculated with different pathogens, obtaining comparable results to commercial fungicides in all cases. Moreover, extracts obtained from *Zuccagnia punctata* and *Solanum pilcomayense* displayed similar results when inoculated fruits were treated with them. Finally, cytotoxicity assays against Huh7 liver cells showed that these natural products were less cytotoxic than commercial fungicides carbendazim and imazalil. It is concluded that phytochemicals have great potential for controlling postharvest diseases in Argentina's fruit production; however, further studies on the standardization of actives in extracts, dosages, and application technologies are needed.

A3
XENOGRAFTS: A VALUABLE TOOL FOR BREAST CANCER RESEARCH

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Experimental studies in cancer research require models that reproduce cancer biology as closely as possible. Cell cultures are useful for analyzing physiological and molecular changes in an in vitro context, allowing for genetic and epigenetic manipulation, as well as providing insights into molecular behavior in response to specific treatments. However, to analyze cell behavior in a physiological or tumor context, or in response to experimental treatments, it is essential to study the interrelationship with the environment, stroma, vasculature, and immune system. In these cases, in vivo studies using animal models are required. Syngeneic studies have the limitation of using cells from the same species as the host animal. Xenograft experiments, on the other hand, allow the use of human tissues for the implantation and development of tumors in easily manipulable animal models, such as rats and mice. To avoid graft rejection by the host's immune system, the animals used for xenografting must have specific mutations that lead to deficiencies in the immune system (generally in T cells). In this presentation, I will showcase examples of using xenografts for breast cancer research through different approaches. First, I will demonstrate the differential tumor behavior of genetically modified cells. Then, I will explore the response of various tumor models to specific treatments. I will also address, at a more physiological level, the impact of tumor environment localization and its interrelation with tumor cells. Finally, I will illustrate how xenograft models can be used with patient-derived samples (Patient-Derived Xenografts, PDXs), whether for diagnostics or research. I will present the generation of PDX tumors resistant to therapies and their use in seeking new treatments. Since the mice used in xenografting are immunocompromised, I will mention the clear limitations of these models and the new options that animal production laboratories have developed to address them (humanized mice).

A4
SCIENCE AND ENVIRONMENTAL COMMITMENT: STRENGTHENING TECHNOLOGICAL SERVICES, EDUCATION AND RESEARCH IN A UNIVERSITY LABORATORY FOR THE STUDY OF ENVIRONMENTAL POLLUTANTS

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It is well known that environmental changes have significant, though not fully understood, effects on human health. Environmental pollutants have become a global concern due to rapid urbanization and industrialization. In our region, environmental challenges arise from the growing industrialization around large urban centers, making dynamic management necessary for sustainability and environmental protection. For several years, the National University of Rosario (UNR) has taken an active role in addressing this issue by creating the Environmental Studies and Sustainability Platform (PEAS), where research groups from various fields work in an interdisciplinary scientific, technological, and academic environment. Thanks to the national and regional funding and grants from the UNR, the PEAS Laboratory (LPEAS) was established to respond to the demands of our society and the private sector. Our aims are: a) To provide environmental measurements for public health and environmental pollutant monitoring in various types of samples using cutting-edge equipment; b) To improve the scientific production of environmental and health research groups by providing state-of-the-art technologies; c) To contribute to the advancement of scientific knowledge in environmental sciences, in the context of the provincial General Environmental Law and the global environmental policy of the 2030 Agenda for Sustainable Development. Equipped with gas chromatography coupled with mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), and atomic absorption spectrometry (AAS), LPEAS can perform precise and sensitive analyses for the identification and quantification of substances and traces present in various environmental (air, water, soil), industrial, food, and biological matrices. Some of our tests include measurements of heavy metals, pesticides, herbicides, and hydrocarbons. This allows us to become a strategic partner in the evaluation and prevention of chemical and environmental disasters, and the impact of agrochemicals on health and biodiversity. Additionally, the laboratory plays an active role in educating and training professionals. This strengthens local capacities in science and technology and fosters inter-institutional collaboration between the university, government, and other entities. The merging of these actors can contribute to more efficient, science-based management tailored to local needs, benefiting human health and helping meet the United Nations Environment Programme's Sustainable Development Goals.

A5
OMICS TECHNIQUES AS TOOLS TO TACKLE QUESTIONS IN BIOREMEDIATION

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Omics techniques originated from the need to study and understand the complexity of biological systems at the molecular level, becoming fundamental tools for comprehensively analyzing large sets of biological molecules, such as genes and proteins. Advances in technology and data analysis have enabled these techniques to be applied in various fields of study. In bioremediation research, metagenomics—one of the first expansions of genomics—allows for examining the total genetic material in environmental samples, providing a detailed view of microbial communities in their natural habitat without the need for laboratory culture. This technique facilitates the identification of genes and metabolic capacities in microorganisms, revealing the primary players in contaminant degradation. On the other hand, proteomics allows for the characterization of the full set of proteins expressed in an organism under specific conditions. In bioremediation, proteomics is key for studying proteins expressed in response to contaminants, offering crucial data on metabolic pathways and mechanisms of tolerance or degradation of toxic compounds. This makes it a fundamental tool for understanding how organisms metabolize contaminants and adapt to hostile environments. The present work aims to present experiences in the application of omics techniques in bioremediation research in the Province of San Luis, highlighting their relevance in analyzing sites affected by anthropogenic activities. Results obtained from the application of metagenomics to study eukaryotic and prokaryotic microbial communities at a site impacted by acid mine drainage in La Carolina, San Luis, are presented. Additionally,

advances in the use of proteomics to understand the molecular mechanisms involved in contaminant removal in native organisms isolated from affected areas are shared, demonstrating the potential of these techniques for developing research in local contexts. Together, metagenomics and proteomics expand the scientific community's ability to explore and leverage biological systems in environmental contexts, with a direct impact on the further development of bioremediation strategies.

A6
ARTIFICIAL INTELLIGENCE APPLIED TO BIOLOGICAL DATA

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El presente trabajo describe las distinciones entre inteligencia artificial (IA) y aprendizaje automático (ML), con un enfoque en sus aplicaciones en la investigación biológica. La IA se refiere a sistemas capaces de realizar tareas complejas que requieren una inteligencia similar a la humana, mientras que el ML, un subconjunto de la IA, implica algoritmos que aprenden de los datos para hacer predicciones otomar decisiones sin programación explícita. La presentación incluye métodos clave de ML: aprendizaje supervisado para clasificación y regresión, aprendizaje no supervisado para agrupamiento y descubrimiento de patrones, y aprendizaje por refuerzo, los cuales se aplican cada vez más en tareas biológicas como la identificación de subtipos de cáncer, la predicción de resultados de enfermedades y el descubrimiento de biomarcadores para la medicina personalizada. Además, el ML contribuye al modelado ecológico, apoyando la clasificación de especies, el monitoreo de la diversidad, y la predicción de las respuestas de los ecosistemas a cambios ambientales, entre muchos otros. También se enfatiza el papel fundamental de la calidad de los datos y el análisis previo de los mismos para lograr resultados confiables enlos modelos de ML. Datos precisos y bien preprocesados son esenciales para construir modelos sólidos e interpretables en biología, donde datos ruidosos o incompletos pueden distorsionar los resultados. Las habilidades esenciales para aplicar y comprender los modelos de MLen este campo incluyen un sólido dominio de estadística, lógica, programación (a menudo en Python o R), y conocimiento biológico específico, así como la capacidad de interpretar y validar los resultados de los modelos. Además, se reconocen los desafíos éticos y de interpretabilidad como aspectos cruciales para la integración efectiva y responsable de la IA y el ML en biología.
Este trabajo destaca el potencial de la IA y el ML para transformar la investigación biológica al acelerar el análisis de datos y mejorar las capacidades predictivas. Los desafíos relacionados con la calidad de los datos, la interpretabilidad de los modelos y las consideraciones éticas también se reconocen como áreas clave para el desarrollo continuo en este campo interdisciplinario.

BIOCELL, Vol.49, Suppl. 3, 2025
ISSN 0327-9545
ISSN 1667-5746 (online version)

SYMPOSIUM

Symposium 1: COMPREHENSIVE AND MULTIDISCIPLINARY APPROACH TO NEGLECTED TROPICAL DISEASES ¨

A7
NEW GEOSPATIAL STRATEGIES FOR EPIDEMIOLOGICAL SURVEILLANCE OF A ZOONOTIC PARASITE

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Canine soil-transmitted helminth (cSTH) parasites need specific environmental conditions to complete their life cycle. *Toxocara* spp is the most important zoonotic cSTH, since it is the causal agent of human toxocariasis. This zoonotic disease is among the top 5 neglected parasitic diseases worldwide. In this study, the presence of cSTH in canine feces was evaluated in 34 crowded public parks and squares from San Juan Province (Argentina). Fecal samples were collected during different seasons in 2021-2022 and analyzed by standard coprological methods. InfoStat 2020 and OpenEpi V. 3.01 were used for statistical analysis and QGIS 3.16.10 for mapping. Weather data from the nearest weather station, San Juan Airport, seven variables were retrieved: mean temperature, diurnal temperature variation, accumulated precipitation, air humidity, cloud cover, solar energy, and wind speed. The correlation between *Toxocara* spp eggs presence and a composite remote sensed index, which can be identified as a proxy for tree shadow, was analyzed. This new index, specifically created for this study, was named the Tree Magnitude Index (TMI) and it is calculated through the multiplication of the Topographic Index Position obtained from a Digital Surface Model and the Normalized Difference Vegetation Index (NDVI) obtained from satellite imagery. The TMI was treated as a response variable and as an explanatory one, using the difference between the observed and expected value of positive cases, assuming a homogeneous distribution. From a total of 1121 samples collected, 100 (8.9%) were positive for at least one intestinal parasite and three cSTH species were detected: *Toxocara* spp*, Toxascaris leonina* and *Trichuris vulpis*. The most prevalent cSTH species was *T. vulpis* (64/1121; 5.7%), while the least prevalent was *T. canis* (19/1121; 1.7%). The detection of *Toxocara* spp eggs was significantly higher during autumn ($\chi 2$=16.34, df=3, $p < 0.001$), where higher humidity values were also recorded. Using simple linear regression, the TMI significantly predicted *Toxocara* spp prevalence ($R2 = 0.67$, $F(1, 10) = 23.2$, $p < 0.01$), with the following fitted regression model: Δ Observed-Expected Value = 0.54 + 0.81*(TMI). This is the first study in San Juan to identify environmental contamination of canine STHs in public areas. The specific localization of squares and parks with the presence of cSTH eggs aim to provide information to design strategies to lower the cSTH infection burden in dogs and to provide information to direct serological screening of the human population for *Toxocara* spp. Given the zoonotic nature of these cSTHs we hope this information will help to reinforce activities of control programs, focusing on the "One Health" approach.

A8

EXPLORING SAN JUAN MOSQUITO POPULATIONS AND THEIR SYMBIONTS: CONSIDERATIONS FOR THEIR CONTROL

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Several species of mosquitoes from the Culicidae family affect people and animals worldwide. Synanthropic species can cause diseases in humans and domestic animals, making them highly significant in public health. Some of these species have been recorded in San Juan province. In 2011, an outbreak of San Luis Encephalitis, a disease associated with *Culex quinquefasciatus*, was reported, resulting in numerous cases and two confirmed deaths. The presence of *Aedes aegypti* in the province is more recent, with autochthonous cases of dengue reported for the first time in 2020. During the 2023-2024 season, the largest dengue epidemic was recorded in the province, due to a significant outbreak in Argentina and neighboring countries. The study of particular characteristics of native populations is essential for developing appropriate control strategies. In this context, we detected and molecularly characterized a strain of the endosymbiotic bacteria *Wolbachia pipientis* in native populations of *C. quinquefasciatus*. We analyzed the susceptibility of *Wolbachia*-carrying individuals to different entomopathogenic bacteria: *Bacillus thuringiensis* subsp. *israelensis*, *Bacillus wiedmannii* biovar *thuringiensis*, and *Lysinibacillus sphaericus* under laboratory conditions. The results showed that *Wolbachia*-carrying insects are less susceptible to the different entomopathogenic strains, particularly when treated with *B. thuringiensis*. These findings should be considered when applying commercial formulations of these bacteria in the field. Additionally, we studied potential breeding sites for *A. aegypti* populations in two departments of the province of San Juan, characterized by its arid climate, predominant in the Monte eco-region. Based on field observations, we suspect that the irrigation ditches, which constitute the artificial irrigation system for public trees, could be used as a resource for the development of their immature stages, despite the extreme physicochemical conditions that characterize these sites. Surprisingly, we found that *A. aegypti* would be using these unfavorable bodies of water. The significant expansion of irrigation systems and their poor maintenance in urban areas justify the adoption of urgent preventive measures in this region.

A9

MOLECULAR TOOLS FOR THE SURVEILLANCE OF ZOONOTIC PARASITES IN LOW-COMPLEXITY LABORATORIES

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Cystic Echinococcosis (CE) is a parasitic zoonosis disease caused by species of the *Echinococcus granulosus sensu lato* (Egsl) complex. Dogs can serve as definitive hosts (DH) by consuming raw viscera from infected intermediate hosts (IH) containing hydatid cysts. The IH (primarily livestock) and humans (accidental hosts) acquire the infection through the ingestion of eggs present in the environment. CE impacts both human and animal health, leading to significant economic losses and diminished quality of life for patients, thus having a profound effect on the economies of low-resource countries. Control programs for CE involve surveillance activities in DH, which have included *post-mortem* techniques (necropsy), the use of anthelmintics (arecoline test), copro-antigen detection (copro-ELISA) , and DNA detection (copro-PCR and copro-LAMP). Loop-mediated isothermal amplification (LAMP) reactions show promise as a point-of-care (POC) tool for DNA detection, offering a low-cost methodology that does not require sophisticated equipment. In this study, two LAMP reactions were developed for the detection of Egsl DNA, employing two different molecular targets: *EgG1HaeIII* (LAMPEGSL 2.0) and *ITS-1/18S rRNA/5.8S* rRNA (LAMPEGSL 3.0). After *in silico* development and validation, each reaction was optimized with different concentrations of betaine, temperatures, and incubation times, using malachite green dye as a low-cost, real-time result indicator. To determine the limits of detection (LOD), serial dilutions of DNA from Egsl species were employed, and analytical specificity was analyzed with DNA samples from helminth parasites and DH. Finally, the two reactions developed in this study, with a previously developed reaction (LAMPEGSL), were evaluated with DNA obtained from different matrices from hosts infected with Egsl. The reactions developed in this work exhibited LODs between 100 fg and 100 zg and 100% specificity against DNA from other parasites and DH. In both cases, the use of malachite green dye enabled direct visualization of the results. Comparison of the three reactions demonstrated better performance for the LAMPEGSL 3.0 reaction. This study provides a new low-cost tool for the detection of Egsl DNA in low-complexity laboratories in endemic areas. Recent studies compared the efficiency of LAMPEGSL 2.0 and 3.0 with samples previously evaluated by copro-PCR and copro-ELISA, aiming to develop a unified algorithm for surveillance activities of canine echinococcosis at municipal, regional, and national levels.

BIOCELL, Vol.49, Suppl. 3, 2025
ISSN 0327-9545
ISSN 1667-5746 (online version)

Symposium 2

SEED GUARDIANS: NATIVE SPECIES CONSERVATION AND PRODUCTION ¨

A10
IMPACT OF SALINITY DUE TO CHLORIDES AND SULPHATES ON SOUTH AMERICAN NATIVE HALOPHYTES: COMPARATIVE BIOCHEMICAL, PHYSIOLOGICAL AND MOLECULAR STUDIES OF SALT TOLERANCE

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Native halophytes occurring in extreme saline environments in Argentina represent promising experimental models to study salt tolerance in plants. They can be explored for the mining of salt-tolerant genes and then tested for their efficacy to improve the limit of salt tolerance in crops. Studies performed in our laboratories demonstrate high salinity tolerance in two native halophytes Strombocarpa strombulifera (Lam.) A. Gray and Lycium humile Phil., growing and surviving up to 1 M NaCl. Lycium humile is endemic of the Altiplano-Puna region with multiple salt tolerance mechanisms such as accumulation of the phytohormone abscisic acid, the increase of the antioxidant capacity and proline content, together with the development of a large leaf water-storage parenchyma that allows Na + accumulation and an efficient osmotic adjustment. This species represents one of the most salt-tolerant halophytic Solanaceae. The analysis of growth and accumulation of specialized metabolites were conducted in hairy root (HR) cultures obtained by infection of young seedlings with Rhizobium rhizogenes, showing a significant increase in total phenolics and total flavonoids under 190 mM NaCl, and an important accumulation of alkaloids in control conditions. Strombocarpa strombulifera is a halophytic shrub found in highly saline soils in Argentina and Chile, with high tolerance against NaCl but strong growth inhibition by Na 2 SO 4 . The adaptive success of S. strombulifera in high NaCl conditions is related to a balance between Na + accumulation (and its use for osmotic adjustment), the maintenance of high K + /Na + discrimination, normal Ca 2+ levels, differential modulation of the antioxidant system (polyphenols and other ROS detoxifying compounds, in joint action with the enzymatic antioxidant system), maintenance of a normal photosynthetic rate and the expression of specific genes responding to increased salinity. Gene expression of the ion transporter NHX (Na^+/H^+ exchanger) gene was investigated in leaves and roots showing that Na_2SO_4 is eliciting a stronger stress response than NaCl, likely due to the unique chemical properties and toxicity of Na_2SO_4. NHX antiporters are crucial for maintaining the balance of Na^+ and K^+ in plants, particularly under high salinity. Finally, our findings highlight the potential of these halophytes as a valuable source of bioactive compounds with high antioxidant activity and potential health benefits.

A11
RESILIENT SEEDS: KEYS TO ADAPTATION AND MOLECULAR BASIS OF NATIVE SPECIES IN XERO-HALOPHYTIC ENVIRONMENTS OF SAN LUIS

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Native xerohalophyte species inhabiting extreme saline environments in Argentina represent key experimental models for investigating salinity tolerance in plants. Several studies carried out in our laboratories have examined the germination behaviour of several native species under high salinity and drought conditions, such as *Vachellia atramentaria*, *V. aroma*, *V. visco*, *V. caven*, *Neltuma alba*, *N. nigra*, *N. flexuosa*, *N. caldenia*, *N. chilensis*, *Bulnesia retama*, and *Parkinsonia praecox*. The results show that both germination and early growth of the mentioned species are severely affected by drought, although some show tolerance to desiccation, such as *Parkinsonia praecox*, which presents 91% germination after 72 hours. In contrast, salinity affects native species more severely, as observed in Bulnesia retama, where germination was influenced by temperatures between 18 ± 2 ºC and 32 ± 2 ºC. In *Neltuma* spp, it was observed that seed dormancy is due to the impermeability of the testa, which requires mechanical scarification to promote germination. On the other hand, the study

of osmotic tolerance showed that the seeds of some species, such as *Bulnesia retama*, withstand osmotic potentials up to -1 MPa, indicating tolerance to drought, but not to salinity. Species such as *Parkinsonia praecox* proved to be more resistant to low water availability than to salinity, being able to germinate in NaCl solutions up to 0.3M and with osmotic potentials of up to -1.2 MPa. In the field of conservation, both in situ and ex situ strategies were developed to protect endemic halophyte species, such as *Sarcocornia neei*, *Atriplex crenatifolia* and *A. lampa*. Work was done on the genetic characterization of *Sarcocornia neei* using SCoT molecular markers, which are dominant and do not require prior genomic sequences. Through the optimization of the DNA extraction protocol, high-quality genomic DNA was obtained from individuals from two experimental sites in the province of San Luis, which allowed genetic diversity analyses to be performed. Six SCoT markers were tested and polymorphic amplification patterns were obtained. This work contributes to the knowledge on the physiology, genetics and conservation strategies of endemic plants from extreme saline environments in Argentina, with a focus on improving tolerance to salinity and drought, as well as on optimizing molecular techniques for their study.

Symposium 3: INVITED BIOLOGY SOCIETES

A12
EDITING FISH FOR RESEARCH, DEVELOPMENT, AND INNOVATION (R+D+I)

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Fish are a vital source of high-quality protein for much of the global population; however, wild fish stocks are under increasing pressure due to overfishing, climate change, and environmental degradation. In response, aquaculture has become the fastest-growing sector in animal production, projected to supply 75% of global fish consumption within the next 15 years. While aquaculture presents a promising solution to meet the rising demand for fish, the challenge remains to develop commercially viable, sustainable, and disease-resistant species.

In this context, gene editing technologies, particularly CRISPR/Cas9, are emerging as transformative tools in aquaculture research, development, and innovation (R&D&I). Unlike traditional artificial selection methods, which can take decades to yield results, gene editing allows for faster and more precise improvement of key traits in fish species. For instance, CRISPR has been used to enhance growth rates in Atlantic salmon by introducing genes that accelerate somatic growth, reducing the time needed to reach market size and improving production efficiency. Gene editing is also being applied to improve disease resistance in farmed fish, such as tilapia. By editing immune response genes, researchers have developed tilapia strains that are more resistant to viral infections like Tilapia Lake Virus (TiLV), reducing losses in aquaculture and promoting sustainability. Furthermore, CRISPR has been used to induce sterility in species like carp, preventing them from reproducing in the wild and potentially disrupting local ecosystems. Another significant application is the modification of the fatty acid profile in fish. For example, genetic changes in salmon and tilapia have led to higher omega-3 fatty acid content, enhancing their nutritional value and health benefits. These advancements demonstrate how gene editing can accelerate the development of fish with improved growth, disease resistance, and nutritional profiles, making aquaculture more sustainable, efficient, and adaptable to future challenges. Moreover, gene editing offers the potential to bypass some of the regulatory complexities associated with genetically modified organisms (GMOs), facilitating faster adoption in commercial aquaculture practices. The main goal of this presentation will be to explore the potential of genomic tools to revolutionize aquaculture, highlighting current research examples and discussing the future implications of gene editing technologies for the industry.

A13
MELATONIN DELIVERY SYSTEMS: A PRECLINICAL STUDY IN A RETINAL DEGENERATION MODEL

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Melatonin (Mel), a naturally occurring indolamine primarily produced by the pineal gland, is recognized for its antioxidant and antiapoptotic properties, making it a promising candidate in neuroprotection therapies. Its application in treating retinal diseases, including central serous chorioretinopathy, diabetic retinopathy, and retinitis pigmentosa, has shown positive results when administered orally. However, oral administration poses a significant challenge due to Mel's limited ability to penetrate ocular tissues effectively. To address this issue, we have explored the use of protein nanoparticles (Np) for drug delivery, focusing on human serum albumin (HSA) and ethylcellulose nanocapsules (NCEC) as carriers for Mel. These nanoparticles are known for their biocompatibility and their capacity to encapsulate a wide range of drugs, making them suitable candidates for enhancing Mel's therapeutic efficacy. In vitro and ex vivo studies demonstrated that NCECMel and albumin-based nanoparticles (Np-ASH-Mel) not only promote ocular permeation but also provide sustained drug release over time. These findings suggest that these nanoparticles could potentially overcome the limitations of oral Mel administration by enhancing its ocular bioavailability. To further assess the therapeutic potential of Mel in vivo, a retinal degeneration (RD) model was developed using New Zealand rabbits. This model involved the intravitreal administration of a combination of glutamate (GLUT) and buthionine sulfoximine (BSO), which induces oxidative stress and cytotoxicity, particularly affecting retinal ganglion cells (RGCs). This method effectively mimics the progressive degeneration observed in ocular neurodegenerative diseases, providing a relevant platform for evaluating neuroprotective strategies. Subsequent studies investigated the neuroprotective effects of Mel solution delivered via intravitreal injection and using NCECMel and NpASH-Mel topically and subconjunctival administration respectively. The results demonstrated that both delivery methods effectively protected RGCs from oxidative stress induced by the RD model. The nanoparticles not only enhanced cell viability but also significantly reduced apoptosis in retinal cells compared to a control solution of Mel. These findings highlight the potential of Np-ASH-Mel and NCECMel as safe and effective strategies for treating neurodegenerative eye diseases. The successful application of these nanoparticle-based delivery systems opens new avenues for developing innovative therapies aimed at improving the quality of life for patients suffering from these challenging conditions.

A14
ADVANCES IN REPRODUCTIVE BIOTECHNOLOGIES IN SOUTH AMERICAN CAMELIDS: "OMIC" PERSPECTIVES

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2024 has been declared the International Year of Camelids (A/RES/72/210 UN). For millions of households across over 90 countries, these species are not only of deep historical and cultural value but are also a key resource for subsistence, contributing to food security, nutrition, and economic growth even under climate extreme conditions. However, there is still a need to raise awareness about the untapped potential of these species and promote research that develops new capabilities, practices, and innovative technologies. South American camelids (SAC) are emblematic species of South America, with global recognition for their livestock value (fiber and meat) and scientific-technological value (nanoantibodies). Argentina holds the third largest population of SAC—guanacos, llamas, and vicuñas—worldwide. One of the limiting factors in the production, use, and even conservation of wild species is the reproduction of SAC. Reproductive biotechnologies, commonly used in other domestic species, have limited application in SAC and are considered inefficient: artificial insemination (AI, 2.4-21.7%), *in vitro* fertilization (IVF, 16.5%), and embryo transfer (ET, 17-40%). This is partly due to the incomplete knowledge of the reproductive physiology of these species, which have unique characteristics. In our group "Molecular Biomarkers in Domestic Animals," we aim to enhance and promote the production of SAC as native livestock by applying molecular biology and proteomics techniques to assess reproductive molecular mechanisms and innovative reproductive biotechnologies based on SAC physiology. Specifically, we focus on: 1) the search for pregnancy and/or uterine fertility

biomarkers by evaluating early preimplantation pregnancy, as approximately 50% of embryo losses occur within the first month of pregnancy. Identifying pregnancy markers would facilitate the early, and efficient diagnosis of pregnancy and the management of non-pregnant females, allowing them to undergo new AI or ET cycles. 2) The study of alternative protocols for induced ovulation for SAC, at the uterine functionality level, using nerve growth factor (NGF, which induces ovulation in SAC) or seminal plasma (which contains NGF), could improve AI or ET success rates, as these protocols, based on SAC physiology, are more tailored to their reproductive needs. 3) The evaluation of follicular fluid or oviductal fluid supplementation in sperm capacitation media for IVF, with the goal of identifying SAC-specific proteins derived from these biological fluids to be used in commercial protocols. These developments in reproductive biotechnologies, based on “omic” approaches, will optimize reproductive efficiency in SAC and strengthen their conservation and sustainability, promoting biotechnological progress that meets the needs of an ever-evolving world.

BIOCELL, Vol.49, Suppl. 3, 2025
ISSN 0327-9545
ISSN 1667-5746 (online version)

BRIEF COMMUNICATIONS

GENERAL, CELLULAR AND MOLECULAR BIOLOGY

A15
CIRCADIAN CLOCK SYNCHRONIZATION AND ITS ROLE IN VEMURAFENIB RESPONSE IN BRAF V600E MELANOMA CELLS

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Cutaneous melanoma (CM) is one of the most aggressive and treatment-resistant cancers with escalating incidence worldwide. Approximately 50% of diagnosed CMs express the BRAF V600E mutation which drives uncontrolled activation of ERK (extracellular-signal-regulated kinase) pathway, leading to melanocyte proliferation. Although specific BRAF inhibitors such as vemurafenib (Vem), are used to treat these CMs, their efficacy is often limited by the development of resistance. Interestingly, most CM exhibit a reduced expression of clock genes, indicating a dysfunctional circadian clock compared to the normal skin. Even in immortalized cell lines, circadian clocks regulate physiological processes in a time-dependent manner, driving transcriptional and metabolic rhythms, including cell proliferation. In this study, we investigated whether the human melanoma cell line Lu1205 (BRAF V600E) retains a functional molecular clock and whether clock synchronization affects the response to Vem. We first characterized experimental culture conditions to achieve clock synchronization. Cells grown to 50% of confluence in 5% fetal bovine serum (FBS)-Dulbecco's modified Eagle's medium (DMEM) were subjected to a 30-min shock of 100 nM dexamethasone (DEX) and maintained in an FBS-free medium post-treatment. After 12h of DEX shock, the BMAL1 and Tubulin protein levels were determined by Western blot every 4h over a 48h period. Time point data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey post hoc test. Further, chronobiologic statistics were used for validating temporal changes as rhythms. Thus, each series of data were analyzed by Cosinor method. Our results show that Lu1205 cells exhibit significant temporal variation in BMAL1/Tubulin protein levels, with a rhythmicity of ~60%. BMAL1 expression peaked approximately 30-h after synchronization, with nadirs occurring around 17- and 42-h post-DEX treatment. To assess the impact of synchronization on the response to Vem, we analyzed BMAL1, pERK (phosphorylated ERK), total ERK, and tubulin (loading control) protein levels. Notably, Vem treatment increased BMAL1 expression compared to control cells, and synchronization elevated pERK/ERK ratios without affecting the cells' response to Vem. These findings suggest an interaction between ERK signaling and BMAL1 expression, highlighting the potential influence of circadian clock synchronization on melanoma progression in BRAF V600E cells. Our study suggests that clock synchronization may modulate ERK signaling in melanoma cells, potentially affecting tumor growth or progression. This provides new insights into exploring circadian clock enhancement as a strategy to improve melanoma treatment outcomes.

A16
ACTIVATION OF HYPOXIC PATHWAYS IN BRONCHIAL CELLS: A FOCUS ON PULMONARY MUCO-OBSTRUCTIVE DISEASES

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Pulmonary muco-obstructive diseases (PMOD) are characterized by excessive mucus production, which obstructs airflow through the lungs. These diseases present common symptoms such as difficulty breathing, coughing, and wheezing. The intracellular mechanism activated by mucus accumulation is not well understood. PMOD predisposes individuals to bacterial infections capable of forming biofilms and evading the immune system. Infection and inflammation damage the lungs, leading to structural changes that cause respiratory complications. We hypothesize that in PMOD, the hypoxic response activated by the mucus in the airways contributes to bacterial colonization and persistence. This makes it difficult to eradicate the infection, contributing to chronic lung disease. The project's overall objective is to investigate intermediates

potentially activated by excessive mucus production during PMOD, such as HIF-1 alpha, and carbonic anhydrase (CA). To achieve our goal, we use two types of human bronchial epithelial cell lines: normal cells (16HBE14o-), and cystic fibrosis epithelial cells with ΔF508-CFTR mutation (CFBE41o-). Understanding the behavior of these intermediates will allow us to identify new therapeutic targets for PMOD. First, we analyzed the expression of HIF-1 alpha in both cell types under normoxic and 3 hypoxic times (2h, 4h, 6h) by Western Blot. Our results indicate that in HBE cells, HIF-1 alpha expression increases under hypoxic conditions compared to the normoxic control. CFBE cells showed elevated levels of this intermediate, with even higher expression under hypoxia. Furthermore, we studied the presence of three CA isoenzymes using qualitative PCR: CAII (cytoplasmic isoform), CAIV and CAXII (plasma membrane isoforms). Our results suggest that both HBE and CFBE bronchial epithelial cells express CAII and CAXII. However, CAIV was not present in either cell type. In conclusion, the increased expression of HIF-1 alpha and CA suggests the activation of the hypoxic cellular pathway due to decreased O_2 caused by excess mucus. These data indicates that both HBE and CFBE cells present CAII and CAXII, but lack CAIV. These findings could provide new insights for treating PMOD, a condition often complicated by bacterial infections that are multi-resistant to a wide range of currently available antibiotics.

A17
HIGH-PURITY EXTRACELLULAR VESICLES USED AS A BRAIN BIOPSY IN NEURODIAGNOSTICS

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Neurodegenerative diseases, such as Alzheimer's and Parkinson's, are characterized by the progressive accumulation and spread of mutated or wild type misfolded proteins, which results in neurodegeneration and functional decline. Recent advances suggest that these proteins can propagate pathogenicity by transferring via extracellular vesicles (EVs) that cross cell boundaries. Our research focuses on the role of EVs, derived from microglial cells, as carriers of these misfolded proteins, potentially serving as a "liquid biopsy" for neurodegenerative conditions. We have developed an approach using immortalized murine microglial BV2 cells exposed to tau protein fibrils (dGAE T40) to simulate the cell environment of a proteinopathy. Conditioned media from these cells undergo gradient centrifugation and flotation assays, yielding high-purity EVs. Preliminary results demonstrate the transfer of misfolded proteins through EVs. To evaluate the disease-propagating capacity, HEK293T sensor cells, which have been engineered to report the nucleation and aggregation activity of pathological proteins, are incubated with EVs loaded with dGAE T40 fibrils. Our results hold significant promise for clinical applications, offering a less invasive alternative with the potential for early diagnosis through blood-based biomarkers of neurodegenerative and other brain diseases. Furthermore, understanding the dynamics of EV-mediated protein transfer could illuminate new therapeutic targets to disrupt the pathological progression of neurodegenerative diseases.

A18
MYO1C-MEDIATED ACTIN REORGANIZATION: A KEY PLAYER IN *Chlamydia trachomatis* INFECTION

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Chlamydia trachomatis (Ct), the leading cause of bacterial sexually transmitted infections, manipulates host cell cytoskeletal components to support its intracellular lifestyle. Among these, actin dynamics are crucial for the bacterium's entry, intracellular development, and exit. The myosin superfamily plays a key role in the modulation of the actin cytoskeleton. However, the role of host myosins in these processes remains underexplored. Our study focuses on Myosin 1C (MYO1C), a class I myosin, and its involvement in modulating the actin cytoskeleton during Ct infection. We demonstrate that MYO1C is actively recruited to the chlamydial inclusion membrane, where it plays a central role in organizing the actin cage surrounding the inclusion. Loss of MYO1C, either through siRNA-mediated depletion or pharmacological inhibition with the myosin-specific inhibitor pentachloropseudilin (PClP), results in a destabilization of this actin structure. This disruption severely impairs Ct bacterial development and reduces the efficiency of the non-lytic extrusion process, a key exit mechanism for *Chlamydia*. Mechanistically, we show that MYO1C functions as a dynamic tether for actin at the inclusion membrane, facilitating actin cage formation. We demonstrated that overexpression of a non-actin-binding mutant, MYO1CΔABL, fails to organize the actin cage around the inclusion, highlighting the specific role of MYO1C-actin interactions in this process. Additionally, using in vitro reconstitution assays, we find that MYO1C is necessary and sufficient to organize actin around artificial membranous vesicles, mimicking the inclusion environment. Our findings identify MYO1C as a critical host factor hijacked by Ct to maintain actin cage stability, which is essential for bacterial survival and exit. These results provide new insights into the molecular mechanisms of host-pathogen interactions and suggest MYO1C as a potential therapeutic target to disrupt *Chlamydia* infections.

A19
IBUPROFEN-L ARGININE REDUCES OXIDATIVE STRESS AND PREVENTS MACROPHAGE POLARIZATION TOWARD M1 PHENOTYPE

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A nebulizable hypertonic solution of Ibuprofen (Ibu) with L-arginine (Ar), was designed for the treatment of inflammatory respiratory diseases. Both inflammation and oxidative stress are involved in the onset of these pathologies, so we aimed to investigate the effects of Ibu-Ar on Reactive Oxygen Species (ROS) generation and macrophage polarization. RAW264.7 macrophages were induced to M1 phenotype by lipopolysaccharide/interferon-γ
(LPS/ IFN-γ) stimulation, and treated with different concentrations of Ibu-Ar. DHE staining was used to evaluate ROS, flow cytometry was employed to detect the expression of CD206 and inducible nitric oxide synthase (iNOS); gene expression of inflammatory (TNF-alfa, MCP-1), pro-oxidant (NOX-2) and pro-fibrotic (TGF-beta) markers were determined; ELISA was used to quantify the levels of IL-6 and TNF-α; and Western blotting was used to evaluate the p-STAT3 and p-ERK1/2 protein pathway. Ibu-AR molar ratios greater than 1 and lower than 6.5 generated a marked synergic effect on the inhibition of LPS-increased ROS generation. Ibu-AR administration markedly reduced NOX-2, MCP-1, iNOS, and TGF-β levels when compared with LPS/ IFN-γ, like the effect produced by Hydrocortisone (HC). Also, TNF-alfa expression was drastically inhibited by Ibu-AR ($p< 0.01$). To detect whether the activated cells in M1 modulated to M2, the expression of CD206 was assessed and was found indetectable level compared with HC treatment. Modulation of macrophage polarization is triggered by STAT-3 and ERK1/2 pathways. Ibu-AR significantly reduced LPS/ IFN-γ-mediated phosphorylation of STAT-3 and ERK1/2, reinforcing its ability to modulate macrophage polarization. In conclusion, Ibu-AR acts synergistically by decreasing oxidative stress and modulating M1/M2 polarization of macrophages, thus playing antioxidant and anti-inflammatory roles. Therefore Ibu-AR may be an effective therapeutic strategy in treating inflammatory respiratory diseases.

A20
EFFECTS OF NARINGIN ON LYSOSOMAL ALTERATIONS AND CYTOTOXICITY IN BREAST CANCER CELLS

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Lysosomes are essential organelles involved in intracellular trafficking and macromolecules degradation, playing a key role in cellular function. The expression of most lysosomal proteins is regulated by transcription factor EB (TFEB), a critical regulator of lysosomal biogenesis. Maintaining lysosomal membrane integrity is crucial for preserving lysosomal function and cellular homeostasis. Lysosomal membrane permeabilization (LMP) can lead to the release of lysosomal content into the cytoplasm, resulting in lysosomal-dependent cell death. Naringin (Nar), a flavonoid, has been reported to induce LMP in gastric cancer cells, but its effects on breast cancer cells remain less understood, prompting us to investigate its impact in this context. In this study, MCF-7 (tumoral) and MCF-10A (non-tumoral) mammary cells were incubated with 2 mM Nar for 3, 6, 12, 24, and 48 hours. The acidic compartment index (Ica) was measured using Lysotracker™ Red DND-99, and the LAMP-1 positive compartment index was calculated using indirect immunofluorescence for LAMP-1 (a marker of late endosomes and lysosomes). The impact of Nar on lysosomal biogenesis was evaluated through TFEB immunoblotting. Cell viability was assessed using trypan blue exclusion. Preliminary results showed that Nar significantly increased lysosomal diameter and Ica after 6 hours, correlating with reduced TFEB levels in post-nuclear supernatants of MCF-7 cells and a significant decrease in cell viability. No significant changes in viability were observed in MCF-10A cells. These findings suggest that Nar induces death in breast cancer cells through a lysosomal-dependent mechanism, with a notable specificity for cancer cells. This selective effect underscores the relevance of Nar as a potential therapeutic agent targeting lysosomal pathways in cancer treatment.

A21
STUDY OF THE COOPERATIVE EFFECT OF PPARγ AGONISTS AND VALPROIC ACID ON A COMBINATION OF THERAPEUTIC TARGETS SUCH AS β AND γ-SECRETASES AND BDNF/TRKB IN AN EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is an irreversible neurodegenerative disorder. The balance of biogenesis/clearance of Aß peptides is altered in AD. Aβ is derived by sequential cleavage of the amyloid precursor protein (APP) by β/γ-secretases. Synthetic PPARγ agonists have been shown to improve cognitive performance in patients with AD. It also down regulates β secretase (BACE-1) expression. Valproic acid (VA) alleviates memory deficits and attenuates amyloid-β deposition in transgenic mouse model of Alzheimer's disease. Taking into account these observations, the objective of this study was to evaluate the effect of Pio/VA, on the 24h rhythms of of Aβ; β and γ-secretases, BDNF/TRKB expression in the hippocampus of Aβ-injected rats. Four-month old males Holtzman rats were used in this study. Groups were defined as: 1) control 2) Aβ-injected 3) Aβ-injected treated with Pio-VA. Rats were maintained under 12h-Light: 12h-Dark conditions. β and γ-secretases, and BDNF/TRKB transcript levels were determined by RT-PCR and Aβ levels were analyzed by immunoblotting in hippocampus samples isolated every 6h throughout a 24-h period. We found that the treatment of Pio-VA modified the daily oscillation of β and γ-secretases and reestablished rhythmicity of BDNF/TRKB. These findings might constitute, at least in part, molecular and biochemical basis of effect of Pio-VA on circadian rhythmicity in neurodegenerative disorders.

A22

EFFECT OF MELATONIN AND RESVERATROL ON MAST CELL DEGRANULATION INDUCED BY SECRETAGOGUES ACTIVATING DIFFERENT MOLECULAR TARGETS

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Our laboratory studies the pharmacological regulation of mast cell activation. Mast cells are specialized cells of the immune system that play a crucial role in the inflammatory response since, when activated by both immunological and non-immunological stimuli, they release pro-inflammatory mediators such as histamine and β-hexosaminidase. This release regulates the cellular inflammatory response. Among the compounds we are currently studying as possible regulators of mast cell activation are two antioxidant molecules: melatonin and resveratrol. Melatonin is a hormone secreted by the pineal gland, fundamental for regulating the circadian rhythm and the wake-sleep cycle. In addition to synchronizing sleep, it modulates the immune system. On the other hand, resveratrol is a molecule in grapes and their derivatives known for its antioxidant and anti-inflammatory properties. However, it is not known whether these compounds regulate mast cell activation. The present work aims to evaluate the effect of melatonin and resveratrol on mast cell degranulation induced by secretagogues that activate different molecular targets. Peritoneal mast cells were isolated and purified from male Wistar rats (n=20). Mast cells were incubated with various solutions that were distributed into the following experimental groups: 1) Basal, 2) Secretagogue (compound 48/80 that activates the G protein-coupled MrgX receptor or the calcium ionophore A23187 that increases intracellular calcium concentration), 3) Melatonin (increasing concentrations) + secretagogue and 4) Resveratrol (increasing concentrations)+secretagogue. After 30 min total incubation, the concentration of β-hexosaminidase released and remaining in the cells was assessed by a colorimetric method. Cell viability was analyzed by exclusion with trypan blue. Melatonin did not inhibit mast cell activation induced by stimulation of MrgX G protein-coupled receptors or increased intracellular calcium at any of the concentrations used. Resveratrol inhibited the action exerted by compound 48/80 at the concentration of 200 µg/ml, but did not inhibit the activation induced by the calcium ionophore A23187, suggesting the involvement of signaling molecules activated by the MrgX receptor. These results constitute a starting point for future studies aimed at deepening the bioactivity and mechanisms of action of these antioxidants at cellular and molecular immunology levels.

A23

STRUCTURAL STUDY OF *Trypanosoma cruzi* PROTEINS FOR RATIONAL DRUG DESIGN

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Chagas's disease is an anthropozoonosis caused by the flagellated protozoa *Trypanosoma cruzi*. This disease is transmitted by the insect vector *Triatoma infestans*. It has a high overall prevalence (6-8 million cases) and 65-100 million people are at risk of contracting this infection. Medications used for the disease treatment show undesirable side effects and, currently, there are no available vacines. The identification of new targets for chemotherapy is very important and their three-dimensional structure resolution provides essential information. Nucleoside diphosphate kinases (NDPKs) and adenylate kinases (ADKs) enzymes play a key role in the energetic parasite metabolism. Previously, the TcNDPK1 three-dimensional structure by X-ray crystalligraphy was resolved in our laboratory and a quinary multi-hexameric structure was proposed for a first time for NDPKs. The objective of this work is the structural study of TcNDPK2 and TcADK1 proteins of *Trypanosoma cruzi* by X-ray crystallography and complemetary bioinformatics tools. Homology models of both proteins have been built using the Modeller program and another tools such as Chimera. Additionally, three-dimensional models

were obtained with Alphafold. The models obtained were validated by RAMPAGE, PDBsum and WinCoot. The oligomerization for these proteins was also studied by performing docking tests using different bioinformatic tools, such as ClusPro, HADDOCK, Alphafold, ChimeraX, PDBsum and PIC tools. The TcAdK1 protein was satisfactory overexpressed and purified for crystallographic assays. Recently, TcAdK1 crystals were obtained under different conditions. These results will allow us to advance in crystallographic studies.

A24
STRUCTURAL BASES OF THE INTERACTION BETWEEN CYCLOOXIGENASE-1 AND *LITHREA MELLOIDES* NATURAL COMPOUNDS

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Structural and functional analysis of proteins is crucial to understand their mechanism of action and interactions with ligands. The Protein Data Base (PDB) offers structural three-dimensional details of all proteins solved to date, principally by x-ray cristallography. This structural information can be used to study molecular docking to proteins using several bioinformatic tools. In this work, the extraction of secondary metabolites from *Lithrea melloides*, a plant known for its medicinal properties, was carried out by aqueous and methanolic extractions. Chromatographic profiles form diferent phenological fases plant extracts were identified using high-performance liquid chromatography with diode array detector (HPLC-DAD). Some extracted metabolites were evaluated by bioinformatic molecular docking studies to evaluate their binding with the cyclooxygenase 1 protein (COX-1), a key enzyme in inflammatory processes. Autodock Vina and Chimera programs were used in this step. The molecular details of interactions on protein-ligand complexes were studied using PLIP bioinformatic server and Pymol program and another tools. *Lithrea melloides* plant methabolite collection and extraction conditions were determinate for different phenological phases using HPLC-DAD. Methabolite docking assays to COX-1 protein shows that several metabolites, such as ferulic acid and caffeic acid, have a high affinity for COX-1 active site region, suggesting that they could act as inhibitors of the enzyme. These results suggest that these natural compounds interact with COX-1 and they are potentially inhibit its activity.

A25
HORMONAL REGULATION OF LYSOSOMAL PROTEIN TRANSPORT IN HUMAN ENDOMETRIAL EPITHELIAL CELLS

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The endometrium is a key tissue for reproduction. The steroid hormones, estrogen (E2) and progesterone (P) govern morphological and functional transformations during the menstrual cycle, converting the endometrium into a receptive state, increasing adhesiveness and protein secretion, highlighting the importance of intracellular trafficking to allow implantation. Inadequate adhesive capacity of the endometrial luminal epithelium leads to inadequate blastocyst attachment and implantation failure. Defective endometrial adherence is one of the main causes of implantation failure in infertility, while the mechanisms contributing to this remain poorly defined. In recent years, lysosomes have been recognized for their involvement in numerous physiological processes. Lysosomal proteases, including cathepsins, have been implicated in several physiological processes such as menstruation, implantation, and placenta formation, as well as in different diseases such as cancer or endometriosis. Despite its important role in key reproductive processes, cathepsin D's mechanism and intracellular regulation have not been studied in depth in endometrial epithelial cells. We used a human endometrial epithelial cell line to investigate whether the expression and/or localization of cathepsin D and its carrier proteins could be influenced by sex hormones. Using fluorescence microscopy and Western blot techniques, we observed that the presence of E2 and especially P increases the expression of cathepsin D and its carrier protein, the cation-dependent mannose 6-phosphate receptor (CD-MPR), whereas Sortilin expression was not increased in the presence of sex hormones. Our results demonstrate that the hormonal microenvironment regulates the intracellular transport of cathepsin D. Unraveling the

molecular mechanisms involved in the intracellular transport of cathepsin D modulated by E2 and P will shed further light on the function of the endometrium. These findings could open new scenarios justifying the development of new approaches to improve reproductive outcomes.

A26
AI-ASSISTED DESIGN OF A NOVEL PROTEIN INHIBITOR TARGETING TOPBP1 INTERACTIONS

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Cell cycle regulation involves complex networks of protein-protein interactions, among which TopBP1 (Topoisomerase IIβ-binding protein 1) plays a crucial role. TopBP1 is essential for DNA replication initiation and damage response, interfacing with critical mediators such as 53BP1 and MDC1 to coordinate repair mechanisms and checkpoint activation. In this study, we employed artificial intelligence-driven protein design to develop a novel protein inhibitor that targets a specific interaction within this regulatory network. This inhibitor, a de-novo-designed protein that does not exist in nature, was engineered to modulate key cellular mechanisms by disrupting the interaction between TopBP1 and its partners. The soluble nature of the designed protein facilitates its expression from a synthetic gene, ensuring stable and efficient production under standard experimental conditions. The design process involved the computational generation of 2,000 unique protein backbones, each assigned five potential sequence variants, resulting in a library of 10,000 candidates. These candidates were then analysed using AlphaFold, a deep learning algorithm for protein structure prediction, to assess their ability to form the desired complex with TopBP1, ensuring structural compatibility and predicted stability. From this analysis, we selected the most promising inhibitors based on their predicted binding affinity and structural integrity. By focusing on pathways involving p53-dependent cell cycle regulation, we aim to explore how specific disruptions in protein interactions can modulate cellular responses to DNA damage. Through this AI-driven approach, we are investigating how synthetic molecules can influence the intricate balance between DNA repair and cell cycle progression. We expect the designed inhibitor to effectively bind to TopBP1, potentially altering its interaction with 53BP1 and MDC1, thereby impacting the DNA damage response. Future work will involve detailed biochemical and cellular analyses to elucidate the effects of the inhibitor on cellular processes and to assess its potential as a tool for studying cell cycle regulation and as a lead compound for drug development.

A27
EXPLORING THE DNA-BINDING CAPACITY OF DONSON

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DNA replication is a critical process for cellular proliferation and genomic integrity, making its precise regulation essential to prevent genomic instability and associated diseases. DONSON, a recently discovered scaffold protein, has emerged as a key player in DNA replication, particularly in the assembly of the CMG (CDC45-MCM2-7-GINS) helicase complex and in the recruitment of replication initiation factors such as GINS and TOPBP1. Loss of DONSON disrupts CMG formation, leading to replication stress, stalling of replication forks, and eventual cell death. However, the precise molecular mechanisms by which DONSON ensures the proper execution of DNA replication still need to be completed. In this study, we investigated whether DONSON regulates replication through direct interactions with replisome components and DNA. To explore this, we expressed and purified a human DONSON variant lacking its disordered N-terminal region and generated multiple mutants based on disease-causing mutations found in microcephaly that affect the initiation complex.

Using Electrophoretic Mobility Shift Assays (EMSA), we evaluated how these DONSON variants interact with DNA. We found that the wild-type DONSON variant has a direct affinity for DNA, while specific mutations, notably R385D, significantly reduce this interaction. These findings indicate that DONSON can directly bind DNA and that specific mutations affect this interaction. Our results highlight the importance of studying DONSON's molecular interactions with DNA and replisome components to understand its role in DNA replication and genome maintenance fully. This knowledge could also help explain how mutations in DONSON contribute to developmental disorders like microcephalic dwarfism, where replication stress plays a central role. Future directions for this work include studies to understand how these interactions influence the initiation and progression of DNA replication, providing a more comprehensive understanding of DONSON's role in safeguarding genomic integrity.

VEGETAL BIOCHEMISTRY, PHYSIOLOGY, PATHOLOGY AND PRODUCTION

A28
DEEP DIVING INTO THE ROLE OF GUANOSINE TETRAPHOSPHATE AS A NOVEL REGULATOR OF GROWTH AND STRESS RESPONSES IN *ARABIDOPSIS THALIANA*

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As sessile organisms, plants face constantly-changing environments. Thus, they should induce and activate many signalling molecules and pathways in order to adapt to the challenging conditions imposed by environment. In bacteria, guanosine tetraphospate and guanosine pentaphosphate (collectively named (p)ppGpp or alarmone) have been described as a signal in response to stringent conditions. This hyperphosphorylated nucleotide has been described in plants and algae and can be located both in chloroplast and cytoplasm. In the plant model *Arabidopsis thaliana,* the metabolism of (p)ppGpp is controlled by RelA SpoT Homologue (RSH) enzymes: RSH1, RSH2, RSH3 and cRSH. Alarmone has been reported as a chloroplast gene expression negative regulator which impacts in chlorophyll metabolism and photosynthetic parameters. However, it is not very well known what repressed growth mechanisms are implicated in alarmone signalling; the impact in non-photosynthetic tissues nor the impact in nucleus gene expression. The aim of this study was to evaluate which transcriptional, growth, and stress responses can be modulated by (p)ppGpp in *A. thaliana*. In order to achieve this goal, we used a RSH3 overexpressor line (RSH3OX, alarmone over-producing plants) and a rsh1rsh2rsh3crsh mutant line (rshq, alarmone non-producing plants). Different physiological parameters such as root length, number of lateral roots, hypocotyl length, cotyledon greening, and seedling size were tested. For abiotic stress responses we assayed NaCl sensitivity, temperature and light intensity stress. In the case of biotic stress, we carried out infections with different UFC/mL of *Pseudomonas syringae* Pst DC3000. Finally, we performed total RNA sequencing to understand the (p)ppGpp signalling-regulated gene expression and alternative splicing. We found that RSH3OX plants have overall growth defects in comparison to wild type and rshq mutants. In the case of abiotic stress, we found RSH3OX plants are hyposensitive to salt stress. Besides, RSH3OX plants showed hypersensitivity to *P. syringae* while rshq mutants were hyposensitive to bacterial infection. Finally, we found some transcripts that are alternatively spliced over all the transcriptome of these plants. These results are relevant to understand the importance of this novel molecule on plant growth and adaptation to challenging conditions in a context of climate change.

A29
EFFECT OF SIMULATED WATER STRESS ON GERMINATION AND EARLY GROWTH OF *LEPTOCHLOA CRINITA* (POACEAE) UNDER A CLIMATE CHANGE SCENARIO

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Leptochloa crinita is a natural component of the grasslands of the province of San Luis. This species is of interest for restoration activities of degraded environments due to its tolerance to defoliation, drought and salinity. In this work the germination and early growth processes of *L. crinita* exposed to water stress were studied. The seeds were placed in pots with vermiculite as a substrate. Each pot was watered at 20%, 40%, 60%, 80% and 100% of field capacity, designated as T1, T2, T3 T4 and T5 respectively, in a random design. The number of germinated seeds was recorded, and the germination percentage (GP) was calculated per treatment. When no further germination was recorded, the pots were watered at 100% of field capacity to evaluate the GP of the non-germinatable fraction. At the end of the trial, the survival rate of the seedlings under water stress, leaf emergence rate, shoot and root length, dry weight (DW) and leaf area of the seedlings were measured. The statistical treatment was performed using ANOVA, and the means were compared with tukey´s test ($p=0.005$) finding significant differences in the PG in T1 and T2, with respect to T3, T4 and T5, which were lower. The average root length was greater for T2 followed by T1, and in descending order T3, T4 and T5, on the other hand, the average length of the shoot was greater for T1, followed by T2 with little variations in millimetres with respect to the rest of the treatments T4 and T5, respectively. These differences were significantly greater than T3. This pattern is repeated in the calculation of the leaf area, with the difference that in this case T2 was the largest. It is concluded that germination and early growth of *L. crinita* increase with higher water availability in the medium. PROICO 2-3918.

A30
FLORAL BIOLOGY OF *MINTHOSTACHYS VERTICILLATA* ('PEPERINA CORDOBESA'). NEWS ABOUT THE SEX OF THE FLOWERS ON EACH FOOT

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Minthostachys verticillata, or 'peperina cordobesa' is a species of the Lamiacea family, native to northwestern and central Argentina, widely used as an aromatic and medicinal species, and a fundamental part of the 'yuyera' culture of the highland region. Currently, its wild populations are in frank decline, due to the fragmentation and deforestation of the native forest and extreme harvesting. This highly fragile situation increases the interest in the study of this species, for its potential cultivation and preservation in the wild, where the study of floral biology represents an essential field for its management and conservation. Like other genera of the family, this species presents gyno-dioecia. In preliminary studies we have analyzed the structure of the unisexual flowers present in the female foot, and the bisexual flowers present in the hermaphrodite foot. As a result of the present work, the flowers are characterized in terms of their dimensions and stage of floral maturity, and the distribution of sexes in the different feet is described. Respect to flower dimensions, flower width and length were measured both at the flower bud stage and at anthesis. The average dimensions of the unisexual flowers were 2.34 cm long and 0.82 cm wide at the flower bud stage, and 3.1 cm long and 0.88 cm wide at anthesis. The average dimensions of the bisexual flowers were 2.23 cm long and 0.8 cm wide at the flower bud stage, and 3.3 cm long and 0.88 cm wide at anthesis. The ANAVA showed that the differences were significant for the variable 'maturation stage' in both attributes (width and length) but not for the variable 'sex' in both attributes. Also, it was found that there was a significant interaction for the variables sex and maturity, and their contrast in the anthesis stage. Therefore, although the differences were not significant, flower width and length at the anthesis stage were found to be related to flower sex. As for the sex of the flowers, the presence of both unisexual female and bisexual flowers was detected in the hermaphrodite foot, an aspect that had not been found in previous studies. Based on this novelty, it can be established that the species has a female foot, with unisexual flowers, and a polygamous hermaphrodite foot, with bisexual flowers and female unisexual flowers. It is considered that the findings regarding the distribution of sexes in the plants of this species are of great importance for the knowledge and management of this species, particularly in relation to its potential cultivation, and as relevant aspects in the studies of seed production, germination, and phytochemical composition and variability. All this confers special characteristics that could influence the uses and organoleptic aspects of this species, determining critical aspects at the time of its reproduction and use. Likewise, the correct knowledge and management of the species in the productive sphere is fundamental for the sustainable use and conservation of this species in its natural environment.

A31
COMPARATIVE STUDY OF ESSENTIAL OILS PRODUCED BY FEMALE AND BISEXUAL PLANTS OF *MINTHOSTACHYS VERTICILLATA*

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Minthostachys verticillata, commonly known as peperina, is an iconic native plant from the Sierras de Comechingones region in the provinces of San Luis and Córdoba, associated with the "serrana" identity and widely used in the yerba mate industry. In this study, we compare the yields and chemical profiles of essential oils produced by female (FP) and bisexual (BP) plants. Specimens were selectively harvested from an experimental plot at FTU-UNSL (n = 13 FP and n = 17 BP), then grouped into two random samples for each condition. The essential oils (EO) were obtained through hydro distillation using a Clevenger trap on material previously dried to a constant weight. The average essential oil yield, expressed as volume/weight percentage (% EO), was calculated, and the chemical profile was obtained through qualitative analysis by gas chromatography coupled with mass spectrometry (GC-MS). The relative amount of each compound is expressed as the percentage of each peak area relative to the total chromatographic area. Differences were observed in both parameters. The % EO was 2.0% in FP and 2.5% in BP. The chemical profile corresponded to the pulegone (PU)/menthone (ME) chemotype typical of this region. However, a difference in the amount of PU was observed, being considerably higher in BP (50.2%) compared to FP (32.3%). Conversely, ME was found in greater amounts in FP (7.9%) than in BP (6.9%). Additionally, FP showed an increase in the production of 4-methylacetophenone (MAF) (7.1% in FP versus 1.0% in BP), a metabolite generated through an alternate biosynthetic pathway. This difference in the chemical profile could be related to different ecological strategies employed by FP and BP in their interactions with herbivores and pollinators. It has been suggested that PU and MAP play a major role in herbivore protection, while ME serves to attract pollinators. To compensate for the need for chemical defense, FP may produce more MAP to offset the lower production of PU. Additionally, this difference in the main components of the essential oils (EO) could be due to the selective production of secondary metabolites in the plant's vegetative and reproductive tissues. As found in previous studies, EOs enriched in ME are present in the flowers, while PU predominates in the leaves. This study suggests that FP and BP of *M. verticillata* have developed specific chemical adaptations to respond to interactions with herbivores and pollinators. These differences could impact both the development of commercial products with specific properties and the selection and domestication criteria for the species' cultivation.

A32
ANALYSIS OF BIOMASS PRODUCTION AND SEED GERMINATION TESTS IN FEMALE AND BISEXUAL PLANTS OF *MINTHOSTACHYS VERTICILLATA*

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Peperina (*Minthostachys verticillata*) is a wild aromatic plant from the Lamiaceae family that grows in the Comechingones Sierras of Argentina. It is commercially utilized by companies that manufacture yerba mate. This study aimed to determine differences in biomass (BM), fruit and seed production, as well as germination potential (GP) between female (F) and bisexual (B) plants of this species. The specimens used in this study were obtained from a crop cultivated in the FTU-UNSL field. At the peak fruiting stage, 13 F plants and 17 B plants were harvested and dried separately. Average plant weight was calculated as the quotient of the total dry matter weight by the number of plants. Fruits were cleaned and seed was separated by manual friction. Seeds were grouped as Selected (S) and Random (R) based on seed coat color to differentiate between selected and unselected seeds. Germination tests were conducted with four replicates of 25 seeds each in a germination chamber set at 22°C, with counts taken at 7 (GP7) and 10 (GP10) days. The average dry weight per plant was estimated at 30.2 g for F plants and 47.0 g for B plants, while fruit-seed weights were 5.6 g and 4.1 g, resulting in a BM allocation to fruit of 0.18 in F plants versus 0.09 in B plants. The PG values obtained for F plants were: A 66.7%, S 77.0%, at 7 days, and A 70.7%, S 84.0%, at 10 days. In contrast, B plants showed GP values of: R 36.0%, S 59.0% at 7 days, and R 42.0%, S 73.0% at 10 days. In all cases, the S seed groups demonstrated higher germination potential ($P>0.01$), indicating that pre-evaluation color selection is an effective criterion for improving germination outcomes. On the other hand, the percentages of F plants were higher ($P>0.01$) than those for B plants at both times evaluated. This differential vegetative growth between F and B plants likely corresponds to reproductive specialization in the species. These findings are relevant for understanding the species' ecological behavior, domestication potential, and possible applications in cultivation systems.

A33
ENDOGENOUS COMPETITION BETWEEN CADMIUM AND ZINC INDUCE MORPHOLOGIC CHANGES AND OXIDATIVE DAMAGE IN SOYBEAN ROOTS

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The increasing contamination of agricultural soils by cadmium (Cd) has caused serious concerns worldwide, mainly due to human activities such as agriculture and mining. Zinc (Zn) is a vital plant micronutrient that plays a role in several physiological functions of plants. The chemical similarity of Zn to Cd and the same carriers for root entry allow Zn to counteract the toxicity of Cd in plants. The root surface of plants could counteract Cd toxicity, due to the addition of Zn to the soil. To evaluate the suitability of using Zn to decrease the absorption and toxicity of Cd in soils contaminated with this metal, the Cd/Zn doublet in roots of *Glycine max* (L) was studied. The root absorption and transport, as well as changes in the oxidative profile, were evaluated. The determinations were made using plant roots that had been developed and adapted for 6 days under hydroponic conditions with Hoagland nutrient solution. The plants were exposed to Cd and Zn ions for a period of 7 days. The Zn concentrations added were 0.6 and 4.8 mM, while the Cd concentration remained constant at 40 µM. Morphological parameters, including endogenous Cd and Zn, H_2O_2, carbonyl groups, lipid peroxidation, phenols, flavonoids, and proline, were determined. Microscopic root analysis treatments with Zn [0.6] and [4.8] without Cd did not show any significant differences in root anatomy. The presence of a band or layer of cells in the cortex before reaching the endoderm observed during treatment with Cd could be due to the deposition of heavy metals in the parenchymal cells. When Zn concentration was increased, the absorption of Cd decreased significantly ($p<0.001$), but Zn uptake did not change significantly in the presence or absence of Cd in the root. There was a significant increase in the content of H_2O_2 and carbonyl groups in both Zn [4.8] treatments (with and without Cd) ($p<0.0001$ and $p<0.005$). Lipoperoxidation was significantly increased with Cd in absence of Zn, and Zn [0.6] without and with Cd ($p<0.0001$). The content of phenols decreased by 58 and 62% in Zn [4.8] treatments without and with Cd ($p<0.001$). Flavonoids exhibited a significant increase of 8 and 12% in treatments with Cd when Zn was absent, and Zn [0,6] without Cd. Contrary Zn [4,8] without Cd, a significant decrease of 5% was observed. The decrease in proline was significant ($p<0.0001$) in all treatments with Zn [0.6] (without and with Cd), and Zn [4.8] (without and with Cd). Based on the results, it has been shown that the Cd/Zn doublet induces changes in antioxidant activity and damages to macromolecules, particularly at higher Zn concentrations. This could be explained by a root reaction to Cd/Zn doublet toxicity. Additionally, it was observed damage at the anatomical level in xylem and phloem. This could be explained for Cd uptake by symplast pathway through the root epidermis. In addition, we can observe that the Cd/Zn doublet at low doses of Zn could be improved Cd-tolerance to soybean plants, but at high Zn concentrations a major oxidative damage is generated at root level.

A34
CHANGE ON MORPHOLOGY, ENDOGENOUS ION CONTENT AND OXIDATIVE BALANCE CAUSED BY IONS CADMIO-ZINC IN *GLYCINE MAX* (L) MERR. LEAVES

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The heavy metals have attracted attention around the world due to its zero biodegradability and its high bioaccumulation beings, which represents a threat to the ecosystems and humans. Zinc (Zn) is an essential microelement for plants, however, in excessive concentrations it causes damage and alterations. Cadmium (Cd) is a heavy metal not essential for living beings and it causes alterations in physiological, biochemical and molecular processes also in very low concentrations. These metal ions share some chemical characteristics that make them use the same transporters to enter the cell, thus competing for their input, transport and use in plants. This competition generates changes in oxidative stress parameters and could be visualized at the histological level. The study objective was to compare the morphoanatomy through microscopic analysis and to

determine stress tolerance mechanisms mediated by Cd^{2+} and Zn^{2+} ions and the oxidative damages causes in the *Glycine max* (L) Merr leaves. They were obtained after 6 days of plant development under hydroponics in Hoagland's nutrient solution conditions and subjected to contamination with the two ions (Zn and Cd) for 7 days. $ZnCl_2$ concentrations added in the study were 0.6 and 4.8 mM, and $CdCl_2$ 40 µM as a constant concentration. The morphoanatomy of the leaves was compared by microscopic analysis. Morphophysiological parameters were analysed, including contents of Zn and Cd endogenous, proline, phenols and flavonoids and glutathione reductase (GR) activity. In the microscopic analysis, an increase in the size of intercellular spaces was observed in chlorenchyma with Cd (c/Cd). Palisade parenchyma and spongy parenchyma less compact, more disorganized and with larger intercellular spaces by Zn 0.6 mM (Zn [0.6]). Then, Zn [0.6] c/Cd, loss of mesophyll structure, increase in intercellular spaces in chlorenchyma and decrease in chloroplasts number was observed. When Zn 4.8 mM (Zn [4.8]) was added compact chlorenchymas and loss of characteristic dorsiventral structure, with trichomes in both epidermises. Regarding in Zn [4.8] c/Cd vacuolation of the spongy chlorenchyma fewer chloroplasts was observed with trichomes on both sides. Endogenous ions leave content showed a significantly decrease of Cd absorption by increment in Zn concentration added ($p<0.001$), and Zn uptake had decrement in the presence of Cd (c/Cd) ($p<0.001$). The results showed a significant increase ($p<0.001$) in proline content in both treatments with Zn [4.8]; phenol and flavonoid content showed a significant increment in all treatments compared to the control ($p<0.01$ and $p<0.001$ respectively). In GR activity a significant increase was observed in both treatments of Zn [4.8] ($p <0.001$) with respect to the control. According to these results, we can conclude that the Zn and Cd ions presence in the nutritive medium alters not only the biochemical and physiological parameters, but also the antioxidant and pro-oxidant activity. Mainly in Zn high concentrations, changes in anatomical structure of the leaf could be considered a plant response against the toxicity generated by these two ions. More studies are necessaries for expand this conclusion.

A35
EFFECTS OF A MICROBIAL CONSORTIUM ON TOMATO (*SOLANUM LYCOPERSICUM*) GROWTH AND YIELD: STUDIES IN DIFFERENT CULTIVATION SYSTEMS

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A plant biostimulant has been defined as a fertilizing product applied to plants with the aim of modulating their physiological functions and thus improving crop productivity, nutrient use efficiency, products quality and stress tolerance. Plant biostimulants, according to this definition, include various substances with bioactive properties as well as plant growth-promoting microorganisms such as *Trichoderma* and plant growth-promoting rhizobacteria (PGPR). Plant biostimulants based on microbial consortia may include bacteria from different species, while others may include both beneficial bacteria and fungi with diverse mechanisms of action to provide a broad spectrum of benefits to plants. Thus, the objective of this work was to analyze the potential of a consortium based on *Trichoderma harzianum* ITEM 3636 + *Pseudomonas putida* PCI2 to promote tomato growth under different field conditions. The assays were carried out between 2019 and 2024 in 3 different locations: an experimental field located in the National University of Río Cuarto, a field dedicated to organic production (under semi-cover) and a field for traditional tomato production (under semi-cover). Except for the experimental field, the other sites were located in the periphery of Río Cuarto. The following treatments were used: (1) Control without inoculation; (2) inoculation with ITEM 3636; (3) inoculation with PCI2 and (4) inoculation with ITEM 3636 + PCI2 (consortium). At the time of transplanting, seedlings were inoculated by immersion of their roots in the corresponding suspension, at a concentration of $1x10^5$ conidia ml^{-1} for ITEM 3636 and $1x10^6$ CFU ml^{-1} for PCI2. In general, we observed that inoculation in all its forms was beneficial for tomato plants. When focusing on inoculation with the consortium, increases in different parameters were observed. Significant increases of 20-25% were observed in yield (Kg/m^2) in the three field assays carried out in the experimental field located in the National University of Río Cuarto. On the other hand, significant increases of 50% and 28% were observed for foliar area and fruit diameter, respectively, in the location for traditional tomato production. Finally, a significant increase of 250% in number of fruits per plant was observed in the organic establishment. Several works have suggested that microbial consortia can generally perform activities better than individual strains and interactions between different microbial species within a consortium can lead to enhanced functionality and can provide better resistance to stress and improve nutrient uptake compared to individual strains. Hence, our results support using microbial consortia is suggested by such studies when aiming at increasing plant health and growth.

A36

PRELIMINARY STUDY ON THE INFLUENCE OF INOCULATION OF TOMATO (*SOLANUM LYCOPERSICUM*) PLANTS WITH A MICROBIAL CONSORTIUM ON SOIL BACTERIAL COMMUNITIES

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Recent studies demonstrate the potential of microbial consortia, rhizobacteria and rhizofungi, as plant biostimulants. On the other hand, plants depend on microorganisms to use soil as a growth medium, and synergy between the two is important for their survival. They are allies of plants and govern several fundamental processes related to their growth. Inoculation these microoganisms can cause changes in the structure of the native microbiota, which is crucial to be considered with respect to the safety of introducing microorganisms into the environment. Certain groups of microorganisms can be increased, others can be inhibited, and in some cases, the application of plant growth-promoting microorganisms does not cause any change in microbial populations. The objective of this work was to carry out a preliminary evaluation of the effect of inoculating tomato (*Solanum lycopersicum*) plants with the biostimulant consortium based on *Trichoderma harzianum* ITEM 3636 and *Pseudomonas putida* PCI2 on the structure of soil bacterial communities. The following treatments were carried out: (1) Control without inoculation; (2) inoculation with ITEM 3636; (3) inoculation with PCI2 and (4) inoculation with ITEM 3636 + PCI2 (consortium). Total DNA was extracted from soil samples collected before sowing and at harvest during a tomato plant inoculation assay. The obtained 16S rRNA gene sequences were analyzed using Qiime2 to process and visualize microbiome data, with noise reduction achieved through DADA2. Sequence variants were classified as amplicon sequence variants (ASVs). Beta diversity metrics known as weighted UniFrac distance and unweighted UniFrac distance were calculated using the q2-diversity plugin. Taxonomic composition was determined using a Naive Bayes classifier, and PICRUSt2 was employed to predict phenotypes and functional abundances based on marker gene sequences. The 3 most frequent bacterial phyla found were: Actinobacteria, Gemmatimonadetes and Proteobacteria. In pre-sowing soil, the relative frequency of Actinobacteria was higher while those of Gemmatimonadetes and Proteobacteria were lower, compared to the rest of the treatments. Additionally, the relative frequencies of these phyla were balanced in the inoculation treatments at harvest, compared to the control. The weighted Unifrac distance analysis indicated that there was a dissimilarity in the composition of the bacterial communities between the pre-sowing stage and the rest of the treatments, particularly the inoculation treatments, at the time of harvest. Finally, by exploring some of the functional genes predicted with PICRUST2, it was observed that inoculation with the consortium has the potential to up-regulate functional genes related to phosphorus cycling, compared to the other treatments. Thus, we observed that inoculation with the consortium enhances tomato yield and has potential to positively influence the availability of soil phosphorus.

A37

IMPACT OF EMERGENCY VARIABILITY ON INDIVIDUAL CORN PLANT YIELD

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The production of maize grains in kg per ha is conditioned by the spatial and temporal unevenness generated by poor seed planting, as they are placed at non-uniform distances and depths, resulting in irregular crops and the possibility that maize plants may encounter an excess or deficit of resources. During the 2022/2023 campaign, an experimental field trial was conducted at the Facultad de Ingenieria y Ciencias Agropecuarias of the Universidad Nacional de San Luis (FICA-UNSL), located in the city of Villa Mercedes (SL). To determine the impact of temporal variability on maize emergence on the size of the ears as a yield component, a factorial design was carried out according to the generated staggering in which the ears were classified according to their origin from dominant (D) plants that emerged on the 1st day, or those from the 3rd day, called dominated or recessive. As a second experimental factor, the impact of this variability in space (at the plot level) was considered, with 3 levels of variability: homogeneous situation or T0 = 100% sown on the same day, T1 = 50% sown on the 1st day and 50% on the 3rd day, T2 = 70% sown on the 1st day and 30% on the 3rd day. Each combination of factors had 3 randomly distributed repetitions, consisting of plots of 3 lines of 10 m long spaced 0.52 m apart, to achieve a density of 60,000 plants*ha^{-1}. The emergence related to the sowing date was guaranteed by high-quality seeds, and cultural work

of cleaning and application of phytosanitary products necessary to ensure the success of the trial were carried out. The plots were irrigated by sprinklers to maintain the soil moisture required by the crop, and the furrows where the seeds were placed at two different times were delimited with a no-till seeder to maintain uniform depth. The ears were harvested by hand and weighed to obtain the yield per plant, identifying them according to their origin by plant type (D or R) and the variability situation (T0, T1, or T2). Normality and homoscedasticity of the data set were analyzed, and ANOVA was applied. No significant differences ($p>0.05$) were found between the mean yields of the T0, T1, and T2 plots; being for T0 58.11±2.28 g; T1 56.48±1.86 g and for T2 55.32 ±1.86 g. Highly significant differences ($p<0.01$) were identified in the size of the ears according to the temporal variability in plant emergence, with approximate weight differences of 17% among the three days considered, and weights of D: 59.51 ±1.46 g and R: 51.59 ±1.86 g. It is concluded that minimal temporal variability in the emergence of maize plants in our region significantly affects the individual yield of maize plants, regardless of the level at which this variability is expressed at the plot level. Thus, the importance of defining an optimal sowing date for high germination potential seeds is highlighted.

A38
HEIGHT GROWTH AND AERIAL YIELD OF TOPINAMBUR CROP *HELIANTHUS TUBEROSUS* L. FERTILIZED AND UNFERTILIZED

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Topinambur, *Helianthus tuberosus L.* is a crop that belongs to the family Asteraceae, with high potential to expand as a commercial product in different areas because of its multipurpose nature: horticultural use, industrial production mainly to obtain inulin (which has great nutritional benefits), or even its use as forage; in this case, tuber or aerial parts are consumed. This crop sprouts during the spring and between weeks 14th and 16th the stages of tuber formation begin. In autumn, senescence begins and the translocation of nutrients to the reserve organs takes place. The tubers are the most well-known part of this species, but the aerial organs could be used for livestock production. There are few antecedents on this species in San Luis, and for some time trials have been conducted to characterize the crop and its productive response in the semiarid region. The objective of this work was to evaluate the effect of nitrogen fertilization on aerial growth of a "white" topinambur crop for two exploratory situations: fertilized (CF) and unfertilized (NF) during a crop cycle in the semiarid region of San Luis. Plant height and weight were characterized to estimate forage yield in kg DM.ha^{-1}. A comparative yield trial was conducted in the experimental field of Agricultural Sciences of the FICA-UNSL, Villa Mercedes, San Luis, with a completely randomized design with 4 replications in each case (totaling 8 furrows). Planting was carried out at the middle of December 2023, in 2.5 m long furrows spaced at 0.70 m, totaling 1.75 m^2. Fertilization was done when plants reached 15 cm of height (at January 8, 2024), with 200 kg.ha^{-1} of urea. At the end of the cycle, the total plant height was measured and the aerial material was harvested for drying in ovens at 60 °C until constant weight. Data analysis by t-test showed highly significant differences ($p<0.01$) for the two variables considered, and a non-normal distribution. The height of CF was 1.36 m ±0.20 with a production of 5270 ±764 kg DM.ha^{-1}, while topinambur NF reached 0.99 m ±0.28 m in height and a production of 3850 ±1107 kg DM.ha^{-1}. The application of an exploratory dose of nitrogen fertilizer generated increases in the order of 37%, and it is concluded that the white variety showed a favorable response in both aerial growth and DM production when nitrogen was applied at the beginning of the cycle. The promising results of this experience encourages further evaluation of different doses and timing of application to adjust the response of topinambur in this environment.

A39

GERMINATION OF *VACHELLIA AROMA* AND *SCHINUS FASCICULATUS* FOR THE RESTORATION OF THE NATIVE FOREST OF THE CONLARA VALLEY (SAN LUIS).

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According to the National Plan for the Restoration of Native Forests of Argentina, the conversion of natural ecosystems to agricultural lands, inadequate livestock and forestry management practices, the introduction of invasive exotics species, illegal trafficking of species and all this in the climate change scenario are the main causes of degradation of ecosystem functions and the loss of the productive potential of our native forests. To find alternatives to improve the state of these areas is relevant repopulation with native species. For this it is necessary to obtain quality seedlings produced from local genetic material. The objective of this work was to study the responses to different pre-germinative treatments of the species *Vachellia aroma* and *Schinus fasciculatus*, in order to advance the knowledge of their ecological dynamics and contribute to their sustainable use and conservation. The study area consists of 200 hectares of forest under extensive livestock use in an agricultural establishment located at the parallel 32°57 48.82 S - 65°36 27 13 W at 12 km from the town of Naschel, San Luis. Fruits were collected directly from the woody tree from which the seeds were obtained for further analysis. All the controlled and treated seeds were rinsed with distilled water and distributed in petri boxes with double absorbent paper moistened with 3 ml of distilled water and incubated in germination stoves at 30 ºC, in darkness. The germination percentage (PG) was determined, the seed with a radicle greater than 3 mm was considered germinated. 10 treatments were performed: T0: control; T1: scarification with sandpaper; T2: total elimination of tegument; T3: boiling water 1'; T4: alternation in hot and cold water 5'; T5: concentrated H2SO4 3'; T6: 40% NaClO by 30'; T7: concentrated HCl 7'; T8: concentrated HCl 15'; T9: Ethyl alcohol 20'; T10: Acetone 30'. The experimental design was completely randomized with 3 repeats of 10 seeds each. The data were analyzed using non-parametric statistics (Kruskal Wallis test) using InfoStat statistical software. The results in *V. aroma* showed that T5 (70%) and T8 (10%) had the highest PG and had significant differences between medians. The results in *S. fasciculatus* showed that although no significant statistical differences were found between treatments at a global level, when individual contrasts were performed, both T2 (15%) and T1 (20%) differed from the rest. The *V. aroma* values indicate that uniform germination in the field requires the use of H2SO4 concentrated per 3' or HCl concentrated per 15'. In *S. fasciculatus*, however, the tegument must be removed or sanded before sowing.

A40

RELATIONSHIP BETWEEN MORPHOMETRIC TRAITS OF THE AERIAL PART AND ANATOMICAL ROOT IN *VICIA SATIVA* (LEGUMINOSAE) GROWN ON THREE DIFFERENT SOILS

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Different agricultural practices lead to soil degradation affecting crop productivity. In the experimental field of the UNRC Faculty of Agronomy and Veterinary, the growth and development of forage species, including *Vicia sativa*, is measured in different soils. The objective of this study was to compare the morphometric and anatomical characteristics of *V. sativa* in three contrasting soils: conventional tillage, direct seeding and pristine soil. The variables analysed were fresh weight (g), plant height (cm), main root diameter (µ), vascular cylinder diameter (µ), number of cell layers in the cortex, vessel membersdiameter (µ) and number of vessel members.surface^{-1}. The design of the trial was completely randomized with three repetitions. The evaluation was carried out on seedlings collected at 75 days from sowing. For the observation and measurement of radical characters, cross-sections were made using conventional techniques of fixation, inclusion, cutting and colouring of organs. The observationswere done with a Zeiss microscope and photographed with Motic Images Plus 3.0. Data obtained was analysed by ANOVA and Fisher's LSD test. Among the morphometric characters evaluated, significant differences were found in fresh weight, seedling height and root vessel member diameter. Plants grown in pristine soil reached the highest values for these variables, 3.79 g ($p=0.01$), 29.83 cm ($p=0.01$) and 2.74 µ ($p= 0.03$), respectively.In both conventional tillage and direct seeding soil, the correlation (Pearson coefficient) between diameter and number of members, height and fresh weight of seedlings was high, ranging from 0.83 to 0.98. These results would indicate that in the early stages of plant development of *V. sativa*, the optimal differentiation of the xylematic tissue in relation to the diameter of the vessels in pristine soil favours water and nutrient uptake allowing a greater growth in height and fresh weight production. In the two remaining soils, seedling development is also associated with xylematic development but in relation to the diameter and number of members.surface^{-1} pot, although presenting lower productive parameter values than pristine soil.

A41
MORPHO-ANATOMICAL AND PHYSIOLOGICAL VARIABILITY IN *Adesmia bicolor* (LEGUMINOSAE) UNDER TWO CULTURE CONDITIONS

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In the study of a forage species, it is important to analyze morphological, physiological and anatomical components, and interaction that exists between them. *Adesmia bicolor* has a high forage potential and is suitable for forage use in the central area of Argentina during the winter season. The aim of this study was to evaluate the variability of nine populations from the provinces of Córdoba, San Luis and Entre Ríos. The populations were grown in the field and seven of them were also grown in pots in a completely randomized experimental design. The morphometric variables number of stolons.m^{-2} (ST), internode length (cm) (IL), leaflet length (LL) and width (cm) (LW), number of inflorescences.m^{-2} (NI) and number of flowers. Inflorescence-1(FI) were evaluated in both growing conditions. The anatomical variables measured were transverse height of the leaflet at wing (µm) (HLW) and at central vascular bundle (µm) (HLB), total transverse area (mm^2) (TAL), mesophile transverse area plus vascular bundles (mm^2) (MA) and central vascular bundle transversal area (mm^2) (BA) of leaves; total stem transverse area (mm^2) (SA), xylem tissue transverse area (mm^2)(XA), xylem tissue transverse area.total transverse area^{-1} ratio (XA/SA), vascular tissue transverse area (mm^2)(VAS) and vascular tissue transverse area.total transverse area^{-1} ratio (VAS/SA); total transverse area (mm^2) (TAR), vascular transversal area (mm^2) (VAR), cortex transverse thickness (µm) (CT) and transversal area of the vascular system.total transverse area^{-1} ratio (TAR/VAR) of the root. Aerial (AB), underground (UB) and total biomass (TB) (kg.ha^{-1}), total soluble carbohydrates (SC) (mg.g sample^{-1}), total nitrogen (N) and crude protein (%) (CP) were also quantified. Data was analyzed using ANAVA and Fisher's LSD test. A principal component analysis was performed to determine the relationship between variables and the response of the populations to the growth conditions. The first two components explain 58% of the variability observed in pot condition. VAS, VAS/SA and TAR contributed more to the main component 1 (CP1) while the main component 2 (CP2) was mainly constructed by the variables LL, FI and IL. In the field-grown populations, the first two components explained 66% of the variability. N, CP, LL, LW, FI, AB, TB and SC had the greatest contribution to CP1, while HLB, BA, VAS, HLW and XA were more relevant to CP2. These results show that field environment is the optimal condition if the aim is biomass production, since it relates to the morphological characters, responsible of dry matter production, in conjunction with those responsible for development physiology, such as carbohydrates concentration and mobilization.

A42
HEAT PRIMING INDUCES MORPHO-HISTOLOGICAL CHANGES IN MAIZE ROOT APEX

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Agricultural production is among the sectors that directly depend on climate and weather conditions, making it crucial to study their impacts. Seed germination, a fundamental stage in the life cycle of a plant, can be delayed or inhibited by adverse environmental conditions. The objective was to evaluate the morpho-histological changes in the post-germinative stage of *Zea mays* L (maize) seeds previously subjected to sublethal heat shock of varying duration and intensity. The seeds of the NS 7818 VIP3 maize variety, kindly provided by Syngenta Agro were pretreated at 40 or 50 °C for 3 and 7 days. The seeds, both with and without heat-priming, were superficially disinfected and germinated on cotton and paper towel wetting at 28 °C for 96 h. The determinations were made using the apical 2 cm of the roots. Histologically, a tendency toward increased root diameter was observed as the temperature and/or exposure time increased in the maize seed pretreatments, with a significant increase at 50 °C for 3 ($p<0.01$) and 7 d ($p<0.05$), compared to the control. Regarding the area occupied by the central cylinder in the roots, a tendency to increase was observed as temperature and/or exposure time increased in the pretreatments, with a significant increase in the roots of seeds exposed to 50 °C for 3 ($p<0.01$) and 7 d ($p<0.001$). This could be due to an increase in the number of cells in the vascular tissue as well as a larger area of the xylem conductive elements. Similarly, in the root cortex area, a significant increase was observed in the pretreatment at 40 °C for 7 d ($p<0.05$)

and in both pretreatments at 50 °C for 3 ($p<0.01$) and 7 d ($p<0.05$). This would be caused by an increase in the size of the cortical parenchymal cells. Morphologically, a significant decrease in root length was observed in the pretreatments at 40 °C for 7 d ($P<0.05$) and in both pretreatments at 50 °C ($P<0.001$), all compared to the control. Our results suggest that heat priming in maize seeds generates changes in post-germinated roots, resulting in an increase in root size and/or generating stimuli for the differentiation of both vascular and cortical parenchymal tissues. All of this suggests that thermo priming induces structural cellular changes that, along with results presented in previous studies showing modifications in antioxidant defense mechanisms and physiological changes, could improve the plant's response during the early stages of its development.

A43
TRICHODERMA HARZIANUM ITEM 3636: ITS EFFECT ON THE GROWTH OF TOMATO SEEDINGS (*SOLANUM LYCOPERSICUM)*

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The cultivation of tomato (*Solanum lycopersicum L.*) is widespread worldwide and with a production of more than 187 million tons in a planted area of 5 million hectares in 2023. It is one of the most important crops that are carried out under cover and a current challenge is to maintain or increase production levels using technologies that are friendly to workers, consumers and the environment. Along these lines is the use of biofertilizers, formulated with bacteria and fungi that promote the growth and health of crops. Disinfected seeds were sown in Petri dishes containing Murashige and Skoog medium + 1% agar and 0.6% sucrose at pH 7. After germination they were inoculated with aliquots of a suspension of *T. harzianum* ITEM 3636 spores and incubated for 5 days. A greater root volume was observed in tomato seedlings treated with the fungal strain, with an increase in the number of secondary roots compared to the control treatment. The average density values in the presence of the fungus considerably exceed those of the control, being the same as 1.16 mm and 0.23 mm respectively.
The effects of ITEM 3636 were also evaluated under salinity conditions, adding different concentrations of NaCl to the previous medium, and no statistically significant differences were observed, with respect to the uninoculated treatments, on the length of the main root and the length of the seedlings of tomato. Thus, inoculation with *T. harzianum* ITEM 3636 can be beneficial for tomato crops since it promotes plant growth in adequate water conditions, but it would not be a tool capable of reversing the effect caused by salt stress situations.

A44
INITIAL GROWTH OF *VICIA SATIVA* L. AND X *TRITICOSECALE* WITTMACK IN A SOIL WITH DIFFERENT MANAGEMENT CONDITIONS

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Soil management generates both differences in plant growth and the development of different rhizospheric microorganisms, which causes different microbiomes to form. These studies strengthen ecosystem services associated with increasing the yield and resilience of crops, as well as the recovery process of soils intended for agriculture. The objective of this work was to compare the initial growth of the species *Vicia sativa* L. and x *Triticosecale* Wittmack in a soil with three management conditions. The experiment was carried out in the Experimental Field of the Faculty of Agronomy and Veterinary Medicine, UN of Río Cuarto. The typical Hapludol soil from the Pozo del Carril establishment (La Aguada) was obtained with three different managements: pristine soils (SP), conventional tillage (LC) and direct sowing (SD). The design was completely randomized (n=4). Twelve shots of two seeds were sown in a 10-liter pot with 8 kg of soil. The growth of both species was evaluated through the following variables: fresh weight (PF), total plant height (AT), number of branches (NR), number of leaves (NH), leaf length (LH) and leaf width (AH), on four sampling dates: 41, 54, 61 and 75 days after sowing (DDS). In the variables PF, AT and NR it is observed that initially no statistically significant differences are found between soil management, but at 75 DAS significant differences are observed, highlighting SP over LC and SD. When

analyzing the soil-plant relationship, it is observed that the *V. sativa* species stands out in SP, while *Triticosecale* does not thrive, developing better in LC and SD. In the NH variable, initially there are statistically significant differences between SD with respect to SP and LC. Subsequently, SP shows significant differences with respect to the other soil management conditions. When analyzing the soil-plant relationship, the same trend is maintained as in the aforementioned variables. In the AH variable it is observed that initially it presents the same behavior as in the PF, AT and NR variables, but later LC stands out with respect to SP and SD. In the LH variable, LC stands out from the beginning, maintaining it over time. When analyzing the soil-plant relationship in the AH and LH variables, it is observed that *Triticosecale* performs better in LC and SD, while in *V. sativa* no statistically significant differences are found. The results at the time of implementation reflect that *V. sativa* presents better performance in SP while *Triticosecale* in anthropic soils such as LC and SD, which could be due to the type of legume vs grass species. On the other hand, it could be considered that SP has a different nutritional composition and rhizospheric community than the soils intervened by man, reflected in the differences described above.

A45

INITIAL GROWTH OF TRITICALE (*X TRITICOSECALE* WITTMACK) INOCULATED WITH *Azospirillum argentinense* IN DIFFERENT SOIL CONDITIONS

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Associations initiated within the sperm sphere have lasting impacts on plants as they are crucial for the future implantation of the plant rhizosphere, which significantly influences plant growth and crop yield. In triticaceae, *Azospirillum argentinense* establishes and feeds on the plant root while supplying nitrogen and improving plant growth. Additionally, it synthesizes phytohormones that promote growth and cause morphological and physiological changes in the plant root, which result in better use of water and nutrients and a consequent increase in yield. The objective of this work was to compare the effects of the strain *Azospirillum argentinense* Az39 on the initial growth of triticale in contrasting edaphic conditions. For this, 24 seeds per pot of cv. Tizné-UNRC with and without *A. argentinense* Az39 inoculation were sown in 10-liter pots with soil exposed to the following conditions for 20 years: direct seeding (DS) conventional tillage (CT) and pristine soil (PS). Pots were placed in a phytotechnical cage in a randomized complete blocks experimental design with three replicates. The variables total biomass (g/pl), plant height (cm), number of leaves per plant, leaf length (cm) and leaf width (mm) were measured at 41, 54 and 61 days after sowing. On day 61, total biomass presented statistically significant differences ($p=0.0398$) between inoculated and uninoculated plants (67.00 ± 26.94 and 45.07 ± 27.80 g, respectively). Leaf length also showed differences ($p=0.024$) among inoculated (12.07 ± 2.10 cm) and uninoculated (8.57 ± 2.18 cm) triticale. Plant height varied between soil conditions ($p=0.0182$), as plants were taller in CT and PS at 54 and 61 days after sowing, without any effect of *A. argentinense* Az39 inoculation over plant height. Neither *A. argentinense* Az39 inoculation nor soil conditions had any influence on the number of leaves, while leaf length presented variations at 41 and 54 days for the soil conditions ($p=0.0152$ and 0.0013, respectively), as CT exhibited greater leaf length (10.30 ± 1.30 cm) at day 41 while PS (10.76 ± 1.30 cm) and CT (10.60 ± 1.30 cm) were superior at day 54. On day 54, the leaf width presented differences among the different soil conditions ($p=0.0002$), as PS and CT presented significantly wider leaves (4.07 ± 0.36 and 3.83 ± 0.34 mm, respectively). PS and CT had favorable effects on plant height, leaf length and leaf width. *A. argentinense* Az39 inoculation produced more biomass and longer leaves at more advanced stages of the crop.

A46
PHYTOCHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF *AUSTROBRICKELLIA PATENS*, AN ENDEMIC SPECIES FROM CENTRAL-SOUTHERN ARGENTINA

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The Asteraceae family is renowned for its vast diversity of species with medicinal and nutraceutical properties. Within this family, *Austrobrickellia patens* (Hook &Arn.) R. M. King & H. Rob. is an endemic species of central-southern Argentina that has been poorly studied. Despite its restricted geographical distribution, this plant has been traditionally used in folk medicine, suggesting an unexplored pharmacological potential. The scarcity of scientific information on *A. patens* motivated this study, aimed at contributing to the understanding of its chemical composition and evaluating its biological activity, thus opening new avenues for the development of value-added natural products. Samples of *A. patens* were collected in Anguil, La Pampa province, and *Salvia rosmarinus* (rosemary) was used as a reference. The plants were dried, and secondary metabolites were extracted using solvents of different polarity (hexane, ethyl acetate, and ethanol). Phytochemical assays were performed on the extracts to evaluate secondary metabolites using the following tests: Mayer, Dragendorff, and Wagner for alkaloids; NH_3, Shinoda, H_2SO_4, and Zn for flavonoids; fluorescence, Erlich, and KOH for coumarins; $Mg(CH_3COO)_2$, H_2SO_4, and KOH for quinones; afrosimetric test, Molisch, and Lieberman-Burchard for saponins; legal, Baljet, and $FeCl_3$ for sesquiterpene lactones; DPPH Scavenging Activity; Folin-Ciocalteau for total phenolics content; and a differential pH test for anthocyanins. The results revealed the presence of alkaloids, flavonoids, aurones, catechins, coumarins, quinones, saponins, α, β-unsaturated lactones, and santonins in the ethanol and ethyl acetate extracts, also evidencing antioxidant capacity. In contrast, hexane extracts only showed the presence of alkaloids, flavanone's, coumarins, and α, β-unsaturated lactones. These findings indicate that *A. patens* is a rich source of bioactive compounds, highlighting the importance of solvent selection in extraction processes. This preliminary study reveals the potential of *Austrobrickellia patens* as a source of bioactive compounds with diverse properties, as flavonoids and phenolic compounds in general possess antioxidant and anti-inflammatory activities of interest to the food and pharmaceutical industries. The presence of alkaloids suggests a possible therapeutic potential. These findings open new perspectives for future research and the development of natural products with applications in various fields, such as pharmaceuticals, food, and cosmetics. Further studies are required to determine their structure and biological activity.

A47
CHLOROPHYLL INDEX AND COMPARATIVE NUTRITIONAL QUALITY OF MAIZE SILAGE FROM SOUTH OF SAN LUIS

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Forage silages obtained from crops such as maize provide volume to a diet on strategic or systematic way, in intensified establishments. The conditions of the crop of origin are often left in the background but conditioned the quality of the resulting silage and its impact on production level. Under sufficiency conditions of N and P, the S and Zn nutrients are that most frequently limit corn crop yield, especially in soils with low levels of organic matter such as those of the semiarid region. The role of these elements in plant nutrition is varied, among which its intervention in protein synthesis, CO_2 fixation in photosynthesis and in crop defense systems related to chloroplast protection. The greenness or chlorophyll index (CI) is an important indicator of the quality of crops, directly related to foliar nitrogen content. Healthy corn with a high chlorophyll index tends to result in better quality silage, which is more nutritious and beneficial for livestock. Values of IC less than 35.3 are critical for N content, while IC>50.0 are indicated as adequate. With the aim to evaluate the nutritional status of a corn crop in the south of the province and to contrast the quality of the resulting silage, CI measurements were taken at the end of the crop cycle and the resulting quality values were compared with the average of the last 6 years, provided by the laboratory that analyzed the samples (core zone, Santa Fe). Three paddocks were monitored in southern San Luis, Buena Esperanza (BE), under nitrogen fertilization with urea and foliar fertilization with zinc, to cover their requirements. The CI was measured with a chlorophyll meter Minolta SPAD-512 in the upper third of the plant, from where the greatest contribution of photo assimilates to grain filling comes from. A multivariate descriptive analysis from principal components was carried out, which allowed reducing the dimensionality of the situation and subsequently the comparison from T-tests between the values of both environments. Regarding the CI, the 3 paddocks were close to adequate values, although only one of them exceeded 50. This indicates that the crop has some nutritional limitations. Comparison of BE and laboratory-historic silage quality showed significantly higher values ($p<0.01$) in BE for the parameters: Acid Detergent Fiber (ADF), Neutral detergent fiber (NDF), lignin and pH, while the ash content was significantly higher ($p<0.05$), and the N-ammonia equivalent to crude protein (CP) was lower. Despite a lower dry matter content (DM) that could have improved the final quality parameters of BE silage, it is characterized by a higher "cell wall" development and lower quality in terms of protein and mineral content. Regardless of the Kg-production, the CI values of the crop were reflected in the quality of silage, with more fibrous with lower protein content. The CI allows a good approximation, not only to the characteristics of the crop, but can also be a practical indicator for decision making regarding the convenience of making good quality forage reserves.

BIOCELL, Vol.49, Suppl. 3, 2025
ISSN 0327-9545
ISSN 1667-5746 (online version)

A48
ILLUSTRATED KEY OF THE NATIVE SPECIES OF THE GENUS *FESTUCA* OF THE GRASSLANDS AND FORESTS OF THE PROVINCE OF SAN LUIS

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The genus *Festuca* L comprises approximately 500 species, present in savannahs and grasslands of temperate regions in both hemispheres. In Argentina, 46 species have been recorded and for San Luis, 11 species were mentioned to the moment. The present genus species are C3, mostly perennial, caespitose, with rhizomes or stoloniferous herbs. Leaves with flat or conduplicate blades, sheaths with auricles present or not. Spikelets 2-pluriflorous, florets hermaphrodite, lemmas with rounded backs, apex acute or aristate and glumes unequal. Considering recent taxonomic revision of the genus and the difficulties in identifying the species included, the aim of this work was to build an illustrated key to the native species present in the province of San Luis. In this key, reproductive and vegetative characters were considered easily recognizable in the laboratory and eventually in the field by students, producers and professionals interested in the subject. For this purpose, the collections of the Herbarium of Agricultural Sciences (VMA) and VMSL herbaria were reviewed, as well as specimens of recent incorporation. Specimens were identified by traditional botanical methods, and deposited in the VMA. It should be noted that adventitious and/or naturalized species such as *Festuca bromoides, Festuca megalura, Festuca myuros* as well as *Festuca arundinacea* though is grown as a forage or turf grass are not included in the key. The results of this study were that the following native species are illustrated and included in the key: *Festuca acantophylla, Festuca dissitiflora, Festuca fiebrigii, Festuca hieronymi, Festuca lilloi, Festuca octoflora, Festuca pampeana.* The latter is endemic from Buenos Aires and San Luis provinces and has the status of endangered species.

A49
LITHRAEA MOLLEOIDES: BOTANICAL CHARACTERIZATION AND ANTIMICROBIAL STUDY OF HYDROLATS AND ESSENTIAL OIL

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Antibiotic or pesticide resistance is a public health concern, as antibiotics or pesticides used in animal or crops treatment leads to increased doses. In recent years, alternatives to the use of antibiotics have been proposed, including the use of probiotics, prebiotics, symbiotics, and plant-based products. *Lithraea molleoides*_ (Vell.) Engl. (Anacardiaceae), known in Argentina as "molle", "molle de beber", "molle blanco", is a tree which grows in South America, especially in Argentina, Brasil and Uruguay. Botanic studies are important in order to establish the appropriate parameters to perform an effective quality control of plant. For this reason, the objective of the present work was to characterize ethnobotany and obtain oil from *Lithraea molleoides* leaves to determine its antimicrobial activity. The botanical description of *L. molleoides* was performed by micrographic parameters determination. The studies were illustrated by photomicrographs. In the present work both species from the pharmacobotanical point of view, through various characters micrographic qualitative and quantitative were characterized. For this porpoise aerial parts were collected, herbalized and preserved in acetoalcoholic formalin or dried at 45°C. Samples were diafanized. The qualitative micrographic characters were stomata type, leaf structure, presence of trichomes, presence of oleiferous glands, etc. Regarding the assessment of quantitative micrograph parameters, the following were taken into account: stomata number (SN), stomatal index (SI), palisade ratio (PR), nerve terminals number (NTN) and islets number (IN). The species distinguished both for their exomorphological, anatomical and quantitative micrographic characters. This study contributed to the quality control of these vegetal drug, especially when the product is finely ground or powered antimicrobial activity of hydrolats and essential oil against *Colletotrichum acutatum* (a fungal phytopathogenic), and *Escherichia coli* and *Pseudomonas auriginosa* were evaluated by means of the diffusion well method. The fungus was plated onto potato dextrose agar (PDA) while bacterias onto Luria-Bertani agar (LBA) by spreading a volume (100 µL) inoculum. Then, 6 mm diameter holes were punched aseptically, filled with 100 µl hydrolats or essential oil. Hydrolats showed antifungal activity while essential oils showed antibacterial activity.

A50
EVALUATION OF TWO GERMINATION TREATMENTS OF THE NATIVE SPECIES *PHYSALIS VISCOSA* L., 'CAMAMBÚ,' WITH FOOD POTENTIAL

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Physalis viscosa, 'camambú', is a native, perennial, spontaneous herb belonging to the Solanaceae family whose orange berries have been mentioned as edible as well as *Physalis peruviana* 'uchuva', which is currently cultivated and marketed as a fruit. Studies on germination methods of *P. peruviana*, as well as our own studies on seed viability using the tetrazolium method, were taken as background information. The aim of the present work was to evaluate the most suitable seed germination method for *Physalis viscosa* under controlled laboratory conditions The fruits were harvested in the field at maturity and under appropriate phytosanitary conditions. In the laboratory, 400 healthy seeds were obtained from the fruits and divided into two batches of 200 seeds each. Seeds were taken from one lot immediately after harvesting ('SM') and from the other lot after one week of maceration with fruit residues ('M'). Half of the 100 M and 100 SM (mashed seeds) were placed in Petri dishes with blotting paper and placed in the culture oven at 25 °C. The other half of the seeds, 100 M and 100 SM, were placed in the culture oven. The alternation used was 8 hours of light at 30 °C and 16 hours of darkness at 20 °C. In both situations, irrigation was controlled periodically. As a result, only 2 of the 100 seeds germinated in the oven for the SM treatment. And in the M treatment only 1 seed germinated. On the cultivation table, 70 out of 100 seeds germinated in the SM treatment and 35 of 100 seeds germinated in the M treatment. Exploratory statistical tests were carried out as the treatments could not be repeated in the following season. Although no significant differences were found, it could be inferred that the most suitable germination method would be without prior maceration under alternating light and temperature conditions. Therefore, laboratory studies will continue to obtain statistically significant results, given that this is a native species adaptable to arid conditions with a potential as a fruit crop.

A51
PHYSIOLOGICAL EFFECTS OF CADMIUM STRESS ON *MEDICAGO SATIVA* L. VAR. CW660

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Heavy metal pollution, particularly cadmium (Cd), has become a critical problem in urban and productive ecosystems of the 21st century. The objective of the present study was the impact of Cd on biomass production and the synthesis of photosynthetic pigments and proline in Medicago sativa L. Alfalfa seeds of the CW660 variety were sown in sterilized perlite/soil (1:1) with a completely randomized design, with a control group irrigated with sterile water and others exposed to solutions with concentrations of 75, 100, 150, 225, 300 and 375 µM of $CdCl_2$. After a period of 30 days, all parameters were evaluated in triplicate: growth including length (L), fresh weight (PF) and dry weight (PS) of roots (R) and aerial parts (A). Chlorophylls (a and b) and carotenoids were quantified with spectrophotometer and proline following standardized methods. The results were analyzed using a one-way ANOVA statistical method with $p \leq 0.005$. No significant differences were detected in chlorophyll b, on the other hand chlorophyll a decreased and carotenoids increased significantly at 300 and 375 µM $CdCl_2$. The increase in carotenoids is a physiological response to protect the photosynthetic apparatus against extreme stress. In the growth study, only PFA decreased significantly at these two concentrations. The other parameters PFR, PSA, PSR, LA and LR remained unchanged in all treatments. At the cellular level, an increase in proline was observed with significant differences in proline at all Cd concentrations, indicating that the response pathway of this compatible osmolyte is activated by Cd stress. These findings underline the importance of investigating the mechanisms that regulate the biosynthesis and accumulation of these compounds in various environmental contexts, which could have significant implications for understanding adaptation strategies. In this case, the different mechanisms that the M. sativa var. CW660 develops to tolerate Cd stress were demonstrated.

A52
ARSENIC ABSORPTION BY *SALVINIA MINIMA* L. IN SHORT TIMES IN CONTAMINATED WATERS

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Among the naturally occurring pollutants in the environment, we find the presence of the semimetal Arsenic (As), which in its inorganic state is a confirmed carcinogen and is one of the main water pollutants. It is reported that in the southern area of the province of San Luis there are concentration higher than those recommended for human consumption, being a HACRE (Chronic Regional Endemic Hydro Arsenicism) area. The objective of this study was to evaluate whether *Salvinia minima* L., with great vegetative reproduction, can behave as a bioremediation species. The plants were washed with distilled water and a 10mM EDTA solution and grown in batch-type hydroponics, using a modified Hoagland solution, with artificial lighting (190µ E.S-1.m-1), 16/8 photoperiod (light/dark) and a temperature of 25 +/- 2ºC, for 7 days of adaptation. As exposure was performed with raw water and 0.2 ppm of Sodium Arsenate added. As absorption was analyzed in short period of time (42 h), by extracting three samples of water and three samples of plant every 3 hours. The leaf and root homogenates were subjected to acid mineralization (HNO3) by wet method in microwaves using hermetic Teflon reactors with a pressure valve. The water samples were extracted at the same time periods and acidified with 1% HNO3. The determination of the As concentration was performed on the internal controls over time by ICP-Mass, with an ultrasonic nebulizer due to its high sensitivity and selectivity. Statistical analyses were performed using one-way ANOVA, with ***$p \leq 0.001$. The variation of As throughout the sampling intervals analyzed in water showed a significant decrease within the period of 12 h to 42 h compared to the period of 1, 3, and 6 h with a p ***$p \leq 0.001$ and in the homogenate of *S. minima* a significant increase was observed at times 36 and 42 h in relation to 1, 6, 12, 18 and 24 h ***$p \leq 0.001$. The fresh and dry weight of the plant did not suffer significant variations throughout the treatment with As. *S. minima* has a good behavior at absorbing As within the first 42 hours of exposition. *PROICO 02-2523 y PROICO 02-3423*

A53
IN SEARCH OF GERMOPLASM OF A FORAGE SPECIES (*MEDICAGO SATIVA* L.) TOLERANT TO SALINITY

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New biotechnologies aim to obtain plant germplasm with tolerance to dry and saline environments. Theo bjective of this study was to evaluate 5 varieties of alfalfa provided by the Albert Seed Company, in their tolerance to salinity. The experimental design was completely randomized with a factorial arrangement using seeds of M. *sativa* (Var. 1553, Var. 1580, Var. 1581, Var. 1501, Var 1501 Bioseed) and six levels of NaCl (0, 50, 100, 200, 250 and 300 mM). The tests were carried out in Petri dishes with double filter paper with 5 ml of distilled water as a control and NaCl solutions with two weekly irrigations. 100 seeds were placed per box with 3 replicates each, in an oven at 25ºC and darkness. On the 3rd day, the Germinative Energy (GE) was calculated and on the 7th day, the Germination Power (GP) Germination percentage (%) = Number of germinated seeds / Total number of seeds x 100. The results obtained were analyzed with Graph Pad Prism Version 8.0.2 (263) using the analysis of variance (one-way ANOVA) according to the Tukey test ($p \leq 0.05$). The varieties 1553, 1580, 1581 and 1501 Bioseed had no significant differences in GE and GP at 50 mM and 100 mM NaCl, resulting in tolerance to these saline concentrations. At 200 mM NaCl they had a significant decrease with respect to the control, with the GE with values between 68.33 and 80 % and the PG between 69.33 and 86 % in these varieties. At 250 and 300 mM the levels are low o null. Var 1501 turned out to be the most sensitive with a level of 54% (GE) and 54.67% (GP) at 200 mM NaCl and at 250 and 300 mM NaCl, It was null. In conclusion we can say that the varieties 1553, 1580, 1581 and 1501bioseed the tolerance limitis 200 mM NaCl and for variety 1501 is 100mM NaCl. Higher salinity levels are not tolerated by these varieties of *M. sativa* L.

A54
RHIZOBACTERIAL ISOLATES FROM THE RHIZOSPHERE OF ALFALFA WITH PLANT GROWTH-PROMOTING *ACTIVITY*

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The present study aimed to select microorganisms from the rhizosphere of alfalfa with Plant Growth-Promoting activity (PGPM). Soil samples were collected from an alfalfa crop with no prior history of inoculation, located in a semi-arid area of La Pampa Province. Nine rhizospheric soil samples were obtained. Microorganism isolation was carried out using PCA for total microorganism counting and a selective medium (NBRIP) for the initial screening of strains showing phosphate-solubilizing ability (first screening). Based on this, 142 isolates were selected, and subsequently, 36 isolates were further studied based on their phenotypic characteristics regarding other PGPM properties, such as the production of IAA (indole-3-acetic acid) and siderophores. In order to reduce redundancy, genetic characterization of the isolates was performed using ERIC-PCR, resulting in 23 different band-patterns.
From the 23 selected isolates, it was found that 43% exhibited a high proportion of phosphate solubilization, 30% produced IAA at concentrations greater than 20 ug/mL, and 40% displayed a high capacity for siderophore production. The results obtained allow us to infer the possibility of generating regional bioformulations from native strains with activity in promoting the growth of alfalfa crops, serving as a key tool to enhance the specific agricultural system of the province. Therefore, it is essential to learn how to combine technologies to improve production benefits and preserve the agroecosystem.

A55
ISOLATION AND CHARACTERIZATION OF NATIVE BACTERIA SYMBIONISTS OF MEDICAGO SATIVA IN SOILS OF THE SEMI-ARID PAMPEANA REGION

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Soils of the Semi-arid Pampas Region in Argentina present limitations for agricultural production, being nitrogen and water availability the most significant constraints. Despite this, recently, there has been a shift in crop cultivation towards regions with less fertile soils. This situation has led to the intensive use of fertilizers and the erosion of the fertile soil layer. In this context, microbial biofertilizers, as a partial alternative to the use of chemical fertilizers, have become of great importance. One crop that has expanded its cultivation in the region for forage production is alfalfa, which establishes a symbiotic relationship with *Ensifer meliloti*. Commercial inoculants recommended for this crop are formulated with the *Ensifer meliloti* B399 strain. The objective of this study was to obtain native strains better adapted to the region, capable of nodulating and efficiently fixing nitrogen, for use in the formulation of regional inoculants. Native strain isolations were conducted from nodules of alfalfa plants cultivated in the region of interest. Characterization of the strains present in the nodules from these trials using BOX-PCR revealed the presence of eight different native strains apart from *Ensifer meliloti* B399, capable of nodulating alfalfa and in which the nodC gene could be amplified. Kinetic characterization and infectivity tests on plants were conducted for each isolation. Two of them exhibited higher nodulation capacity and showed no differences in efficiency compared to the recommended strain, while in the remaining isolations, no differences were found compared to the control. These isolated microorganisms will be evaluated in non-sterile microcosm trials with the aim of considering their use in inoculants for crops in the region.

A56
EVALUATION OF A MICROBIAL CONSORTIUM FOR BIOCONTROL OF *ALTERNARIA ALTERNATA* IN TOMATO

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Alternaria alternata is a pathogenic fungus that affects tomato plants. It is a significant concern in agriculture and controlling its spread is crucial. The use of fungicides has been the primary method for controlling *A. alternata*, but there is growing consumer concern about pesticide residues in food. As a result, researchers have been exploring alternative methods, including biological control and environmentally friendly compounds. Microbial consortia composed of carefully selected and compatible beneficial microorganisms, including bacteria and fungi, have been shown to effectively control foliar diseases in tomato plants. These microbial consortia display an extended functionality and versatility in the biocontrol of a wider range of plant diseases, including foliar diseases. They can control pathogens through diverse mechanisms and application methods, such as direct antagonism or induced systemic plant resistance. The objectives of the present work were to evaluate the biocontrol effect of *Trichoderma harzianum* ITEM 3636 and *Pseudomonas putida* PCI2, applied at the root level, on the severity of tomato leaf blight caused by *A. alternata,* and to determine if the inoculated plants show a strengthening of the production of defense enzymes. Plants were grown in a growth chamber. After four weeks, they were transplanted into pots and transferred to a greenhouse. Two weeks after transplanting, 2 ml per plant of a suspension of PCI2 (1×10^6 cfu ml^{-1}) mixed with ITEM 3636 (1×10^5 conidia ml^{-1}) was inoculated in the area adjacent to the roots. One week after inoculation, *A. alternata* spores (1×10^4 conidia ml^{-1}) were sprayed on the leaves. A pathogenicity control (plants treated only with *A. alternata*) was included. At 1, 3, 5 and 7 days post infestation, leaf samples were collected and used as a source of enzyme to estimate peroxidase (PO), polyphenol oxidase (PPO) and β-1, 3 glucanase. The severity of leaf blight was recorded two weeks after infestation with the pathogen. Pathogenicity control plants showed an average percent disease index (PDI) of 45. Pre-inoculation with ITEM 3636 + PCI2 significantly reduced the average PDI to a value of 10. Plants inoculated with ITEM 3636 + PCI2 showed a 40% increase in PO activity at 5 days post infestation, compared to the patogenicity control. On the other hand, inoculation did not modify the activity levels of PPO, compared to the pathogenicity control. Finally, it was observed that, after 7 days in the presence of *A. alternata*, the leaves of the co-inoculated plants showed a significant increase in *β*-1,3-glucanase activity. Thus, we can conclude higher levels of PO and *β*-1,3 glucanase, caused by inoculation with the microbial consortium, may have contributed to plant resistance against this foliar pathogen.

A57
TOMATO GROWTH STIMULATION BY A MICROBIAL CONSORTIUM

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Individually, strains of *Trichoderma* spp. And plant growth-promoting bacteria (PGPB) can be effective as biocontrol and crop growth promotion agents. However, combinations of different *Trichoderma* species with PGPB have been shown to be more effective than a single microorganism. Numerous studies point towards a synergistic interaction of this type of consortium, given that this combination enhances the benefits on plants. Thus, it is essential to harness this intricate network of natural interactions to create microbial consortia that substantially and consistently benefit plant growth and health, increase crop production and decrease the use of chemical compounds. The objective of the present study was to analyze the potential of the strains *Pseudomonas putida* PCI2 and *Trichoderma harzianum* ITEM 3636 to promote the growth of tomato plants under commercial field conditions and to eventually control diseases affecting this crop. The field assays were carried out in an organic production greenhouse, located in Tres Acequias (33°01 ‘33.2 “S 64°25’ 53.7”W), and in greenhouses intended for traditional tomato production, which were located in the 37eriphery of Río Cuarto (33°04’46.6”S 64°20’ 37.2”W), during the 2022-2023 production period. The following treatments were carried out: (1) Control

without inoculation; (2) inoculation with PCI2; (3) inoculation with ITEM 3636 and (4) inoculation with PCI2 + ITEM 3636. At the time of transplanting, seedlings were inoculated by immersion of their roots in the corresponding suspension, at a concentration of 1 x 10^5 conidia ml^{-1} for ITEM 3636 and 1 x 10^6 CFU ml^{-1} for PCI2. In the organic greenhouse, with focus on the consortium inoculation treatment, we observed statistically significant increases in leaf area (74%) and number of fruits per plant (225%), compared to the controls. Also, we observed that the inoculated treatments were always in a more advanced phenological stage than the control treatment, which was left behind due to competition with weeds. Thus, inoculation also contributed to the rapid establishment of the crop. On the other hand, in the traditional production greenhouses, we observed that the consortium inoculation treatment caused statistically significant increases in leaf area (68%), internode length (28%), equatorial diameter of fruits (20%) and polar diameter of fruits (28%), compared to the control. No disease occurred in any of the greenhouses. PGPB and fungi such as *Trichoderma* are examples of microbial players within the rhizosphere that can enhance nutrient availability, modulate phytohormones, provide biocontrol, and improve biotic and abiotic stress tolerance in plants. We can conclude that inoculation of tomato roots with PCI2 + ITEM 3636 favored the growth, yield and health of tomato plants, compared to the controls, under two different production systems.

BIOTECHNOLOGY AND GENETICS

A58
TERPENES AS ANTIFUNGAL AGENTS FOR THE PREVENTION AND TREATMENT OF GRAPEVINE WOOD DISEASES

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The disease of the vine known as “Grapevine wood decay,” caused by ligninolytic fungi, is considered one of the main global threats due to its potential to cause the death of grapevines. In the province of San Juan, where wine production is the most important economic activity, any disease affecting the yield and quality of the vine has a significant economic impact. These diseases are caused by a group of ligninolytic fungi responsible for conditions such as Eutypa dieback, Esca, Petri disease, Black Dead Arm (BAD), and Malvón leaf. Recently, *Lasiodiplodia theobromae* has been reported as a causal agent of irreversible destruction in this disease. The increasing resistance of fungal pathogens to conventional antifungal agents has prompted the search for alternative treatments. Among the various compounds investigated for their antifungal properties, menthol and terpineol have emerged as notable candidates. As part of our group's work, ligninolytic fungi were isolated from grapevines in San Juan. After conducting morphological and molecular analyses, the presence of *L. theobromae* was confirmed. To evaluate the antifungal activity of menthol, terpineol, and their 50% mixtures (using thymol as a positive control and also in combinations: menthol-terpineol, thymol-menthol, terpineol-thymol), the minimum inhibitory concentration (MIC) was determined through several bioassays. The final concentrations evaluated were 5, 4, 3, 2, 1, and 0.5 mM for each compound/mixture, with three replicates per treatment. Additionally, a bioassay was conducted to determine whether the compounds acted as fungicides or fungistatic when no mycelium was observed in the MIC assay. The results indicated that the MICs for thymol, menthol, terpineol, menthol-terpineol, thymol-menthol, and terpineol-thymol were 1, 5, 5, 5, 2, and 2 mM, respectively. Furthermore, it was confirmed that they acted as fungicides at concentrations of 3, 5, >5, >5, 5, and 3 mM respectively based on visual inspection in the fungicide/fungistatic assay. Thymol is the compound with the highest antifungal activity at the lowest concentration, followed by its combination with terpineol for the MIC and demonstrating equal effectiveness for both in fungicidal activity. This is the first assay of synergistic activity between these terpenes. The notable ability to act as fungicides at relatively low concentrations offers a sustainable and effective alternative to current treatments, opening new avenues for crop protection and reducing significant economic losses. Therefore, it is justified and crucial to continue studying these compounds and their combinations to optimize their application in managing vine diseases.

A59

A SPECTROPHOTOMETRY TECHNIQUE FOR DETERMINATION OF VIABILITY *SPIROGYRA* ALGAE AFTER CRYOPRESERVATION

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In recent years, interest in microalgae as a source of plant proteins has grown. Spirogyra, which owes its name to the helical arrangement of its chloroplasts, contains more than 20% (w/w) protein in its dry form. Cryopreservation is a crucial first step to produce proteins from this alga, as it provides cells for the development of an efficient bioprocess in biomass production and protein extraction. Therefore, it is essential to have an adequate technique to evaluate cell viability. This study presents a colorimetric method to determine the proportion of live and dead cells in *Spirogyra* algae cryopreserved for 40 days in a freezer -40°C with 12% sucrose solution, using methylene blue (MB) uptake. This technique identifies dead cells, since the dye only penetrates cells with damaged membranes, allowing viability to be evaluated. A 0.1 M sodium triphosphate buffer and 0.155 mM MB were used as a dye solution, with readings at 664 nm, using the buffer as a blank. When living cells come into contact with methylene blue (MB), it diffuses through the cell wall and interacts with the membrane. Prolonged exposure causes oxidative damage and membrane rupture, allowing MB to penetrate the cell. Two peaks were observed in the absorbance curve: the first due to the dye's diffusion into the cell wall and the second due to the dye's absorption in the membrane and its rupture. Therefore, to avoid damage, an initial diffusion time of 1.75 min and an exposure time before the membrane rupture of 2.25 min were determined. A calibration curve was developed to determine the viability percentage. The viability percentage was determined by microscopy as the percentage of living cells (unstained cells) over the total count of cells. Therefore, the absorbance value was related to the viability percentage obtained by microscopy. The value of 0% viability or 100% dead cells was determined by long-term contact of a fresh sample with MB until obtaining a constant absorbance value. The 100% dead cells were confirmed by microscopy. Then, 1 mg of fresh sample was exposed to the colorant solution for 2.25 min and filtered, obtaining an absorbance value of 0,415±0,010 corresponding to 75% viability. Different combinations of fresh and treated samples with 75% and 0% viability, respectively, were used to determine the intermediate points of the calibration curve. The linear equation obtained for the calibration curve was: % of viability = 364.35 * (Absorbance) – 72.614 (R^2 = 0.9915). Therefore, the steps of the optimized technique were mix 1 mg of the fresh sample and 3.5 ml of colorant solution and then leave to rest for an exposition time of 2.25 min. The sample was filtered to extract algae, and the absorbance was measured. The viability percentage was obtained from the absorbance value and the linear equation. Therefore, from this work, it was possible to optimize a colorimetric method to determine the algae viability percentage through a technique with a low cost and with a simple, easy, fast, and precise execution.

A60

USE OF INTRACELLULAR AND EXTRACELLULAR CRYOPROTECTIVE AGENTS FOR PRESERVING THE THREE-DIMENSIONAL STRUCTURAL CONFORMATION OF NATIVE SPIROGYRA FROM SAN LUIS, ARGENTINA

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Green algae, like charophyta *Spirogyra*, contains over 20% (p/p) of proteins on a dried basis. Therefore, algae protein extraction research is of technological interest for the food industry. In this sense, algae preservation is crucial for efficient development and obtaining adequate biomass for protein extraction. Consequently, it is essential to obtain starter cultures with adequate plasma membrane integrity, cell viability, and proper functionality. This work aimed to study the cryoprotective effect of the extracellular agent sucrose at 12% (w/w) combined with the intracellular agent glycerol at 20% (w/w) each in its respective final concentration, and then combined in the algae *Spirogyra*, compared with sucrose at 12% (w/w) and a control sample without a cryoprotective agent. The samples were prepared with a concentration of algae of 10% (w/v) in combination with the different cryoprotective agents in hermetically sealed containers of 15 ml. Subsequently, the samples were freezing at a speed of 0.50±0.10 °C/min at -40 °C for 40 days. The samples were unfrozen in a thermostatic bath at 37 °C. The membrane structural integrity was analyzed with Lugol´s solution at 40% (v/v) by optical microscopy (40x), and digital images were taken for each sample. The value of the plasmatic membrane contraction inside the cellular

wall was analyzed and was expressed as the percentage non-occupied by the helical or spiral structure of chloroplasts due to the membrane collapse (contraction percentage). The images of eight replicates per sample were analyzed with Image-Pro Plus 6.0 software, and the statistical analysis was performed with Graph Pad In Stat software. For the calculation of the contraction percentage, the images were segmented into two zones: the area occupied by the cellular membrane, cytoplasm, chloroplasts spiral, and pyrenoids (black), and the area no occupied inside the wall cell (white). The contraction percentage was calculated as a percentage of the white area to the total area. The results showed that the control sample without a cryoprotective agent after unfreezing presented a contraction percentage of the cell membrane of 59.98±3.76% (P<0.05), and the samples with sucrose at 12% (w/v) generated a contraction percentage of 39.58±3.56% (P<0.01). The results showed that combining the intra and extracellular agents decreased the contraction percentage, obtaining a value of 33.26±3.06%. Therefore, the combination of the cryoprotectants sucrose and glycerol could interact with the polar head groups of the lipid bilayer outside and inside of the membrane, preserving in a higher proportion the cell-membrane structural integrity or obtaining a loss of the spatial conformation inferior compared to the control sample (P<0.01) and the sample with only the extracellular agent (sucrose 12%, w/w). Hence, combining sucrose 12% (w/w) and glycerol 20% (w/w) as cryoprotective agents improved the viability after freezing, preventing membrane destabilization by dehydration and ice crystal formation.

A61
RECOMBINANT *BST* POLYMERASE PRODUCTION: A CRITICAL INPUT FOR THE DEVELOPMENT OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) TESTS

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Loop-mediated isothermal amplification (LAMP) techniques have several advantages among other traditional diagnostic methods such as simpler procedures, shorter processing time, greater versatility and lower costs while maintaining real-time PCR sensitivity and specificity. The *Bst* polymerase, a large fragment of DNA polymerase I from *Geobacillus stearothermophilus*, is the most commonly used enzyme in these assays. It presents a strong strand displacement activity which enables amplification at a constant temperature. However, it is obtained from international suppliers. The development and fine-tuning of a biotechnological method to obtain this enzyme is essential to the subsequent development of low-cost infectious disease diagnostic kits per sample unit. Production of *Bst* polymerase Large Fragment (BstLF) was evaluated in mediums LB and M9 modified (M9m). *E. coli* expression strains ER2566 and BL21 were transformed using an expression vector (EV) pET15b containing the BstLF gene followed by a His-Tag sequence for future purification steps. A donated EV containing the same gene was used as control. Protein expression was induced by overnight incubation at 20°C±1 with IPTG 4 mM under stirred conditions, non-induced cultures were used as control. Processed samples were analyzed performing a sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) and protein bands were only observed for M9m cultures. Production of BstLF in larger volume was carried out inducing M9m cultures of ER2566 and BL21 as mentioned before. The recombinant BstLF obtained was purified by immobilized metal affinity chromatography (IMAC) using a nickel resin and the extracts were analyzed by 10% SDS-PAGE gel. The amount of protein obtained (5 mg/mL) was quantified by the Bradford Protein Assay and the purity was determined to be around 70%. The activity of the BstLF enzyme was measured by comparing the produced DNA bands with those produced by the commercial enzyme in LAMP reactions. Results showed that BstLF was able to amplify a DNA sample successfully. Further activity assays are needed in order to determinate optimal reaction conditions for the efficient performance of the recombinant BstLF.

A62
MOLECULAR IDENTIFICATION, ENHANCEMENT AND LIQUID FORMULATION OF A YEAST STRAIN FOR CRAFT BEER PRODUCTION

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Craft beer has experienced remarkable growth as an industry, where brewers strive to establish distinctive and recognized brands. To achieve this, they constantly seek innovation in the pursuit of new flavour and aroma profiles in their beers. Non-conventional yeasts, meaning those different from the ones traditionally used in beer production, are a valuable tool for exploring new flavours and aromas. The aims of this study were performing a molecular identification, conducting adaptive improvement treatments, and design a liquid formulation of native yeast from San Luis for craft beer production. For molecular identification, genomic DNA from yeast using a commercial kit, followed by PCR amplification of conserved genomic regions with primers ITS1 and NL4. Subsequently, the fragments were sequenced by Macrogen. The obtained sequences were edited and aligned using MEGA v11 software. The identification was conducted via BLASTn searches against NCBI database. The improvement treatments were conducted in 100 mL micro-fermenters with beer wort, inoculated with $1x10^6$ cells/mL of native yeast Sc3, incubation at 20°C for 7 days, and the fermentative power was verified. The yeast was then recovered, for start a new fermentation. The same procedure was repeated for 12 cycles. The liquid formulation was designed after recovering the yeast from the 12^{th} cycle and inoculated it into a bioreactor with beer wort to multiply the yeast and achieve a concentration suitable for craft beer production. To brew beer, was used the British Golden Ale style, beer wort was prepared and inoculated with the designed formulation. After the fermentation, maturation, and bottling process, a blind tasting was conducted with untrained personnel. As a control, a craft beer brewed using a commercial dry yeast was used. The native yeast was identified as *Saccharomyces cerevisiae*, designated Sc3 (GenBank accession number PP510222). The improvement process showed enhanced fermentative power after 12 cycles, with *S. cerevisiae* Sc3 exhibiting greater fermentative power, evident in the consumption of sugars during fermentation. The initial sugar density was 1046 g/cm3, and it ended at 1019 g/cm3. The parameters of the beer fell within the standards established for the beer style described in the 2021 Beer Judge Certification Program (BJCP), including foam presence, color, and alcohol content. Results from the blind tasting with untrained participants indicated that 52% of people preferred the beer brewed with native yeast Sc3 compared to 48% for the beer brewed with commercial yeast. The improvement processes allowed *S. cerevisiae* Sc3 to adapt to ferment beer wort, and the designed formulation enabled the production of beer preferred by an untrained audience, indicating its potential for further study and transfer to the local craft brewing industry.

A63
COMPARATIVE STUDY OF CRAFT BEERS MADE WITH DIFFERENT *SACCHAROMYCES CEREVISIAE* YEAST LIQUID FORMULATION

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Beer is one of the most consumed alcoholic beverages worldwide. It is a fermented beverage made with natural ingredients such as water, barley, hops, and yeast. Currently, craft brewers are trying to differentiate themselves in the market through innovation, aiming to incorporate liquid yeasts into the brewing process, as these generate a different sensory profile in the final product. The objective of this work was to design two liquid formulations using the yeast *Saccharomyces cerevisiae* to produce craft beer and verify if sensory changes were achieved. The yeasts used were commercial *S. cerevisiae* called Safeale-US-05 and native *S. cerevisiae* isolated from the province of San Luis, called SC-3. Each liquid formulation was designed using beer wort as a culture medium to multiply the yeasts in a bioreactor for 24 hours at 24±2°C, with aeration at 5L/m and agitation speed at 150 rpm. The quality of the formulations was verified under a microscope. To make beer, beer wort was prepared following the recipe for the British Golden Ale style, designed by the Beer Judge Certification Program (BJCP) 2021. The beer wort was placed in two fermenters and inoculated with Formulation 1 (F1-Safeale-US-05) and Formulation 2 (F2-SC-3). After 14 days (7 days of fermentation and 7 days of maturation), the resulting beers were bottled and naturally carbonated. For the sensory study, the beers were evaluated by the brewmaster. The results of the formulation indicated that optimal concentrations for beer production were obtained, F1 ($3.8x10^8$ cel/mL) and F2 ($1.5x10^8$ cel/mL). Both formulations were of high quality, as they showed 100% viability and no contaminants. The sensory test results indicated that the beer produced by F1 had a bright golden yellow appearance, with low turbidity, while the beer produced by F2 had

an amber-yellow color, slightly darker. With respect to the aroma, the beer made with F1 did not exhibit a noticeable hop or malt aroma, whereas F2 had a predominant intense hop aroma. Both beers were within the parameters established for the beer style described by the BJCP. In conclusion, we can say that both formulations are optimal for brewing beer and that the differences observed in each beer are due to the fact that each formulation was designed with two different yeast types. F2, designed with native yeast from the province of San Luis, could be promising for transfer to the craft brewing sector.

A64

CHARACTERIZATION AND ENCAPSULATION OF BACTERIAL CONSORTIA, PLANT GROWTH PROMOTERS ISOLATED FROM AROMATIC PLANTS (*Salvia Rosmarinus* and *Origanum vulgare*)

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Sodium alginate, is a natural polymer, widely used method for encapsulating microorganisms, due to its ability to form gels in the presence of calcium ions. This study aimed to analysed, on the one hand, various properties of Plant Growth Promoting Bacteria (PGPB) isolated from rosemary (*Salvia Rosmarinus*) and oregano (*Origanum vulgare*) and on the other, optimize conditions for efficient encapsulation of a consortium formulated from a selection of these isolates. The bacteria were evaluated for their ability to solubilize phosphorus, produce indole-3-acetic acid (IAA) and siderophores, produce acyl-homoserine lactones (AHLs), compete with phytopathogens, and produce extracellular hydrolytic enzymes (pectinases and cellulases). The encapsulation method involved a droplet-based gelation process, where a sodium alginate solution containing the bacteria was extruded into a calcium chloride solution. Various concentrations of sodium alginate (0.037-0.5% w/v) and calcium chloride (2-11.1 g/L), as well as different agitation speeds (1000-2000 rpm), were tested to achieve optimal capsule size and homogeneity. The microcapsules containing the microbial consortium were stored in cryovials with and without water, at 4°C. Cell viability within the capsules was assessed at 15, 30, 60, and 90 days, using serial dilution and plate counting. Results showed that all six isolates could solubilize phosphorus and produce IAA, with isolate 2 being the highest producer (38.2 µg AIA/ml). Only four isolates produced siderophores, and no AHL production was detected. Biocontrol activity against *Fusarium oxysporum* and *Fusarium graminearum* was observed in only one isolate, and pectinase activity was detected in strains 4 and 21, while strain 2 exhibited cellulase activity. The optimal encapsulation conditions involved 0.5% w/v sodium alginate, 7.5 g/L calcium chloride at agitation speed 1000 rpm, resulting in uniform, spherical capsules of approximately 4 mm of diameter. Viability tests demonstrated that the encapsulated bacteria remained viable for up to 90 days, with counts decreasing from 10^8 CFU/ml to 10^6 CFU/ml. These findings support the use of sodium alginate and calcium chloride for encapsulating PGPB isolated from rosemary and oregano, providing new opportunities for sustainable agriculture. This methodology could be a significant advancement in the development of microbial-based biofertilizers, promoting practices that seek to minimize environmental impact and encourage sustainability.

A65

IDENTIFICATION OF RESISTANT MICROORGANISMS AND MICROBIAL ACTIVITY IN CONTAMINATED SOILS UNDER THREE BIOREMEDIATION APPROACHES

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The success of a bioremediation process primarily depends on the intrinsic ability of the system to create and maintain conditions to promote the biodegradation of contaminants at a sufficiently high rate. Strategies to accelerate the biodegradation of hydrocarbons and other compounds in the soil include stimulating indigenous microorganisms (bio-stimulation) by optimizing factors such as the inoculation of a mixed microbial culture in the soil (bio-augmentation). The aim of this work was to identify effluent-resistant strains isolated from a landfarming and to evaluate three bioremediation approaches for a soil contaminated with glycols. For this, a series of laboratory-scale experiments were carried out with different experimental conditions: natural attenuation, biostimulation and bioaugmentation with previously selected native strains. The strains selected in previous studies were cultivated into LB-glucose (g L-1): NaCl 5.0; yeast extract 5.0; peptone

10.0; Glucose 10.0. The identification was realized by molecular techniques: DNA was obtained using a biology kit; PCR amplification with universal primers the DNA concentration in the PCR products was determined using Epoch (Biotek) and the integrity of the samples was evaluated through 1% Agarose gel electrophoresis. The PCR products were sent to CERELA (Tucuman-Argentina) for their purification and sequencing. The sequences were edited with Molecular Evolutionary Genetics Analysis (MEGA v7.0) and were analyzed with BLASTn using NCBI databases (www.ncbi.nlm.nih.gov). For the microbial activity test, 70 grams of soil were weighed in glass jars. Then, 4 ml of distilled water and 3 drops of Bromothymol Blue1 indicator were placed in the test tubes. The assembly of the devices was completed and then a "control" jar was made without the soil, to ensure that the change produced in the indicator was due to microbial respiration. The selected microorganisms were identified as coming: *Penicillium, Bacillus and Acinetobacter*. In the microbial activity test, different shades from yellow to green pH (6.7/7.4) could be seen: the one that received the mixed crop sowing presented lower pH values, indicating higher concentrations of CO2 coming from microbial respiration, followed by the biostimulation process and finally the natural attenuation process. Therefore, bioaugmentation and biostimulation increased microbial activity, indicating improved landfarming performance.

A66
BIOCHEMICAL CHARACTERIZATION OF DIFFERENT CYANOBACTERIAL SPECIES ISOLATED FROM SAN LUIS PROVINCE SOILS

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Cyanobacteria are photoautotrophic organisms that thrive in the upper layers of soil, acting as a primary source of carbon and nitrogen for heterotrophic microorganisms in the soil. These organisms are recognized for their beneficial effects on soil fertility and crop production, primarily due to their capacity to produce a diverse range of bioactive compounds. This study aimed to characterize the biomolecules—specifically photosynthetic pigments, proteins, and carbohydrates—found in various cyanobacterial species from the soils of San Luis province. Three local strains were collected from different areas within San Luis province, cultivated, and subsequently analysed for carbohydrate content using the Nelson-Somogyi method. We also assessed chlorophyll and phycobiliprotein composition (including phycocyanin, allophycocyanin, and phycoerythrin) through spectrophotometric methods, as well as protein levels using the Bradford method. Additionally, an artificial consortium composed of different cyanobacterial species was biochemically tested and compared with the individual strains. The results revealed that among the different cyanobacterial species studied there were no significant differences in protein and carbohydrate levels or chlorophyll content. Similar findings were observed when comparing the protein and carbohydrate content of these species to that of the artificial consortium. However, when measuring phycobiliproteins, *Nostoc sp* exhibited the highest concentration of pigments (1.63±0.6 µg/gram of biomass), while *Phormidium sp*. and the artificial consortium displayed the lowest levels (0.11 and 0.09 µg/gram of biomass, respectively). Notably, in *Nostoc sp*, phycocyanins comprised the majority of the total phycobiliprotein content (1.3±0.2 µg/gram of biomass), accounting for nearly 80% of all pigments. In conclusion, these findings highlight the significance of evaluating.

A67
MICROPROPAGATION OF *ALIMENAEA INTEGRIFOLIA* (GRISEB.) N. O'LEARY & P. MORONI

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Alimenaea integrifolia, "incayuyo", is a species with aromatic-medicinal value with economic impact on the families that collect and sell it. This native resource has been overexploited, with the risk of genetic erosion and reduction of natural populations. Therefore, in order to promote the domestication and genetic improvement of the species, biotechniques were used to multiply this species on a large scale and in the shortest possible time. *In vitro* growth is influenced by genetic factors (plant tissue) and environmental factors (light, nutrition, temperature, humidity). The effect of light, its quality and intensity have been studied in various *in vitro* cultivation investigations, finding that the visible spectrum affects plants metabolically, reproductively, and morphogenetically. In the present work, the rate of sprouting and rooting was evaluated

as a function of conventional light conditions and LED lights. The experiment began with binodal explants of *A. integrifolia*. Five treatments were evaluated with 20 repetitions each: T1 fluorescent lamps-without growth regulators, T2 fluorescent lamps-with growth regulators (0.5 mg/l kinetin and 0.5 mg/l naphthaleneacetic acid), T3 LED I (red: 40% - blue: 30% - white: 30%), T4 LED II (red: 60% - blue: 20% - white: 20%), T5 LED III (red: 80% - blue: 10% - white: 10%). The cultures were incubated at 24±1°C and photoperiod 16 h light-8 h dark. Measurements (rate number of leaves/explant and roots/explant) were made every seven days for 49 days. The vitroplants were acclimatized in a substrate composed of: perlite (10%), worm humus (40%), river waste (20%), and sand (30%), in a controlled humid environment. It was determined that the T5 LED III presented significant differences with a higher average production rate of the number of leaves/explant (38+/-0.83 leaves/explant) and number of roots/explant (6+/-0.78 leaves/explant) during the *in vitro* culture of *A. integrifolia*. These results correspond to the physiological response of the plant sensor system that captures the different light spectra (red), and emits a biological response. LED lights, for this species, allow obtaining a greater number of shoots and roots than fluorescent lights, presenting the advantages of low energy consumption, long useful life and minimum heat emission. For *A. integrifolia*, the use of growth regulators can be replaced with LED lights with light spectra similar to those evaluated in this work, reducing micropropagation costs and allowing the availability of *in vitro* foliar material for the extraction of aromatic-medicinal principles. Conduct research using LEDs at different intensities and combinations of spectra in conducting research with the use of LEDs at different intensities and combinations of spectra results in a contribution to energy efficiency in a commercial laboratory. PROICO 14-1923 (CyT UNSL).

A68
ACCLIMATIZATION ANALYSIS OF *CLINOPODIUM GILLIESII* (BENTH.) KUNTZE UNDER NEW LIGHT TECHNOLOGIES

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Currently, there has been a significant increase in interest in addressing the knowledge of native plant resources. The paradigm shift is driven by changes in food, medicine and cosmetics, with the demand for natural and functional products. In this global context, several native species of the South American continent have been revalued and have begun to be mass produced. In Argentina, there are native resources with medicinal and aromatic value that possess bioactive compounds responsible for antioxidant, anti-inflammatory, anti-tumor, and antibiotic properties. *Clinopodium gilliesii* (muña muña) is used as an infusion for stomach problems, menopause, altitude sickness, female infertility, heart problems, and as a vasodilator. This species has a high variability in chemical composition and quantity of secondary metabolites and has a low reproductive capacity, which, added to excessive extraction for commercial purposes, determines that it is at serious risk of extinction; determining the need to implement its sustainable use and establish efficient propagation methodologies. In this work, a new lighting technology is evaluated to induce a high acclimatization rate in *C. gilliesii* as the final stage of micropropagation and crucial to determine the final number of cloned plants. A technological alternative to traditional lighting systems was implemented during *in vitro* cultivation, with the following treatments: T1 fluorescent lamps and growth regulators, T2 LED I (100% white), T3 LED II (red: 40% - blue: 30% - white: 30%), T4 LED III (red: 60% - blue: 20% - white: 20%), T5 LED IV (red: 80% - blue: 10% - white: 10%), After 90 days of *in vitro* culture, the acclimatization process was started. Micropropagation has been widely used for the efficient multiplication of many plant species. However, its use is restricted by the high percentage of losses or damage when plants are transferred to *ex vitro* conditions. A high survival rate was observed in plants from T3 LED II with significant differences from the other procedures. The plants showed greater root development, shorter internodes and a balance in the ratio of root length to stem length. The treatment with a higher percentage of blue light, combined with red and white light, favors the stimulation of photosynthetic pigment synthesis, vigorous growth and less water stress. These morpho-physiological aspects favor the *ex-vitro* adaptation of micropropagated plants. A contribution is made to the technological development for the production of a species demanded by consumers and with the potential to diversify production systems in the region. PROICO 14-1923 (CyT UNSL).

A69
USE OF RESPONSE SURFACE METHODOLOGY TO OPTIMIZE INVERTASA PRODUCTION BY *ASPERGILLUS NIGER*

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The enzyme β-fructofuranosidase, also known as Invertase, produce the hydrolysis of sucrose into its constituent glucose and fructose. Invertase is widely used in candy-making to create liquid centers because it helps maintain moisture in baked goods and contributes to smoother textures. Invertase is produced by various organisms including yeast, fungi, bacteria, higher plants, and animals. *A. niger* which is approved for safety by FDA is discovered as naturally producing larger titers of invertases and efficiently hydrolyze sucrose into glucose and fructose, resulting in inverted sugar syrup. For biomass production, *A. niger* was inoculated on Potato Dextrose Agar (PDA) plates and in a culture oven for 120 hours at 28ºC. The resulting spores were transferred to 70 mL of Sabouraud Dextrose Broth (SDB), Potato Dextrose Broth (PDB) and Sabouraud Broth Supplemented with Sucrose (SBS), at concentrations of 5 to 50 g/L. These cultures were maintained under agitation at 150 rpm for 96 hours at 28ºC. Subsequently, 2.5 g of fungal biomass were placed in extraction buffer (0.1M acetate in 0.5M NaCl) and a 10 g/L sucrose solution to stimulate further enzyme production, and they were kept under agitation at 150 rpm for 24 hours at different temperatures: 22°C, 24°C, 26°C, and 28°C. Additionally, a similar protocol was followed, but with the temperature maintained at 28°C while varying the pH to 3, 4, 5, and 6 of the both solution. The cultures were centrifuged for 10 minutes, and the supernatant was taken as the enzyme extracted. Enzymatic activity was evaluated using the indirect technique of measuring reducing sugars with 3,5-dinitrosalicylic acid (DNS), incubating the samples at 100°C for 10 minutes and measuring the absorbance at 570 nm. The Response Surface Methodology (RSM) was used to determine the optimal conditions for enzyme production. The data were analyzed with the STATISTICA 8.0 Stat Soft Inc. software. A complete second degree model like the one shown below was proposed: where bij represents regression coefficients of the model, found by the least squares method, Xij are the scaled factors (X1 = (Temperature - 27.1)/2; X2 = (pH-4.7)/1.5; X3 = ([Sucrose] -5)/5), and ε is the statistic error or residual. The effects of Temperature, pH and [Sacarose] on [Glucose] were investigated. With the experimental results obtained, it was possible to fit a linear regression model with good predictive capacity, from which an optimal mean [Glucose] of 24.910 ± 3.241 g/L (95% confidence interval) was estimated model for a Temperature = 28 °C; pH = 6 and [Sacarose] = 10 g/L. The ANOVA indicated that the pH and Temperature factors are the ones that have the greatest effect on [Glucose], these effects are predominantly linear with a small curvature. The model indicated that a new experimental region should be explored, which would allow for more [Glucose].

HUMAN CLINICAL ODONTOLOGY

A70
ANXIETY, STRESS AND PHYSICAL ACTIVITY IN MIDDLE LEVEL STUDENTS OF SAN LUIS

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Stress and anxiety are common problems in students, on a biological and psychological level. Physical activity and sun exposure, among others factors, influence coping mechanisms necessary for managing the changes inherent to this stage of life. We investigated four schools in San Luis, from different geographical locations: Volcán, Chile (suburban) and San Martín and Pringles (urban). Objective: To investigate perceived stress (PE), anxiety and physiological parameters in high school students. Methodology: PE, anxiety, physical activity, Body Mass Index (BMI) and sun exposure were investigated. The sample was made up of 90 students in total, Volcán school (7 women, 7 men), Chile school (8 women, 12 men), San Martín school (9 women, 23 men) and Pringles school (16 women, 8 men); mean age (m)= 16.01 years, SD=0.8, range: (15-19). 0.000). Results: Anxiety at Pringles school, m: 2.37, SD: 1.09; Chile, m: 2.40, SD 1.04; Volcan, m: 2.28, DE 1.13; San Martín, m: 2.41 DE: 0.86. EP in Volcano, m: 2.14, SD: 0.77; Pringles, m: 2.41, SD: 0.82. Chile, m: 2.50, SD 0.68; San

Martín, m: 2.42 SD: 0.56. At the Volcán school, the greater the physical activity, the lower the anxiety p=0.08; San Martín school: the greater the physical activity, the less anxiety, p= 0.08; Chile school: the greater the physical activity, the lower the PE, p=0.009. At Volcán school, Pringles and Chile schools, women presented higher levels of anxiety than men ($p \leq 0.05$). At the Volcán and Pringles school, women also express higher levels of PD ($p \leq 0.05$). Physical activity: In Volcán and San Martín schools, the greater the frequency of physical activity, the lower the anxiety levels ($p \leq 0.08$). In Chile school, the higher the frequency of physical activity, the lower PE ($p \leq 0.05$). BMI and PE at the Volcán school, students with higher BMI presented higher levels of PE ($p \leq 0.00$). Sun exposure: At Chile school, students who were not exposed to the sun had higher levels of anxiety ($p \leq 0.05$). At San Martín School, those who were not exposed to the sun showed higher levels of PD ($p \leq 0.05$). Positive correlations were found between anxiety and PD at Volcán school ($p \leq 0.01$), San Martín school ($p \leq 0.005$) and Pringles school ($p \leq 0.000$). Conclusions: The results reveal that physical activity, BMI, gender and sun exposure are factors associated with anxiety and PD in students. These findings underscore the need for interventions to promote mental health and physical well-being in school settings.

A71
MICROSCALE ADAPTATION OF THE REFERENCE COLORIMETRIC TECHNIQUE FOR THE MEASUREMENT OF URINARY IODINE

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Iodine is an essential element for metabolic and endocrine human and animal physiology. Mendoza is considered an endemic area of goiter, due to the natural deficiency in iodine. For this reason, iodine must be supplemented in table salt to reach the proper nutritional requirements. The insufficient intake of this microelement generates metabolic disorders mainly in patients seeking pregnancy. Urinary iodine excretion is currently the most convenient laboratory marker of iodine deficiency. The WHO establishes a healthy level of iodine in urine >100 ug/L in adults. The reference methodologies used by the WHO are ICP-MS and the Sandell-Kolthoff reaction. Both methodologies have major disadvantages. The first method uses complex equipment that is not accessible general population. The second method involves laborious digestion and a large volume of reagents that are harmful to the environment. Therefore, the objective of this work, was to adapt the Sandell-Kolthoff colorimetric technique to a microscale, thereby reducing the volume of reagents and allowing a greater number of samples to be analyzed simultaneously. For this purpose, the Sandell-Kolthoff reaction was developed in a microplate using reference standards. Next, a prospective study was carried out on a cohort of 82 women aged 18 to 42 years, who attended the Instituto de Medicina Reproductiva of the Mendoza province. These patients were given a brief survey on iodine intake and finally a urine sample was requested. With this, the dietary habits and salt consumption of the patients could be known in order to relate them to the urinary iodine dosage performed by the microscale technique. Firstly, the microscale technique allowed us to reduce the volume of reagents by almost ten times, since 24 determinations can be carried out at the same time and with a correlation R=0.99. Using this methodology, we found that 24.3% of the population studied had suboptimal levels of iodine. The analysis of consumption showed that there is a significant correlation (p=0.019) between iodine and the amount of salt consumed by the patients. In turn, we observed that 34% of the patients do not consume fish, 12% do not consume dairy products, 7% are vegetarians and 5% do not consume eggs. Finally, we found that 16.7% of the patients consume salt with iodine levels below the limit established by law. This study allows us to conclude that the microscale technique reduces the volume of reagents harmful to the environment and speeds up the determinations, which makes it a suitable technique for population control. On the other hand, considering the results from patients, we arrive to the conclusion that greater monitoring of iodine consumption and access to information on its nutritional importance is necessary, especially for women of childbearing age seeking for pregnancy.

A72

STUDY OF THE SECONDARY STRUCTURES OF TOOTH ENAMEL IN RELATION TO ADHESIVE SYSTEMS USING A FATIGUE MACHINE FOR SAMPLE PREPARATION

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The research work we are working on is based on the analysis, evaluation and comparison of the different restoration biomaterials using state-of-the-art adhesives. In this same line, the objective was to describe the performance of a fatigue machine to obtain fragments of dental crowns that enable the study of the enamel microstructure and its interrelation with adhesive systems. Each sample was placed on the rupture testing machine, ensuring that it was properly aligned to apply the load evenly. An increasing load was applied in a controlled manner until each sample was broken. The speed of application of the load was constant for all samples. This technique also allowed us to analyse the cost-effectiveness of these adhesion systems in relation to the primary and secondary structures of the enamel under the Scanning Electron Microscope. Healthy and fresh teeth (upper premolars) extracted with orthodontic and/or periodontal indication were used, cavities were carved with rotary instruments of the same pattern, causing a cavity of 3 mm on an extension side, with the respective coronary fractures due to high-impact force. The samples were restored with biomaterials (composite) through state-of-the-art adhesives. Subsequently, the experimental units were cut with the slot/fracture technique, preparing them for immersion in the fatigue machine, then they were subjected to the action of a continuous pressure cycle force until fracture. The maximum load supported by each sample before the rupture of the tooth fragment was recorded. The description of the rupture tests in which 15 samples were used yielded results of detachment of the shear fragment with an average maximum force of 450 Newtons. This technique allowed us on the one hand to know the minimum force necessary for the fracture and on the other hand shows the work plan to be followed for the development of the research.

A73

DETECTION OF CERVICAL CYTOLOGICAL ALTERATIONS AND HUMAN PAPILLOMA VIRUS IN RELATION TO VAGINAL CONTENT UNBALANCE

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The vaginal tract is populated with various types of microorganisms, collectively called the vaginal microbiome (VB). Recent evidence suggests that VB may play a functional role in the acquisition and persistence of Human Papillomavirus (HPV), and subsequent development of cervical cancer. The aim of study was to evaluate cervical cytologic status, HPV presence and its association with basic vaginal states (BVS) in women attending gynecologic service. It was performed in a public primary care hospital in Juana Koslay (San Luis, Argentina) between July 2023 -2024; included 300 healthy women (18-70 years), sexually active and non-vaccinated for HPV. Exclusion criteria: patients who had received antibiotics or corticosteroids, pregnancy or sexual intercourse 48h prior to the study. After consent was obtained, a standardized questionnaire was collected. Three groups (G) were considered: G1 (18-24 years) n=86, G2 (25-50 years) n=189 and G3 (>50 years) n=25. The BVS were established according to the Balance of the Vaginal Content (BAVACO) methodology that evaluates microbiota and vaginal inflammatory reaction. Five BVS can be recognized: normal microbiota (I), normal microbiota plus inflammatory reaction (II), intermediate microbiota (III), bacterial vaginosis (IV), and non-specific microbial vaginitis (V). Endocervix and ectocervix samples for cytology and for HPV-DNA detection were collected. Cervical cytology abnormalities were reported by the Bethesda classification. The detection of high-risk HPV (hr-HPV) was performed by real time PCR (Cobas 4800). The prevalence of cervical intraepithelial lesions among our study participants was 11.7% (35/300), of which 85.7% (30/35) were low-grade squamous intraepithelial lesions (L-SIL). Five women (1.7%) were diagnosed with high-grade squamous intraepithelial lesion/cervical cancer (H-SIL/CC). The frequency of BVS of unbalance (III, IV and V) in L-SIL was: G1: 60% (3/5); G2: 76.2% (16/21) and G3: 100% (4/4). The frequency BVS of unbalance in H-SIL/CC was: G1: no cases; G2: 50% (2/4) and G3: 100% (1/1). The frequency of hr-HPV infection in those of >25 years was 29% (62/214). The frequency of basic vaginal states of unbalance in hr-HPV positive sample was: G2: 66.7% (36/54) and G3: 100% (8/8). In all groups, the most prevalent BVS in patients with HPV infection or squamous intra-epithelial lesions was the nonspecific vaginitis. A greater unbalance of VB was observed in women with abnormal cervical cytology or who were hr-HPV positive. Further research is needed to elucidate the complex interactions between host genetics, environmental factors, and microbial communities in order to establish a causal connection between the enriched bacteria and cervical carcinogenesis.

A74
PREVALENCE OF ANEMIA IN ELDERLY DIABETIC SUBJECTS ATTENDING A PRIMARY HEALTH CARE CENTER

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Anemia is a frequent clinical finding in patients with diabetes mellitus (DM), particularly in older adults. The pathogenesis of anemia in DM is multifactorial and is associated mainly with the presence of chronic inflammation, diabetic nephropathy and nutritional deficiencies. Although anemia in elderly people is an independent risk factor for increased morbidity and mortality, it often goes unrecognized and not treated. This study intended to determinate the prevalence of anemia and its related factors in people aged ≥65 with diagnosis of DM. The retrospective analysis included 321 patients (152 women and 169 men) attending in a public primary care hospital in Juana Koslay city (San Luis, Argentina) from July 2022 to July 2024. Participants were excluded from the study if they had received a blood transfusion recently, they were on treatment for nutritional causes of anaemia or has history of hereditary anemias. All the individuals who participated in the study provided informed written consent. Anemia was defined according to World Health Organization criteria as a hemoglobin (Hb) level <13 g/dL in men or <12 g/dL in women, and classified as mild, moderate, or severe. Glycated hemoglobin, blood glucose level, red blood cells count, hemoglobin (Hb) concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and serum creatinine of subjects were evaluated. Glomerular filtration rate (GFR) was estimated using the modified MDRD-4 formula or calculated by the measurement of creatinine clearance, as appropriate. Each patient provided an early morning spot urine sample to measurement urine albumin-creatinine-ratio (UACR). Presence of chronic kidney disease (CKD) is defined as persistent GFR <60 mL/min/1.73m^2 or albuminuria (UACR >30 mg/g). The mean age was 72.2±6.2 years, and 52.6% were men. The overall prevalence of anemia was 31.2% (95% CI: 26.1-36.0%), predominating in women (36.8% vs 26.0%; p< 0.01), among older people (44.2% in >75 years old group vs 26.4% in 65-75 years old group; p< 0.01) and in those with CKD, measured by a decreased GFR (42.0% vs 21.7%; p<0.01) but not by the presence of albuminuria. The majority of anemia (80%) was normocytic; anemia was macrocytic in 12% and microcytic in 8% of patients. Mild anemia was the most prevalent (57.0%); the others were moderate anemia in 35.0% and severe in 8%. Moderate anemia and severe anemia were more common in female patients as compared to male patients (46.4%. vs 20.5%; p<0.01) and (12.5% vs 2.3%), respectively. This study describes a high prevalence of anemia in older adults with DM, particularly in women and in the presence of CKD, highlighting the clinical importance of this coexistence. Therefore, early detection and proactive management of anemia in the elderly is justified to avoid its impact on patients' quality of life.

A75
ROLE OF OXIDATIVE STRESS ON POTENTIALLY CARCINOGENIC ORAL LESIONS: LOOKING FOR MARKERS OF MALIGNANCY

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Oral potentially malignant lesions (OPML) are defined as morphologically altered tissues in which cancer is more likely to develop. OPML can remain stable for long periods and the causes determining their transition to malignancy are unknown. It has been shown that increased oxidative stress increases the expression of γH2AX, a phosphorylated histone that appears in the DNA-breaks chain. The effect of oxidative stress on DNA along with the expression of some genes probably influences the via these lesions will follow. In patients with OPML, we aimed to determine the expression of γH2AX, the variations in oxidative stress, and the gene expression of RAD51, a DNA repair protein, a marker of poor prognosis in various types of cancer. In addition, the evolution of the lesions with medical treatments and the clinical responses were evaluated. The study included 10 patients (6 women/4 men) diagnosed with leucoplakia treated with tretinoin or imiquimod cream for six months. Before treatment, the expression of histone γH2AX was evaluated in patient biopsies by immunohistochemistry. Samples were taken from the lesions and the healthy area before the start, one month, and six months after treatment. Superoxide anion (SOA) generation and 8-Oxo-2'-deoxyguanosine (8-oxo-dG) expression were determined by flow cytometry. The expression of RAD51 mRNA was evaluated by real-time qPCR. In 3/7 biopsies of patients with OPML, an increase in γH2AX expression was observed before treatment. Local medical treatments demonstrated a clinical improvement in potentially malignant lesions of the oral mucosa. A significant decrease in the generation of SOA in the diseased area was found one month after treatment, and a significant reduction at six months after treatment (p=0.001). Only two patients showed a significant decrease in the expression of 8-oxo-dG (p < 0.05 and < 0.0001). 50% of the patients showed an increase in the expression of 8-oxo-dG, and 30% did not modify the expression of this

marker. No expression of RAD51 was observed in the patients studied after six months of treatment. This work demonstrates that oxidative stress is present in OPML and varies in response to the treatments applied. In our study, the increase in DNA adducts did not augment the malignancy of the lesion, as determined by RAD-51. It is important to find early and/or prognostic markers to adopt appropriate therapeutic behaviors to prevent oral cancer or treat it in its early stages.

A76
DEVELOPMENT OF NEW THERAPEUTIC STRATEGIES BASED ON NATURAL COMPOUNDS FOR *CHLAMYDIA TRACHOMATIS* TREATMENT

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Chlamydia trachomatis (Ct) is an obligate intracellular bacterium, responsible for the most common sexually transmitted infection in Argentina and worldwide, representing a serious public health problem. The bacteria mainly infect young women and cause significant acute morbidity and long-term sexual and reproductive health issues, such as chronic inflammation, preterm labour, spontaneous abortions, and infertility. Most Ct infections are asymptomatic, leading to delayed diagnosis and facilitating the spread of the pathogen. Since conventional antibiotic treatments fail to eliminate the infection and associated inflammation, it is crucial to explore, identify, and develop alternative therapies to prevent and eradicate the pathogen. In this study, we continue to analyse the antibacterial effect of Quercetin (Q) and the phenolic extract (PE) from olive pomace, both of which have strong antioxidant and anti-inflammatory properties in Ct-infected HeLa cells using a microscopy approach. Previous studies have shown that both compounds exhibited anti-chlamydial activity in infected HeLa cells but did not reduce Ct entry when added at the time of infection. The combination of Q and PE was not effective in reducing bacterial development and growth after 24 hours of infection. Since the main component of PE is hydroxytyrosol (HT), the anti-chlamydial activity of this compound was analysed exclusively. We observed that HT was able to inhibit the development and growth of Ct, as well as the number of infected HeLa cells. Altogether, these results suggest that Quercetin, the phenolic extract, and hydroxytyrosol, which is the main component of the phenolic extract, exert an anti-chlamydial effect on infected HeLa cells.

MICROBIOLOGY AND IMMUNOLOGY

A77
EFFECT OF *ZINNIA PERUVIANA* PLANT EXTRACTS ON THE GENERATION OF OXIDATIVE STRESS IN MICROBIAL CELLS

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The mechanisms by which compounds with antimicrobial activity inhibit the growth or cause the death of microorganisms depend on the interaction of the antimicrobial with its target of action. However, other metabolic events such as the generation of reactive oxygen species (ROS) could act as a cofactor of the biocidal effect. This study aimed to evaluate the influence of antimicrobial extracts from *Zinnia peruviana* on ROS production by NBT reduction in *Staphilococcus aureus*, *Staphilococcus epidermidis*, *Listeria. monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. The compounds tested were: ziniolide (10, 0.312, and 0.156 mg/ml), acetone root extract (10 and 0.156 mg/ml), acetone leaf extract (10 and 0.156 mg/ml), and acetone flower extract (10 and 0.156 mg/ml). Conventional antibiotics, ciprofloxacin (10 mg/ml) and gentamicin (10 mg/ml), were tested for comparison. The production of ROS was also determined by

chemiluminescence with luminol as a luminescence intermediate, using root extract and ziniolide at concentrations of 0.156 and 10 mg/ml. Treatment with bactericidal concentrations of extracts from *Z. peruviana* leaves, flowers, and roots induced intracellular ROS generation in Gram-positive and Gram-negative bacteria as well as yeasts. No significant changes were observed at bacteriostatic concentrations. Contrary to expectations, treatment with ziniolide, the major metabolite of root extract, did not significantly increase ROS levels, unlike the effect observed with the root extract itself. This suggests that said compound would not be involved in the intracellular increase in superoxide anion observed in the presence of the root extract at the same concentrations. The generation of ROS induced by ciprofloxacin and gentamicin was significantly lower than that observed in the presence of plant extracts. Chemiluminescence analysis detected increases in free radicals at microbiostatic concentrations of *Z. peruviana* root extract and ziniolide. Based on our results, the intracellular ROS increase could indicate a metabolic imbalance within the cells, potentially leading to microbial death.

A78
ANTIMICROBIAL ACTIVITY OF TRIAZOLES OBTAINED BY SYNTHESIS

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Triazole derivatives obtained through chemical synthesis are nitrogen-containing heterocyclic compounds of importance in the design of new drugs. These compounds have been shown to be effective against bacteria, fungi and viruses due to their ability to interfere with vital microbial processes. The structure of this chemical group allows easy modification, which facilitates the optimization of its antimicrobial activity and the reduction of side effects, positioning them as promising candidates for the development of new therapeutic agents.
The antimicrobial activity of four triazole-derived compounds: TZA1, TZC1, TZC5 and TZC4 was evaluated against strains of *Staphylococcus*, *Listeria* and *Candida albicans* by broth microdilution. TZC1, TZC4, and TZC5 inhibited the growth of *Staphylococcus* and *Listeria* species between 0.5 and 0.8 mg/ml. The 3 compounds presented an inhibition effect for *C. albicans* in a concentration range of 0.4 to 1 mg/ml, with TZC1 being the compound with the highest activity (0.415 mg/ml). The triazole TZA1 was not active at concentrations lower than 1 mg/ml. The mixtures of triazole compounds did not enhance the antimicrobial effect; however the combination of TZC5 with an extract derived from *L. divaricata* generated a synergistic effect, enhancing the growth inhibitory activity of the microbial strains tested. The generation of reactive oxygen species could enhance the antimicrobial activity of the triazoles tested. Using an NBT reduction assay, it was determined that the most active triazole (TZC5) generated an increase in the superoxide anion of 12.6% for *C. albicans*. The results encourage continued studies given the potential that these compounds offer as antimicrobial agents.

A79
ASSOCIATION BETWEEN SEGMENTED FILAMENTOUS BACTERIA (SFB) COLONIZATION AND THE USE OF ANTIBIOTICS IN THE GUT IMMUNE RESPONSE OF *TNFR1*$^{-/-}$ MICE

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Segmented filamentous bacteria (SFB) are potent microbial stimuli of the gut mucosal immune system, including IgA and IL-17 production. In the ileum of mice, SFB appear shortly after weaning, and then quickly decreases. Antibiotic use has been shown to create an imbalance in the intestinal microbiota, called dysbiosis, which induces inflammation that can lead to several chronic pathologies. TNF receptor 1-deficient (*TNFR1*$^{-/-}$) mice develop reactive arthritis (ReA) after oral infection with *Yersinia enterocolitica* (Ye) serotype O:3. This study aimed to evaluate the association between SFB colonization and antibiotic treatment on the intestinal immune response in TNFR1-deficient mice. First, we confirmed the presence of SFB in the mice in our laboratory. For this purpose, SFB determination was carried out by qPCR in fecal samples. In addition, SFB presence was analyzed by Gram staining in the ileum lavage of 21 to 35-day-old (weaning time) male C57BL/6 wild-type (WT) and *TNFR1*$^{-/-}$ mice. Then, we compared the number of IgA-producing cells in the intestinal lamina propria by immunofluorescence. Finally, lymphocytes, neutrophils, total macrophages and CX3CR1^{+} macrophages (cells that maintain gut homeostasis) were evaluated in mesenteric lymph nodes (MLN) by flow cytometry. qPCR results were positive for SFB in fecal DNA extracts. In addition, Gram staining showed high number of SFB in both WT and *TNFR1*$^{-/-}$ mice at the weaning

time. We observed higher number of IgA^+ cells in lamina propria of *TNFR1*$^{-/-}$ mice ($p<0.05$, compared with WT mice). Slight increase of $CX3CR1^+$ macrophages was detected in *TNFR1*$^{-/-}$ mice ($p<0.05$). After the treatment of the mice with antibiotic (vancomycin), macroscopic changes (inflammation) were observed in the ileocecal valve (ViC) of WT mice. In addition, we observed a decrease of IgA^+ cells in both groups of mice ($p<0.05$) and an increase of the difference between *TNFR1*$^{-/-}$ and WT mice ($p<0.0001$). Similar to WT mice, significant increases of neutrophils, macrophages, $CX3CR1^+$ macrophages, total T lymphocytes, $CD4^+$ T lymphocytes and $CD8^+$ T lymphocytes were detected in the MLN of antibiotic-treated 35-day-old *TNFR1*$^{-/-}$ mice compared with *TNFR1*$^{-/-}$ mice without antibiotic treatment ($p<0.01$). Our results demonstrate that both the lack of TNF receptor 1 and antibiotic treatment impact on the development of the intestinal immune response. More results are needed to understand the mechanism behind these effects.

A80
ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM *Neltuma flexuosa* SEED PODS

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Flour obtained from *Neltuma* spp. seed pods (*Neltuma alba*, *Neltuma flexuosa* and natural hybrids) has historically been used for human consumption. Flour is energy-dense food due to its high content of simple and soluble natural sugars, in addition is a source of high biological value proteins and micronutrients: iron, calcium, magnesium, manganese, zinc, copper, phosphorus and potassium, as well as vitamins A, B1, B2 and E and antioxidants such as anthocyanins and tannins. This food has particular nutritional properties due to fibres (lignins, galactomannans and pectins) that contribute to the presence of beneficial intestinal microbiota. In order to produce fermented food based on seed pods flour, pods from *N. flexuosa* were harvested in Balde de Escudero, San Luis. Cultures in MRS broth medium were performed from the samples for the purpose of detecting the presence of lactic acid bacteria (LAB) to be used as fermentation starters. Of the total of isolates achieved, fifteen were characterised as LAB. The isolates, identified as strains 2 and 11, were selected for their ability to coagulate milk proteins. This study aimed to determine the antimicrobial activity of strains 2 and 11 using the liquid medium method against pathogens or food contaminants: *Listeria monocytogenes*, *Candida albicans*, *Yersinia enterocolitica* and *Staphylococcus aureus*.Three consecutive cultures of LAB strain were executed every 24h in MRS medium in order to activate antimicrobial substances production. Cell-free supernatant (CFS) was obtained using sterile 0.22 µm syringe filters. Suspensions of 6×10^8 cells ml^{-1} (OD of 0.08-0.1 at 625 nm) of each microorganism antimicrobial activity indicator were prepared. Antimicrobial activity assays were performed by liquid medium technique in Tryptein Soy Broth (TSC), by addition of indicator microorganism suspension to CFS. Simultaneously, an indicator of growth control (blank) was prepared without CFS. Following incubation for 6 h at 35ºC, O.D. of sample (Am) and blank (A0) was measured at 700 nm and percentage of inhibition was calculated. The results obtained from tests of both BAL strains showed significant differences ($p<0.0001$), between the controls and those treated with CFS. A very important reduction in the growth of the pathogens was observed by treatment with CFS from strain 2 and CFS from strain 11. In the case of *S. aureus* and *Y. enterocolitica* the inhibition was superior to 90%. CFS from strain 2 inhibited *L. monocytogenes* growth by 53% and *C. albicans* growth by 42%, while when antimicrobial activity of CFS from strain 11 was tested, an inhibition of 60% was observed with both indicator microorganisms. As a conclusion of this work, we can consider strain 2 and strain 11 as valuable microorganisms with the capacity to ferment flour from *N. flexuosa* seed pods, additionally, have potential as inhibitors of some microorganisms that cause food spoilage and food poisoning.

A81

DETERMINATION OF METHICILLIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS* BY FLOW CYTOMETRY

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Staphylococcus aureus (Sa) is a Gram-positive bacterium that causes major public health problems due to the presence of methicillin-resistant strains (MRSA). The standardized method for determining antibiotic resistance in bacterial strains is the Kirby Bauer method, which requires 24 hours to obtain a result. The aim of this work was to optimize the determination of resistance to methicillin by flow cytometry (FC) in order to reduce the analysis time to 3 hours. For a validation of this method in a Gram-negative bacterium as well, we determined the resistance to Kanamycin (KAN) in *Yersinia enterocolitica* (Ye) YopP deficient (Ye*ΔyopP*) resistant to this antibiotic, using the sensitive strain Ye wild -type (YeWT) as a control. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Cefoxitin (FOX) were calculated for the two Sa reference strains: ATCC43300 (methicillin-resistant) and ATCC29213 (methicillin-sensitive). The MIC and MBC of KAN were also calculated for the YeWT and Ye*ΔyopP*. Moreover, a clinical isolated MRSA strain (Strain 11) was analyzed. For FC, bacterial suspensions of all strains were prepared in saline solution (SS) with a turbidity comparable to the standard 0.5 on the MacFarland scale. Then, 200 µl of a 1:20 dilution was inoculated in 2 ml of Mueller Hinton broth (MHB) with or without the respective antibiotics: FOX (8 ug/ml, for the Sa strains) or KAN (8.81 µg/ml for the Ye strains). Then, they were incubated for 3 hours at 37ºC. After that, the bacteria were centrifuged at 4000 rpm for 10 minutes, resuspended in 500 µl of SS and labeled with 5µl of Propidium Iodide (PI) to be analyzed by FC. The relations between the number of bacteria presents in MHB to the number of bacteria in MHB with the respective antibiotics (FOX for Sa; KAN for Ye) were evaluated. We found that these relations were higher in the sensitive strains ($p<0.05$) for both *S. aureus* and Ye compared to the corresponding resistant strains. We demonstrate that FC is a promising fast and quantitative tool to determine whether a bacterium is sensitive or resistant to a particular antibiotic. Since a rapid detection of antimicrobial resistance of bacteria that cause serious health problems, such as *S. aureus,* is crucial for early initiation of appropriate therapy, these results contribute to the hospital environment.

A82

YERSINIA ENTEROCOLITICA OUTER PROTEIN P (YopP) INDUCES APOPTOSIS AND MODULATES INOSITOL HEXAKISPHOSPHATE (IP6) LEVELS THROUGH FCγRIII: *IN VITRO* AND *IN SILICO* ANALYSIS

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The role of *Yersinia enterocolitica* Outer Protein P (YopP) in inducing apoptosis and the importance of inositol hexakisphosphate (IP6) in its stabilization are well established. However, there are results that reveal an extracellular function of YopP in macrophages modulation. It is known that IP6 levels in macrophages are regulated in response to Fcγ receptors (FcγRs) activation. This study aimed to investigate the role of extracellular YopP in the modulation of macrophages response through FcγRs. Murine peritoneal macrophages (Mφ) were infected with Ye wild-type (Ye wt) or YopP-deficient (Ye ΔyopP) and treated with human IgG (IgGh). We assessed apoptosis by flow cytometry, IP6 levels by colorimetric methods, and the interaction between IP6 and YopP through docking experiments. Additionally, we examined the interaction of YopP, FcγRIII, and IgGh using molecular models and confirmed the binding of YopP to IgGh through immunofluorescence (IF) and Western blot (WB). Our results showed an initial increase in IP6 levels ($p<0.05$) followed by a significant decline in Ye wt-infected Mφ at 45 min post-infection ($p<0.001$), correlating with increased apoptosis ($p<0,.05$). Interestingly, IgGh treatment counteracted YopP-induced apoptosis ($p<0.05$) and increased IP6 levels ($p<0.05$). *In silico* analysis suggests that YopP interacts with FcγRIII and IgGh with moderate and high affinity, respectively. Furthermore, we observed that IgGh has a higher affinity for FcγRIII than YopP. The Yops- IgGh interaction was confirmed by IF ($p<0.05$) and flow cytometry ($p<0.01$) but not by WB, indicating the importanceof the conformational structure in the binding. Our findings suggest that YopP may regulate both apoptosis and IP6 production through interaction with FcγRs. Furthermore, IgG could inhibit YopP-mediated effects by binding to FcγRIII or by directly interacting with YopP.

BIOCELL, Vol.49, Suppl. 3, 2025
ISSN 0327-9545
ISSN 1667-5746 (online version)

A83

ROLE OF MACROPHAGE N-GLYCANS IN INNATE IMMUNITY AGAINST *YERSINIA ENTEROCOLITICA*

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Recent studies suggest that macrophage surface glycans are crucial in immune evasion by pathogens. However, specific interactions between *Yersinia enterocolitica* (Ye) and macrophage glycans remain largely unexplored. We investigated the role of peritoneal murine macrophage surface N-glycans in Ye infections. In this regard, macrophages were obtained, purified, cultured in DMEM medium, and treated (Md) or untreated (M) with PNGase F (N-deglycosidase). Then, the effects of N-deglycosylation were evaluated *in vitro* on Ye-infected M and Md. Superoxide anion production and colony-forming units (CFU) were determined. Additionally, we assessed the *in vivo* effects of N-deglycosylation by performing adoptive transfers of Md into mice orally infected with Ye. CFU, nitrite levels, and inducible nitric oxide synthase (iNOS) expression were measured in Peyer's patch (PP) homogenates five days post-infection. Our results demonstrated that N-deglycosylation did not significantly alter Ye clearance after 1, 2, or 3 hours of infection. However, superoxide anion production increased after 1 hour of infection in M ($p<0.01$) but decreased in Md ($p<0.05$). Mice adoptively transferred with Md and infected with Ye exhibited no significant differences in weight or survival compared to controls. However, they displayed increased nitrite production ($p<0.01$), iNOS expression ($p<0.01$), and enhanced pathogen clearance ($p<0.05$). Our findings highlight a novel therapeutic approach targeting N-glycans to modulate innate immunity against *Y. enterocolitica*.

A84

PREVALENCE OF *bla*CTX-M GENES IN ESBL-PRODUCING ENTEROBACTERIA: IMPACT ON ANTIMICROBIAL RESISTANCE IN MENDOZA (2021-2022)

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Extended-Spectrum Beta-Lactamases (ESBL) include cefotaximases (CTX-M), enzymes that hydrolyze cefotaxime, penicillins, extended-spectrum cephalosporins, and monobactams. Predominantly found among *Enterobacteriaceae*, these enzymes have spread worldwide. The concerning rapid mobilization of *bla*CTX-M genes warrants careful monitoring, as strains carrying these genes often exhibit resistance to other antibiotic families, such as aminoglycosides and fluoroquinolones. This forces carbapenems to become the next line of treatment. Epidemiological surveillance of infections in both hospitalized and outpatient settings must be implemented to develop effective antimicrobial treatment strategies. Our aim was to analyze the prevalence of CTX-M genes associated with the ESBL phenotype using PCR technology. We collected 78 ESBL-producing bacteria isolates from Mendoza's Central Hospital between 2021 and 2022. *Escherichia coli* and *Klebsiella pneumoniae* were the most prevalent *Enterobacteriaceae* among the samples carrying this class of gene. The most common genes during this period were *bla*CTXM-1 and *bla*CTXM-2, with a significant increase in the prevalence of *bla*CTXM-1 ($p<0.001$). Within the samples, 54 patients carried the *bla*CTX-M1 group, while 34 patients carried the *bla*CTX-M2 group. In hospitalized patients, *bla*CTX-M1 group was more prevalent, with 35 cases compared to 19 cases in *bla*CTX-M2 group. In outpatients, *bla*CTX-M1 group had a slightly higher prevalence, with 19 cases compared to 15 cases for *bla*CTX-M2 group. Of the 54 samples in the *bla*CTX-M1 group, 46 were integron-positive and 8 were integron-negative. While in the *bla*CTX-M2 group, of the 34 samples, 26 were integron-positive and 8 were integron-negative. Of the 78 total samples, 23 contained both *bla*CTX-M1 and *bla*CTX-M2 genes. Within the individual groups, 31 samples were positive for the *bla*CTX-M1 gene only, while 11 samples carried the *bla*CTX-M2 gene only. Additionally, preliminary results examining *bla*CTXM-8, *bla*CTXM-9, and *bla*CTXM-25 in these samples showed an increased prevalence of *bla*CTXM-8. This research provides valuable insights into the limited information available in our region regarding the evolution of *bla*CTX-M genes associated with the ESBL phenotype in Mendoza's hospital from 2021 to 2022. Furthermore, it supports the development of appropriate antimicrobial treatments through rapid PCR-based diagnostics, improving health outcomes.

A85
EFFECT OF GLUCOSE SUPPLEMENTATION ON ORAL *YERSINIA ENTEROCOLITICA* O:8 INFECTION

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Glucose is a key metabolite for immune cells and plays a critical role in bacterial infection outcomes. We previously demonstrated that Yersinia enterocolitica (Ye) serotype O:8 induces sustained hypoglycemia in mice during the early stages of infection. Whether this metabolic change confers a protective advantage to the host remains unknown. Here, we examined the impact of glucose supplementation on Ye infection. C57BL/6 mice were orally infected with Ye O:8 and received glucose supplementation twice daily by gavage. Infected and non-infected mice without supplementation served as controls. Body weight, food intake, and serum interleukin (IL)-17 levels were evaluated throughout the infection. Spleen bacterial load was assessed on day 3 post-infection (pi). Both groups showed significant weight loss ($p<0.05$) and reduced food intake ($p<0.001$) compared to non-infected controls. Weight loss was significantly greater in the glucose-supplemented group on day 2 pi ($p<0.01$) compared to non-supplemented mice. Spleen bacterial load was significantly higher in the glucose-supplemented group compared to the non-supplemented group ($p<0.01$). Ye infection induced significant serum IL-17 levels on day 3 ($p<0.05$) in the glucose-supplemented group compared to the non-supplemented group. Our results suggest that the hypoglycemic state induced during Ye infection plays a protective role for the host, while glucose supplementation appears detrimental. These findings suggest that IL-17 has a metabolism-modulating role in addition to its mucosal antibacterial function. Thus, systemic metabolism is coordinated to support the protection against bacterial infection. Further studies are needed to elucidate the long-term effects of glucose homeostasis changes and their relation to infection outcomes.

A86
DETECTION OF SEROTONYLATION AFTER *YERSINIA ENTEROCOLITICA* INFECTION IN A MURINE MODEL OF REACTIVE ARTHRITIS

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Reactive arthritis (ReA) is a spondyloarthritis characterized by sterile inflammation of joints after a mucous membrane infection. ReA-prone mice with a TNF receptor 1 knockout (TNFR1 KO) background have higher basal serum serotonin (5-HT) levels and develop arthritis following Yersinia enterocolitica (Ye) infection. Moreover, reducing 5-HT levels with selective 5-HT reuptake inhibitors (SSRI) and inhibiting transglutaminase 2 (TG2), responsible for protein serotonylation, attenuates ReA severity in the murine model, suggesting serotonylation's involvement in the disease. We have observed that this post-translational modification can be monitored with streptavidin-PE-labeled samples by flow cytometry (FC), using a previously prepared alkyne-functionalized 5-HT derivative coupled to biotin via click chemistry. Accordingly, we propose to evaluate cellular serotonylation levels 5 days post-Ye infection and again 21 days later, when mice have developed ReA. Samples were obtained from TNFR1 KO mice with and without Ye O:3 infection. On day 21 post-infection (pi), mice were euthanized, and FC was performed on the regional lymph node (RLN) and spleen to detect neutrophil infiltrates. Additionally, splenocytes from TNFR1 KO mice with and without ReA were assessed at 5 and 21 dpi for serotonylation using click reactions and FC. Statistical analyses were conducted using one-way ANOVA and Student t-tests. An increased number of serotonylated splenocytes was observed 5 days post-Ye infection ($p=0.0132$), and this difference was maintained 21 days post-infection (7 days after ReA onset). An increase in mean fluorescence intensity (MFI, $p=0.008$) for serotonylated splenocytes was noted 21 days post-infection but not at 5 dpi. We observed a higher number of neutrophils in the spleen 5 days after infection ($p=0.0082$ Additionally, an increased number of neutrophil infiltrates was observed in the RLN 21 days post-infection ($p=0.0074$). Thus, higher serotonylation levels and immune cell infiltration were observed in ReA compared to healthy mice. Our results suggest that serotonylation may be triggered by Ye infection and that, over time, elevated serotonylation levels could sustain inflammatory conditions in TNFR1 KO mice, contributing to ReA development.

BIOCELL, Vol.49, Suppl. 3, 2025
ISSN 0327-9545
ISSN 1667-5746 (online version)

A87
COMBINED EFFECT OF THYMOL AND SURFACTANTS AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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Methicillin-resistant *Staphylococcus aureus* (MRSA), is considered one of the most important nosocomial pathogens, capable of producing significant mortality and morbidity in hospitalized patients (HA-MRSA). In addition, MRSA isolated in community settings (CA-MRSA), is capable of triggering serious infections. In general, these strains present multi-resistance to different antimicrobial. Thymol (2-isopropyl-5-methylphenol), an important component of *Thymus vulgaris* (Thyme) and *Origanum vulgare* (Orégano) essential oils, presents a variety of pharmacological properties including antimicrobial activity. The objective of this work was to evaluate the combined effect of thymol and sodium dodecyl sulfate (SDS) on the growth of methicillin-resistant *Staphylococcus aureus* ATCC 43300. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by the micro-well dilution assay method in trypticase soy broth (TSB), supplemented with 0.01% (w/v) of 2,3,5-triphenyltetrazolium chloride as a visual indicator of bacterial growth. Stock solutions of thymol, SDS (10,000 µg/mL) and a 1:1 mixture from both solutions were prepared. Serial twofold dilutions were carried out in concentrations ranging from 2,500 to 2.44 µg/mL. In each well of the 96-well plates, 100 µL of TSB with the indicator, 100 µL of serial dilutions and 5 µL of inoculum obtained from a suspension of 10^6 CFU/mL, were dispensed. Control wells containing only TSB, strains and compounds were also included. The plates were incubated at 37°C for 24 h under static conditions. MIC was defined as the lowest concentration of each compound, at which no visible growth was observed after incubation (no red colour). MBC was determined by subculturing on trypticase soy agar (TSA), using the last three wells that exhibited no visible bacterial growth. Experiments were performed in duplicate and replicated at least twice. The fractional inhibitory concentration (FIC) and the potentiation radius (PR) were calculated to determine the probable synergistic activity. The MIC/MBC values obtained for thymol, SDS and the combination of both compounds against *S. aureus* ATCC 43300, were the following (µg/mL): 625/1,250, 9.76/2,500, 4.88/4.88. The FICthymol and PR_{thymol} were respectively 0.007, 142 (synergism, FIC $\leq$ 0.5 and PR $\geq$ 2). The thymol-SDS combination showed an important synergistic effect, both inhibitory and bactericidal, with respect to thymol tested individually. The anionic surfactant SDS turned out to be an excellent growth inhibitor but with limited bactericidal activity. The observed effect could be due to the formation of spontaneous emulsions, which increase the permeability and interaction of thymol in the bacterial cell. There is currently great interest in searching for new compounds, or combinations between them, against MRSA and other multidrug-resistant pathogens of clinical importance. This research is an interesting contribution in this regard; however, future studies are required for further development.

A88
STUDY OF EXOPOLYSACCHARIDE PRODUCTION FROM THE YEAST *CRYPTOCOCCUS LAURENTII*

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Microbial exopolysaccharides (EPS) have a wide application in many industrial sectors due to their emulsifying, antioxidant and antimicrobial properties, etc. Particularly, yeasts have been described as potential producers of EPS, however, there are few reports on the production of EPS by *Cryptococcus laurentii*. The aim of this study was to produce and isolate exopolysaccharides from *Cryptococcus laurentii*, in order to study the relationship between EPS production, microbial biomass and glucose consumption at different cultivation times. *Cryptococcus laurentii* BNM 0525, from the laboratory of industrial microbiology (UNSL), was cultivated in Erlenmeyer with 50 ml of culture medium (g/L): glucose: 20; $(NH_4)_2SO_4$: 1.65; KH_2PO_4: 0.86; $MgS0_4.7H_20$: 0.3; NaCl: 0.1; $CaCl_2.2H_20$: 0.01; $MnSO_4$: 0.01; yeast extract: 0.05; and pH 5. The cultures were incubated on a shaker (120 rpm) at 25 °C for 120 h. To isolate the EPS, the cultures obtained were centrifuged at 10,000 G-force/RCF for 20 min to separate the biomass from the supernatant. The supernatants were reduced to 10 % of the volume and two volumes of 96 % ethanol were added at 4 °C for 24 h to precipitate the EPS. The precipitate was filtered, dried and weighed, expressing the results in g/L. Also, microbial biomass was determined, using dry weight, and substrate (glucose), using Enzymatic Glicemia Kit (Wiener Lab.), obtaining g/L at different cultivation times (24 h, 48 h, and 120 h). Then, the yields were calculated: Yx/s (biomass produced with respect to substrate consumed), Yp/s (product obtained with respect to substrate consumed) and Yp/x (product obtained with respect to biomass produced). The assays were carried out

in duplicate. Considering that the culture medium was designed for the production of EPS and to obtain 5 g/L of biomass, using the nitrogen source as the limiting substrate, the results obtained were (g/L): at 24 h (biomass: 2.93; EPS: 1.88; glucose: 7.98), at 48 h (biomass: 3.32; EPS: 2.72; glucose: 0), and at 120 h (biomass: 4.01; EPS: 3.72; glucose: 0). The yields were: at 24 h (Yx/s: 0.24; Yp/s: 0.15; Yp/x: 0.64), at 48 h (Yx/s: 0.17; Yp/s: 0.14; Yp/x: 0.82) and at 120 h (Yx/s: 0.20; Yp/s: 0.19; Yp/x: 0.93). In conclusion, a direct relationship was observed between EPS production and yeast growth, presenting the highest values at 120 h. At this time there were no differences between the values of Yx/s and Yp/s, whereas Yp/x was close to unity. On the other hand, glucose consumption at 24 h was 60 % and after 48 h it was maximum. This demonstrated that glucose is a suitable carbon source for yeast, with rapid assimilation but slow metabolization. Although the results obtained were encouraging, the production of EPS will continue to be studied to optimize the process and physicochemical characterizations of the EPS will be carried out.

A89

PRODUCTION OF LETTUCE SEEDLINGS (*LACTUCA SATIVA L.*) IN A GREENHOUSE WITH APPLICATION OF *KOSAKONIA RADICINCITANS* AS A GROWTH PROMOTER

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The use of agricultural bioinputs with plant growth-promoting microorganisms (PGPM) contribute to unconventional and sustainable production models. *Kosakonia radicincitans* is a microorganism with advantageous capabilities in the field of agroecology, with previously published reports. Objective: To study the effects of applying *K. radicincitans* as a PGPM on lettuce seed germination and development. The bacteria were cultivated in a minimal medium based on industrial waste, the biomass of the microorganism was obtained by centrifugation and washing with sterile distilled water twice. The application consisted of a simple formulation in sterile distilled water from a suspension of the microorganism equivalent to 6 x 10^8 CFU/mL. Lettuce seeds (*Lactuca sativa* L.) of the White Boston variety were used for seedling production. The seeds were placed in plastic sowing trays (in duplicate), with basic solid substrate (30% soil, 50% composted horse manure, 20% sawdust). The seeds were placed in plastic trays for sowing (in duplicate), with basic solid substrate (soil 30%, composted horse manure 50%, sawdust 20%). The trays were treated so that each well received 1 mL of (a) sprayed *K. radicincitans* suspension (6 x 10^8 CFU/mL) and (b) sprayed sterile distilled water (control). Both trays were maintained under the same greenhouse conditions with controlled temperature and supplemental irrigation. After 7 days, the germination percentage was quantified using the formula: % germination = (N°. of germinated seeds/N°. of total seeds) x 100. After 15 days, 10 seedlings were randomly removed from the trays (a) and (b) to quantify vegetative growth as total plant length (cm) and chlorophyll A and B content (ug/g) by spectrophotometry at 646.6 and 663.6 nm. The germination percentage for seeds treated with *K. radicincitans* (a) was significantly higher on average by 11% compared to the control (b) ($p \leq 0.05$). Chlorophyll A and B content varied significantly with microorganism treatment (a) with chlorophyll A concentration equal to 61.7 ug/g and B of 81.3 ug/g; for control (b) chlorophyll A and B was 47. 2 ug/g and 17.0 ug/g respectively ($p \leq 0.05$). Regarding the total length of the seedlings, treatment (a) presented a significant difference of 20% with respect to control (b) ($p \leq 0.05$). The results indicated that the application of *K. radicincitans* at the time of sowing has a positive impact on both germination and vegetative development of the seedlings.

A90

STUDY OF SIDEROPHORE PRODUCTION: INFLUENCE OF CULTURE TIME IN TWO STRAINS OF MICROORGANISMS

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Many microorganisms have developed specialized mechanisms for acquiring iron from the environment, particularly from soil, where soluble iron is scarce. Among these mechanisms, the production of siderophores stands out. Siderophores are low-molecular-weight compounds with a high affinity for iron, acting as chelators that facilitate iron uptake by binding it tightly and transporting it into microbial cells. These compounds can be classified based on their chemical structure into

catecholates, hydroxamates, and mixed siderophores. Siderophores have a wide range of potential applications. In agriculture, they promote plant growth and can help control pathogenic species. In medicine and pharmaceuticals, they are being explored for drug delivery systems and diagnostic purposes. This study aimed to compare the siderophore production of two bacterial strains, ChM90c and ChM120y, isolated from vineyard soils. The focus was on evaluating the influence of culture time on siderophore concentration, specifically catecholate production, at 24, 48, and 120 hours. These strains were selected for their siderophore-producing capabilities. Strain ChM90c was chosen for its moderate siderophore production and additional beneficial traits, such as phosphorus solubilization, nitrogen fixation, and indole acetic acid production, making it a promising candidate for sustainable agriculture. Meanwhile, strain ChM120y was selected for its high siderophore output, along with its ability to fix nitrogen and produce indole acetic acid. For the experiment, 10 mL of each strain was inoculated into a low-iron-content medium (Vitamin Free) with a final volume of 100 mL, using a 24-hour inoculum. Samples were taken at 24, 48, and 120 hours, centrifuged at 13,000 rpm for 10 minutes, and the supernatants were separated. Both the inoculum and the cultures were grown in a culture chamber at 24-25 °C and agitated at 120 rpm. Catecholate production was measured using Arnow's assay, with absorbance taken at 510 nm. The results were converted to mg/L using a calibration curve based on enterobactin, a known catechol produced by enterobacteria. Each assay was performed in sextuplicate. The statistical analysis, using repeated measures ANOVA in R with a significance level of 0.05, followed by Tukey and Bonferroni post-hoc tests, revealed significant differences in catecholate production over time. However, no significant differences were observed between the 48- and 120-hour time points, suggesting that siderophore production plateaus after 48 hours. This finding indicates that the assay can be effectively concluded at 48 hours, thus optimizing the protocol by reducing culture times and associated costs.

A91
EVALUATION OF NETTLE AND HORSTAIL EXTRACT COMPOSITION AS A NATURAL ANTIFUNGAL FERTILIZER

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The increasing global demand for food has resulted in an increased use of agrochemical, causing environmental pollution and risks to consumer health. As a sustainable alternative, natural fertilizers from nettle (*Urtica dioica*) and horsetail (*Equisetum arvense*) extracts provide essential nutrients and antifungal properties, reducing the reliance on harmful chemicals. The aim of the present study was to evaluate the influence of the extraction methods on the chemical and microbiological characteristics of an aqueous extract of mixture of nettle and horsetail in a 1:1 ratio. The extracts were prepared by the decoction and maceration methods in a 1:30 ratio of dry plant material and miliQ water, respectively. Then, the chemical characterization of the extracts was carried out through the quantification of the macro and micronutrients by means of atomic absorption spectroscopy. In addition, phenols and flavonoids content as well as antioxidant activity of the extracts were quantified by Folin-Ciocalteu, aluminum chloride and DPPH free radical methods, respectively. Finally, the antifungal activity of the extracts was evaluated in vitro against *Aspergillus niger, Alternaria* sp*, Botrytis cinerea, Fusarium* spp*, Rhizopus* spp *and Penicillium expansum* strains by measuring the inhibition halo. The results showed that the nutrients concentration in the decocted extract, expressed as ppm, were lower than that in macerated extract, depicting values of: 37.04 for K, 0.049 for Cu, 6.012 for Mg, 0.52 for Mn, 0.261 for Zn and 0.192 for Fe for decoction, and 77.96 for K, 0.212 for Cu, 8.297 for Mg, 0.912 for Mn, 0.186 for Zn, 0.345 for Fe for maceration. The total phenolic content was also lower in the decocted extract, with 7.471 mg gallic acid/100 mL sample, compared to 8.674 mg gallic acid/100 mL sample in the macerated extract. While, the total flavonoid content was similar in both extracts, showing 1.023 mg quercetin and 5.605 mg rutin/100 mL in decocted extract, and 1.164 mg quercetin and 5.571 mg rutin/100 mL in the macerated extract. Surprisingly, the decocted extract showed higher antioxidant activity (79%) than the macerated extract (63%). Finally, none of the extracts exhibited inhibitory activity against the phytopathogenic fungi tested in vitro. In conclusion, both extracts show promise as natural fertilizers. The maceration method is particularly effective in extracting essential nutrients for plant growth and is easily appable to horticultural producers. These nutrients enhance plant resistance to abiotic stress and disease. However, the extracts showed limited antifungal activity against the phytopathogens tested.

A92
IMMUNE RESPONSE IN HENS AGAINST *LARREA DIVARICATA* PROTEINS

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The proteins of *Larrea divaricate* (jarilla) are immunogenic in mice. It was observed that the antibodies generated by these proteins not only recognized the plant's proteins but also showed cross-reactivity with proteins from *Pseudomonas aeruginosa*. This finding suggests the potential of jarilla proteins for use in the development of vaccines or immunological therapies aimed at combating bacterial infections. Immunoglobulin Y (IgY) has proven to be valuable in medicine and research, as its use promotes animal welfare by avoiding invasive methods and allows for significant antibody production compared to other animal models. Bird eggs contain nutrients and antibodies that provide passive immunity to offspring, making this approach attractive for investigating immune responses in hens against specific proteins. The objective of the study was to obtain and identify anti-jarilla IgY using proteins from a partially purified aqueous extract (JPCE) by 10 kDa Millipore concentrators (JP10), as the immunogen. Three Hy Line Brown hens (Cabaña Avícola Feller, Entre Ríos, Argentina) were inoculated with an emulsion (0.5 ml) of antigen (75 or 150 µg) and adjuvant (Montanide™ ISA 71 VG) (1:1) via intramuscular injection into the pectoral muscle. Injections were repeated at 15, 30, 45 and 60 days. Eggs were collected before and during immunizations up to 15 days after the last dose. To evaluate the immune response, the level of IgY antigen-specific was determined using qualitative ELISA. JP10 was used as the sensitizing antigen. Then it was incubated with PBS-diluted egg yolk. Rabbit anti-IgY conjugated with peroxidase was used as conjugate (Rabbit Anti-Chicken IgY H&L (HRP) Abcam). Eggs from free-range hens and eggs from pre-immunized hens were used as negative controls. The results indicate that the immunization plan in hens tested in this assay could be improved to obtain specific antibodies against jarilla, serving as a starting point to optimize the levels of IgY antibodies obtained; and subsequently confirm, in this new animal model, the antigenic similarity between *L. divaricata* proteins and *P. aeruginosa*. This approach not only highlights the advantages of immunizing hens but also its potential biotechnological applications in preventive and therapeutic treatments for infectious diseases.

A93
ANTIMICROBIAL EFFECT OF *BACILLUS VELEZENSIS* SL-6 AGAINST *BURKHOLDERIA CENOCEPACIA*

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Burkholderia cenocepacia is a member of the *Burkholderia cepacia* complex (Bcc), a group of more than 22 gram-negative non-fermentative bacilli that cause opportunistic infections in both cystic fibrosis and the immunocompromised patients. Since 2022, ANMAT has included Bcc as objectionable microorganisms in water-based pharmaceutical products. Additionally, *B. cenocepacia* is naturally resistant to different classes of antibiotics used in clinical practice, and remains viable in several biocide formulations. *Bacillus velezensis* SL-6 secretes many antimicrobial metabolites that control bacteria, yeasts and phytopathogenic fungi. The present study describes the antimicrobial and antibiofilm activities of *B. velezensis* SL-6 against *B. cenocepacia* ATCC 25608. The cell-free supernatant (CFS) of *B. velezensis* SL-6 was obtained by batch culture, centrifugation and filtration. Bioactive metabolite extraction was subjected to acid precipitation, followed by ten-fold methanolic concentration (ME10X). The antimicrobial activities of CFS, the organic and aqueous fractions were determined using the agar well diffusion method. The diameters of the inhibition zones were recorded, and the activity was quantified in arbitrary units per milliliter (AU/ml) as the reciprocal of the highest two-fold dilution factor, yielding a clear inhibition zone. To allow biofilm formation, *B. cenocepacia* was grown in Petri dish with Mueller-Hinton Broth, and a glass surface was added for adherence. After 24 h of incubation at 37 °C, the evolution of planktonic and biofilm cultures was evaluated by viable count and Gram staining. Thin-layer chromatography of ME10X was performed using chloroform-methanol-water (65:25:4, v/v/v) as the mobile phase. The bands were visualized under ultraviolet light (UV 254 nm) and developed using water and H_2SO_4 5%. The bioactivity was detected by contact bioautography. The undiluted CFS and ME10X (20 µl) showed an inhibition zone of 16.24±0.73 mm and 25.93±1.66 mm, respectively. The extraction system concentrated active metabolites from the supernatant, increasing the activity from 200 to 1600 AU/ml in the methanolic extract (ME10X), with no activity in the acidic aqueous phase. The effects of CFS (200 AU/ml) on planktonic and biofilm cultures were evaluated. Significant decrease in the count of viable cells in treated compared to the untreated *B. cenocepacia*

cultures were observed. Different morphologies in the treated (coccoid forms and short bacilli) and untreated (long bacilli) cultures were visualized using light microscopy. The chromatogram showed several bands under UV light, recovering a single bioactive fraction (Rf 0.3-0.5) positive with H_2SO_4 reagent, and the reaction with water was inconsistent. These preliminary chromatographic results could indicate that the bioactivity was coincident with the Rf values referenced for iturin family compounds. However, subsequent studies should be conducted to purify and confirm the chemical structure responsible for the anti-*Burkhloderia* activity, and to examine its potential as an alternative approach to counteract the problem of resistance.

A94
NEW BONE FORMATION IN REACTIVE ARTHRITIS INDUCED IN TNFR1-DEFICIENT MICE

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Reactive arthritis (ReA) is an aseptic synovitis caused by gastrointestinal or genitourinary infection. ReA belongs to Spondyloarthritis (SpA), a second most frequent chronic inflammatory arthritis characterized by inflammation and progressive new bone formation. The SpA pathogenesis is not completely known. Previously, we have demonstrated that TNFR1-deficient (*TNFR$^{-/-}$)* mice develop chronic ReA after intragastric infection with *Yersinia enterocolitica* (Ye) O:3. To assess similarity to human SpA we performed a detailed characterization of this murine model focusing on the articular pathology. Male and female C57BL/6 wild-type (WT) and C57BL/6 *TNFR$^{-/-}$* mice were infected with Ye O:3. We monitored survival, body weight and arthritis development, which was assessed by clinical score, radiographic images and grip strength. Joints were histologically evaluated using hematoxylin-eosin, Masson Trichrome (TM) or safranin- O/fast green staining. As serum alkaline phosphatase (ALP) is considered a potential biomarker of bone formation, we assessed ALP in sera of WT and *TNFR$^{-/-}$* mice. We found that *TNFR$^{-/-}$* mice reduced significantly survival rate (50-67%) and body weight on day 14 post-infection (pi) ($p<0.001$, compared with WT mice), with no gender bias. In contrast with female, male *TNFR$^{-/-}$* mice developed chronic ReA. From day 21 to 63 pi, male *TNFR$^{-/-}$* mice exhibited higher clinical score in all limbs ($p<0.001$, compared with male WT mice), which resulted in loss of grip strength ($p<0.05$). On days 21 and 56, changes at posterior calcaneus and knee joints as well as thickened metatarsal bones and phalanges were detected in radiographic images of hind limbs of male *TNFR$^{-/-}$* mice. Histopathological examination showed synovitis, increased number of mesenchymal cells, fibrosis and foci of hypertrophic chondrocytes. TM and safranin -O staining showed chondrocytes within osteophyte proliferation area, suggesting endochondral new bone formation. In line, higher serum ALP levels were detected in male *TNFR$^{-/-}$* compared with WT mice ($p<0.01$ and $p<0.05$ on days 28 and 56, respectively). Our findings support a central role of stromal cells in SpA immunopathology.

A95
EFFECT OF DEHYDROLEUCODINE AND XANTHATIN ON MAST CELL ACTIVATION INDUCED BY *HELICOBACTER PYLORI*

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Gastric *Helicobacter pylori* infection induces a strong immune response with infiltration of inflammatory cells into the mucosa, leading to chronic gastritis, peptic ulcer, MALT lymphoma, and gastric cancer. Among these cells, the mast cells (MC) interact with pathogens, recruit leukocytes, release histamine which induces vasodilation and edema, and play a fundamental role in the initiation of the inflammatory reaction. In previous work we purified active compounds from regional plants that could modulate the inflammation caused by *H. pylori*. The objective of this work was to study the effect of Dehydroleukodine (DhL, isolated from *Artemisia douglasiana* Besser) and Xanthatin (Xt, isolated from *Xanthium*

cavanillesii Schouw) on the *H. pylori*-induced MC activation. *H. pylori* strains NCTC 11638 and HP661 were cultured on Mueller Hinton agar for 48 hours in microaerophilic atmosphere. The cells were resuspended, and then sonicated. Peritoneal MCs were obtained from adult Wistar rats and incubated with 48/80 compound (10 µg/ml, MC activator), 100 µg/ml sonicates, 250 µg/ml DhL or Xt and 2 µM sodium cromoglycate or ketotifen (MC stabilizers). β-hexosaminidase released from mast cells was measured as a marker of activation. The sonicates of both strains potentiated the release of β-hexosaminidase induced by 48/80 (NCTC $p=0.0015$ and HP661 $p=0.0043$). DhL and Xt, in individual or combined assays, inhibited the release of β-hexosaminidase and MC activation compared to reference stabilizers ($p<0.0001$). In conclusion, we have shown that the natural compounds DhL and Xt inhibit the activation of MC induced by *H. pylori* and could reduce gastric inflammation in the initial stage of infection. Future *in vivo* studies are necessary to demonstrate the anti-inflammatory effect of these compounds and their potential use as a complementary treatment of infection.

A96
VALIDATION OF OPTIMIZATION AND EVALUATION OF CULTURE MEDIUM COST FOR THE PRODUCTION OF BIOSURFACTANT FROM *BACILLUS ATROPHAEUS*

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Surfactants are amphipathic molecules with surface-active properties, widely used in various industrial processes. However, most synthetic surfactants are toxic and only partially biodegradable. In response to the need for sustainable solutions, biosurfactants (BS), produced by microorganisms such as Bacillus, have gained interest for being biodegradable and less polluting. However, their high production cost remains a challenge for their mass use, so optimizing their production is key to improving their efficiency and reducing costs. In previous work it was determined that the determining factors were the carbon source (sucrose) and the nitrogen source (peptone). The objective of this work was to experimentally validate the optimized culture medium and evaluate the efficacy of the biosurfactant, antifungal activity and to analyze the medium cost. The microorganism used in this work was *Bacillus atrophaeus*. Optimization of the composition of the medium was carried out with central composite design (CCD) by Design-Expert version 11. The response the bio emulsifying activity of the cell-free supernatant (CFS) of *B. atrophaeus*, was measured by the stable emulsion index at 24 h (IE%) with kerosene in relation 1:1 and the agar diffusion method to measure the antifungal activity against phytopathogenic fungi. The results indicated that the optimal concentrations were (gL^{-1}): peptone 8.0, sucrose 0.5 and NaCl 9.0. CFS obtained from the optimized medium was compared with the reference medium, standard nutrient-peptone (gL^{-1}): peptone 18.0, glucose 1.0 and NaCl 6.0. With both CFS obtained, *B. atrophaeus*, showed a maximum IE% at 72h. Regarding antifungal activity, *Fusarium graminearum* and *Botrytis cinerea* were sensitive to the optimized CFS, while *Penicillium roqueforti* showed lower sensitivity in relation to the CFS obtained from the reference medium. The optimization of the medium allowed to reduce costs, approximately 50% with respect to the cost of the reference medium, without compromising the efficacy of the IE%, thus improving its efficiency, underlining the relevance of chemometric tools to improve biotechnological production. However, the optimized medium was not favorable for antifungal activity.

A97
PRODUCTION AND CYTOTOXICITY ANALYSIS OF EXOPOLYSACCHARIDE (EPS) PRODUCED BY *BACILLUS ATROPHAEUS*

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Exopolysaccharides (EPS) produced by bacteria have demonstrated significant potential for the bioremediation of heavy metals due to their retention capabilities. Previous studies evaluated the production of EPS in the presence of Cu(II) and Cr(VI), revealing high efficiency in Cu(II) retention. In this study, the objective is to assess the Cu(II) retention capacity of EPS by increasing its concentration and investigating the effects of structural changes induced by the presence of Cr(VI) in the growth medium. Additionally, the environmental cytotoxicity of EPS is evaluated using *Artemia salina*.
EPS were produced in *Standard Nutrient* (SN) medium (g/L: peptone 15.0, yest extract 3.0, glucosa 1.0 and NaCl 6.0). Six flasks containing 60 mL of Cu(II) solution at a concentration of 70 ppm were prepared, with dialysis tubes containing 5 mL of EPS at concentrations of 67 mg/L and 560 mg/L. The EPS were produced in standard media with and without Cr(VI)

present. A control flask containing distilled water was also included. The samples were agitated for 24 hours, and Cu(II) concentration was measured using atomic absorption spectroscopy.
For the cytotoxicity assay, *Artemia salina* eggs were incubated in saltwater at 27°C for 48 hours until the nauplii hatched. The nauplii were exposed to SN and EPS at different concentrations. $K_2Cr_2O_7$ was used as a positive control, while saltwater served as the negative control in ELISA plate.
Results showed greater Cu(II) retention by EPS at lower concentrations and in the absence of metals in the growth medium. The nauplii were unaffected by the presence of EPS or SN, indicating low environmental toxicity.
In conclusion, the structural changes induced by Cr(VI) and the increased EPS concentration, reduced Cu(II) retention. However, the results suggest that EPS could be applied in environmental contexts due to its low cytotoxicity

A98
ANTIBACTERIAL ACTIVITY OF NORBELLADINE ANALOGUE ALKALOIDS

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The Amaryllidaceae family of plants has attracted considerable attention due to the diverse biological activities that exhibits its alkaloid constituents, and, their effects on human health have been widely evaluated. However, there is a limited number of studies on the bioactivity of its precursors. The protoalkaloid 4'-O-methylnorbelladine is the common precursor to all Amaryllidaceae alkaloids such as haemanthamine, lycorine and galantamine. The aim of this work was to evaluate the antibacterial effect of two unnatural analogues of 4'-O-methylnorbelladine, against strains of methicillin-resistant Staphylococcus aureus ATCC 43300, Listeria monocytogenes CLIP 74904 and Pseudomonas aeruginosa ATCC 27853. Compounds N-(p-hydroxyphenylethyil)-N-(p-fluor) benzylamine (1) and N-(p-hydroxyphenylethyl)-N-(p-hydroxy) benzylamine (2), were, synthesized through a condensation reaction between commercial aldehydes 4-fluorobenzaldehyde or 4-hydroxybenzaldehyde, and tyramine. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by the micro-well dilution assay method in trypticase soy broth (TSB) supplemented with 0.01% (w/v) of 2,3,5-triphenyltetrazolium chloride as a visual indicator of bacterial growth. Suspensions of 106 CFU/mL were prepared for each strain. Compounds 1 and 2 were dissolved in water, and serial twofold dilutions were prepared in concentrations ranging from 5.000 to 2.44 μg/mL. Each well of the 96-well plates were dispensed with 100 μL of TSB with the indicator, 100 μL of serial dilutions of compounds, and 5 μL of the inoculum. Control wells of TSB, strains and compounds were also included. The plates were incubated at 37°C for 24 h under static conditions. MIC was defined as the lowest concentration of the compounds, at which no visible growth was observed after incubation (no red color). MBC was determined by subculturing on trypticase soy agar (TSA), using the last three wells that exhibited no visible bacterial growth. Experiments were performed in duplicate and replicated at least twice. MIC and MBC (MIC/MBC) values for compounds 1 and 2 against the evaluated microorganisms were as follows respectively (μg/mL): S. aureus 1250/2500, 2500/5000; L. monocytogenes 625/1250, 1250/1250. However, no antimicrobial activity was detected against P. aeruginosa, (MIC-MBC > 5000 μg/mL for both compounds). In this work, we observed that norbelladine analogues tested were more active against Gram-positive bacteria than Gram-negative ones, particularly P. aeruginosa. However, S. aureus exhibited less susceptibility compared to L. monocytogenes. The lack of activity against P. aeruginosa may be attributed to factors associated with its outer membrane structure. The antibacterial activity of these new synthetic compounds is of increasing interest for the treatment of infections caused by pathogenic bacteria resistant to multiple drugs. Nevertheless, additional studies are necessary to assess their toxicity and determine appropriate routes of administration for these products.

BIOCELL, Vol.49, Suppl. 3, 2025
ISSN 0327-9545
ISSN 1667-5746 (online version)

ECOLOGY, BEHAVIOR AND BIODIVERSITY

A99
THE FIRE'S IMPACT ON THE FLORISTIC COMPOSITION IN THE SOUTHERN END OF "SIERRA DE SAN LUIS"

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Humanity has lived with fire and used it to free up land for cultivation, cooking, heat, energy production, etc. But while it has served its purpose as a means of subsistence, its effects alter the health and survival of ecosystems and their biodiversity. Intentional fires have greatly outgrown natural ones, modifying the fire patterns established over millennia in various parts of the world. These cause variations in plant diversity, affecting especially the structure of ecosystems and their floristic composition, even favoring anatomical and physiological alterations in some plant species. This work aims to know the state of plant communities (floristic composition, physiognomy, morpho-anatomical characters, and metabolite contents). The phytosociology surveys were carried out along transects in the southern end of the Sierra de San Luis including foothills, slopes, peaks, and intermontane valleys comparing affected and fire-free areas. On the local foothills of the Sierra de San Luis there was a bi- or Tri stratified forest with predominance of "algarrobos" (*Neltuma* spp.) and "quebracho blanco" (*Aspidosperma quebracho-blanco* Schltdl.). Deforestation and recurrent fires due mainly to the expansion of urban areas have caused severe changes, drastically reducing vegetation cover in areas very sensitive to erosion. The forest has been replaced by thorny shrubs with predominance of *Senegalia gilliesii* (Steud.) Seigler & Ebinger and *Vachellia caven* (Molina) Molina, that resprouting from the base and then upper branching post-fire , as well as several species of cacti (*Gymnocalycium* sp., *Opuntia sulphurea* Gill. ex Salm-Dyck, *Trichocereus candicans* (Gill. ex-Salm-Dyck) Britt. & Rose) and at the same time there was a large increase in the population of *Hyaloseris cinerea* (Griseb.) Griseb. var. *cinerea*, *Aloysia gratissima* (Gill. ex Hook.) Tronc. var. *gratissima* and *Austroflourensia thurifera* (Molina) J.C. Ospina & S.E. Freire. On the slopes which were strongly affected by the fire, the forest has almost disappeared, severely affecting *Aspidosperma quebracho-blanco* as well as the bromeliad *Dyckia longipetala* Baker. There was a high mortality rate of specimens, loss of their vitality and severe regress of their populations. From the morphoanatomical point of view, the most affected taxon was *H. cinerea* var. *cinerea*, with a conspicuous fasciation and vegetative proliferation. Towards the apex, the fasciate branches were very leafy, and the lateral ramifications were oriented in a distinct way to the ribs of the fasciation. *Digitaria californica* (Benth.) Henrard presented a vigorous, not uniform regrowth due to the carbonization of some of its rhizomes. It has been detected that some taxa exposed to fire have had an increase in secondary metabolites with hyperproduction of tannic substances. All these parameters would provide evidence for the design of an integrated management plan of forest fires in the region under study. Proyecto 2-1023 SECyT-UNSL. [3]PICT 2021-CAT-II-00105 ANPCyT

A100
PRELIMINARY STUDIES TOWARDS THE HERBACEOUS AND SEMI-WOODY SPECIES DOMESTICATION WITH ORNAMENTAL POTENTIAL FROM THE COMECHINGONES AREA, NORTHWEST OF SAN LUIS PROVINCE, ARGENTINA

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The forest mountain ecosystem located between the valley and the Comechingones mountain ranges offers, as a non-timber resource, numerous species with potential ornamental, aromatic, and medicinal uses. This set of species could be integrated into biodiverse gardens, which would enable their conservation (reservoir of genetic variability). And also fulfilling the same functions as in natural ecosystems (biological interactions and ecological niche). To expand knowledge about the domestication of native herbaceous and semi-woody species to be used in the composition of ecosystemic gardens, a study was carried out on the in situ characteristics and ex situ behavior of 10 climbing species *(Amphilophyum cynanchoides, Anredera cordifolia, Araujia brachystephana, Aristolochia argentina, Clematis denticulata, Dolichandra cynanchoides, Ipomea purpurea, Janusia guaranitica, Mandevilla pentlandiana, Passiflora caerulea)* and 10 shrub species *(Abutilon grandifolium, Buddleja cordobensis, Lippia junelliana, Austroeupatorium inulifolium, Vernonanthura nudifora, Collaea argentina, Aloysia gratissima, Lippia turbinata, Salvia cuspidata, Erithrostemun gilliesii*). The study area included a forest, stream ravines, slopes of the "Sierra de los Comechingones", and also urban and peri-urban areas in Merlo City and neighboring towns. In the different study areas, data were taken over a year, on growth habit, regrowth, flowering and fruiting, on 2-3 geopositioned plants (non-probabilistic sampling; 1 km transect). Samples of green material were taken to the herbarium and observed under a magnifying glass in the laboratory. Species identification was made based on a

comparison with existing catalogs and herbariums. Fruits and/or clean seeds were saved and germination tests were carried out in the field, obtaining seedlings of twelve of the twenty species studied. The plants obtained were placed to observe their growth and development in the Ecosystem Garden, on the Barranca Colorada campus (FTU), in Merlo, San Luis. Faced with the increasingly pressing problems of climate change and the loss of wild environments and ecological processes in habitats, the value of the vegetation native to each place is highlighted. Native species have a better adaptation to extreme conditions (winter droughts and extreme temperatures in both winter and summer), provide ecosystem services (habitat and food for animal species) and also have ornamental value (foliage, flowering, fruiting and coverage). The information generated in this study will make it possible to plan the management of these species, in a domestication process for their use in biodiverse gardens, as a comprehensive strategy for the conservation and sustainable use of the native vegetation of this area of San Luis province.

A101
INTERSPECIFIC RELATIONSHIPS OBSERVED IN *ARAUJIA BRACHYSTEPHANA* (GRISEB.) FONTELLA & GOYDER, "TASI", (APOCINACEAE), IN THE DRY CHACO ECOREGION, SAN LUIS PROVINCE, ARGENTINA

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In the Dry Chaco and Espinal ecoregions, there are native species with ornamental potential that could enhance ecosystemic gardens. When developing these types of gardens, it is essential to consider interspecific interactions that support the establishment of biodiverse and sustainable ecosystems. This study aimed to record and identify plant-insect interactions involving *Araujia brachystephana* (Griseb.) Fontella & Goyder, a native climbing species with ornamental value, in the northwest area of San Luis within the Dry Chaco ecoregion. This climbing plant, commonly known as "tasi," is widely distributed across South America, including Brazil, Bolivia, Paraguay, Uruguay, and Argentina. Its ornamental potential stems from its dense, greyish-green foliage, which provides attractive contrast for fences, as well as the distinctive size and shape of its fruits. A preliminary survey of native forest species was conducted in two locations—one in a rural area (a private nature reserve) and the other in an urban area (FTU Campus)—to observe potential interspecific relationships between insects and the plant. A total of five samples were collected. In the first location, samples were taken from mature tasi fruits containing insects in the larval stage, which were then incubated until the insects reached adulthood. In the second location, samples of leaves and stems with insects at various stages of development were collected. Finally, all insect samples were taken to the laboratory for identification. The findings revealed that the larvae colonizing the fruits were legless, with a curculioniform type. Upon completing their life cycle, they were taxonomically identified using keys within the order Coleoptera, family Curculionidae, genus *Rhyssomatus*. The second species sampled included both nymph and adult stages and was identified within the order Hemiptera (suborder Homoptera), family Aphididae, as *Aphis nerii* Boyer de Fonscolombe, a typical aphid associated with plants in the family Apocynaceae. In conclusion, from an ecological perspective, the selection of species for ecosystemic gardens also involves the selection of their natural guests. Additionally, these local studies provide valuable insights for biodiversity management and conservation, suggesting that further investigation into new native plant species with ornamental potential may be beneficial.

A102
NATIVE PLANTS AS STABILISER AGENTS FOR LEAD-CONTAMINATED ENVIRONMENTS

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Pollutants can cause irreversible changes in the environment and controlling their dispersion is essential to avoid further damage. In particular, heavy metal (HM) contamination causes changes in the organisms that inhabit these areas. The study of these communities can be valuable in understanding how they survive in such environments. The objective of this work was to analyze HM accumulation and mycorrhizal associations in the dominant plant species present in lead (Pb) contaminated sites. The study area is located in Bouwer, Córdoba, where a battery recycling plant contaminated the surrounding sites with Pb. Seven sites with different Pb soil contents were selected; site I: 15µg.g^{-1}, site II: 341µg.g^{-1}, site

III: 374µg.g^{-1}, site IV: 248µg.g^{-1}, site V: 979µg.g^{-1}, site VI: 22660µg.g^{-1}, site VII: 17198µg.g^{-1}. In each site, 5 individuals of the most abundant plant were collected. The concentration of Pb in the shoot and root was determined by the total reflection of X-ray fluorescence. The accumulation (BCF: bioconcentration factor shoot/soil) and the translocation capacity of Pb (TF: translocation factor shoot/root) were calculated. Mycorrhizal associations were determined and quantified under the microscope. *Bidens pilosa*, *Jarava aff. ichu*, *Solanum argentinum* and *Zinnia peruviana* accumulated Pb in the root. Metal translocation capacity was low, and differences were observed between plants and sites. Arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) were registered in the roots of all study plants. The proportion of fungal colonization varied between the host plants and the sites. In general, *B. pilosa* and *Z. peruviana* presented the highest AM colonization and lowest DSE colonization while in *S. argentinum* and *J. ichu* the proportion of fungal colonization was similar. According to the plant species, a positive relationship between Pb soil, Pb roots and fungal colonization was detected. These plant/AMF/DSE associations would allow the development of strategies to survive in these environments, including immobilization of the metal in the root system. Considering these strategies in the stabilization of lead-contaminated soils could be essential for the advance of environmental bioremediation practices.

A103

LIQUID FORMULATIONS BASED ON SOIL MICROORGANISMS WITH POTENTIALITIES AS BIOFERTILIZERS

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The ecological damage caused by chemical fertilizers has sparked interest in the search for sustainable alternatives. Microorganisms isolated from soil can be used as microbial inoculants or biofertilizers. The objective of this work was to study the conservation of soil microorganisms with biofertilizer potential in liquid formulations, with the aim of being applied as a bioinput. From 20 pre-selected strains of microorganisms isolated from rhizospheric soil of *Vitis vinifera* plantations (Mendoza) with positive tests for biofertilizers (phosphate solubilization, nitrogen fixation, indoleacetic acid production and siderophore production) and deposited in the Industrial Microbiology Laboratory (N.U.S.L), strains ChM90c and ChM114c were selected to prepare the formulations. The selection consisted of development in 2 culture media: YGM and medium formulated with glycerol and dried yeast (waste from the brewing industry) and two positive biofertilizer tests. To prepare the liquid formulations, a standardized suspension of the microorganism was placed with different preservation media: A) Sterile Physiological Solution (sodium chloride 0.85%), B) Lactose 10%, C) Glycerol 20%, D) Medium 1 (Sucrose 2 g, Glycerol 2 g, $(NH_4)_2SO_4$ 2g, KOH 0.5g, K_2HPO_4 0.2g, $MnSO_4.H_2O$ 0.05g, $CuSO_4.5H_2O$ 0.1g, $ZnSO_4.7H_20$ 0.01g, $MgSO_4.7H_2O$ 0.01g, Malic acid 0.05 g, $CaCl_2$ 0.01 g, Distilled water 1000 mL). Finally, they were placed in sterilized vials (10 mL) and stored at 4 °C and 25 °C for one month. Viable cells were counted (Plate counting technique) at time zero and 30 days. The results expressed in CFU/mL were transformed to Log CFU/mL and the % reduction in viability = (Log CFU/mL at time zero - Log CFU/mL at time 30 days)/ Log CFU/mL at time zero x 100, and % survival = 100 - % reduction in viability, were calculated. Data were analyzed using InfoStat statistical software. Initial counts were 9.11 and 9.82 Log CFU/mL. The ChM90c formulations presented a 92% survival in conservation medium A with no significant differences in conservation temperature, whereas medium B and C were favored at low temperatures (89% survival and 81% respectively, versus 80% and 77%). In the formulations with ChM114c, 100% survival was obtained in Medium 1 without significant differences in the temperatures, for the C formulation at 4°C it presented 95% survival, but at 25°C it was reduced to 58%. Regarding formulation A, there were no differences in temperatures, with a survival rate close to 89%. It was concluded that the strains in formulations A and D presented the highest counts and in some formulations the low temperature favored viability, the ChM90c and ChM114c strains were able to be preserved for 30 days with a survival rate greater than 80%, except for the medium with glycerol at 25 °C, with a survival rate of 76% and 58% for ChM90c and ChM114c respectively. The conservation of formulations will be studied for a longer period of time. Solid formulations will also be studied at the same conservation temperatures.

BIOCELL, Vol.49, Suppl. 3, 2025
ISSN 0327-9545
ISSN 1667-5746 (online version)

A104
ESSENTIAL OILS OF *BACCHARIS SALICIFOLIA* RUIZ & PAVON, (ASTERACEAE) AS OVICIDAL FOR *TRIATOMA INFESTANS* KLUG.

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The use of natural insecticides is a much sought-after option by society, as it is an accessible and low-cost control alternative for the community; therefore, the use of botanical insecticides is an alternative for control in areas infested by triatomines. Likewise, insecticides of plant origin have the advantage of being biodegradable and immediately available. For this reason and considering previous data, isolated oils of *Baccharis salicifolia* Ruiz & Pavon, (Asteraceae) were selected and their ovicidal activity and contact toxicity were determined. The plant material was collected in two regions of the province of San Luis in spring and summer. The essential oils obtained by hydrodistillation were analysed by GC-MS techniques and the following main components α-cubebene, Germacrene D, Germacrene B, Santolina triene, α-Thujene, 6-Camphene, Sabinene, Limonene, Ocimene and ɣ-Terpinene were determined. Additionally, 1H NMR and 13C NMR techniques were used to obtain a general profile of them. Ovicidal and fumigant activity was studied. Ovicidal activity was determined by spraying batches of 10 eggs with 2 µl of each oil in different dilutions, recording egg hatching up to 60 days. The study of the fumigant activity was performed with 20 nymphs, which were placed in plastic containers, a piece of suspended filter paper was placed on the lids of each container and a determined volume of the oils was applied, and mortality was recorded after 24 hours. Each test was performed in triplicate using control batches with acetone, deltamethrin and a blank test. The ovicidal activity was effective for all concentrations with a hatching percentage of 60% in concentrations of 25% and in concentrations of 50% there is a difference according to the locality, 50% hatching in plants collected in Cruz de Piedra and less than 40% in plants of Las Barrancas, 35 Km from the city, south of San Luis., while the controls (blank, acetone and deltamethrin) were 90.00%, 86.60% and 73.3%, respectively. The fumigant activity exhibited a high percentage of mortality at the 50 % concentration (50 µl of essential oil and 50 µl of the diluent). In some cases only a 30% mortality at 24 hs. In the experiments carried out with this essential oil as a fumigant, the activity was null or scarce. It is likely that since they are highly volatile, their efficacy could decay within hours. The results confirm the potential of this plant and encourage its better use. It is of great importance to explore and understand the processes in more depth, and to encourage further research to find an appropriate use for this plant species in the field of Chagas disease vector control. *Triatomine infested* areas, because several plant species, which possess recognized insecticidal activity, grow easily or are native to these geographical areas.

A105
EFFECT OF CROP ROTATION ON THE ABUNDANCE AND ARBUSCULAR MYCORRHIZAL FUNGI COMPOSITION IN AN AGROECOSYSTEM

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Soil microorganisms include diverse communities that interact differently with plant species. These communities are essential to agroecosystems, influencing soil fertility, crop productivity and stress tolerance. Crop rotation is an agricultural practice that can benefit soil microbiota by successive cultivation of different plant species in the same plot. Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs and may benefit from rotations that minimize bare soil periods. The objective of this study was to assess AMF abundance and richness in soils with a history of continuous monoculture and three crop rotations. The crop rotation treatments were arranged in randomized complete blocks with three replicates, consisting of soybean-soybean (S-S), rye-soybean (R-S), vetch+rye-soybean (V+R-S), and corn-soybean-wheat/soybean (C-S-W/S). Composite soil samples were collected at a depth of 10 cm in each treatment for AMF spores extraction and analysis of soil chemical properties, including pH, electrical conductivity, total organic carbon, nitrate, phosphorus, and sulfate-sulfur. AMF spores were extracted from 50 g of fresh soil using the decantation wet sieving method followed by sucrose gradient centrifugation. Spore abundance and richness were determined using an optical microscope. For taxonomic identification, spores were compared to reference isolates described by the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM). The spore abundance ranged from 59 to 333. The highest total

spore abundance was recorded in S-S treatment, showing a significant difference compared to R-S and C-S-W/S. A total of 10 species was identified, with no significant variation in species richness across rotations. However, a distinct distribution patterns were observed among genera. In S-S, *Funneliformis* was the dominant genus, followed by *Claroideglomus*, whereas in R-S, V+R-S, and C-S-W/S, *Rhizophagus* and *Acaulospora* were more prevalent. Across all treatments, over 50% of spores belonged to the Glomeraceae, represented by 8 species. The significant differences observed in spore abundance and dominance of certain genera in monoculture compared to crop rotation suggest that agricultural practices have an effect on AMF communities, and as a consequence, their interactions with cultivated plants.

A106
EVOLUTION OF STRUCTURAL CHARACTERISTICS OF A *THINOPYRUM PONTICUM* PASTURE, DURING EIGHT YEARS OF RESTORATION PROCESS AFTER PROLONGED OVERGRAZING

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Tall wheatgrass (*Thinopyrum ponticum*) is a temperate perennial grass, which although it does not provide abundant autumn production in San Luis, it expresses its maximum production at the end of winter, as a rapid response to the occurrence of rainfall. It is characterized by its adaptation to soils with certain salinity and even sodium content, with fluctuating saline water tables and periodic flooding. Although this pasture cannot be considered a resource to be systematically implanted in the semiarid region, it has characteristics that make it of outstanding productive and ecological interest for incorporation in marginal environments, where it finds its "niche" in the semiarid region of the province. The purpose of this analysis is to compare the evolution of the number of plants and their size from basal area (cm^2) and aerial cover of a tall wheatgrass pasture in the eighth year of restoration from a prolonged condition of livestock overutilization. The information accumulated in the monitoring of a pasture seeded in 1996 in a slightly saline lowland of the campus of the FICA UNSL (at the north of Villa Mercedes, SL) was analyzed. This pasture had a decade of excessive and continuous livestock overgrazing (2006-2016), which practically generated its disappearance, but by removing livestock its evolution was monitored during 2017, 2019, 2023 and 2024, compared to 1997. Density was counted, while basal area was estimated from the average major and minor diameter of individual plants and cover by visual estimation. Regarding the plant stand, they differed significantly at the different years, observing that at the beginning of the pasture restoration, the density was lower than that of the implantation, which increased until 2023 as the emergence of new individuals was recorded, and in '24 it decreased notably while signs of degradation and replacement at the level of clumps (subdivision) were observed, with a very low presence of new individuals. Contrary, the basal area of individual clumps shows a tendency to increase in the period evaluated, although the decrease is significant towards 2024 ($p<0.01$). Also, the aerial cover of the pasture duplicated between the 1st and 3rd year (2019) and is currently above 80%. This data provide evidence that the wheatgrass pasture started a vigorous restoration process since reseeding, with values similar to those of the initial seeding 20 years before. In the 6th year, the pasture was almost stabilized, while in the 8th year it showed signs of natural renewal of clumps. Understanding the evolution of the different structural characteristics of a pasture allows to generate strategies to monitoring its restoration after unfavorable management practices.

A107
SCORPIONS AND SOLIFUGES FROM THE VICINITY OF SIERRA DEL GIGANTE, SAN LUIS, ARGENTINA

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Sierra del Gigante is part of the Natural Protected Areas System of the Province of San Luis (Provincial Law IX-0309-2004). It stands out due to its remarkable geological and paleontological features and is of great interest as it lies in an ecotone between the Arid Chaco and Monte de Llanuras y Mesetas ecoregions. Arachnids remain one of the least understood taxonomic groups, with many species still undescribed, undetected, or poorly understood in terms of their distribution. The order Solifugae is particularly unique within the class Arachnida and also one of the least studied. Although they range from southern Canada to Patagonia, little is known about the diversity of solifuges coexisting in specific areas. Conversely, the scorpion fauna of Argentina is better documented, though many regions still require further exploration. No prior studies of

solifuges have been recorded in San Luis province, and existing scorpion data in national museum databases come from sporadic collections. As such, creating local collections is crucial, both to inventory the region's biodiversity and to track potential biodiversity loss in specific habitats. This study aims to address this knowledge gap by conducting an inventory of scorpions and solifuges in the vicinity of the Sierra del Gigante. Samples were collected in late spring 2018 and late autumn 2019. Six sampling sites were selected, and at each site, 12 pitfall traps were placed along three 100-meter linear transects. Collected specimens were recorded, then classified into families, genera, or species using available taxonomic keys. A digital image database was created, featuring photographs of somatic traits that aid in distinguishing between families and genera. A total of 58 arachnids were captured—29 solifuges and 29 scorpions. The solifuges were represented by two of the three families found in Argentina: Ammotrechidae and Mummuciidae, with the latter being more abundant. The presence of *Oltacola cf. gomezi* was identified. Among the Scorpiones, the study recorded one of the two families found in Argentina: Bothriuridae, the most diverse and widely distributed scorpion family in the country. Three of its six genera were identified in this study: *Bothriurus*, *Timogenes*, and *Brachistosternus*, with *Timogenes* being the most abundant. The predominant species were *T. elegans* and *Brachistosternus ferrugineus*. This inventory provides the first data on solifuges and scorpions in the vicinity of the Sierra del Gigante. It contributes to the understanding of the region's biodiversity and offers a foundation for future research.

A108

ISOLATION OF NATIVE EDAPHIC CYANOBACTERIA FROM SOILS IN SAN LUIS PROVINCE, ARGENTINA CENTRAL-EAST REGION

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Cyanobacteria are Gram-negative, photoautotrophic prokaryotes found in diverse environmental habitats. Considering the challenges related to agroecosystems and the environment, cyanobacteria possess unique characteristics, including their ubiquity, short generation time, and ability to fix atmospheric N_2, making them potential biotechnological resources in various areas. This study aimed to isolate native cyanobacteria from soil samples collected from fields in Las Isletas, a rural area of General Pedernera Department, San Luis province, Argentina. Specifically, four composite soil samples were taken from different zones of the "Las Margaritas" establishment, stored in nylon bags, and processed. From the collected material, 10 g of homogenized dry soil were placed in Petri dishes with 20 ml of selective Watanabe medium for cyanobacterial growth. Primary cultures were incubated at 27°C with a 12:12 photoperiod for 60 days. Some colonies showing suspected cyanobacterial growth were selected for aseptic subculture on semi-solid medium (1.5% agar) containing Watanabe and BG-11 media, and were grown at 27°C with a 12:12 photoperiod for 15 days or until colony growth is observed on the culture plate. Taxonomic identification of the obtained colonies was performed using an Olympus BX 50 optical microscope through micro and macroscopic observations of bacterial structures. Differences in selectivity for cyanobacteria and cellular morphology were observed among the used media. Predominantly, *Nostoc* sp., *Cylindrospermum* sp., and *Phormidium* sp. colonies were isolated. The obtained native cyanobacterial isolates serve as a source for future assays as bioactive microorganisms with biotechnological potential applicable to soil fertility, crop production, and environmental quality.

A109

FIRE INTENSITY AND ITS EFFECT ON CYANOBACTERIA PRESENT IN BIOLOGICAL SOIL CRUSTS (BSC) IN THREE MOUNTAIN SITES IN THE CENTRAL REGION OF ARGENTINA

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Soil cyanobacteria present in BSC are affected by forest fires. This condition depends on the thermal intensity reached. The objective of this research was identifying the resistance of soil cyanobacteria in order to use them to recover soils affected by forest fires is crucial in the central mountain region of Argentina, where these occur year after year. Soil losses due to runoff caused by rainfall following the occurrence of fires are of great importance since the soils are shallow, with little development and with significant slopes. The effect of different thermal intensities on the Cyanobacteria present in the CBS of soils in three different mountainous regions was compared: Quebracho Ladeado (QL), La Calera (LC) and Los Lobos

(LL). The samples taken were subjected to 5 thermal intensities controlled in the laboratory (100°-200°-300°- 400° and 500°C) for a period of 15 minutes. They were sown in triplicate in specific culture medium for cyanobacteria and a weekly percentage count was performed under an optical microscope at 40X magnification, for 9 consecutive weeks. Cyanobacteria were differentiated into Nitrogen-fixing (F) and Non-fixing (NF). At the end of the 63-day period from the start of the test, it was observed that at 100°C, QL presents 30% NF and 56% F; LC 22% NF and 63% F and LL 21% NF and 55% F. At 200°C the results showed for QL 0% NF and 71% F; LC 16% NF and 75% F and LL 41% NF and 27% F. At 300°C, QL presented 0% NF and 85% F; LC 12% NF and 75% F and LL 35% NF and 40% F. At 400°C QL presented 0% NF and 99% F; LC 10% NF and 87% F and LL 14% NF and 51% F. Finally, at 500°C QL the results showed 0% NF and 100% F; LC 3% NF and 97% F and LL 21% NF and 63% F. These results demonstrate that the effect of the different thermal intensities is greater on the NF species, and that at intensities higher than 300°C they tend to disappear, except in the samples taken in the town of Los Lobos. For species belonging to the F group, however, trend shows that as the intensity of burning increases, they are present in greater proportion, which indicates a priori their greater resistance to severe fire impacts. Given the evident degradation of the soil system at high temperatures, its recovery through the use of Nitrogen-Fixing Cyanobacteria would be the best proposal, while in mild to medium severity events, the use of a consortium of Cyanobacteria containing NF and F species could be a better alternative.

A110
NEW RECORD OF *TITYUS* (SCORPIONES; BUTHIDAE) FOR THE PROVINCE OF SAN LUIS

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The genus *Tityus* is the most representative and diverse of the Buthidae family. Its importance lies in the toxicity of the poison for humans. The presence of seven species has been recorded in Argentine territory, of which only four are potentially lethal: *T. bahiensis, T. carrilloi*, *T. confluens* and *T. serrulatus*. *T. carrilloi* is the species of greatest toxicological importance in the country, with several fatal accidents recorded; *T. confluens* is the second species with toxicological relevance with several cases of scorpionism recorded. These species are considered synanthropic, since they can live in urban environments and have been favored by human activity, and as a result they have expanded their distribution area, settling in most cities in central and northern Argentina. Although the province of San Luis is located in the central region of the country, information about scorpions is very scarce. The only reports on this group are from accidents reported by government institutions such as the National Ministry of Health and the epidemiology area of San Luis province, as well as from some informal online reports. Considering the rise in scorpion poisoning cases in recent years, sampling was planned in key locations within the departments of Ayacucho and Junín. The sampling took place during the summer months of November and December 2023 and January 2024.At each site, scorpion specimens were collected, which were fixed and preserved in 70% alcohol to later be identified and classified in the laboratory. The presence of *Tityus* was recorded for the first time in the towns of Candelaria, Quines, Lujan and Lafinur. The examined specimens were deposited in the collection of the Zoology chair, at the Universidad de Los Comechingones, waiting to be classified at species level by the specialist taxonomist. The discovery of *Tityus* specimens in the major urban centers in the northern region of the province presents a public health issue that requires comprehensive management to reduce scorpionism cases.

A111

DESIGN OF A SOLID MEDIUM FOR ISOLATING FUNGAL PATHOGENS FROM TUBERS OF THE JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS*)

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Jerusalem artichoke (*Helianthus tuberosus*) is characterized by its high fiber content in the form of inulin. This fructooligosaccharide is classified as a prebiotic since it reaches the colon without degradation and serves as food for beneficial intestinal microbiota. During its cultivation period, Jerusalem artichoke is attacked by many pathogens, some of which can affect the integrity of the tubers and render them unfit for fresh consumption or planting. The objective of this study was to evaluate the formulation of a solid medium for the isolation of fungi in fresh Jerusalem artichoke tubers. Tubers of the white variety were cultivated in Villa Mercedes (San Luis, Argentina) and harvested in June 2024. They were stored at 4°C until processing. Fungal isolation was performed in the laboratory using Jerusalem artichoke tubers and potato tubers (*Solanum tuberosum*) as controls. The tubers were divided into three sections (basal, middle, and apical), from which slices of 10-12 mm thickness were extracted using a knife. These were washed with tap water and submerged in 3% sodium hypochlorite for 2 minutes. The slices were rinsed three times with sterile distilled water and drained on filter paper. Subsequently, they were divided into 1 cm^3 sections and submerged in 70% alcohol for 1 minute. Then, they were placed in Petri dishes with sterile agar potato dextrose (APD) medium, and the variation of the previous medium using Jerusalem artichoke instead of potato was evaluated, referred to as ATD. The dishes were incubated at 28±2°C for seven days until fungal colony development was observed. A description of the isolates was made through micro and macroscopic observations of the cultures in a binocular microscope, evaluating spore, conidium, and hypha production. Based on this characterization, it was possible to identify different presumptive fungal genera by comparing with reference literature keys. From the collected tubers, fungal isolates and characterizations were obtained from 6 presumptive fungal strains, and differences were observed in microbial growth between APD and ATD culture media, concluding that the formulation of the medium based on Jerusalem artichoke tubers allows the selection of fungi that do not grow in APD medium.

A112

EVALUATION OF THE A NATIVE STRAIN OF S*CENEDESMUS OBLIQUUS* PHYCOREMEDIATION POTENTIAL AND GROWTH IN IMIDACLOPRID PRESENCE

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Microalgae, as primary producers in aquatic ecosystems, play a crucial role in maintaining ecological balance. Their ability to grow under various environmental conditions makes them key organisms in pollutant mitigation. The intensive use of pesticides in agriculture, such as imidacloprid (IMI), poses a significant threat to the environment. Its toxicity affects both non-target organisms and microalgal communities, disrupting the ecosystem services they provide. In this study, the growth and phycoremediation capacity of a native strain of *Scenedesmus obliquus* was evaluated. The strain was exposed to IMI concentrations of 25 mg/L (C1), 50 mg/L (C2), and 75 mg/L (C3), along with two controls: a microorganism control (CMO) consisting of Bold Basal Medium (BBM) and a microalgal inoculum, and a substrate control (CS) containing BBM with 50 mg/L of IMI. Daily measurements were taken to assess growth through absorbance at 750 nm, and phycoremediation capacity was determined by quantifying IMI degradation every 48 hours. Chlorophyll a, b, and carotenoids were measured as pigment indicators of photosynthetic activity. After 29 days, the results showed that IMI removal was most efficient in C1 (54%), while treatments C2 and C3 achieved removals for only 32% and 33%, respectively. In terms of growth, all three pesticide treatments surpassed the control, with final absorbance values of 0.41 in C3, 0.4 in C2, 0.36 in C1, and 0.32 in CMO. These findings demonstrate the potential of *Scenedesmus obliquus* as a phycoremediation agent, as it not only tolerates IMI exposure but also contributes to its partial degradation in aquatic environments. This study underscores the vital role of microalgae in developing effective phycoremediation strategies to combat pesticide contamination and protect aquatic ecosystems.

A113
ECOSYSTEM FUNCTIONALITY OF MULTI-STRATA LIVING FENCES WITH NATIVE SPECIES.

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Living fences play a crucial role in housing by delineating property boundaries. They serve as visual, sound, and physical barrier, mitigating wind and dust, while also acting as environmental filters. When designed with ecosystem gardening principles, they present an opportunity to create micro-corridors of biodiversity in urbanized areas. In this context, a three-year-study is being worked on with spontaneous vegetation plots along a perimeter fence, proposing a strategy based on design and landscape management, aimed at developing ornamental value and strengthening the ecological and functional value. To achieve this, we implement two treatments. The first involves multi-layered fences, which focus on establishment and managing native species categorized into functional layers (structural, ephemeral, ground cover, and seasonal). The second treatment monitors the spontaneous growth of the fence and records the emergence of species that fit these layers. Ornamental and functional variables (coverage, color, shape homogeneity, rhythm, maintenance time, cost, and establishment time) and ecological variables (soil characteristics and faunal diversity) are assessed. For the latter, the abundance and diversity of: soil arthropods -using pitfall traps-; and pollinators and birds -both through visual recording over a specified period- are documented. These variables are also quantified in living fences with exotic species -mainly monospecific- to compare the associated fauna in fences with different compositions of plant species and structure. Based on the preliminary results, the field evaluation of different native species, and validation through bibliographic references, the following species were decided for the different layers of treatment 1: in the structural layer, *Schinus fasciculata*, *Aloysia gratissima*, and *Lippia turbinata*; in the seasonal layer, *Salvia cuspidata, Solidago chilensis, Grindelia pulchella, Chromolaena arnottiana, Lantana grisebachii, Ipomoea hieronymi, Passiflora caerulea*, and *Mandevilla laxa*; in the ephemeral layer, *Gomphrena pulchella* and *Zinnia peruviana*; and in the ground cover layer, *Melica argyrea, Commelina erecta, Oxalis conorrhiza, Modiolastrum malvifolium,* and *Acaena myriophylla*. In treatment 2, spontaneous species of interest that would meet the multi-layer characteristics were recorded: in the structural layer, *Geoffroea decorticans* and *Baccharis flabellata*; in the seasonal layer, *Clematis montevidensis and Cestrum parqui*; in the ground cover layer, grasses of the genera *Nassella* and *Setaria*; and in the ephemeral layer, *Glandularia peruviana* and *Verbena litoralis*. Monitoring these trials will help select useful species for fences that aling with region's ecological characteristics. It will also take into account the aesthetic parameters necessary in gardening.

DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

A114
HORMONAL REGULATION OF LOBULOALVEOLAR DEVELOPMENT IN A PSEUDOPREGNANT MOUSE MODEL

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The development of the mammary gland is very dynamic and passes through two major phases, one during puberty and the other at the onset of pregnancy. With each pregnancy, the mammary gland begins a process of growth, functional differentiation and involution that is mainly controlled by hormonal stimuli. In particular, estrogens stimulate ductal development after puberty, while progesterone and prolactin participate in lobuloalveolar development during pregnancy. In addition, various local factors are involved in fine-tuning growth, development and regression. The aim of this study was to characterize the lobuloalveolar development in genetically modified female mice producing human chorionic gonadotropin (TG). These mice are infertile and exhibit elevated levels of estradiol, progesterone and prolactin. This hormonal environment mimics that of pregnant females, creating a unique model for studying pseudopregnancy. For this purpose, three-month-old TG and wild-type (WT) female mice were used to analyze the mammary gland development by whole mount carmine staining, mammary cellular architecture by hematoxylin-eosine staining, apoptosis by TUNEL, and the key marker of cell proliferation PCNA (proliferating cell nuclear antigen) by immunohistochemistry. The gene expression of *Lalba* (lactalbumin) and *Prlr* (prolactin receptor) were also determined by RT-PCR. These studies showed that TG females developed a prominent lobuloalveolar architecture, with extensive branching and differentiation of mammary epithelial cells into alveolar structures full of cytoplasmic lipid droplets, resembling a pre-term pregnant mammary gland. A reduced level of apoptosis and an increased number of PCNA-positive nuclei was detected in the epithelium of TG mammary glands compared to WT ($p<0.05$). The expression levels of *Prlr* and *Lalba* were also significantly increased in TG females ($p<0.05$). These findings demonstrate the proliferative activity of mammary epithelia in this pseudopregnant mouse model and pave the way for further research into hormone-mediated mammary remodeling.

BIOCELL, Vol.49, Suppl. 3, 2025
ISSN 0327-9545
ISSN 1667-5746 (online version)

A115
PROGESTERONE TREATMENT MODULATES EXPRESSION OF NEUROINFLAMMATORY FACTORS IN A MURINE MODEL OF INDUCED ENDOMETRIOSIS

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Endometriosis (EDT) is a chronic, estradiol-dependent gynecological disease characterized by the abnormal presence of endometrial tissue outside the uterus. These endometriotic lesions respond to sex hormones, leading to microbleeds that produce a constant inflammatory-oxidative focus, contributing to persistent pain. Progestin-based therapy is the first-line treatment for pain control in most women with EDT. This effect is mediated by suppression or attenuation of ovulation, although its mechanism of action is not yet fully elucidated. The aim of this study is to investigate the effect of progesterone on inflammation, neurogenesis and oxidative stress in a murine model of induced EDT. The EDT was surgically induced in C57BL/6 mice by autotransplanting 3 pieces of right uterine horn to the intestinal mesentery. From day 1 after the operation, the animals of the control group (n= 7) received 100 µl sterile corn oil, while the animals of the experimental group (n= 7) received 50 µg/100 µl of Progesterone (P0130, Sigma-Aldrich, USA) subcutaneously, administered daily for 1 month. Peritoneal fluid was collected for analysis of protein levels of TNF-α, IL-1β (pro-inflamatories cytokines) as well as TGF-β1 (a cytokine associated with pain) by ELISA. The lesions were removed and stored at -80 °C for subsequent analysis of mRNA expression of *Pgp9.5* (general marker of nerve fibers), *Tac1* (tachykinin precursor 1) and *Cgrp* (proinflammatory markers for sensory nerve fibers) using RT-qPCR. Additionally, the activity of antioxidant enzymes (CAT, SOD, and GPX) and the concentration of TBARS-MDA (lipid peroxidation marker) were measured through spectrophotometric analysis. The data were statistically analyzed by the Student t test ($p<0.05$). Progesterone administration did not modify IL-1β protein levels, *Cgrp* expression, or oxidative stress markers. However, it reduced protein levels of TNF-α ($p<0.01$) and TGF-β1 ($p<0.05$), as well as the mRNA expression of *Pgp9.5* ($p<0.01$) and *Tac1* ($p<0.05$). The results show that progesterone modulates the expression of neuroinflammatory factors associated with the development of EDT. Therefore, this strengthens the scientific evidence for using progestins as a treatment for alleviating the hallmark symptoms of EDT, such as pain and inflammation.

A116
ALTERATION OF TFEB EXPRESSION IN THE EPIDIDYMIS OF CASTRATED RATS

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The mammalian epididymis is a crucial organ in the male reproductive system, where sperm mature and acquire motility as they transit through it. The lumen of the epididymis contains a variety of proteins, notably lysosomal enzymes, which are synthesized and secreted by the epididymal epithelium and may play a key role in sperm maturation. This organ is highly influenced by hormones such as androgens. Castration, used as a model for circulating testosterone depletion, has shown morphological and biochemical changes in the epididymis. In our laboratory, we have observed that castration in rats induces an increase in the size of epididymal lysosomes, as well as greater expression and secretion of certain lysosomal proteins. This phenomenon could be related to the action of transcription factor EB (TFEB), which, when activated, translocates from the cytoplasm to the nucleus, promoting lysosomal biogenesis. In the context of prostate cancer, it has been shown that androgen depletion can lead to a reduction in TFEB expression and activity, contributing to tumor progression. Based on this background, we hypothesized that the regulatory mechanisms of TFEB activity in the epididymis might differ from those observed in prostate cancer. Therefore, the aim of our study is to evaluate the impact of testosterone deprivation on TFEB expression and activity in three regions of the epididymis: caput, corpus, and cauda. We used 90-day-old Sprague Dawley rats, divided into control, castrated, and castrated groups with testosterone replacement (1 mg/kg/day). The animals were sacrificed 48 hours after the treatments began, and their epididymides were processed for immunoblot analysis. We observed a trend toward decreased cytoplasmic expression of TFEB in all three epididymal regions of testosterone-deprived rats. However, despite this reduction, lysosomal biogenesis was found to be increased. Our preliminary data suggest that, unlike in the prostate, castration may increase TFEB activity (increased phosphorylation and nuclear translocation), resulting in enlarged lysosomes and higher expression of lysosomal proteins. This effect suggests

that reduced testosterone levels in the epididymis may activate mechanisms that promote lysosomal biogenesis, implicating TFEB as a key regulator in the cellular response to hormonal changes.

A117
ASSOCIATION BETWEEN POST-COVID-19 CONDITIONS AND SEMEN QUALITY IN MENDOZA

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More than four years after the emergence of COVID-19, the effects of the virus on the male reproductive system continue to be a subject of debate. However, there is widespread concern about the possibility that COVID-19 is contributing to the pre-existing decline in male fertility. In this prospective study, initiated in mid-2021, our objective was to investigate the sequelae of COVID-19 on sperm quality and, consequently, on fertility. We recruited and analyzed two sample populations. The first semen samples were processed immediately after being obtained from healthy donors (without a history of the disease) and from donors who had recovered from COVID-19 (up to 12 months [-12] and more than 12 months [+12] after infection). A total of 66 donors participated: 18 controls (C) and 48 recovered from COVID-19 (4 from the [-12] group and 44 from the [+12] group), with an age range of 20 to 45 years. We evaluated macroscopic and microscopic parameters, obtaining the following ranges (minimum and maximum) for each group: volume: C = 1.8-3.1 ml; -12 = 2-3.1 ml; +12 = 1.6-2.9 ml; pH: C = 7.2-7.5; -12 = 7.3-7.5; +12 = 7.3-7.5; liquefaction: C = 30-60 min; -12 = 30-60 min; +12 = 30-60 min; total cell count = 32-230 million; vitality = 78-96%; morphology = 4-10%; and progressive motility = 35-80%. Macroscopic and microscopic values were found to be normal according to WHO andrological consensus. No significant differences were observed in intracellular calcium levels between groups, indicating that all responded to progesterone stimulation with a significant increase in cytoplasmic calcium. The absolute percentages of AR in response to progesterone were C = 9-15%, -12 = 8-14%, and +12 = 9-14%, with no significant differences between groups. The samples were subjected to the "swim-up" sperm selection method, obtaining a recovery percentage of between 43% and 52% of the progressive motile fraction in all groups. The second sample population was donated by patients who underwent assisted reproductive treatment, allowing us to evaluate three variables of the semen analysis during the most critical years of the pandemic: 2019 (n= 61), 2020 (n = 314), 2021 (n = 383), and 2022 (n = 223). All patients performed at least two semen analyses in different years. The evaluated variables were: volume, cell count and progressive motility. Although previous studies have suggested that COVID-19 may affect male reproductive function, no significant effects were observed in this study. As with other viral infections, a transient decrease in spermatogenesis during the acute phase of the infection cannot be ruled out; however, the long-term effects of COVID-19 on spermatogenesis appear to be minimal.

A118
ROLE OF MYELOPEROXIDASE IN THE MODULATION OF INFLAMMATORY RESPONSES IN ANTERIOR PITUITARY IN RAT WITH POLYCYSTIC OVARY

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Polycystic Ovary Syndrome (PCOS) is a heterogeneous functional endocrine-gynecology disorder that affects many women of childbearing age. It is characterized by hyperandrogenism and infertility. In addition to neuro-endocrine alterations, other mechanisms including oxidative stress and low-grade inflammation are related to the pathogenesis of PCOS. Previously, we have demonstrated an immune-endocrine interaction, through of the effect of spleen macrophage secretion, in anterior pituitary (AP) of rats with polycystic ovary (PCO). These macrophages secretions from PCO rats increased the expression of pro-inflammatory interleukin (IL) genes, as IL1β and tumor necrosis factor (TNFα), and nitric oxide release (NO). Myeloperoxidase (MPO) is an enzyme released from immune cells as macrophages are involved in the pathological processes of diseases mainly through the oxidation of biomolecules, which promotes inflammation and oxidative stress. Thus, the aim of this work was to study, in AP from PCO-induced rat model, 1) the presence of MPO and its relationship with the expressions/release of inflammatory markers and 2) the effects of macrophage secretion on MPO content and its oxidant. The PCO condition was induced in adult female Holtzman rat with estradiol valerate (i.m injection 2 mg/rat) and

2 months later animals were sacrificed. PCO and Control pituitaries (PCO-AP and C-AP) were incubated with RPMI medium (basal value) and PCO macrophage secretions (PCO-MϕS), for 3h in metabolic bath. In AP homogenates, MPO and its oxidants: nitrotyrosine (nitrosative stress) and chlorotyrosine (HOCl induced protein oxidation) and iNOS were determinate by ELISA assay. In culture supernatant, levels of TNFα and IL6 release were measured by ELISA and their genic expressions were analyzed by RT-PCR. In basal condition: MPO, nitrotyrosine and chlorotyrosine concentration were increased in the PCO-AP group compared with C-AP ($p < 0.05$). No difference in these parameters was observed in presence of PCO-MϕS, both in C and PCO-AP. The TNFα and IL-6 release, as well as its mRNA levels, were higher in PCO-AP vs C-AP ($p<0, 01$). To the best of our knowledge, this is the first study investigating MPO in rat PCO-AP. Our results provide evidence of a link between MPO and increased inflammation in this tissue, with the subsequent damage and dysfunction, probably enhanced by the androgenic environment. Therefore, strategies to decrease MPO- mediated inflammation may influence reproductive outcomes.

BIOCHEMISTRY, PHYSIOLOGY AND NEUROCHEMISTRY

A119
EFFECT OF HISTONE DEACETYLASE INHIBITOR VALPROIC ACID ON SPATIAL MEMORY IN AN EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE

Coria Lucero C, Ledezma C, Britos A, Ibanez J, Torales P, Sanchez Vela M, Cortez M, Alba R, Golini R, Toledo F, Alfonso J, Lopez M, Delgado S, Anzulovich A and Navigatore Fonzo L
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Alzheimer's disease (AD) is accompanied by memory loss due to neuronal cell death caused by toxic amyloid β-peptide (Aβ) aggregates. Acid Valproic (AV) is a widely used antiepileptic drug and exhibits anti-inflammatory and neuroprotective effects in a rat model of ischemic stroke. In the present study, we examined the effect of AV treatment on the cognitive impairment in an experimental model of Alzheimer's disease. The effect of VA treatment on spatial learning was assessed using a modified version of the Barnes maze test and by object recognition test. Four-month-old males Holtzman rats were divided into three groups defined as: (1) control (saline solution), (2) Aβ-injected (Aβ aggregates-10 μg), (3) Aβ-injected treated with Aβ aggregates and AV (200 mg/kg, in the drinking water by 15 days). Rats were injected into the lateral ventricle (coordinates: AP: −1 mm, L: 1.5 mm, and DV: −3.5 mm). Rats were maintained under 12 h-light:12 h-dark conditions and received water and food ad libitum. Barnes' test modified was performed to test cognitive functions of the rats during day. The parameters assessed were as follows: (a) number of errors; (b) escape box latency; (c) % exploration in the goal sector; (d) % exploration in the goal region; (e) Path length (f) recognition index. Our results demonstrated that Aβ-injected rats showed an increase in the number of errors, exhibited a notable increase in the latent period, explored less frequently the target sector, and showed a significantly higher exploratory activity in comparison with control group. Treatment with AV improved these cognitive parameters. Our encouraging results provided a new basis for using VA in treatment for AD.

A120
EFFECT OF AN I.C.V. INJECTION OF AGREGATED BETA-AMYLOID (1-42) ON THE DAILY LEVELS OF IL-6, TNF-α IN THE RAT HIPPOCAMPUS

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Alzheimer's disease (AD) is one of the common forms of dementia. In the progression of AD, it has been demonstrated that accumulation and aggregation of Aβ peptide in the brain, initiates a neuroinflammatory response, involving inflammatory cytokines, including interleukin (IL) IL-6 and tumor necrosis factor (TNF-α). Besides cognitive deficits, AD patients show alterations in their circadian rhythms. The objective of this study was to investigate the effects of an intracerebroventricular (i.c.v.) injection of amyloid beta peptide (1-42) on daily patterns of IL-6, TNF-α levels, as well as of Bmal1 expression in the hippocampus and on temporal profiles of cognitive performance. Groups were defined as: control (CO) and Aβ-injected (Aβ). Rats were maintained under 12 h-light:12 h-dark conditions and received food and water ad libitum throughout the

entire experimental. For the Novel Object Recognition (NOR) test, the experimental findings were evaluated by analysis of ANOVA with repeated measures followed by Bonferroni's post-hoc test for specific comparisons, a $p<0.05$ was considered to be significant. Daily rhythms of IL-6, TNF-α levels were determined in the serum of both CO and Aβ groups by ELISA. Bmal1 levels was determined by qPCR (ΔΔCt method) in hippocampus samples isolated every 6 h during a 24 h period. Total RNA was extracted from hippocampus samples using the Trizol. All RNA isolations were performed as directed by the manufacturers. Statistical differences throughout the 24-h period were analyzed by one-way ANOVA, followed by a post-hoc test, to confirm statistical differences between zeitgeber time (ZT) within each group; chronobiological statistics were used to confirm the presence of rhythm and Student t test to compare rhythm's parameters (acrophase, mesor, and amplitude) between groups. We observed that IL-6, TNF-α levels vary throughout a 24 h-period in the serum control rats and peaking around the middle of the day. We found that the Aβ injected group showed a phase delay in the IL-6 levels ($p<0.01$). Unexpectedly, Bmal levels do not display a rhythmic variation throughout a 24-h period. The injection of Aβ (1-42) abolished the rhythmic patterns of daily TNFα levels ($p<0.05$). In addition, an intracerebroventricular injection of Aβ aggregates altered the temporal profiles of cognitive performance specifically decreased the recognition rate ($p<0.05$). Thus, elevated Aβ peptide levels might affect the temporal patterns of inflammatory cytokines and behavioral performance, probably by altering the expression of clock-activating factor.

A121
INFLAMMATION AND OXIDATIVE DAMAGE BIOMARKERS TEMPORAL PROFILES ARE MODIFIED BY AN INTRACEREBROVENTRICULAR INJECTION OF AB-AMYLOID (1-42) AGGREGATES IN ALZHEIMER 's DISEASE RAT MODEL

Torales P, Ibanez J, Rodriguez Brito A, Sanchez Vela M, Cortez M, Alba R, Golini R, Toledo F, Pellerin N, Garraza M, Delsouc B, Casais M, Ramirez D, Gomez Mejiba S, Delgado S, Anzulovich A and Navigatore Fonzo L
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Alzheimer's disease (AD) is the major neurodegenerative cause of progressive dementia in the elderly. The accumulation of Aβ in the brain plays a key role in the pathogenesis of AD. Elevated levels of Aβ causes an increase in oxidative damage and exacerbates neuroinflammation. Besides cognitive deficits, AD patients show alterations in their circadian rhythms. Previously, we found that an intracerebroventricular (i.c.v) injection of Aβ(1-42) modified the daily patterns of antioxidant enzyme activity in the rat temporal cortex. Taking into account these observations, the objective of this work was to investigate the effects of an i.c.v. injection of Aβ(1–42) on 24 h patterns of biomarkers of inflammation and oxidative damage in the rat temporal cortex. Four-month-old male Holtzman rats were divided into two groups defined as: control (CO) and Aβ-injected (Aβ). Rats were maintained under12 h-light:12 h-dark conditions and received water and food ad libitum. Tissues samples were isolated every 6 h during a 24 h period. Daily rhythms of nitrites, iNOS, chlorotyrosine and nitrotyrosine levels were determined by ELISA. Statistical differences throughout the 24-h period were analyzed by one-way ANOVA, followed by a post-hoc test, to confirm statistical differences between ZTs within each group; chronobiological statistics were used to confirm the presence of rhythm and Student t test to compare rhythm's parameters (acrophase, mesor, and amplitude) between groups. We found nitrites levels vary significantly and rhythmically throughout a day in the CO group, with rhythms' acrophases occurring at the middle of the light period. The nitrites rhythm's acrophase was shifted at middle of the night and mesor was increased in the Aβ-injected animals ($p <0.01$). In addition, we also found that iNOS, chlorotyrosine and nitrotyrosine levels displays a rhythmic pattern in the hippocampus of the control group, with maximal levels occurring at the middle of the night. Interestingly, iNOS, chlorotyrosine and nitrotyrosine levels do not display rhythmic variation throughout a 24-h period in the hippocampus of Aβ-injected rats. Given the relevant role of oxidative stress and neuroinflammation and the consequent alterations in expression of factors related to cognition on the pathophysiology of the disease Alzheimer. We would expect that elevated Aβ peptide levels alter temporal patterns of inflammation and stress-related parameters and, consequently, would negatively affect cellular clock activity and the transcription of their target genes. These alterations in the daily rhythmicity of markers of oxidative stress might be responsible for changes in the temporal organization of the redox state in this brain area.

BIOCELL, Vol.49, Suppl. 3, 2025
ISSN 0327-9545
ISSN 1667-5746 (online version)

VETERINARY SCIENCES: ANATOMY, HISTOLOGY AND PHYSIOLOGY

A122
HEPATIC AND PLASMA EVALUATION IN C57BL/6 J MICE TREATED WITH *CANNABIS SATIVA* L OIL RICH IN CANNABIDIOL (CBD)

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Cannabidiol (CBD) is one of the main pharmacologically active phytocannabinoids. Despite the many therapeutic effects studied for CBD, hepatotoxicity has been reported as one of the side effects associated with high doses of this compound. The aim of this work was to evaluate the effect of chronic consumption of a full-spectrum cannabis oil rich in CBD, on liver function, and plasmatic and hepatic oxidative parameters in C57BL/6 J mice. A total of eighteen 6-week-old male mice were divided into 3 groups and treated: Control: orally administered olive oil (5 µL/day for 21 days); D5: orally administered CBD-rich cannabis oil (5 µL/day for 21 days); D10: orally administered CBD-rich cannabis oil (10 µL/day for 21 days). Body weight (BW) and water and food intake were measured. After sacrifice, blood was collected to determine glycaemia (G), total protein (PT), triacylglycerol (TAG), malonyldialdehyde (MDA), total antioxidant capacity of plasma (FRAP), nitric oxide (ON), alkaline phosphatase (AP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. In liver, MDA, carbonyls and superoxide dismutase (SOD) and catalase (CAT) activity were determined. When compared to control, water intake increased in D5 and D10 and food intake decreased in D10 ($p=0.0001$), although no differences were found in BW gain between groups. No changes were observed in G, PT and TAG. FA levels were higher in D10, and no changes were found in AST and ALT. MDA and SOD increased in D5 and D10 and with no changes in carbonyls levels and CAT. In plasma no modifications in ON, FRAP and MDA were recorded. Increased AP may indicate impaired liver function, although no significant changes were observed in ALT and AST, which are important markers of liver damage. Increases in MDA and SOD suggest that chronic CBD consumption may induce oxidative stress in liver, although there was no evidence of severe oxidative damage to proteins. Chronic consumption of CBD-rich cannabis oil had moderate effects on liver function and oxidative status, with no evidence of systemic damage or severe alterations in liver enzymes.

A123
EFFECTS OF GLYPHOSATE-BASED HERBICIDE ON MORPHOLOGICAL ASPECTS OF SEMINIFEROUS TUBULES AND TESTOSTERONE CONCENTRATION IN WISTAR RATS

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Recent studies have suggested that glyphosate has an endocrine disrupting effect in hormone-dependent tissues, such as testicles. This results have generated debates and taken attention to the study of the reproductive toxicity induced by glyphosate-based herbicides. The aim of this work was to determine the effect of a glyphosate-based herbicide on morphological aspects of seminiferous tubules (ST) and testosterone concentration in Wistar rats. 24 eight-week-old male Wistar rats were divided into 4 groups, which had access to different solutions of acid glyphosate (Roundup®) in water, administered oral via: 1) 0 mg/kg/day (control group), 2) 10/kg/day, 3) 50 mg/kg/day, 4) 100 mg/kg/day. Animals were treated for 365 days, and then euthanized. Blood was obtained by cardiac puncture, and plasmatic testosterone levels were measured by ELISA. Also, testicles were excised for histological evaluation. After hematoxylin and eosine stain, testicles histological preparations were photographed using a digital camera, and the images obtained were analyzed using the program Axio Vision LE. 5 fields per ST were observed using light microscopy, and total area, perimeter, total diameter, total tubule lumen area, lumen perimeter, lumen diameter, thickness, number of tears and cellularity were determined. Groups 3 and 4 showed a decrease in testosterone levels when compared to control group ($p\leq 0.05$). In addition, ST total area, perimeter and diameter were lower in groups 3 and 4. An increase of ST lumen area and perimeter was observed in groups 2 and 3, while group 4 showed a decrease, when compared to the control group. Moreover, a reduction of ST thickness and lumen diameter was found in group 4, while a marked increase of tears and lack of cellularity was observed in groups 3 and 4. Diminished levels of testosterone and morphologic alteration found in ST in groups treated with glyphosate could lead to a reduction in sperm quality, and hence, fertility, endangering the survival of populations living in areas contaminated with this component.

A124
DIET MANAGEMENT EFFECTS ON HEAVY LAMB CARCASS QUALITY

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Heavy lamb is an alternative to reach a continuous offer to ovine meat consumers. To study the effects of diet management, may improve product quantity and quality. The objective of this work was evaluating feeding effect and breeds on weights, yield and subjective classification of heavy carcasses. 48 heavy lambs of two breeds, Hampshire Down (HD) and Corriedale (CORR) were compared. The group was under two feeding managements, A: natural resources and supplementation with alfalfa hay, B: alfalfa or green pasture supplemented with corn and commercial feed. The slaughter was carried out according to official regulations, when animals exceeded 30 kg of body weight. In abattoir were determined slaughter weight at origin or final weight (FW: kg), cold carcass weight (CCW: kg) and commercial yield (CY: %), carcasses were scored according to subject evaluation as conformation (CONF) and fattening (FAT). Data was analyzed using ANOVA. Feeding method influenced significantly all characteristics evaluated ($p<0,001$), supplementation with corn and commercial balanced obtained higher means (FW 33.8 kg vs 43.0 kg; CCW 13.7 kg vs 20.4 kg; CY 43.5% vs 47.5%; FAT grade 2.1 vs 3.1; CONF score 2.0 vs 2.7). Breed didn't influence significantly any parameter. It was observed a genotype-environment interaction in the carcasses fattening, lambs HD had greater classification with a high nutritional level (grade 2.92 vs 3.45), in feedings with energy restriction CORR fattened the carcass more than HD. We concluded that with better quality diet and greater energy intake are obtained improved weights and yields, with superior muscularity carcass and adequate fattening; likewise, it was observed a differential carcass fattening in each breed, according to supplementation received, HD reacts with a higher carcass fattening at higher levels off energy in diet, while CORR respond under a low nutritional level.

A125
HISTOPATHOLOGICAL EFFECTS OF IMIDACLOPRID ON THE LIVER OF THE EARED DOVE (*Zenaida auriculata*)

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Imidacloprid (IMI), a neonicotinoid insecticide, exerts its toxic effects on insects by selectively binding to nicotinic acetylcholine receptors in the central nervous system, leading to disruption of neural transmission, paralysis, and ultimately death. Its widespread use is attributed to its efficacy in controlling a broad spectrum of pests, particularly through applications like seed coatings to protect crops. Although neonicotinoids are designed to exhibit lower toxicity in vertebrates, increasing evidence suggests that exposure to these compounds can result in adverse hepatic effects. IMI has been reported to induce oxidative stress, mitochondrial dysfunction, apoptosis, and inflammation within the hepatic parenchyma. The objective of this work was to assess the toxicological effects of IMI on eared doves (*Zenaida auriculata*) liver subjected to chronic exposure at sublethal concentrations that are environmentally relevant. A total of 18 adult eared doves were randomly distributed into three groups: one control group and two experimental groups. Each bird underwent intermittent gavage administration daily at a consistent time between 08:00 and 09:00 a.m. throughout a 21-day exposure period. The control group (G0) received only the vehicle (water), while the experimental groups G1 and G2 were administered IMI solutions in water at concentrations of 3.93 mg/kg and 11.80 mg/kg of body weight, respectively. After the exposition, the birds were euthanized, and a portion of liver was collected for light microscopy. Histological sections were stained using hematoxylin-eosin, periodic acid-Schiff (PAS), Perl's Prussian Blue, and Masson Trichrome. The G0 group exhibited a relatively normal liver with typical histological characteristics. In contrast, the G1 group showed marked damage, with the presence of steatosis, inflammatory infiltrates, and areas of possible infarction. The G2 group displayed the most severe damage, characterized by zones of necrosis, leukocytic infiltrates, numerous hemosiderin deposits, and alterations in the epithelium of the bile ducts. Both G1 and G2 presented increased sinusoidal dilation, vascular congestion, and enlargement of central veins, suggesting progressive impairment of hepatic blood flow. Dislodged hepatocytes were observed within the central veins, indicating a detachment from the surrounding parenchymal tissue, potentially as a result of hepatotoxic stress or vascular injury. Additionally, G2 showed an increased amount of collagen fibers, particularly surrounding central veins and even forming bridges between portal areas. No PAS-positive structures were identified in the parenchyma. The histopathological analysis revealed hepatic damage in the experimental groups, characterized by steatosis,

necrosis, inflammatory infiltrates, vascular alterations, and early-stage fibrosis, particularly in the group receiving the higher concentration of IMI. These findings highlight the potential risks associated with IMI exposure used in agricultural practices. The observed alterations in the liver histoarchitecture of eared doves suggest that even sublethal concentrations can result in detrimental effects on wildlife health. Supported by PROIPRO 02-3723-UNSL to VP Filippa.

A126
CHRONIC EFFECTS OF IMIDACLOPRID ON THE INTESTINAL MORPHOLOGY OF THE EARED DOVE (*Zenaida auriculata*)

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Neonicotinoid insecticides have become crucial to global agriculture due to their efficiency and versatility. Among them, imidacloprid (IMI) is one of the most widely used, particularly as a seed treatment for crops such as soybeans, maize, and wheat. This extensive application has resulted in ecosystem contamination, with IMI residues being detected in soil, water, and various wildlife species. The aim of the present study was to evaluate the IMI toxic effects on the proximal intestine (PI) of eared dove (*Zenaida auriculata*) chronically exposed to sublethal and environmentally relevant concentrations. Adult eared doves (n = 18) were randomly divided into three groups, one control and two experimental. They were administered by intermittent gavage each day at the same time 08:00-09:00 a.m. during 21-day exposure period (EP). The control group (G0) received only the vehicle (water), while the experimental groups G1 and G2 received an IMI solution in water at concentrations of 3.93 mg/kg and 11.80 mg/kg of body weight, respectively, administered once daily. After the EP, the birds were euthanized, and a portion of the PI was collected from each for light microscopy processing. Hematoxylin-eosin staining, and periodic acid-Schiff (PAS) histochemical staining were performed on the histological sections. In the G0 group, conserved villi with tall enterocytes, numerous goblet cells (PAS+), and a prominent brush border PAS reaction were observed. Lymphocyte accumulations were frequently found in the mucosa, and the Lieberkühn crypts displayed normal morphological characteristics. In contrast, morphological changes were observed in G1 and G2 compared to G0. These included epithelial detachment, enterocytes of varying heights, and frequent apoptotic cells, indicating increased cellular stress. In addition, an apparent decrease in goblet cells, and a weaker PAS+ reaction in the brush border, suggesting reduced mucin production. The Lieberkühn crypts showed a reduced size, and some appeared collapsed with intensely stained cells, possibly undergoing degeneration. These alterations indicate damage to the intestinal mucosa, possible modifications in cell proliferation and differentiation, and sparse evidence of loose lymphatic tissue presence. Additionally, differences in the response to IMI treatment were noted between the experimental groups. The G2 group exhibited more severe mucosal damage and morphological alterations than G1. In conclusion, the histological findings suggest that IMI exposure has a deleterious effect on the proximal intestine, primarily causing mucosal damage in a dose-dependent manner, potentially compromising intestinal absorption and barrier functions. Further studies are needed to quantify these morphological changes and assess potential long-term functional consequences. Supported by PROIPRO 02-3723-UNSL to VP Filippa.

A127
BEHAVIORAL EFFECTS INDUCED BY SUBANAESTHETIC DOSES OF KETAMINE IN THE OPEN FIELD AND THE FORCED SWIMMING TESTS

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Non-competitive antagonism of the NMDA receptor produced by ketamine is used as an antidepressant. In previous studies of our laboratory, we observed changes in the locomotor activity of rats in the Hole Board test with ketamine subanaesthetic doses at 3 minutes after its intraperitoneal (IP) administration. In the present work, we studied the movement pattern at 30 minutes after IP administration in the open field test (Opto Varimex, OVM) and in the forced swim test (FST). Male Holtzman rats (240-290 g) were used. Significance was studied by ANOVA I followed by Student-Newman-Keuls. In all cases, a $p<0.05$ value (two-tailed) was considered significant. In experiment 1 (OVM) the animals were divided into 4 groups that received ketamine (2.5, 5 and 10 mg/kg) or saline (control) IP (n=10) 30 min before the OVM, and the test lasted 5 min. The parameters measured were: horizontal, ambulatory, non-ambulatory movements and total number of movements. We observed a significant decrease $F (3/32) = 2.417$, $p<0.0845$) with the highest dose in the number of

horizontal movements ($p<0.05$) in the open field test. In experiment 2 (FST) the rats were divided into 4 groups. They received ketamine (2.5, 5 and 10 mg/kg) or saline (control) IP (n=15) 30 min before the FST. After 30 min they were placed in the forced swim test for 5 min. A significant decrease (ANOVA, $F(3/58) = 5.058$, $p<0.0035$) was observed in resting with the dose of 10 mg/kg ($p<0.05$). From both experiments we conclude that there are findings compatible with a sedative effect in the open field test and an antidepressant effect in the forced swim test with the highest dose used.

A128
DELAYED EFFECT OF KETAMINE SUBANESTHETIC DOSES IN THE FORCED SWIMMING TEST AND METABOLIC ACTIVITY OF THE AMYGDALA IN RATS (PRELIMINARY STUDY)

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There is currently evidence of antidepressant effects of ketamine. The aim of this study was to study the effect of subanaesthetic doses of ketamine administered after the pre-test in the Forced Swimming Test (FST), 24 hours before the test and its correlation with the metabolic activity of the amygdala. Male Holtzman rats (240-290 g) were used, divided into 4 groups (n=10). They received saline (control) or ketamine (1.25, 2.5 and 5 mg/kg) immediately after the pre-test session. After 24 hours, the FST test was performed. At the end of the test, the amygdala was removed (n = 5 per group) and the MTT test was performed to determine metabolic activity. Resting, climbing, swimming and diving were evaluated. Significance was studied by ANOVA I followed by Student-Newman-Kewls. In all cases a value of $p<0.05$ (two-tailed) was considered significant. A significant decrease in resting time ($F_{(3/41)}=67.79$, $p<0.0001$) was observed with all doses studied ($p<0.0001$). A significant decrease in climbing time ($F_{(3/41)}=241.4$, $p<0.0001$) was found with the lowest dose (1.25 mg/kg, $p<0.0001$) and an increase with the highest dose ($p<0.0001$). An increase in swimming time was found ($F_{(3/41)}=144.5$, $p<0.0001$) with the doses of 1.25 and 2.5 mg/kg ($p<0.0001$). No significant changes were observed in diving time. The metabolic activity of the amygdala did not show a statistically significant difference at 24 hours. We conclude that the doses studied present modifications in the behavioural parameters indicative of an antidepressant effect at 24 hours, with no alterations being found in the cerebral metabolism of the amygdala during this time.

PHARMACOLOGY AND TOXICOLOGY

A129
ANTIINFLAMMATORY ACTIVITY OF *PROSOPANCHE AMERICANA* IN WISTAR RATS

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The inflammatory reaction is a physiological response that protects our body against tissue damage or microorganisms aims. Medicinal plants have been the main remedy to treat various ailments for a long time and nowadays, many drugs have been developed from traditional medicine. The main advantages of using herbal medicines are the low cost, affordability and usually fewer side effects. *Prosopanche americana* (R. Br.) Baillon is a perennial hemiparasitic native plant distributed in Argentina (Córdoba, Santiago del Estero, Mendoza, La Rioja, San Luis), belonging to the family Hydnoraceae. Commonly known as “guaycurú santiagueño”, “huaycurú”, “huáchar”, “guacharo”, “flor de fierro”, “flor de la tierra” o “espinazo de lagarto”. The fruit is edible and rhizome is used in folk medicine as vulnerary, homeostatic, expectorant, anti-inflammatory, cardiac disorders and antidiabetic. Specimens were deposited in the Herbarium of the Universidad Nacional de San Luis under registration UNSL#560 (Del Vitto LA & EM Petenatti). Infusion to 10% was prepared following the methodology outlined in the VII Ed Argentine National Pharmacopoeia and then lyophilized to preserve it. The aim of this study was to evaluate the anti-inflammatory activity of the *P. americana* rhizome lyophilized aqueous extract (PALE) in rats. The proposed method used male adult rat with a body weight range from 100-180 g in a randomized manner and assigned into

groups (n=6-8). All experiments were following ANMAT No. 9236/2023 for animal care guidelines. Experimental protocols were approved by the Laboratory Animal Care and Use Institutional Committee (CICUAL) of Faculty of Chemistry, Biochemistry and Pharmacy, Universidad Nacional de San Luis (Protocols: F-404/22, F 431/23). Inflammation induced by cotton pellet in rat: granuloma was induced in all animals by subcutaneous (s.c.) implant of a sterile cotton pellet in the dorsal area of anesthetized rats. Animals were divided into four groups and received: negative control (vehicle), reference group (dexamethasone, 4 mg/kg, s.c.), PALE 750 and 1000 mg/kg, v.o., respectively. Acute inflammation model with carrageenan-induced rat paw edema: animals were divided into five groups and received v.o.: negative control (vehicle), reference group (indometacin, 10 mg/kg), PALE 500, 750 and 1000 mg/kg, respectively. For the measurement of plantar edema, the Digital Water Plethysmometer was used. The experimental data obtained were expressed as the mean ± S.E.M. The significance of the results was determined using analysis of variance (ANOVA) with later comparison by Tuckey–Kramer. A probability of $p<0.05$ was considered significant. In the chronic inflammation model, PALE induced a reduction in granuloma weight (0.65 ± 0.03 g) at dose 1000 mg/kg vs negative control (0.77 ± 0.04 g, $p<0.05$). However, evaluation of the anti-inflammatory effect on the acute inflammation model (carrageenan-induced rat paw edema) did not produce statistically significant changes. *P. americana* at dose 1000 mg/kg in the chronic model displayed significant anti-inflammatory activity. Further phytochemical investigations will be necessary for analysis of involucres metabolites.

A130
EFFECT OF *TRIPODANTHUS FLAGELLARIS* ON DIURETIC ACTIVITY IN WISTAR RATS

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Tripodanthus flagellaris (Cham. & Schldlt.) Tiegh. (Loranthaceae) is popularly known as “liga blanca” or “liguilla”. It is used in folk medicine by their hypotensive properties, as well as abortive, emmenagogue and oxytocic effects. This study was aimed to investigate the effect of *T. flagellaris* on diuretic activity in rats. Specimens were deposited in the Herbarium of the Universidad Nacional de San Luis under the registration UNSL#8553 (Del Vitto LA & EM Petenatti). Infusion was prepared to 10% from the dried powdered plant material, separated by filtration and the aqueous extract was concentrated and lyophilized to preserve it. *T. flagellaris* was administered, *p.o.*, in concentrations: 0 (control group), 62.5 mg/kg, 125 mg/kg and 250 mg/kg. The proposed method used adult rat with a body weight range from 150-180 g in a randomized manner and assigned into groups (n=6-8). All experiments were conducted in accordance with ANMAT No. 9236/2023. Experimental protocols approved by the Committee for Animal Care and Laboratory Use (CICUAL) of the Faculty of Chemistry, Biochemistry and Pharmacy, Universidad Nacional de San Luis (Protocol F-435/23). Lipschitz *et al*. method was used. Control (saline solution), furosemide and hydrochlorothiazide (reference drugs) groups were established. Urinary volume was measured at 15 min intervals for 3 hours to determine urinary volumetric excretion (UVE). The urine samples showed normal chemical parameters in all the cases. Urine Na^+ and K^+ contents were analyzed. Urinary density and pH were measured. Rats treated with infusion showed a significant diuretic effect (UVE: 62.5 mg/kg: 74.23 ± 7.13 ($p< 0.05$); 125 mg/kg: 88.97 ± 3.68 ($p< 0.0001$); 250 mg/kg: 75.98 ± 4.24 ($p< 0.001$)) respect the control (57.74 ± 1.49). Excretion followed values from between 25-55% compared with the control group. Urinary sodium excretion was significantly increased at doses 125 and 250 mg/kg, ($p<0.001$ y $p<0.05$, respectively), while urinary potassium excretion increased significantly at all three doses (62.5 mg/kg ($p<0.0001$), 125 y 250 ($p<0.01$) respectively vs negative control. Urinary pH was significantly increased at doses of 62.5 and 125 mg/kg; however, urinary density was similar to negative control. This diuretic activity could be due, in part, to the presence of flavonoids in this plant. Flavonoids are responsible for diuretic effect in other species. This diuretic had important effects on the excretion of water and Na^+, providing scientific support to the traditional use of this plant.

A131
EFFECT OF *VACHELLIA CAVEN* IN HEPATOTOXICITY PRODUCED BY PARACETAMOL

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Vachellia caven (Vc) is a plant popularly known as "espinillo". This plant was selected for the study because it is one of the most common and abundant tree species in San Luis. The liver damage produced by paracetamol is a widely used model for the study of hepatoprotective substances that, when metabolized, produce free radicals that cause liver damage. Biochemical tests are useful for studying and treating an organism with impaired liver function. Today it is possible to analyze liver function and from there infer the results of a pharmacological study. The aim of this research was to evaluate the effect of the infusion of fresh pollen *Vc* (250 mg/kg and 500 mg/kg) on glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in the injury produced by paracetamol. The plant material was collected in the province of San Luis. Wistar rats of both sexes, 150-180 g, were used. Experiments were conducted in accordance with ANMAT No. 9236/2023. Experimental protocol approved by the Committee for Animal Care and Laboratory Use (CICUAL) of the Faculty of Chemistry, Biochemistry and Pharmacy, UNSL. Liver injury was caused by oral administration of 640 mg / kg of paracetamol. Biochemical determinations were carried out with manual analysis techniques using Wiener lab reagents. The experimental animals (n=20) were administered v.o. 10 ml/kg/day (during 3 days) of physiological solution (group 1), physiological solution + paracetamol one hour after the last dose (group 2) and infusion of *Vc* (250 mg/kg and 500 mg/kg) + paracetamol one hour after the last dose (group 3). One day later, blood samples were obtained for the biochemical determination of GOT and GPT. The toxic dose of paracetamol (640 mg / kg) produced severe liver damage, as indicated by the significant increase in GOT and GPT values compared to the control group (66.15±4.61 vs. 44.11±1.75, $p < 0.05$ and 26.76±1.42 vs. 20.79±1.16, $p < 0.05$, respectively). Pretreatment with the infusion of fresh pollen *Vc* (250mg/kg) produced significantly decreased GPT and GOT activity (13.53±1.17 vs. 26.76±1.42, $p < 0.0001$ and 46.93±3.49 vs 66.15±4.61, $p < 0.05$; respectively). The results showed that the infusion of fresh polen *Vc* (250 mg/kg) presented hepatoprotective activity, evidenced by the decrease in the enzymatic activity of GPT and GOT. Future studies will be oriented towards the specific study of the mechanism of action involved. New studies will also be developed to determine the hepatoprotective metabolites of the infusion.

A132
ACACIAIN PEPTIDASE GASTROPROTECTION: ROLE OF SULFHYDRYL, PROSTAGLANDINS AND ENDOGENOUS NITRIC OXIDE IN RATS

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Acacia caven (Mol.) Molina is an arid and semi-arid area native tree of South America, which belongs to the Mimosaceae (Leguminosae) family. It is a very widespread species in Argentina, Chile, Bolivia, Uruguay, Paraguay and Brazil. Non-wood forest products, defined as "goods of biological origin other than wood, coming from forests", have experienced a sustained increase in their consumption in recent decades, especially foods and medicinal species, valued for their condition as goods of wild, natural and/or organic origin, with a significant impact on health and in the context of healthy eating. The objective of this work was to evaluate the role of sulfhydryl groups (SH), prostaglandins (PG) and endogenous nitric oxide (NO) in the gastroprotective effect of the purified proteolytic extract of *Acacia caven* (Mol.) Molina pollen. The crude extract and the purified proteolytic fraction (acaciain peptidase) of *A. caven* pollen were obtained according to Barcia et al. (2019), using an FPLC unit (Akta Prime Plus, General Electric), and concentration and vacuum drying (SpeedVac™ Vacuum Concentrator SPD1030/2030). All experiments were conducted in accordance with ANMAT No. 9236/2023. Wistar rats (180-200 g; n=6-8), having fasted for 24 h, were employed. N-ethylmaleimide (NEM; 10 mg/kg, s.c.), indomethacin (10 mg/kg, s.c.) and N^G-nitro-L-arginina (L-NNA, 40 mg/kg, i.p.) were used to evaluate the role of SH, PG and NO respectively. Thirty min after the pretreatment, the oral treatment was applied (acaciain peptidase, 65 mg/kg). Sixty minutes later, 1 ml of absolute ethanol (necrotizing agent) was given to each rat, being eutanized after 1 h, with CO_2 and the

stomachs excised. The stomach scanned images were analyzed using Image J software (NIH). Ethanol caused gastric ulcers in all animals, while pretreatment with the extract at doses 65 mg/kg prevented the formation of gastric lesions induced by ethanol ($p<0.001$ vs ethanol). The pre-treatment with NEM (a blocker of SH) and indomethacin (a PG synthesis inhibitor) did not alter the acaciain peptidase-induced gastroprotection of ethanol-induced gastric lesions, thus excluding the role of SH and PG in mediating the protective effect of extract. The gastroprotection of acaciain peptidase was counteracted by pretreatment with L-NNA (L-NNA-acaciain peptidase-ethanol: 65.09±14.41 mm^2 vs acaciain peptidase-ethanol: 24.75±6.40 mm^2; $p<0.05$). NO is involved in the modulation of gastric mucosal integrity and is important in the regulation of mucus secretion and gastric mucosal blood flow. The results suggest that, at least in part, NO system contributes to the gastroprotective effect of acaciain peptidase against ethanol-induced damage.

A133

ROSUVASTATIN REDUCES ALLOSTATIC OVERLOAD DUE TO MECHANICAL STRAIN: A NEW FINDING ON VASCULAR REMODELING LINKED TO HYPERTENSION

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Chronic hemodynamic overload due to hypertension produces cardiovascular remodeling, where mechanical stretch (MS) deformation, neurohumoral factors and/or chronic interaction promote a harmful allostatic overload. Previously, our laboratory demonstrated that rosuvastatin modulates the NO-Hsp70-WT1 pathway during MS at the renal level, and other authors have reported that some statins inhibit the proliferation of rat vascular smooth muscle cells (VSMC) induced by MS. Therefore, this study aims to evaluate, in VSMC culture, the possible modulation of rosuvastatin on NO-Hsp70-WT1 signaling linked to MS and its impact on vascular remodeling due to hypertension. After 10 weeks of life, we perform cultures of mesenteric VSMC of spontaneously hypertensive (SHR) and Wistar Kyoto (WKY) rats. Previous to the culture, systolic blood pressure (SBP) in SHR was (180 ± 10 mmHg) and WKY (125 ± 8 mmHg). Eight groups were established: SHR/WKY with and without MS for 48 hours (Flexcell®); and subgroups treated or not with rosuvastatin (10-5 mol/L). Apoptosis was evaluated by flow cytometry, fibrosis (TGF-β levels), NO levels, as well as WT1 and Hsp70 expression. SHR cultures without MS vs WKY without MS, showed higher apoptosis/fibrosis and low NO, WT1, and Hsp70 ($p < 0.01$). Of interest, WKY with MS was similar to SHR without MS. Furthermore, when comparing SHR with MS vs SHR without MS, we verified more significant apoptosis/fibrosis with lower NO, WT1, and Hsp70 ($p < 0.01$). However, rosuvastatin reduced these differences, promoting the restoration of the altered parameters. Hence, rosuvastatin would reduce vascular remodeling and allostatic overload during hypertension by decreasing MS-associated apoptosis/fibrosis in mesenteric VSMC and regulating the NO-Hsp70-WT1 axis.

BIOCELL, Vol.49, Suppl. 3, 2025
ISSN 0327-9545
ISSN 1667-5746 (online version)

A134
NANOPHARMACOLOGY: THE NEW FRONTIER IN THE TREATMENT OF HYPERTENSION

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Anandamide (AEA) has antihypertensive effects that are poorly explored due to its characteristics and side effects. Encapsulation in nanocarriers could overcome these limitations. The present study aimed to encapsulate AEA in poly-ε-caprolactone/Pluronic® F127 nanoparticles (AEA/PCL/PF127 NPs) by electrospraying to characterize their physicochemical properties, cytocompatibility and to evaluate their effect in an *in vivo* model of cardiovascular remodeling caused by hypertension. AEA/PCL/PF127 NPs were characterized in terms of morphology, size, polydispersity, Z potential, hydrophilicity, thermal and spectroscopic properties. Encapsulation efficiencies, loading and *in vitro* release of AEA were also analyzed. AEA/PCL/PF127 NPs (700-1000 nm) showed adequate cytocompatibility. For cardiovascular remodeling studies, normotensive (WKY) and hypertensive (SHR) male rats (N=10 per group) were treated or not with AEA/PCL/PF127 NPs (5 mg/kg, intraperitoneal injection) weekly for 1 month. CICUAL 137/2018. Inflammatory markers and hemodynamic, structural and cardiac function parameters were monitored. In SHR, treatment with AEA/PCL/PF127 NPs reversed all altered cardiovascular markers and parameters ($p<0.05$). Therefore, we conclude that nanoformulated AEA proved to be effective for the treatment of hypertension and its comorbidities, especially cardiovascular remodeling.

A135
AQUATIC ENVIROMENT INFLUENCES ON *Rhinella arenarum* TADPOLES ONTOGENY

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A distinctive feature of amphibian ontogeny is the "openness" of all its stages to the influence of the environment. The typical amphibian life cycle consists of a gelatinous egg, deposited in the water, an aquatic larval stage, and a terrestrial adult form following metamorphosis. Both stages differ in morphology, physiology, locomotion, behavior, diet, and, in general, occupy different ecological niches, evolving largely independently. The separation of life stages among habitats can lead to differences in the risk and fitness payoff, in terms of survival, in each stage. In certain instances, a dangerous environment will favor strategies that opt for slower growth in safer habitats or vice versa. Unfortunately, the Embalse La Florida, an artificial lake in the arid region of San Luis province, Argentina, is contaminated with the heavy metal Cadmium (Cd), also detected in adult anuran amphibians collected from its shores. On the other hand, Spirulina has been recognized by its high nutritional value. It is known that spirulina has positive effects on the toxicity induced by metals such as Cd. However, relatively few studies have experimentally examined the effects of Cd contamination or spirulina of aquatic reservoirs on *Rinella arenarum* (Argentine toad) physiology. This study aimed to evaluate whether Cd or enriched spirulina environment affect the ontogeny of *R. arenarum* tadpole and whether it constitutes a biomarker of pollution and its bioremediation. We collected amphibian larval stages (IV-V according to Martin et al.) of *R. arenarum* in springtime in San Luis city. Fifteen specimens were exposed by triplicate to different Cd concentrations (0.5 and 0.25 mg/L) for a month. Anuran larvae are microphagous and thus feed largely on alga and blended plants. In this assay, we used equal amount of blanched spinach, obtained by chopping the leaf. The Cd groups were feed *ad libitum* with dry chopped spinach. Other groups of tadpoles were subjected, in the same conditions and time, to 5, 15, 30 and 100% of spirulina. All of them received equal amount (mgr) of food every day along the experiment. At the end of the first week, we observed that exposure to Cd increased the amount of aliment ingested. This alteration on the daily consumption of food correlated to larvae growth alterations, (weight, length and size body) of Cd treated tadpoles, that were significantly superior than controls in a dose dependent manner. In contrast, larvae fed only with spirulina ended with significant lower weight. The longitude of the tadpoles from head to tail and the size of the body were measured using the Image J program, from NIH page. Spirulina treatment delays the growth of the tadpoles. Interestingly, the percentage of individuals completing metamorphosis and

acquiring the hind and front legs indicates that Cd exposure accelerates metamorphosis while spirulina slows tadpoles development. Together, our results point out the need of further investigations of spirulina as bioremediator and the use of ontogeny of *R. arenarum* as a biomarker of healthy or contaminated aquatic ecosystems.

A136
A STUDY OF THE CYTOTOXICITY OF METAL-ORGANIC FRAMEWORKS BASED ON BI-11 BISMUTH METAL IONS

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Metal Organic Frameworks (MOFs) are formed by the self-assembly process between metallic ions and organic linkers, giving rise to open 3D structures. Due to their versatility, MOFs materials could be useful for optics, gas sorption, catalysis, and drug delivery, especially for antimicrobial agents. In the present work, a bismuth-based MOF was synthesized by solvothermal technique, and their cytotoxicity was analyzed. The compound, named as **Bi-11** with formula $[Bi_2(2,6\text{-}NDC)_3(1,10\text{-}phen)_2]$. $2H_2O$ (2,6-NDC=2,6-naphtalendicarboxylate), consists in a 2D structure that crystallizes into the $P2_1/n$ triclinic space group. One unique nonacoordinated bismuth center is surrounded by six oxygen atoms belonging to 2,6-NDC ligands and two nitrogen atoms from one 1,10-phenanthroline ligand. We started by using RAW 264.7 culture cells, a murine macrophage line cell to accomplish our aim. Monolayers of RAW were cultured in clear 24-well microtiter plates for 24, 48, and 72 h in DMEM-high glucose medium containing 10% fetal calf serum and antibiotics (complete medium, CM). After this incubation cell monolayers were rinsed and incubated in CM containing different concentrations of **Bi-11** (25-300 μg/ml). After 24, 48, and 72 h of incubation, nitrite concentration in the medium was measured using the Griess assay. The cells were rinsed with pre-warmed sterile saline and exposed to 1mg/ml MTT, after 30 min of incubation at 37 °C, formed formazan crystals in living cells were dissolved with DMSO. The absorbance was read at 570 nm in a plate reader as a viability measurement. The preliminary results promise answers to the possibility of including **Bi-11** in the cluster of materials with biomedical interest (MIBM), improving the choices when applying antimicrobial therapies.

A137
CYTOTOXICITY OF METAL-ORGANIC FRAMEWORKS BASED ON BI-9 BISMUTH METAL IONS

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Metal Organic Frameworks (MOFs) are formed by the self-assembly process between metallic ions and organic linkers, giving rise to open 3D structures. Due to their versatility and potential cavities, MOFs materials could be useful for optics, gas sorption, catalysis, and drug delivery, and biological properties, especially for antimicrobial agents. In the present work, one bismuth-based MOF was synthesized by solvothermal technique, and its cytotoxicity was analysed. The compound, with the formula $[Bi_2(1,4\text{-}NDC)_3]$. 2DMF (1,4-NDC=1,4-naphtalendicarboxylate) (namely as Bi-9), crystallizes into the $P2_1/n$ monoclinic space group. Besides, each bismuth center nonacoordinated by oxygen atoms belonging to 1,4-NDC ligands. One unique nonacoordinate bismuth center is surrounded by six oxygen atoms belonging to 2,6-NDC ligands and

two nitrogen atoms from one 1,10-phenanthroline ligand. To accomplish our aim, we started using RAW 264.7 culture cells, a murine macrophage line cell. Monolayers of RAW were cultured in clear 24-well microtiter plates for 24, 48, and 72 h in DMEM-high glucose medium containing 10% fetal calf serum and antibiotics (complete medium, CM). After this incubation cell monolayers were rinsed and incubated in CM containing different concentrations of Bi-9 (25-300 µg/ml). After 24, 48, and 72 h of incubation, nitrite concentration in the medium was measured using the Griess assay. The cells were rinsed with pre-warmed sterile saline and exposed to 1mg/ml MTT, after 30 min of incubation at 37°C, formed formazan crystals in living cells were dissolved with DMSO and the absorbance was read at 570nm in a plate reader as a viability measurement. The preliminary results obtained in this study promise answers to the possibility of including the MOFs studied in the cluster of materials with biomedical interest (MIBM), improving the choices when applying antimicrobial therapies.

NUTRITION AND HEALTH

A138

RISKS IN THE CONSUMPTION OF DIETARY SUPPLEMENTS FORMULATED FROM NATURAL ORIGIN RAW MATERIALS LABELLED AS *SPIRULINA* AND *CHLORELLA*

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The consumption of dietary supplements (SD) has become very important in recent years, especially those formulated with natural drugs such as microalgae of the *Chlorella* genus and cyanobacteria distributed under the vernacular name "Spirulina". Theses requires rigorous controls to ensure the safety, efficacy and quality of these products. SDs are defined in the Argentine Food Code (Art.1381) and are regulated by ANMAT through the INAL (National Institute of Food). In order to provide information on the quality of SD delivered in the region, SD were analyzed under different pharmaceutical forms (hard gelatin capsules, powders and tablets). They were evaluated through their macroscopic characters, optical microscopy (OM) and scanning electron microscopy (SEM), together with biomass determination using Neubauer's camera. In addition, molecular techniques were applied to confirm the identity of species present in the SD. The samples (M_1 to M_8) were reduced to dust and the Method of successive quartering established in Argentinian Pharmacopeia, 7th Edition. was applied until obtaining the laboratory samples. Macroscopic characteristics: physical (colour, texture, surface) and organoleptic (aroma and taste) were determined. The optical microscopy allowed us to identify morphological characteristics and detect possible impurities or adulterations. The surface characteristics of the samples were analyzed through the SEM. Cell count allowed us to estimate the active content per gram of sample. Overall, the results indicated that the formulations showed a deterioration of macroscopic and microscopic characters, as well as a decrease in biomass, with the addition of excipients and with the compression process. The samples distributed as tablets had a surface and granular texture that could be confirmed by SEM analysis. By molecular identification techniques, the presence of species of *Arthrospira* in samples labeled "Spirulina" and *Chlorella vulgaris* in the sample labeled *Chlorella* was confirmed. Two of the samples tested showed adulteration: M_3 was shown to be macroscopically different from the rest, as in the other tests applied; while M_5 presented contamination by the presence of cyanobacteria of the genus *Microcystis*, producing microcystins (hepatotoxic toxins). Our results suggest the changes need in regulations for the production and sale of dietary supplements formulated with natural drugs, stressing the importance of standards that unify growing conditions for cyanobacteria and microalgae intended for human consumption. Dietary supplements potential as a strategy to reduce the risk of chronic non-communicable diseases is highlighted, provided they are produced and consumed in an appropriate manner.

A139

EVALUATION OF TWO NITROGEN SOURCES IN HEIFERS REARING SUPPLEMENTATION ON DEFERRED PASTURE (*DIGITARIA ERIANTHA*)

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The forage base of livestock systems in the semiarid region consists of natural pastures and perennial summer grasses. The use of summer growth as deferred forage is a common practice in the forage chain. It is well known that the crude protein levels provided by this forage mass do not meet the nutritional requirements of replacement heifers, making protein supplementation an alternative to achieve desirable daily weight gains in this category. The objective of this trial was to evaluate the effect of two protein supplementation types, differentiated by nitrogen source: non-protein nitrogen (NPN) and true protein (TP), in a replacement heifer herd. The study was conducted at the EEA INTA San Luis over a 64-day period, preceded by a 15-day adaptation phase. The heifers grazed on deferred summer growth of a *Digitaria eriantha* pasture. Forage availability was 2602 ± 1572 kg DM/ha of deferred material at the animal entry time. The crude protein (CP) content, neutral detergent fiber (NDF), and dry matter digestibility (DMD) of the deferred pasture were 4.8%, 77.3%, and 49.4%, respectively. The protein supplement contained 29% CP and minerals according to the label: calcium 10.5%, phosphorus 5%, chlorine 27%, sodium 18%, and copper 0.35%. The pellet was formulated with ground corn, soybean expeller, salt, wheat bran, calcium carbonate, and urea, containing 24% CP, 8% crude fat, 2.5 Mcal ME, 1.25 Mcal NE, and 67.6% TDN. The 42 heifers were divided into four groups; two groups (10 and 9 heifers each) received commercial pellet supplementation, and the other two groups (11 and 12 heifers) were supplemented with protein salt. The four groups were randomly assigned to four deferred Digitaria plots, each with an area of 3 ha. Live weight was recorded every 21 days. Statistical analysis was conducted using Analysis of Covariance (Infostat/P 2020), employing initial weight as a covariate. This model was used to measure the initial entry weight of each animal in the trial, as it influences daily gains and consequently final weight. Results from the comparative analysis of nitrogen sources show that final weights (FW) and average daily gains (ADG) were significantly different ($p<0.05$) between the two supplement types, with FW of 376.5 ± 3.6 and ADG of 560 ± 410 for TP (pellet); and FW of 364.4 ± 3.2 and ADG of 420 ± 130 for NPN (protein salt). These results indicate that nitrogen sources from true proteins yield higher ADG and result in a higher final weight at the end of the evaluation. It is concluded that the assessment of these two protein supplementation types is useful information for deciding which supplement ~~to~~ would be used during winter periods in grazing systems on deferred pastures with low nutritional quality.

A140

STUDY OF TWO ORGANIC MATRICES FUNCTIONALIZED WITH CuNi NPs FOR ANTIMICROBIAL FOOD PACKAGING

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Consumer demands for healthy foods free of synthetic preservatives and environmental concerns, associated with non-degradable plastic packaging waste polluting the ecosystem, have led to the development of alternative bio-based packaging materials. In this work, the effect of matrix changes of organic films with CuNi nanoparticles (NPs) with proven antimicrobial activity is investigated. The matrices studied were agar and pectin. The NPs were obtained by the citrate-gel method. Agar films were prepared according to the methodology of Kumar et al (2019). Pectin films were synthesized using commercial citrus pectin 2% w/w and glycerol 1% w/w dissolved in distilled water with magnetic stirring at 250 rpm at 25°C. The liquid mixture was poured into plates and dried at 40°C for 48 h. The films were characterized by scanning electron microscopy (SEM), microanalysis (EDS), colorimetry and textural mechanical properties. The results obtained from the characterization by SEM and EDS show a surface of the Agar-NPs films with relatively low roughness, except in the center, where a particle with a different morphology was observed. The upper superstructure corresponds to an organic formation. EDS analysis detected the presence of carbon (C), oxygen (O), nickel (Ni) and copper (Cu), which confirms the incorporation of Cu and Ni NPs in an agar matrix. For the pectin-NPs films, secondary electron images show a smooth surface without irregularities even at resolutions of the order of 500 nm. The backscattered electron images show the presence of irregularly distributed particles. EDS analysis shows the presence of the elements Cu and Ni corresponding to

the NPs. In addition, the constituent elements of the pectin (N, O and C) and their distribution in the films obtained, whose thickness is of the order of 75.3 µm, were characterized. The analysis of the mechanical properties allowed obtaining two parameters of greater relevance; the tensile strength (TS) and percentage of extensibility (%EAB). The test results allowed us to conclude that the Pectin-NPs Films are mechanically more resistant and have greater extensibility. The colorimetric analysis showed that the pectin films are clearer and more transparent than the agar films where one of the causes could be attributed to the difference in thickness between them due to the agar films being slightly thicker. According to the results obtained, it can be concluded that both matrices are suitable and potentially attractive for the design of a variety of applications, among which this investigation pursues, the elaboration of a Film-NPs with pronounced antimicrobial activity, which could be used for the reduction of microbial loads in food packaging.

A141
COMPARISON OF THE ANTIFUNGAL ACTIVITY OF KEFIR AND GARLIC EXTRACT AGAINST BREAD SPOILAGE FUNGI

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In previous studies, the antifungal capacity of kefir, an ancient, fermented beverage, was evaluated. In the present work, 6 strains of fungi isolated from spoiled breads were identified and the antifungal activity of garlic extract against these fungi was evaluated and compared with the antifungal activity of kefir. The kefir beverage was prepared by inoculating a total of 3 g of kefir grains in 30 mL of sterile brown sugar aqueous solution (5 g%). It was incubated at 30°C with an orbital shaker at 140 rpm. The sample was taken after 72 h and the cell-free supernatant (CFS) was obtained by centrifugation at 10,000 x g for 10 minutes. It was sterilized by filtration using a 0.22-micron membrane. The garlic extract was prepared by macerating garlic (8 g) in a mixture of ethanol and water in equal parts. After one week, it was centrifuged at 6000 xg for 20 minutes. The fungi isolated were cultivated into Sabouraud medium for 24 h. The identification was realized by molecular techniques: DNA was obtained using a biologic kit; PCR amplification with universal primers were made; DNA concentration in the PCR products was determined using Epoch (Biotek) and the integrity of the samples was evaluated through 1% agarose gel electrophoresis. The PCR products were sent to CERELA (Tucuman-Argentina) for their purification and sequencing. The sequences were edited with Molecular Evolutionary Genetics Analysis (MEGA v7.0) and were analyzed with BLASTn using NCBI databases. The isolated were identified belong the genus *Penicillum*, *Aspergillus* and *Paecilomyces*. The Minimum Inhibitory Concentration (MIC) was determined for each fungi. Serial dilutions of kefir and garlic were prepared. A volume of 300 µL of each dilution was placed into sterile Eppendorfs containing 620 µL of 2x Sabouraund medium. Each tube was inoculated with 620 µL of spore suspension of 1/100 dilution of an original suspension of $OD_{600\ nm}$ 1.4 of each strain. Spores were obtained from a 7-day-old plate on glucose potato agar. Positive controls (inoculated medium without the addition of kefir or garlic) and negative controls (uninoculated medium with the addition of kefir or garlic) were included. They were cultured for 72 h and observed every 24 h. The results showed that garlic extract has greater antifungal activity than kefir. The most sensitive were fungi of the *Penicillium* genus, while the strains most resistant to them were *Aspergillus niger* and *Paecilomyces variotii*. In future, the synergies and stability over time of kefir and garlic extract will be studied, in order to advance their use as natural preservatives in bread.

A142
MINDFULNESS AND LIFESTYLE: A HEALTHY BOND IN YOUNG UNIVERSITY STUDENTS

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Brain well-being in young university students is an area of interest in health research, especially in a framework where academic demands and emotional challenges are constant. Dispositional mindfulness, also known as trait mindfulness, is a personality trait that involves paying attention to the present moment, without judgment and with acceptance. This has been linked to several benefits, including reduced emotional intensity, enhanced emotional recovery, in learning, improving mental health, and cognitive functions. Furthermore, another aspect is to lead a healthy lifestyle, which includes adequate

stress management, regular sleep patterns, and a balanced diet. All of them are crucial to maintaining and optimizing brain plasticity. Therefore, a mindfulness trait would positively influence the habits and decisions that make up a person's lifestyle. The goal of this study was to analyze the relationship between disposition to mindfulness and lifestyle; in UNSL students. A descriptive non-experimental design with correlational methodology was used. Pearson/Spearman Correlation and t test were used. The non-probabilistic sample included 131 UNSL students from different academic units (26% men, 74% women), were evaluated through virtual surveys. The instruments were the FFMQ, to measure mindfulness attitudes and FANTASTICO questionnaire for lifestyle. The analysis was carried out with descriptive tables and correlations. From the descriptive statistical analysis, FFMQ and FANTASICO values were obtained: $\overline{x}$= 122 ± 17.3 and $\overline{x}$= 68.4 ±12.4 (mean ±SD, respectively). The correlation between the total scores of the FFMQ and FANTASTICO was r = 0.674 ($p \leq 0.001$). The dimensions: Act with awareness and description; showed significant correlations with FANTASTIC. Sleep and Stress and Introspection; showed significant correlations with FFMQ. The results show a positive correlation between mindfulness traits and a healthy lifestyle in university students, highlighting improvements in stress management, sleep quality and introspection. These findings, consistent with previous findings, highlight the importance of promoting mindfulness education in academic settings to encourage more balanced lifestyle habits.

A143
EXPERIMENTAL MODEL OF NUTRITIONAL OBESITY AND THE DAILY VARIATION OF SOME BIOMARKERS OF OXIDATIVE STRESS. POSSIBLE EFFECTS ON RAT LIVER

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During the process of oxidative damage several biomolecules are altered in the cell, which is why diets with the potential to restore metabolic homeostasis and internal temporal order could be a novel strategy for the treatment of free radical imbalance caused by obesity (OB). Our aim was to evaluate histological parameters and oxidative stress in adult rat liver in a model of OB. Two-month-old male Wistar rats were fed for 14 weeks on a normocaloric (NC) maintenance diet (AIN-93M; control group, NC) and another group on a high saturated fat diet (HFM, modified AIN-93M 1; OB group). For the following 8 weeks, half of the animals in each group were fed the chronodiet (modified AIN-93M 2; CRNC and CROB groups). All animals were maintained under 12h-light:12h-dark and 22-24°C conditions, with water and feed ad-libitum. For chronobiological studies, four (4) animals from each group were sacrificed at zeitgeber times (ZT): ZT2, ZT8, ZT14 and ZT20. All the experiments were performed following national and international guides for the care and use of laboratory animals and were approved by the CICUA (UNSL). Biomarkers such as TBARS, Catalase Activity (CAT), and proteins levels were determined spectrophotometrically in liver homogenates. Statistical differences over the 24 h period was analyzed by two-way ANOVA, Chronos-fit and Cocinor, as appropriate. As a result, we observed that CAT (enzyme oxide-reductase) concentration presented a significantly lower mesor in the OB group compared to the NC group ($p<0.01$). A similar behavior was observed for TBARS (MDA) quantification in the OB group compared to the NC group ($p<0.001$). Both determinations showed an acrophase towards the end of the activity period of the animals. When studying the oxide-reductase enzyme concentration and TBARS levels in the CROB group, we observed that both mesor and acrophase presented a significant rhythmic variation when compared to the CRNC group ($p<0.05$). From the above, we can conclude that chronobiologically based diets are able to partially restore the levels of markers altered in the OB group, including the rhythmic patterns of oxidative stress, approaching the values observed in the NC group. Thus, a chronobiologically based diet would have a positive impact on the health status of individuals with OB, through the restoration of internal temporal order. *Proyecto de Unidad Ejecutora (PUE). Resolución N°-2018-930-APN (IMIBIO-SL-CONICET)*

A144
EVALUATION OF ANTIFUNGAL ACTIVITY AND CYTOTOXICITY OF ADDITIVES USED IN AGRICULTURE AND THE FOOD INDUSTRY

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Food losses due to the presence of microorganisms, such as fungi, are a major concern worldwide, especially with a constantly growing world population. The development of these organisms leads to sensory defects in food ranging from visual deterioration to noticeable changes in smell, taste or texture, but they can also have negative impacts on health due to the production of mycotoxins by some species. The objective of this work was to evaluate antifungal activity on fungi of the genus *Aspergillus* in the presence of Cu, Calcium Propionate and Potassium Sorbate, which are used as antifungals in agriculture and the food industry. The cytotoxicity also was evaluated. Fungi isolated and identified in previous work from spoiled bread were used: *Aspergillus niger* and *A. tritici*. The proposed methodology included different concentrations of sodium propionate and sorbate (0.15–2 % w/v) in the presence of the microorganism under study in liquid Luria-Bertani (LB) culture medium. On the other hand, the antifungal activity of different additives was determined by measuring mycelial growth on extract-glucose agar (EG), with and without the additives. For the copper effect, a similar methodology with 400 ppm. For the cytotoxicity assay, Artemia salina eggs were incubated in saltwater at 27°C for 48 hours until the nauplii hatched. The nauplii were exposed to the additive solution. The results showed inhibition of the growth of both fungi species with the different additives solutions used. Propionate inhibited the growth of A. niger at a concentration of 2%, while for *A. tritici* was more resistant but a decrease in growth was observed, compared to the positive control. With potassium sorbate it was observed that at concentrations of 0.5% and 1% no growth of *A. niger* and *A. tritici* was observed, respectively. Finally, the experiments carried out in solid medium showed that *A. niger* did not showed mycelial growth in the presence of 1.5% calcium propionate and copper, while with potassium sorbate the growth was not significant. In cytotoxicity assay, copper presented total inhibition and all nauplii died, followed by propionate y sorbate. These results shows that the additives are effective in fungi controlling in concentrations higher than those allowed by the argentine food code and they are cytotoxic, so it is important to look for other additives more effective and less toxic alternative.

A145
ZINC CONCENTRATION IN HAIR IN UNIVERSITY STAFF. PROPOSED AS AN EARLY BIOMARKER OF MINERAL DEFICIENCY

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It has been shown that the analysis of minerals in hair samples allows the evaluation of mineral imbalances (deficiencies), as well as excesses of toxic elements that accumulate in the body. The measurement of zinc in hair represents an advantage in allowing the analysis of the contribution of the micronutrient to the body over long periods of time and added to the fact in terms of sample taking, which is non-invasive and widely accepted by patients. The technique of sampling and measuring zinc in hair was standardized, making time, costs and reagents feasible in said procedure. The concentration of zinc in the samples was measured by AAS (Atomic Absorption Spectroscopy). Subsequently, samples were taken from university teaching and non-teaching staff, from 40 years of age. The results obtained from the analysed samples were mostly between 81 mg/kg and 771 mg/kg of zinc, that is, within the normal ranges, even higher, compared to the standards currently found from other countries and in populations with different characteristics. This may be due to the marked differences in diet between different countries and cultures, mainly variations in zinc and phytate content. It should be noted that the lowest values of hair zinc correspond to individuals with diets zinc deficient and/or high in phytate, older adults, overweight individuals, who reported suffering from stress and with pathologies such as type 2 diabetes, cancer and cardiovascular diseases. Likewise, the highest values of hair zinc were associated with individuals with adequate zinc in diets, because use micronutrient supplements. Reference ranges of zinc concentration in hair were able to be established in the population analysed (according to sex and age). Based on the obtained results, the determination of zinc in hair is proposed as an early biomarker of micronutrient deficiency, mainly in cases of moderate or initial deficiencies (caused by deficiencies in the

quality of the diet and/or by pathophysiological processes). In addition, it is important to have a simple and effective technique and an easily accessible sample such as hair, which is very useful for measuring minerals and determining the relationship between them and their importance in health.

A146
IMPACT OF CHRONONUTRITION ON BIOCHEMICAL AND ANTHROPOMETRIC PARAMETERS OF OVERWEIGHT AND OBESE INDIVIDUALS

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Currently, the incidence of overweight and obesity is an epidemic and represents a global public health problem. These pathologies are related to a greater probability of developing chronic diseases, which are increasing and represent a significant burden for the health system. In the context of weight gain, the study of chrono nutrition has been developed to elucidate the relationship between obesity and the desynchronization of biological clocks. A diet synchronized with the circadian cycle (CC) and based on natural foods with a low glycemic index and healthy fats and the practice of intermittent fasting (IF) have gained considerable scientific and popular repercussion and are being used as effective practices for the treatment of excess weight and its comorbidities. The objective of this work was to analyze the impact of synchronizing the circadian cycle on metabolic health by fasting for 16 hours. To do so, monitoring was carried out through biochemical and anthropometric parameters of overweight and obese people who were invited to participate voluntarily and signed the informed consent. A correlational, longitudinal study was conducted in which 80 individuals of both sexes, aged between 18 and 59 years, participated for ten months. The sample consisted of 32.5% men and 67.5% women. 96% of the individuals registered weight loss (100% of men and 92% of women). Regarding the percentage of body fat, 60% of men and 50% of women started with a diagnosis of "very high", and at the end of the period it was reduced to 30% in both cases. 80% of men and 90% of women performed strength and aerobic physical activity. 75% of men and 60% of women managed to lower their blood sugar (this value was reduced from 108 mg/dL to 90 mg/dL in men and from 105 mg/dL to 92 mg/dL in women). 75% of men started with a “very high risk” waist circumference diagnosis and changed to 55%; and in women, these values were reduced from 70% to 50%. Regarding total cholesterol, 40% decreased their value compared to the initial value (50% of men and 30% of women) and regarding LDL, 85% of men and 80% of women decreased their value to values within the normal ranges ($\bar{X}$=192 mg/dL to 125 mg/dL in men and $\bar{X}$=190 mg/dL to 130 mg/dL in women). Regarding triglycerides, 95% of men and women modified their values positively. Feeding synchronized with CC would promote weight loss, decrease abdominal fat mass and positively influence lipid biochemical parameters and glycemia in overweight and obese individuals analyzed, in male individuals ($p<0.05$).

A147
BODY MASS INDEX AND ITS RELATIONSHIP WITH THE CONSUMPTION OF FOODS SOURCE OF ZINC IN SCHOOL CHILDREN WHO ATTEND THE INSTITUTO NUESTRA SEÑORA DEL CARMEN, IN THE YEAR 2023

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Childhood overweight and obesity have increased alarmingly in recent times. This problem has predisposed a high prevalence of micronutrient deficiencies, even in populations with apparently healthy individuals with normal weight. In this scenario, zinc (Zn) as a micronutrient is essential, its deficiency compromises developing individuals, since it plays an important role in the regulation of cell growth and differentiation. The objective of this study was to evaluate the body mass index (BMI) and its relationship with the consumption of Zn-source foods in school children from 6 to 12 years of age who attended the Our Lady of Mount Carmel Institute, during the months of September to December of the year 2023. A

descriptive, correlational, cross-sectional study was carried out, with a total sample of 60 children from 6 to 12 years of age who underwent anthropometric measurements of weight and height to determine the Body Mass Index according to age (BMI/ AGE) together with a qualitative food frequency consumption survey oriented to food sources of Zn. To make the association between the BMI/AGE variables and Zn consumption, the non-parametric Chi-Pearson test and the SPSS version 22 statistical program were used. It was found that there was no significant association between the BMI/AGE and the consumption of food sources of Zn. However, it was evidenced that the highest percentage of the sample (58%) presented a deficit in the consumption of the trace element. The aforementioned findings raise the need for nutritional intervention from an early age and the importance of including a varied, balanced and balanced diet in this population.

A148
MICROBIAL PHYTASE INFLUENCE ON MONOGASTRIC ANIMAL BONE DENSITY

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Phytases are enzymes present in fungi, yeasts, and bacteria that have the ability to hydrolyse phytic acid by releasing phosphate ions in solution. These active enzymes are used as additives to avoid antinutritional phytate effect in feeds, which reduces some essential minerals availability such as zinc, what are required for development. Femur is the body longest and strongest bone and its morphology can be influenced by various factors, including diet and nutritional supplements. This study aimed to evaluate the effect of phytase supplementation in monogastric animals through general morphological and biochemical characteristics and femur structural changes. Wistar rats from third generation (F_2) raised with phytase supplement were used for this purpose. Rats (N= 15, males and females) were separated into four groups: control females (CF) and control males (CM) fed normal diet (ND) and phytase females (PhF) and phytase males (PhM) fed ND + phytase. Enzyme extraction from yeasts (*Saccharomyces cerevisiae*) was performed at the end of the fermentation process in brewing. Cholesterol, triglycerides, uric acid, and glycemia biochemical analysis were determined. Also urine phosphate was estimated. Femur width and length were measured likewise body weight was weekly monitored. ANOVA analysis was performed. No significant differences were found between treatments in either biochemical parameters and body weight. When determining phosphate in urine, rats treated with phytase had significantly greater phosphate value (5.23+/- 0.1 mg/L) than control (4.79 +/- 0.1 mg/L) ($p<0.05$). Female (3.17 cm +/- 0.02 vs 3.7 cm+/- 0.10) and male (3.0 cm +/- 0.0 vs 3.95+/- 0.05) femur length was significantly higher in phytase fed rats compared to control ($p<0.001$). No significant differences were found in F_2 female (5.17 mm +/- 0.25 vs 5.25 mm+/- 0.27) femur width, while in males (4.0 mm +/- 0.0 vs 6.0 mm+/- 0.05) it was significantly higher in phytase fed rats compared to control ($p<0.001$). Possibly, improving essential nutrient bioavailability, would optimize phosphorus bone metabolism and consequently phytase could enhance bone remodelling, by an increment in sized and thickness.

A149
CHAÑAR BREA GUM IMPACT ON THE DIET OF WISTAR RATS: NUTRITIONAL EFFECT STUDY

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Chañar Gum (GCB) is a hydrocolloid extracted from the legume tree *Parkinsonia praecox*, native to central, western and northwestern Argentina. Its ability as a thickener, gelling agent, emulsifier and stabilizer has sparked interest in its use as a substitute for imported additives, such as arabic gum, in the food industry. It has been shown that GCB can modify the rheological properties of food, and in this study, its potential impact on metabolic health is explored. The present study aims to investigate the effects of chañar brea gum (GCB) use as additive food in the Wistar rats' diet, evaluating its impact on body weight and biochemical parameters, such as: glucose, triglycerides and cholesterol. Male Wistar rats, 21 days old, with an N=13 were used, which were divided into three groups: Group I (Control), Group II (supplemented diet with 5% with GCB) and Group III (supplemented diet with 10% GCB). Weight was monitored weekly in all animals of the different treatments. After two months of treatment, the animals were slaughtered, prior to which blood was extracted by heart puncture to evaluate glucose, total cholesterol, triglycerides, LDL and HDL. Results showed that blood glucose decreases significantly ($p<0.01$) in the same proportion with both GCB concentrations. On the other hand, total cholesterol ($p<0.05$) and triglycerides ($p<0.01$) decrease significantly in the case of GCB al 10%; while with GCB at 5% triglycerides only

declines significantly ($p<0.01$). As for HDL and LDL cholesterol, no significant differences were seen between the groups, the same happened with uric acid, whose values were not modified with any treatment. These initial findings indicate that GCB has the potential to positively influence the management of some lipid parameters, as well as an effect on glycaemia, which opens the door to future research in this field.

A150
LIQUID MEDIUM ISOLATION AND BASIC NUTRITIONAL CHARACTERIZATION OF EDAPHIC CYANOBACTERIA

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Cyanobacteria are Gram-negative bacteria that perform oxygenic photosynthesis, producing organic matter from CO2 and sunlight. They are responsible for oxygen production in the Earth's atmosphere and are considered one of the most morphologically diverse groups of prokaryotes. Cyanobacteria play crucial roles in nutrient cycling, particularly nitrogen fixation. Due to their potential applications in biotechnology, environmental monitoring, and food production, cyanobacteria have become a promising resource. They contain significant levels of macro and micronutrients, making them suitable for human consumption and protein supplements. They are versatile microorganisms with high growth rates and survival capabilities in severe environmental conditions. This study aimed to isolate and biochemically characterize edaphic cyanobacteria from natural consortia in San Luis Province, Argentina. 50 mL of sterile liquid media (Watanabe and BG-11) were inoculated with 10 mL of three different natural consortia (I, II, and III) and incubated at 27°C with a 12:12 photoperiod for 30 days. Taxonomic identification was performed using an Olympus BX 50 optical microscope. Biochemical analysis of protein, carbohydrate, and phycobiliprotein content was conducted on axenic microbial cultures. Preliminary taxonomic identification revealed the presence of three dominant cyanobacterial genera. Biochemical analysis showed promising results for protein, carbohydrate, and pigment content, varying according to the dominant cyanobacterial type. This study contributes to the biochemical characterization of edaphic cyanobacteria, highlighting their potential applications in biotechnology and food production. Further research is warranted to explore the diverse uses of cyanobacteria.

A151
IMPACT OF HYPERCHOLESTEROLEMIA ON THE FORMATION OF ATHEROGENIC PLAQUES

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Cholesterol is vital for human physiology, however, historically atherosclerosis has been mainly attributed to elevated levels of totalcholesterol (TC) and low-density lipoproteins (LDL). However, hypercholesterolemia as the basic cause of atherogenesis does not explain the reports of patients with acute myocardial infarction and normal LDL cholesterol, so there may be other associated factors in the development of these pathologies. The objective of this study was to determine the incidence of atherogenic plaque formation in people with total cholesterol and LDL above normal values. Individuals who voluntarily attended a nutritional consultation at a private institution during the current year and who signed an informed consent participated in the study. The inclusion criteria were: 1) Adult age. 2) Not having received lipid-lowering medication. 3) Having total cholesterol >200 mg/dl and LDL >130 mg/dl during the last 3 months. 4) Having recently had a carotid doppler performed. In the latter, the following were evaluated: preservation of arterial caliber, presence of images suggestive of plaque, stenosis of the lumen and thickening of the intima. The sample was made up of 10 individuals (8 women and 2 men) with average total cholesterol levels of: 244 mg/dl, and 160 mg/dl of LDL cholesterol and with preserved arterial caliber, in all cases. In 90% of the sample, no image suggestive of plaque was evident, although stenosis

of the lumen was present in 10% of the cases. Regarding thickening of the intima, 8 patients did not present it, while 2 patients did. Although the entire sample had TC and LDL cholesterol levels, 90% did not present images suggestive of atherogenic plaque and 80% did not present pathological thickening of the vascular intima. Thus, although this is a small sample and would constitute the first phase of a study that aims to investigate the factors associated with cardiovascular risk, elevated blood levels of TC and LDL were not related to atherosclerosis, which is why a study with a multivariate approach and on a larger scale could be important to know all the variables involved in this pathology, which would allow preventing cerebrovascular accidents among other pathologies; as it is also important to be able to generate therapeutic tools that target all the variables involved.