

**Abstracts of the  
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## **OPENING LECTURE:**

### **A1**

#### **ALKALINE pH REGULATABLE PROMOTERS: NOVEL PLATFORMS FOR HETEROLOGOUS PROTEIN EXPRESSION IN YEASTS**

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The yeast *Saccharomyces cerevisiae* responds to moderate alkalinization of the medium with an extensive remodeling of gene expression that affects hundreds of genes. These changes occur in response to the activation of diverse signaling pathways, including the Rim101/PacC pathway, the calcium-activated phosphatase calcineurin, the Wsc1-Pkc1-Slt2 MAP kinase, and oxidative stress-responsive pathways, as well as diverse nutrient-signaling pathways such those mediated by the Snf1, Pho85, and PKA kinases. The transcription factors mediating these inputs are also well characterized and there are examples, such as the *ENA1* Na<sup>+</sup>-ATPase or the *PHO89* Na<sup>+</sup>/Pi transporter genes, of a single promoter integrating various positive and negative signals to better shape the appropriate response. We will describe current work aiming to the generation, by both rational design and combinatorial methods, of alkaline pH-driven synthetic hybrid promoters that could serve as novel, simple, and inexpensive platforms for heterologous protein expression in *S. cerevisiae*. Over 25 different constructs including single and multiple combinations of the calcineurin-regulatable CDRE motif, the *PHO* sequence, and the novel Stp1/2 consensus present in the *ENA1* promoter identified recently in our laboratory, were made and tested for GFP production. In some cases, they show a response comparable to the powerful *GALI-10* promoter. Selected constructs are being currently tested for production of industrial enzymes. In parallel, we have defined by RNA-Seq the transcriptional landscape in response to alkaline pH in *Komagataella phaffii* (*P. pastoris*), a yeast widely used in heterologous protein production for pharmaceutical and biotechnological applications. Alkalinization triggers fast changes in the yeast's mRNA levels, resulting in more than 400 genes induced at least 2-fold. Guided by the transcriptomic data, we demonstrated the appearance of oxidative stress and the participation of the calcineurin/Crz1 pathway in the transcriptional response to alkali. We have created yeast strains expressing enzymes of industrial interest from diverse alkaline pH inducible promoters identified in the transcriptomic survey. Our current data indicates that these strains could effectively compete with the commonly used methanol-inducible, *AOX1*-based expression strains. Therefore, our work opens new avenues leading to the generation of novel native or hybrid promoters useful to develop innovative and more sustainable platforms for heterologous protein expression using yeasts as host organisms (work funded by grant 2020-PID2020-113319RB-I00 (MICINN) to JA and AC.)

## **LECTURE 1:**

### **A2**

#### **SALT TOLERANCE AND BIOTECHNOLOGICAL POTENTIAL IN *Debaryomyces hansenii*: A CINDERELLA WITH A HAPPY ENDING**

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*Debaryomyces hansenii* is an ascomycetous non-conventional yeast with high biotechnological potential. This organism has been defined as a salt-loving yeast since it was first isolated from sea water, and it is abundant in salty food, sausages, and dry meat products where it plays an important role during the ripening process. One of the most interesting aspects of *D. hansenii* is its halotolerant/halophilic character. In contrast to the general behavior of most fungi, *D. hansenii* is a sodium includer yeast. Although multiple salt tolerance determinants have been found and analyzed in *Debaryomyces*, their real contribution to the whole halotolerance process remains obscure. Several differential determinants between the model yeast *Saccharomyces cerevisiae* and *D. hansenii* have been reported, such as the existence of ion transporters absent in *Saccharomyces* (Hak1), the high salt tolerance of some *D. hansenii* enzymes (Hal2 or Ppz1) or specific changes in its plasma membrane composition in response to salt. In relation to the effects of high salt concentrations on the metabolism, the group of Prof Peña (México) published a series of works hypothesizing that high salinity affects energy pathways and growth in *D. hansenii* but these aspects remain ambiguous. By following different approaches, we found that NaCl and LiCl trigger completely different cellular responses as shown by metabolite levels, enzymatic activities measurements and transcriptional regulation of genes. In summary, we will show that sodium, but not

lithium up regulates the shunt of the glyoxylic acid in *D. hansenii* and we propose that this is an important metabolic adaptation to thrive in salty environments. As aforementioned, *Debaryomyces* is the most abundant yeast in dry meat products. We have explored its biocontrol potential to try to limit the use of chemical preservatives in those products. From a previously identified collection of 24 strains of *D. hansenii*, isolated from dry meat products of the “Valle de los Pedroches” (Córdoba, Spain), a screening was carried out to determine which strains had inhibitory potential against a battery of fungi belonging to the genera *Aspergillus*, *Penicillium*, and *Candida*. After a series of general trials, we selected and characterized LRC2 strain. In all conditions, Iberian loins inoculated with LRC2 showed fewer mold population than the non-inoculated ones. In this way, the biocontrol capability of the *D. hansenii* LRC2 strain was tested in laboratory conditions against several molds previously isolated from loins. Results demonstrate that LRC2 volatile compounds production inhibited the activity of unwanted fungi in all conditions studied, strengthening the hypothesis of its potential conservative effect in cured meat products.

## SYMPOSIUM I: DIVERSE CLIMATE CHANGE IMPACTS

### A3

#### SURVIVORS OF THE MONTE: CONSERVATION STATUS AND CURRENT SITUATION OF MAMMALS FACING THE ANTHROPOCENE

Cappa FM

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Humans have brought about changes and impacts on environments and biodiversity. This has led to the recent emergence of the term 'Anthropocene'. This period is characterized by various processes such as greenhouse gas emissions, global warming, habitat fragmentation, and changes in land use, among others. As a consequence of several of these aforementioned processes, we find ourselves in what has been termed 'the sixth mass extinction of species'. However, this is not the greatest of problems; the decrease in abundance in each species, preceding extinction, is what generates significant impacts on current ecosystems. Within the animal kingdom, mammals are one of the most vulnerable groups to these disturbances. Therefore, we set out to identify which species in this group are found in the Argentine Monte ecoregion, their conservation status, and the threats they are exposed to. Based on data obtained from the *Categorización de los mamíferos de Argentina (SAREM 2019)*, we found that the Monte ecoregion is home to at least 100 species of mammals belonging to 6 orders. Of these, 75% of the species do not face conservation issues at the moment. However, 13% of the species fall within the three categories closest to extinction, of which 70% are experiencing population decline. The remaining 12% are categorized as 'insufficient data' due to a lack of scientific studies on these species. Among the 15 threats identified for the mammals of the Monte, 'degradation' is the most frequent, followed by 'hunting' and 'livestock farming'. At the other end are 'restricted habitat', 'isolation', and in second last place, 'specialization' along with 'global warming'. As observed, there are both extrinsic and intrinsic factors affecting conservation. However, the most frequent disturbances are related to what defines the Anthropocene, reinforcing the notion of the negative impact of humans on species. Based on

### A4

#### THERMAL SENSITIVITY TO GLOBAL WARMING OF ANURAN TADPOLES IN THE MONTE DESERT: CURRENT STATE AND FUTURE PROSPECTS

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Global anthropogenic change is causing an increase in average and extreme temperatures (heat waves) at an accelerated rate, which has important ecological implications. In recent years, science has focused on the effects of global warming on organisms. Currently the study trends focus on distribution modeling (macro scale), physiological studies (micro scale) and a combination of both, evaluating and predicting organisms' responses to global warming. As ectotherms, amphibian tadpoles are no exception, and are strongly influenced by environmental thermal changes. Currently, it is postulated that organisms near the tropics will be affected, to a greater extent, due to lower acclimatization capability, impact on higher latitudes will depend on the organisms' phenology and its environment. In this context, we present a compendium of the possible impacts of future global warming on tadpoles in the Monte Desert. We detect that so called conservative scenarios are not realistic enough to determine impacts on amphibian tadpoles and may not be suitable for ectotherms in general. For a better approach other stressors such as commonly used agrochemicals should be included. Therefore, we propose that new efforts to model climate change impact

should be multi-target. Correlative macro climatic models (classic approach) should include physiological traits as well as other environmental variables such as pollutants (pesticides and other chemical agent), changes in humidity, among others. The combination of these data will give us more robust, dynamic and realistic predictive models, which can be used as a solid tools for impact assessment.

## A5

### **THE EFFECT OF GLOBAL WARMING ON THE DISTRIBUTION AND ECOLOGICAL NICHES OF TERRESTRIAL ARTHROPODS: THERMOTOLERANCE AS A POSSIBLE EXPLANATION**

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Temperature affects most biological processes, and therefore is one of the most important abiotic factors, especially for ectothermic animals, such as most arthropods. In this talk, I will present work done in the laboratory, where we showed how physiological characteristics such as thermotolerance can limit the distribution of some terrestrial arthropods' species. These include everything from spiders to insect vectors of diseases and invasive species. First, on spiders of the native Atlantic Forest, we tested the specialization-disturbance hypothesis, which predicts that specialist species will be more affected by disturbances than generalist ones. Our results showed a higher tolerance temperature for species living in young plantations than species living in native forest, regardless of their guild, and supporting the hypothesis. Then, I will present results showing how the geographical distribution of seven species of triatomines, vectors of Chagas disease, can be explained, in part, by their thermo-tolerance range. Finally, physiological results from the highly invasive little fire ant, *Wasmannia auropunctata*, showed a modulation of the Critical thermal maximum (CTmax) and minimum (CTmin) by different acclimation temperatures and by seasonal thermal acclimatization. At local scale CTmin limited their foraging activity, and at the global scale, CTmin constitutes a key physiological trait that, when linked with the minimum temperature of the coldest month, could explain the southernmost limit of its native distribution. An eco-physiological approach helps explain current distribution and predict potential spread of populations when there is no certain information about the whole distribution of the species or how they distributed under a changing environment, for example due to global warming. The latter is particularly of great importance when analyzing invasive insects, pests or disease vectors.

## **LECTURES**

## A6

### **FROM THE LABORATORY TO THE COMMERCIAL PRODUCT... WHICH RECORDS OF TOXICITY SHOULD WE CONSIDER?**

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In recent years, worldwide, the scientific system has begun to place particular emphasis on the so-called "translational research", whose objective is to create a bridge between the results of basic research and its application for the benefit of people and communities. As a definition, its use in the field of scientific research is infinite. Limiting the scope of this definition to the field of natural compounds, particularly to studies carried out on plants, the knowledge provided by basic sciences has great potential for application in the food and pharmaceutical industries; as well as, in its area of intersection called nutraceutical, which refers to the exploitation of the medicinal properties of some edibles or foods. In this recent translational context, it is essential to identify those previous antecedents of relevance and toxicological validity, to establish the priorities of future research. Therefore, located in the basic research carried out on plants, the objective of this presentation is to establish which published scientific studies that allow us to estimate the toxicity of plant compounds for their use as food, biopharmaceuticals or nutraceuticals. In accordance with international regulations, determined by international control agencies, the toxic risk assessment must provide information on the identification of the danger presented by a compound, the evaluation of its exposure, the determined effects due to its dose-response relationship and the characterization of the type of risk (risk quantification). Considering these conceptual contributions, coming from general toxicology, finding antecedents of toxicological validity, among available scientific publications, generally aimed at evaluating and describing biological properties (i.e. antioxidant, antimicrobial, cytotoxic, between others), is often a frustrating challenge. In the selection of published antecedents, the first thing need to be considered is the type of study carried out; thus, epidemiological studies, clinical trials and some laboratory experiments are of interest. Among them, those studies that evaluate the toxic properties of the compounds, specifying the route of

contact, the magnitude and the duration of exposure, will be useful. In each of these cases, the characterization of a dose-response relationship is essential. Then, on based of this information, the relevant toxicological information of a product can be certainly established. Concluding, the compound details at least need to include: effects (i.e. nephrotoxicity, hepatotoxicity, neurotoxicity, mutagenicity, reproductive toxicity), the highest non-toxic doses (NOAEL), the lowest doses at which these effects are observed (LOAEL), potency, margin of safety; as well as the characteristics of the different populations subject to risk (i.e. sick people, children, pregnant women).

## A7

### CHALLENGES OF RURAL EXTENSION IN A CONTEXT OF CLIMATE CHANGE

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Agricultural production and its rural environment have new challenges linked to a growing demand for food, a greater requirement for quality meat, fruits and vegetables, a greater concern about contamination processes and the effects of climate change. 30 years ago, there was talk of sustainability; currently, this term implies projects to produce more and better per surface unit in less time, respecting safety criteria and good agricultural practices. Although, viticulture is the main Argentine fruit activity and San Juan is the second province with the largest cultivated area and it is the one with the highest level of diversification towards the production of must, table grapes and raisins. The grape production is on farms smaller than 10 ha, being a family activity with producers of 62 years on average, tractors from 1970, aging vineyard structures and little investment. Climate change adds new challenges: drought, increase in maximum temperatures, change in the distribution of rainfall, greater incidence of heat waves, increase in hailstorms and frequency of late frosts. All these problems have solutions associated with higher costs and investment. The use of drip irrigation, anti-hail mesh, solar panels are one of the investments that become essential. The use of agroecological criteria proposes a change in traditional agriculture under the reduction of the use of phytosanitary products and fertilizers that reduce soil and water contamination, guaranteeing the safety and security of rural workers, these being the strategies that will lead to the new principles of economic, environmental and social sustainability from a rural extension approach with all its actors.

## A8

### TRIPARTITE PLANT-FUNGAL-INSECT INTERACTIONS AND THEIR AGROECOLOGICAL IMPLICATIONS

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Tripartite interactions involving plants, fungi, and insects play a pivotal role in shaping agroecosystems and fostering agricultural sustainability. These intricate relationships involve communication and resource exchange, and fungal entomopathogens (ETP) like *Metarhizium* spp. have gained attention for their potential to enhance biological pest control, disease resistance, and nutrient uptake in plants, ultimately impacting crop health and yield. This study delved into the role of native ETP strains in grapevine (*Vitis vinifera*) cultivation, focusing on their ability to control pests, influence plant physiology, and improve crop health. *Metarhizium* strains were isolated from agricultural soils in San Juan, Argentina. Three promising strains (CEP413, CEP589, and CEP591) were selected based on their physiological capabilities. These 3 strains demonstrated excellent control potential over larval (75%), pupal (99.9%), and adult (85%) stages of *Lobesia botrana* (the European grapevine moth) in laboratory trials. CEP591 stood out for its superior efficacy in controlling *L. botrana* resulting in a remarkable mortality rate. Consequently, CEP591 was chosen for field trials targeting *L. botrana* larvae. The results revealed that larvae remained susceptible to ETP infection across all seasons, with heightened susceptibility observed in early spring (91%) and early summer (81.5%). In addition to pest control, the study examined the impact of ETP strains on the physiological traits of *V. vinifera* plants. Plants inoculated with ETP strains displayed notable differences compared to the control group, including a higher leaf count (34 leaves) and increased plant height (55 cm) compared to control plants (26 leaves and 48 cm, respectively). Furthermore, ETP inoculation led to heightened pigment content in grapevine leaves, with significant increases in chlorophyll a ( $F=15.99$ ;  $p<0.0001$ ), chlorophyll b ( $F=4.59$ ;  $p<0.05$ ), and carotenoids ( $F=13.97$ ;  $p<0.005$ ). Biomass production was also assessed, with ETP-treated plants exhibiting significantly greater above-ground and root biomass compared to control plants ( $F=5.35$ ;  $p<0.005$ ). Leaf area and leaf mass per area (LMA) were likewise affected, with ETP-treated plants displaying larger leaf areas and favorable LMA ratios. Moreover, carbon-to-nitrogen (C/N) ratio analysis revealed significant differences between ETP-treated plants and control plants. ETP-treated plants exhibited higher carbon and nitrogen content, suggesting potential alterations in nutrient assimilation and allocation. In summary, this study underscores the promising potential of native ETP strains, particularly CEP591, in

enhancing pest control, promoting plant growth, and improving physiological traits in grapevine cultivation. These findings emphasize the importance of tripartite interactions in agroecosystems and highlight the potential for sustainable agricultural practices that reduce reliance on chemical compounds.

## A9

### SUSTAINABLE USE OF WASTE FROM THE OLIVE INDUSTRY IN NORTHWEST ARGENTINA: CHARACTERIZATION AND BIOLOGICAL ACTIVITIES

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The olive oil industry generates a residue known as "alperujo," which is considered phytotoxic due to its high phenol content. However, these compounds have a wide range of applications, including potential health benefits. Thus, we conducted an extensive study on the hydroalcoholic extract of alperujo, investigating its chemical composition, antioxidant properties, antipathogenic and inhibitory enzyme activities, ecotoxicity, and acute toxicity. In the quantitative analysis, we found that the total phenolic content was  $30.4 \pm 2.8$  mg GAE/g DW (Gallic acid equivalent). Through HPLC-DAD analysis, we identified 29 phenolic compounds, with the most prominent ones including: kaempferol-3-O-glucoside>4,5-di-O-caffeoylquinic acid>caftaric acid>rutin>gallic acid>isorhamnetin-3-O-glucoside> myricetin>ellagic acid>catechin>quercetin-3-O-galactoside. Furthermore, the alperujo extract exhibited significant total antioxidant capacity, with a value of  $62.1 \pm 1.1$  mg AAE/g DW (Ascorbic acid equivalent). It demonstrated the ability to scavenge ABTS<sup>•+</sup> ( $IC_{50}=64.7 \pm 0.6$  µg/ml), superoxide anion ( $IC_{50}=551.8 \pm 64.7$  µg/ml), nitric oxide ( $IC_{50}=482.6 \pm 8.9$  µg/ml), hypochlorite ( $IC_{50}=18.7 \pm 1.4$  µg/ml), also efficiently reduced Fe<sup>3+</sup> ions ( $RC_{50}= 88.0 \pm 1.7$  µg/ml) and Cu<sup>2+</sup> ions ( $29.8 \pm 2.0$  mg GAE/g DW). Additionally, it displayed iron-chelating abilities ( $CC_{50}= 574.7 \pm 2.5$  µg/ml) and inhibited the proinflammatory enzyme lipoxygenase ( $IC_{50}= 419.4 \pm 3.5$  µg/ml). In toxicity tests, the alperujo extract demonstrated no toxicity at the maximum tested concentration (1000 µg/ml) in the acute toxicity test using the crustacean *Artemia salina* and the ecotoxicity test involving *Lemna minor* and *Armadidium vulgare*. However, in the *Bacillus subtilis* test (another ecotoxicity model organism), a concentration of 1000 µg/ml inhibited bacterial growth by 60%, indicating toxicity toward bacteria. Additionally, the extract significantly reduced biofilm formation by *Staphylococcus aureus* y *Pseudomonas aeruginosa* strains and disrupted a previously formed biofilm by reducing biomass and cellular metabolism. Furthermore, it exhibited Quorum sensing (QS) inhibitory activity against *Chromobacterium violaceum*, limited the spread of *P. aeruginosa* by restricting swarming and swimming movements, and interfered with other QS-controlled phenotypes such as elastase and pyocyanin. The diverse array of phenolic metabolites found in the alperujo extract, coupled with its beneficial biological activities, suggests its potential to promote human health.

## A10

### SPICES AS A NATURAL ANTIPATHOGENIC ALTERNATIVE

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Bacterial biofilms pose a challenge in both the food industry and the healthcare sector. The issue lies in the fact that bacteria that thrive in this lifestyle are much more resistant than those in a planktonic state. Consequently, traditional control methods have become ineffective in combating the development of pathogenic microorganisms. Currently, there is a trend towards embracing natural solutions to combat pathogens. Therefore, this study assessed the biological activity against *E. coli*, *S. enterica*, *P. aeruginosa*, and *S. aureus* of spices—coriander, bay laurel, and paprika—cultivated in Argentina. This research demonstrated the non-toxic ability of coriander, bay laurel, and paprika extracts to reduce biofilm biomass formation, inhibit cell viability in pre-formed biofilms, attenuate the swarming motility of the studied pathogens, and interfere with the *Quorum Sensing* (QS) intercellular communication mechanism. Consequently, these spice extracts offer an alternative for controlling food contamination and/or deterioration, as well as infectious diseases, by targeting both Gram-positive and Gram-negative bacterial biofilms. It is worth noting that the most significant activity observed was anti-biofilm, both in preventing biofilm formation and controlling pre-existing biofilms. The hexane and chloroform extracts were particularly active, especially against *S. aureus*. The determination of the chemical composition of the most active extracts revealed the predominance of fatty acids such as linoleic, hexadecanoic, oleic and linolenic acids with recognized anti-biofilm activity. Taken together, these results demonstrate that these three Argentine spices have added value beyond their use as seasonings and hold potential for biotechnological applications. The potential application of these extracts or their



derived compounds as anti-biofilm agents or for generating anti-adhesive surfaces, in both the food industry and the clinical field, shows promise.

## **SYMPOSIUM II. EDUCATION: INTERDICTING NATURAL SCIENCES TEACHING**

**Chair: Mg. María de los Ángeles Bizzio**

### **A11**

#### **TEACHING AND LEARNING OF NATURAL SCIENCES: THE CHALLENGE OF INCLUSIVE EDUCATION**

*Guirado Ana María*

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Education is considered to have a major and influential role in the development of society, being a fundamental human right to overcome inequalities and to ensure sustainable development. In this sense, it is relevant to promote Inclusive Education, where diversity is an opportunity to promote participation, learning and autonomy for everyone, paying special attention to those groups whose right to quality education has traditionally been violated. Inclusive Education is a principle that values the well-being of students, respects their inherent dignity and autonomy, and recognizes the needs of individuals and their effective capacity to be included in and contribute to society. It is presented as an overcoming instance that guarantees rights from a curricular proposal that is open, flexible, decentralized, revisable and adaptable according to contexts and groups in response to the learners' needs with mobile times and spaces. This implies generating contextualized curricular proposals and institutional conditions that guarantee the learning of all students based on the recognition of their uniqueness and capabilities. Consequently, it is necessary to promote learning processes in the different fields of knowledge, where the teaching of scientific knowledge occupies a central place in the formation of critical and autonomous citizens. In this regard, the teaching of Natural Sciences is a valuable area and a priority for everyone. Learning sciences requires students to have opportunities to inquire, to question, to hypothesize, to predict, to propose explanations, to confront and analyze information from different sources about the natural world around them. For this, it is necessary to think about specific, intentional and planned teaching that allows subjects to appropriate knowledge in a creative way. It also requires reviewing and reflecting on the teaching practices that are developed in the classroom, seeking to overcome traditional methodologies based on the memorization of data, focused only on content, and orienting them towards models that privilege cognitive processes and skills and the resolution of meaningful problems for students. Therefore, the teaching and learning of Natural Sciences has the challenge of promoting educational practices that guarantee an Inclusive Education as the right to an education for everyone.

### **A12**

#### **WHY, WHEN AND HOW DO WE TAKE CARE OF READING AND WRITING WHEN WE TEACH NATURAL SCIENCES?**

*Maturano CI*

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This presentation addresses questions related to reading and writing in Natural Sciences classes. The starting point is to consider the results of numerous investigations that report students' difficulties in reading and writing science texts, to which is added a lack of specific training of teachers and future teachers in this disciplinary area to accompany and guide these processes. The theoretical framework used adhere to the teaching of reading and writing in the disciplines, and is framed in Systemic-Functional Linguistics and teaching models that promote the construction of students' knowledge in the social context of the classroom. A way of working, that emerged from an interdisciplinary team, that combines knowledge about language, the discursive organization of texts from Linguistics, and disciplinary content from each specific field is presented. Firstly, why to deal with language in science classes is considered, assuming that reading and writing are tools for teaching and learning and that the ways of reading and writing are different in each disciplinary area. From this, it would be the science teacher the adequate person to be in charge of mediating these processes in the classroom. Secondly, the question of when it is appropriate to accompany these processes is addressed, considering that reading and writing should be guided while the contents are taught. This involves establishing didactic connections between the disciplinary content that is taught and learned in Natural Sciences and the reading and writing strategies that should be implemented to achieve such educational objectives. Finally, in third place, ideas about how the disciplinary teacher can develop

proposals to support reading and writing are raised. This involves planning the approach, selecting the texts to read or produce according to the disciplinary content, assessing possible comprehension difficulties, considering the discursive forms or genres with which the students will work, making explicit the key linguistic features of the discursive genres, analyzing the semiotic unfolding of multimodal resources that combine verbal, mathematical and visual-graphic resources, posing instructions to guide the tasks that will be proposed to students and designing ways to mediate and provide feedback to these processes according to the educational situation. In conclusion, the need to reflect on the insertion of reading and writing in the disciplines and to generate specific actions to train teachers to develop and implement classroom proposals that explicitly contribute to understanding, written production and learning is highlighted.

### **A13**

## **EXPERIMENTAL ACTIVITIES IN THE TEACHING OF NATURAL SCIENCES**

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The implementation of experimental activities in the teaching of Natural Sciences in general, and Biology in particular, plays a crucial role in the development of essential skills for students to construct and understand different types of content. At the same time, they are also related to the development of scientific vocations and the creation of work habits, which allow students to build confidence in their ability to face various problematic situations. In general terms, some authors agree that experimental practices are activities carried out by students, although with different degrees of involvement in their design and execution. This variation in student participation in activities is associated, among other factors, with the didactic model underlying these practices. It has been shown that more constructivist didactic models of learning. Furthermore, as teachers transmit their perspectives through their speech and practical actions, which are based on these didactic models, each model influences the construction of a specific image of science in students. It is worth noting that shaping this image of science is a relevant issue in both school teaching and learning, since questions related to what science is, how it is constructed, and what it entails are important aspects of scientific literacy. Furthermore, it is important to highlight that, in some cases, the teacher's discourse and teaching practices may not necessarily align with the same teaching model, potentially leading to different images of science. In different contexts, it has been observed that, while some teachers declaratively adhere to more humanistic and contextual perspectives on understanding scientific disciplines, their classroom practices may align with a more elitist and dogmatic conception of science. In such cases, this implicit knowledge could result in laboratory practices with highly structured formats that do not accurately represent scientific activities and do not allow the development of certain skills associated with experimental work. For these reasons, it is crucial to emphasize the need for coherence between speech and action. In summary, experimental activities are a valuable tool for teaching Natural Sciences, but their effectiveness and impact on students depend largely on the underlying didactic model, the image of science associated with that model, and the coherence between the discourse and the teacher's action. Understanding and addressing these dimensions is essential to promoting sound and coherent science education.

## **SYMPOSIUM III. INVITED BIOLOGY SOCIETES**

### **SOCIEDAD DE BIOLOGÍA DE ROSARIO**

**A14**

## **TUBERCULOSIS IN THE TYPE 2 DIABETES MELLITUS CONTEXT, IMMUNE-ENDOCRINE-METABOLIC ALTERATIONS THAT CHARACTERIZE ITS MANIFESTATIONS**

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Lung tuberculosis (TB) is one of the main world public health problems, given that a quarter of the world population would be infected by its etiological agent: *M. tuberculosis* (Mtb). Comorbidity TB- type 2 diabetes mellitus (TB-DBT) has gained relevance in the last decades due to

the increase of the diabetic population, with the highest increases in low- and middle-income countries, where TB is prevalent. Both pathologies present alterations in the two ways communication between the immune system (IS) and the neuroendocrine system with important repercussion on the GCs activity and the metabolism (PPARs) in patients with the comorbidity, and it was compared to voluntary TB patients, DBT and HCo patients. Using blood samples several parameters were analyzed: plasma levels of proinflammatory and anti-inflammatory cytokines (IL-6, IFN- $\gamma$ , IL10), cortisol, DHEA, DHEA-S, GH, prolactin, leptin, adiponectin. CMP specific blastogenic capacity was analyzed and GR $\alpha$ , GR $\beta$ , 11 $\beta$ HSD1, 11 $\beta$ HSD2, PPAR $\alpha$  and PPAR $\gamma$  transcript were quantified. Every TB patient showed higher levels of cortisol, GH and proinflammatory mediators compared to HCo, such as PPAR $\gamma$ . TB+DBT showed even higher results than TB in IFN- $\gamma$ , GH, DBT, IL-6, CRP, cortisol, GH and PPAR $\gamma$ , while IL-10 showed an increment in TB+DBT as well as in DBT. DHEA-S levels decreased in every patient group, differing TB+DBT from DBT. Leptin decreased in both TB groups even more in the patients without comorbidity, that also showed the lowest body mass. Opposite to this, CMP of TB+DBT showed a high proliferative capacity, HCo differing from TB. These patients CMP did not show alterations in the GCs receptor isoforms relation and also showed higher cortisol availability against the 11 $\beta$ HSD1 high level found in this patients' cells. Nevertheless, this potential inhibitory action, in the unbalanced immune-endocrine-metabolic context of these comorbidity patients would not turn off the exacerbated response. Altogether, the analysis of the immune-endocrine-metabolic variables showed that the deregulation in DBT patients was even higher in those with comorbidity, where the proinflammatory response due to the infection was superior to the one developed in TB only patients.

## SOCIEDAD DE BIOLOGÍA DE TUCUMÁN

### A15

#### **“BETA THALASSEMIA MINOR AND FERROPTOSIS: NEW FINDINGS IN THE PATHOPHYSIOLOGY OF A SILENT ANEMIA”.**

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The  $\beta$ -thalassemia trait (BTT) corresponds to a heterogeneous group of genetic defects that lead to decreased  $\beta$ -globin production causing microcytic and hypochromic anemia and reduced amounts of hemoglobin A (HbA). The decrease or absence of the number of  $\beta$ -chains produces a relative excess of free alpha ( $\alpha$ ) chains that form aggregates that autoxidize, denature and precipitate as hemichromes in both mature red blood cells and thalassemic erythroblasts. As a result, reactive oxygen species (ROS), free iron and heme are produced.

ROS, which are also produced from free iron by the Fenton reaction, oxidize lipids, nucleic acids and cellular proteins. Existing evidence strongly suggests that excess free  $\alpha$ -globin chains are the direct cause of hemolysis and ineffective erythropoiesis, which are the two main pathophysiological mechanisms causing anemia in  $\beta$ -thalassemic patients. Oxidative stress (EOx) plays a fundamental role in the behavior and severity of anemia in subjects with BTT.

In the face of a basal redox imbalance, the scavenger systems react in an attempt to mitigate the damage caused by highly reactive and toxic radicals. The compensatory cytoprotective response occurs at different levels in the organism: systemic, thanks to the action of antioxidant enzymes that share a common microenvironment, where different cell types coexist, capable of converting highly reactive and toxic compounds into more innocuous ones. The transcriptional response is characterized by increased expression of genes crucial in the maintenance of redox homeostasis and at the post-transcriptional level, proteins involved in pathophysiological mechanisms important in the development of anemia in BTT patients are released.

To elucidate more clearly the mechanisms involved in the processes of erythroid survival/senescence in BTT, we focused our attention on the study of the behavior of erythroid membrane proteins.

The analysis of membrane proteins has been favored in recent times thanks to advances in functional genomics and the study of the transcriptome and proteome. This allowed a global approach in the identification of proteins and the control of their expression.

In the case of BTT, which is a pathology that mainly affects the erythroid series, the absence of the nucleus in the mature erythroid places proteomic analysis as a fundamental tool for the study of the cytoprotective response in this cell type.

Our advances provide further evidence of ferroptosis as a mechanism of cell death in BTT erythrocytes, and highlight its contribution to the adaptive mechanisms against EOx in this pathology. These findings suggest the possibility of developing personalized strategies for individuals with BTT in situations such as growth, pregnancy or high performance sports practice, which would represent an innovation in the clinical approach to this hereditary anemia.

## SOCIEDAD DE BIOLOGÍA DE CÓRDOBA

### A16

#### SEXUAL DIMORPHISM IN BLOOD PRESSURE REGULATION AND BODY FLUID HOMEOSTASIS

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Clinical and experimental studies indicate that sex matters when it comes to blood pressure regulation, rates of cardiovascular disease, symptoms and risk factors. Although awareness of sex differences in cardiovascular disease is increasing, much of what we know about blood pressure regulation has been derived from studies in males. However, principles learned in male models do not necessarily apply to females, and this addresses the importance of studying in more detail the sources of physiological disparity between sexes.

Angiotensin and vasopressin differentially modulate hydroelectrolyte and cardiovascular responses in male and female. But, are the hormonal factors the only to blame for such differences? Sex steroids can induce organizational (long-lasting or permanent) effect during critical periods of development but can also impart (temporary or reversible) activational effects. Furthermore, males and females differ not only in their sex (males are born with testes- and females with ovaries-hormonal factors) but also carry different sex chromosome complements (XY and XX respectively), and thus are influenced throughout life by different genomes.

In this symposium, we will discuss our studies in which we have dissected the contribution of the sex chromosome complement factor, organizational and activational hormonal effects on sex-related differences in angiotensinergic and vasopressinergic involvement in blood pressure regulation and body fluid homeostasis.

## SOCIEDAD ARGENTINA DE BIOLOGÍA

### A17

#### GENE TARGET DISCOVERY WITH NETWORK ANALYSIS IN *Toxoplasma gondii*

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Toxoplasmosis is a zoonotic disease that affects almost one third of the human population. This condition is caused by the obligate intracellular parasite *Toxoplasma gondii*, which transits a complex life cycle. It develops an asexual phase in mammals and birds where the parasite can adopt a latent and encysted form in the host; finally sexual phase begins when latent form is ingested by members of the *felidae* family, the definitive host. Thus, the passage through the different life cycle stages allows the pathogen to adapt to diverse contexts by modulating its virulence and pathogenic potential. While the stages of the biological cycle of *T. gondii* are well known, the molecular mechanisms that regulate the transitions between them are not completely understood. In this sense, several systematic approaches have been implemented which are based on the application of high-throughput technologies (HTTs) generating a great amount of new biological data. Given the range of experimental conditions and the typical performance of these techniques, a new challenge arises: organize and analyze resulting information from new technologies in a coherent framework. Here we integrate the available transcriptomics data for the *T. gondii* cycle in a systems biology approach with the aim of reconstructing the gene regulatory network (GRN) that explains parasites' life cycle. The GRN is a dynamic system whose states are determined by the expression levels of each gene or groups of genes (nodes), while the edges, or links, between nodes represent regulatory interactions. Once reconstructed it is possible to address a number of different biological and biomedical questions such the study of phenotypes related to health conditions, the development of new therapies and the design of perturbation experiments. Exploring the reconstructed GRN for *T. gondii* we were able to predict genes as master key regulators involved in the life cycle. Additionally we proposed *in silico* perturbation experiments that could be useful for predicting cell destiny with the aim of searching targets for further experimental validation at knockout protocols. On the other hand, our bioinformatics analysis of the network led us to assign biological processes and functions to uncharacterized genes with focus on pathogenic and virulence factors. In this context, our analysis led us to propose new genes for diagnosis, some of which were experimentally validated for human toxoplasmosis. In conclusion, we present a new computational tool for predicting new gene targets of study in the context of toxoplasmosis diagnosis, that can integrate available transcriptomics for the parasite cell at different contexts.

## **SOCIEDAD DE BIOLOGÍA DE CUYO**

### **A18**

#### **HYPERTENSION A PREVALENT AND SILENT DISEASE: NEW PERSPECTIVES IN TREATMENT**

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Hypertension is a chronic disease that affects over 1.28 billion people worldwide, making it the leading cause of premature death. Despite significant advances in diagnosis and treatment, the number of people with the disease continues to rise. This fact is due to many factors, including population ageing, lack of adherence to treatment, the rise of obesity and sedentary lifestyles, and the prevalence of risk factors such as diabetes and chronic kidney disease. In recent years, new strategies have been developed to address hypertension. These include the use of nanotechnology to improve drug delivery and theragnosis, the development of new drugs that target the molecular mechanisms of the disease, and the move towards a more holistic treatment that considers the psychological and environmental factors that contribute to the disease. The new perspectives for the treatment of hypertension include: The development of new drugs that are more effective and have fewer side effects. The use of nanotechnology to improve the delivery of drugs to affected tissues. The development of new diagnostic and prognostic techniques to identify people at higher risk of complications. The development of more effective prevention and treatment programs considering the psychological and environmental factors contributing to the disease. Specific Ideas Projections: It is estimated that the prevalence of hypertension will increase in the coming years due to population ageing and lifestyle changes. Diagnosis: New non-invasive diagnostic techniques, such as optical coherence tomography, are being developed, which could help detect hypertension in its early stages. Prognosis: New biomarkers are being developed that could help to predict the risk of complications from hypertension. Prevention: New prevention strategies, such as dietary modification and exercise, are being investigated. Treatment: More recent agents in development are currently being studied in clinical trials, including dual endothelin receptor antagonists and aldosterone synthase inhibitors that are effective. Incorporating other approved drugs into treatment paradigms, including SGLT2 inhibitors and non-steroidal mineralocorticoids, such as finerenone, is also necessary. Finally, hypertension is a preventable and treatable disease. The new perspectives for the treatment of hypertension offer the opportunity to improve disease control and reduce the risk of complications.

## **CLOSING LECTURE**

### **A19**

#### **EXPLORATION OF THE ARGENTINIAN FLORA CHEMODIVERSITY IN THE SEARCH OF METABOLITES AS NEW LEADS IN DRUG DISCOVERY**

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Plants are an excellent reservoir of biologically active compounds with a significant contribution to cancer and microbial chemotherapy. The great biodiversity of existing plants results in a wide variety of secondary metabolites which enjoy the presence of varied pharmacophores and a high degree of stereochemistry that give them high possibilities of emerging as prominent therapeutic agents. The need of alternative drugs, particularly for the treatment of pathologies resistant to clinical therapies, encourages the search for new substances in the plant world. This bioprospection becomes even more relevant and novel when it involves species from the low explored Argentinian flora.

As part of our continuing search for agents to treat cancer and microbial infections, a panel of 170 extracts from mostly native plants from Argentina and the bioactive metabolites obtained from these, were screened in order to determine their cytotoxic and multidrug-resistance (MDR) reversing potential against sensitive and P-gp-overexpressing cell lines. In addition, their inhibitory effect against efflux transporters expressed in *Candida* species was evaluated, as well as their inhibitory activity on growth of sensitive and resistant pathogenic bacteria. The most effective principles were further studied in view of determining their modes of action. Derivatives of these metabolites were designed and synthesized showing improved activity in comparison to the structural leads. These findings provide important evidence that the identified

compounds may be a promising entities to be further investigated to develop agents able to overcome MDR and to treat resistant tumors and emerging infections.

## **SHORT COMMUNICATIONS**

### **GENERAL, CELLULAR AND MOLECULAR BIOLOGY**

#### **A20**

### **CLONING, OVEREXPRESSION AND PURIFICATION OF PBSs ENZYMES OF *Brucella abortus***

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Bacteria of the *Brucella* genus are the causative agents of brucellosis, a widely distributed zoonotic disease that affects a variety of animal species. This disease can lead to abortions and infertility, causing significant economic losses due to reduced livestock productivity. *Brucella* spp. can be transmitted to humans through contact with infected animals or the consumption of products derived from them, resulting in a debilitating febrile illness. Without proper treatment, it can become chronic, leading to complications such as spondylitis, endocarditis, and encephalitis. These bacteria are facultative intracellular pathogens, and the main structural component of their cell wall is a peptidoglycan (PG) polymer consisting of oligosaccharide chains. These chains contain a periodic disaccharide motif of N-acetylglucosamine (NAG) and N-acetylmuramic (NAM) linked together through attached peptide chains. The complete assembly of PG is a complex process involving approximately 20 enzymatic reactions that occur in both the cytoplasm and the interior and exterior of the cytoplasmic membrane. Key reactions include the activity of a glycosyltransferase (GTase), which polymerizes the oligosaccharide chains, and a transpeptidase (TPase), which catalyzes peptide cross-linking. These activities are performed by Penicillin-Binding Proteins (PBPs). A collaboration was established for the structural characterization of the enzymes involved in the formation of *Brucella* spp.'s PG, including *pbp-2*, *pbp-1B*, *muaA*, *murD*, *murE*, and *murF*. While the Mur proteins were successfully purified, the PBP proteins formed inclusion bodies. In this project, we aim to clone the PBPs into expression vectors that incorporate a solubility tag or facilitate their export to the bacterial periplasm. This approach will allow us to obtain soluble fractions of these proteins, enabling their proper folding and purification.

#### **A21**

### **AN OPTIMIZED PROTOCOL FOR ANGIOTENSIN-CONVERTING ENZYME INSERTION/DELETION POLYMORPHISM GENOTYPING**

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Hypertension (HTA) is known as a chronic and polygenic disease that affects more than 30% of the world's adult population. Several candidate genes involved in blood pressure alteration have been proposed. Among these, we focused on the angiotensin-converting enzyme (ACE) gene. Previous studies have addressed the potential relevance of the ACE-I/D polymorphism in the etiology of HTA, but the results have been inconsistent. In humans, the ACE gene is located on chromosome 17q23. The most commonly identified polymorphism is the insertion or deletion (I/D) of 287-pb Alu repeat sequence within intron 16, resulting in three genotypes (DD and II homozygotes and ID heterozygotes). In this study, we investigated the ACE polymorphism in 397 subjects, 230 hypertensive patients (HTA group) and 167 healthy individuals (control group). Genomic DNA from peripheral blood leukocytes was isolated, and ACE I/D genotyping was performed by Polymerase Chain Reaction (PCR). Mistyping of ID heterozygotes as D homozygotes may occur due to the preferential amplification of the D allele and inefficiency in the amplification of the I allele. To increase the specificity of DD genotyping, a second independent PCR amplification was performed with a primer pair that recognizes an insertion-specific sequence. Thereby, the second PCR was performed for samples with DD genotype. Among the total samples (397), 110 samples were genotyped as D/D homozygotes with the first PCR. The re-evaluation with a second PCR showed an erroneous classification of I/D heterozygotes as D/D homozygotes in 10 % of these samples.



Significant ACE I/D genotype differences between HTA and control groups were found. Our results provided evidence that, by using this optimized protocol, the DD mistyping was decreased. Therefore, to avoid a mistyping of ID heterozygotes as DD homozygotes, all homozygous for D have to be re-evaluated through a second independent PCR to identify possible insertion alleles. In conclusion, to understand the pathogenesis of HTA and its related complications, deep insight into the physiological and genetic aspects of ACE polymorphisms is necessary.

## A22

### SUBCELLULAR DISTRIBUTION OF ARP2/3 AND TUBULIN IN SEMINIFEROUS TUBULES IN NEW ZEALAND RABBITS ON A HIGH-FAT DIET

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Numerous investigations have delved into the impact of high-fat diets (HFD), obesity, and related metabolic issues like dyslipidemia on male fertility. However, the specific influence of HFD on the testes remains largely unexplored. Previous studies on rabbits fed HFD revealed irregularities in sperm development, including asymmetries in acrosome formation and cell elongation, increased cellular cholesterol, and abnormal nuclear condensation. The final shape of spermatozoa hinges on a complex series of steps that involve actin filaments (acroplaxome) and microtubules (manchette) anchored to plasma membranes. Therefore, it is hypothesized that the changes in shape may be correlated with the abnormal distribution of actin-associated proteins (ARP) or irregular tubulin distribution, probably due to elevated membrane cholesterol. The study aimed to examine Actin-Related Proteins 2/3 (ARP2/3) distribution in germ cells of New Zealand rabbits under a high-fat diet. Two groups were studied: one on a balanced diet (CD group) and the other with 14% bovine fat supplementation (HFD group). ARP2/3 and tubulin expression was assessed after 12 months using indirect immunofluorescence (IIF) and a confocal microscope, focusing on seminiferous tubules and considering spermatogenic cycle stages. Our findings revealed distinct protein distribution patterns in the HFD group, particularly in round spermatids, when compared to the CD group. Additionally, variations in Arp 2/3 and tubulin localization were observed across different stages of the seminiferous epithelium. Despite quantifying mean fluorescence intensity (MFI) expression, no statistically significant differences were identified. Thus, we conclude that diet influences the arrangement of ARP2/3 and tubulin in germ cells without impacting their overall expression levels. This association may be related to previously documented sperm malformation within our research group.

## A23

### RADIOPROTECTION OF VASCULAR ENDOTHELIUM IN CANCER TREATMENT: EVALUATION OF EXPERIMENTAL INHIBITORS OF NADPH OXIDASES

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The primary challenge of oncological radiotherapy is to maximize damage to cancerous tissue while minimizing harm to healthy tissue. One of the healthy tissues particularly sensitive to radiation and substantially affected by ionizing radiation (IR) is the vascular endothelium (VE). Reactive Oxygen Species (ROS) play a crucial role in radiation-induced vascular damage. Over time after irradiation, high levels of ROS have persistently been observed to continue damaging DNA regardless of the initial stimulus. This indicates the presence of an endogenous source of ROS that generates chronic oxidative stress. One of the primary contributors to endogenous ROS is the activity of NADPH oxidases (NOX). Based on the aforementioned, we raise the question of whether selective inhibition of NOX could reverse the damage to the vascular endothelium (VE) caused by ionizing radiation (IR). Human umbilical vein endothelial cells (HUVECs) were irradiated with escalating doses of X-rays (0, 4 and 8 Gy). Superoxide generation was measured by fluorescence techniques using 10 uM dihydroethidium (DHE) and flow cytometry. We found that the fluorescence intensity of DHE was significantly and sustained increased after 1, 3 and 6 days after irradiation in a dose response manner (p<0.01). Then, endothelial cells were treated with specific NOX inhibitors, subsequently irradiated with X rays and cell viability (MTT assay) and DNA damage ( $\gamma$ H2Ax foci formation) were measured. NOX inhibitor did not reduced DNA damage but the effect of radiation on cell viability was reverted (p<0.01). While further studies are required to elucidate the underlying mechanisms, the results obtained suggest that NOX-specific inhibitors could serve as radioprotective agents for the VE.

## A24

### THE INHIBITION OF ATR INCREASES CISPLATIN SENSITIVITY IN MISMATCH REPAIR-DEFICIENT COLON TUMOR CELLS

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Hereditary Nonpolyposis Colorectal Cancer is characterized by Mismatch repair (MMR) deficiency which is associated with cisplatin (cPt) resistance. cPt is a chemotherapeutic drug widely used to treat several types of human tumors. The activation of the ATR/CHK1 signaling pathway is critical for cPt-induced DNA damage response (DDR). It plays a crucial role in regulating cell cycle progression, promoting DNA repair, and triggering apoptosis. Previously we demonstrated that Hsp27 interacts with DNA MMR proteins in human colon cancer cells. Hsp27 is a molecular chaperone overexpressed in many tumor types, plays a crucial antiapoptotic role, and has been associated with cPt resistance. Accordingly, the ATR pathway and Hsp27 have become attractive therapeutic targets. This study aims to investigate ATR/CHK1 pathway and Hsp27 roles in cPt-exposed human colon cancer cell lines: HCT116+ch2 (MMR deficient, MMR-) and HCT116+ch3 (MMR proficient, MMR+). ATR was inhibited by VE-821 (VE) and Hsp27 was downregulated with OGX-427 before cPt treatment. Cells were treated with 10  $\mu$ M cPt for 24 h and collected at time 0 (immediately after cPt treatment, T0), 3 (T3), 9 (T9) and 24 (T24) h post-treatment. Cellular viability was determined by CCK8 and the expression of pCHK1 (Ser345),  $\gamma$ H2AX (Ser139, marker of DNA double-strand breaks), and Hsp27 was analyzed by Western blot. Combined therapy with cPt+VE significantly reduced cell viability, especially in MMR- cells ( $p < 0.01$ ). Conversely, MMR+ cells were more sensitive to cPt+OGX-427 owing to the more pronounced reduction in cell viability after treatment. Interestingly, cPt+VE augmented  $\gamma$ H2AX expression in both cell lines (T0), but it decreased during recovery ( $p < 0.01$ ). Cleaved PARP1 and activated caspase-3 were upregulated by cPt+VE, particularly in MMR+ cells ( $p < 0.01$ ). However, senescence was induced (T9,  $p < 0.001$ ) particularly in MMR- cells. In conclusion, ATR modulation through VE inhibitor combined with cPt could be a promising tool to improve cPt-chemosensitivity in MMR deficient tumors.

## A25

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

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Chagas's disease is an anthroponosis 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 vaccines. 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 crystallography 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 complementary bioinformatics tools. Homology models of both proteins have been built using the Modeller program and other 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 satisfactorily overexpressed and purified for crystallographic assays. Recently, TcAdK1 crystals were obtained under different conditions. These results will allow us to advance in crystallographic studies.



## A26

### CHARACTERIZATION OF INTERACTION REGIONS OF THE *Trypanosoma brucei* RIBOSOMAL PROTEIN Tbl19

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*Trypanosoma brucei* is the cause of Sleeping Sickness or African Trypanosomiasis, and it is estimated that it risk 3,000,000 people in Sub-Saharan Africa with a high rate of morbidity. Currently there are no effective treatments against these parasites and approved drugs employees: Eflornithine, Pentamidine, Suramin, Nifurtimox and Melarsoprol for *T. brucei* possess high toxicity with severe side effects and in some cases the development of endurance. For this and other neglected tropical diseases (WHO-CDC), the investment in the development of new drugs and therapies is much lower since it affects populations in the most vulnerable regions, poor of the world. For this reason, the structural and molecular study of potential targets for the design of new drugs is of great relevance. Trypanosomatids diverged early from the rest of the eukaryotes, and several components of their protein synthesis machinery developed differences that gave rise to unique domains. By studying these differences, we found that the *T. brucei* ribosomal protein Tbl19 has differential domains at its C-Terminal end that establish new interactions between the large subunit and the small subunit of the ribosomes. To analyze the region that would be involved in the interaction between Tbl19 with another ribosomal region, we selected the area to explore from the *T. brucei* structure (4V8M) and compared it with the *S. cerevisiae* structure (4V88), both resolved by cryo electron microscopy. We observed a high amino acid sequence identity for the compared sequences up to residue 170, from here on the difference in the length of this protein is due to specific extensions present in trypanosomatids. Several interactions were detected using PDBePISA (EMBL-EBI) (Krissinel & Henrick, 2007), including six hydrogen bonds (HBs) located between residues 166 to 183 in the C terminus of Tbl19 and position 945 to 996 in rRNA. 18S and a salt bridge (SB) formed by residue His4 of the Tbs7 protein and Asp 192 of Tbl19. In addition to the evolutionary divergence, the low sequence identity of these differential structures and the new interactions they generate could be considered as a target for new antiparasitic drugs, since these extensions are specific for *Trypanosoma brucei* and its close relative *Trypanosoma cruzi*.

## A27

### BIOLOGICAL ACTIVITY FROM EXTRACTS OF *Baccharis spartioides*, *Heliantus petiolaris* AND *Calendula officinalis*

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Essential oils and plant extracts cover a wide spectrum of pharmacological effects showing various properties such as anti-inflammatory, antioxidant and anti-cancer. Other biological activities are reported as biocides against a wide range of microorganisms such as bacteria, fungi, viruses, protozoans, insects and plants. In this work, bioassays were carried out under laboratory conditions against *Daphnia magna* (to evaluate toxicity) and *Fusarium oxysporum* (to evaluate antifungal activity). The evaluation of the toxicity of ethanolic extracts of *Calendula officinalis*, *Baccharis spartioides* and *Heliantus petiolaris* was carried out at different concentrations, using potassium dichromate, as a reference toxicant. Acute tests of 48 h duration were carried out. 10 specimens of *Daphnia magna* (less than 24 hours old - neonates) are selected for each concentration, placing one specimen in each chamber, without changing the medium and without feeding. Observations were made every 12 hours. Immobility or death were demonstrated as indicators of effect. The Probit method was used to calculate the LC<sub>50</sub> and its respective 95% confidence limits. To evaluate the antifungal activity of the extracts, Petri dishes containing PDA agar and the different extracts were prepared. Plates were also used without the addition of extract, considered as a positive control. The radial growth rate and the percentage of inhibition of the fungus in the presence of the extracts were analyzed for 10 days. The ethanolic extracts of *Heliantus petiolaris* produce the highest mortality in *Daphnia magna* at a dose of 1,770 mgL<sup>-1</sup> (LC<sub>50</sub>) compared to the reference toxicant with LC<sub>50</sub> at a concentration of 0.25 mg L<sup>-1</sup>. With the *Baccharis spartioides* extract, LC<sub>50</sub> value of 1.23796 mgL<sup>-1</sup> and for the *Calendula officinalis* extract the concentration found is LC<sub>50</sub>: 0.146399 mgL<sup>-1</sup>. Regarding phytopathogenic activity, *Calendula officinalis* and *Heliantus petiolaris* achieved inhibitions of around 50%, while with *Baccharis spartioides* a 30% inhibition of fungal growth was obtained (at the concentrations tested). The results obtained allow us to conclude that the extracts evaluated here could be used as natural biocontrollers to replace the synthetic products present on the market.

## A28

### UNRAVELING THE ROLE OF RAB22A DURING DENDRITIC CELL INFECTION BY *Toxoplasma gondii*

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*Toxoplasma gondii* is an obligate intracellular parasite and the causative agent of Toxoplasmosis, a worldwide spread disease. *T. gondii* has developed multiple strategies to infect most of nucleated warm blood animal cells including dendritic cells (DC). The parasite thrives within a specialized compartment known as the parasitophorous vacuole (PV), which serves as a protective niche for its survival and replication, enabling the parasite to evade host immune responses. Many interactions between the host cell and the PV have been described, but the molecular effectors involved in these connections and the outcome for the parasite growth and survival are still unknown. Previous works from our group have described the small GTPase Rab22a as a critical regulator of cross-presentation, including *T. gondii*-associated antigens. Rab22a is recruited to the *T. gondii* PV shortly after infection, but is not required for successful invasion. The main goal of this study is to elucidate the role of Rab22a in later stages of *T. gondii* infection within DCs. In this context, we show by immunofluorescence and confocal microscopy a sustained recruitment of Rab22a to the PV at 24, 48 and 72 hour post-infection. We silenced Rab22a expression in DCs by using a lentiviral vector containing a shRNA (Rab22a KD) and compared them to control DCs. We assessed the arrival of lipid droplets at the PV at 48 hours post-infection, as well as their quantity and size by fluorescent staining with the probe bodipy. Moreover, by using a fluorescent strain of the parasite and a flow cytometry-based approach, we determined that *T. gondii* is unable to replicate properly after 24 hours post-infection, suggesting an important role for Rab22a in late stages of the infection.

## A29

### NARINGIN AS A POTENTIAL INDUCTOR OF LYSOSOME-DEPENDENT CELL DEATH IN BREAST CANCER CELLS

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Breast cancer is the leading cause of cancer-related death in women worldwide. It is known that some tumor cells exhibit increased lysosomal biogenesis along with alterations in lysosomal integrity and functionality. Various studies have demonstrated that lysosomal membrane permeabilization (LMP) can lead to lysosome-dependent cell death. It has been demonstrated that naringin (Nar), a flavonone mainly found in citrus fruits, possesses anti-proliferative, antioxidant, and anti-carcinogenic effects. In gastric cancer cells, Nar has shown to induce lysosomal membrane permeability, leading to cell death. The aim of this study is to evaluate the possible impact of Nar on lysosomal status in cells derived from mammary ductal adenocarcinoma (MCF-7 cell line). Cells were incubated with 50  $\mu$ M Nar for 3, 6, and 12 hours, in the presence or absence of LysoTracker™ Red DND-99 during the last hour of incubation, and subsequently processed for fluorescence microscopy. Our preliminary results suggest that Nar increases the number of acidic compartments corresponding to late endosomes/lysosomes (by indirect immunofluorescence of the lysosomal protein LAMP-1 and lysosomal labeling using LysoTracker™ Red DND-99) in a time-dependent manner. Although these results should be confirmed, these observations may correspond to a typical response, mediated by the transcription factor EB (TFEB), to lysosomal damage, indicating that endosome-lysosome system could be a potential target for Nar.

## A30

### HORMONAL REGULATION OF INTRACELLULAR TRANSPORT OF CATHEPSIN D IN ISHIKAWA CELL LINES OF HUMAN ENDOMETRIUM

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The endometrium is a crucial tissue for reproduction, playing a key role in normal fertility and reproductive success. In recent years, lysosomes have been recognized for their involvement in numerous physiological processes. Lysosomal proteases, including cathepsins, have been linked to various physiological processes such as menstruation, implantation, and placenta formation, as well as different diseases like cancer or endometriosis. Despite their significant role in key reproductive processes, the mechanism and intracellular regulation of cathepsin D have not been thoroughly studied in endometrial cells. We utilized a human endometrial Ishikawa cell line to investigate whether the expression and/or localization of cathepsin D and its transporter proteins could be influenced by sex hormones. Through fluorescence microscopy and Western blotting techniques, we observed that the presence of estrogen increases the expression and processing of both cathepsin D and its transporter protein, sortilin. Our results demonstrate that the hormonal microenvironment regulates the intracellular transport of cathepsin D. These findings could open new scenarios that justify the development of novel approaches to improve reproductive outcomes.

### A31

#### DESIGN AND EXPRESSION OF A G-QUADRUPLEX BINDING PROTEIN DOMAIN FOR LABORATORY APPLICATIONS

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G-quadruplexes (G-quads) are secondary structures that can form in nucleic acids due to guanine-rich sequences. These structures have gained significant importance recently due to their involvement in regulating various biological pathways, including replication, transcription, and translation. Therefore, a tool for rapidly and precisely identifying G-quads in different DNA and RNA samples would be highly valuable. In addition, the genomes of coronaviruses, including those responsible for diseases like SARS (SARS-CoV and SARS-CoV-2), encode numerous proteins, among which their NSP3 protein stands out due to its exclusive "SARS-unique domain" (SUD) containing two tandem macrodomains (MDs) with G-quad binding capabilities. We aim to use the SUD domain as a valuable laboratory tool for G-quadruplex identification and analysis. We have designed and expressed a construct of the SUD domain from SARS-CoV-2 that initially produced only non-soluble protein. The construct has undergone refinement, with optimized expression conditions and purification processes, currently yielding over 10 mg of purified protein per litre of culture. Our ongoing work centres on generating G-quadruplex structures through custom-made oligonucleotides to corroborate their interactions. By investigating the SUD domain's potential utility as a G-quad binding tool, we will contribute to understanding the role of these structures in various biological processes and their potential applications in nucleic acid research and drug development. This work holds promise for enhancing our knowledge of G-quads and providing a valuable resource for researchers studying nucleic acid structures, understanding changes in the profile of these structures following exposure to molecules that influence DNA topology, and their implications in health and disease.

### A32

#### ANTI-TRYPANOSOMA CRUZI SYNERGISTIC EFFECT OF MENADIONE AND KETOCONAZOLE

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The search for drugs against Chagas disease is continuous and intense since the nitrofurans commonly used against this disease have many adverse effects. In order to reposition drugs in use against parasites we have attempted combinatorial assays against *Trypanosoma cruzi* epimastigotes and trypomastigotes. In this study combinatorial effects of two drugs, the antifungal Ketoconazole and the fat-soluble Menadione (vitamin K<sub>2</sub>) on *T.cruzi* epimastigotes and trypomastigotes. Parasites were incubated with drug combinations in constant ratio (1:1) and synergistic effect was evaluated through software Compusyn, a computer program for quantitation of synergism and antagonism in drug combinations developed by Ting-Chao Chou. We demonstrated that both drugs are active against the two parasite stages and they are synergistic with each other. Then, using the Ellman reaction, we proved that the amount of total reduced thiols decreased in parasites treated with the compounds in combination and separately. This could be due to an increase in intracellular ROS as a consequence of an oxidative stress, depleting the reserves of trypanothione. By transmission electron microscopy we observed that parasites treated with the combination of both compounds displayed mitochondrial swelling accompanied by cytoplasmic vacuolization and abnormal structures as clues of cell death. Future studies will be carried out in order to find the drug combination that selectively affects the parasites and alters their evolution in the hosts.

## VEGETAL BIOCHEMISTRY, PHYSIOLOGY, PATHOLOGY AND PRODUCTION

### A33

#### CHARACTERIZATION AND QUANTIFICATION OF NORDIHYDROGUAYARETIC ACID OF THE ETHANOLIC EXTRACT OF *Larrea divaricata* CAV. by UHPLC-DAD-MS/MS

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*L. divaricata* is a species used in popular medicine to treat different affections, focusing on its use as an antimicrobial agent. The aim of this study was to characterize and quantify nordihydroguayaretic acid (NDGA) from the ethanolic extract of *Larrea divaricata* by UHPLC-DAD-MS/MS. The aerial parts of *L. divaricata* Cav. were collected in February, 2015 in Nogolí, San Luis, Argentina. A voucher specimen was deposited at the Herbarium of the Universidad Nacional de San Luis (UNSL Del Vitto and Petenatti 8432). The vegetable materials were dried in shade at room temperature, then chopped and ground to fine powder in a mechanical blender. An ethanolic extract (EE) was prepared from the aerial parts of *L. divaricata* (300 g) following official methods described by FA VIIEd., (2003). The solid residue was evaporated under reduced pressure, and then dried in an oven (40 °C) to constant weight. (Ethanolic SR= 23.10 g/mL). Its physicochemical parameters were determined. The characterization of the *L. divaricata* EE was performed in an ACQUITY H-Class UPLC equipped with a XEVO TQ-S micro triple quadrupole mass spectrometer (Waters Corp, Milford, MA, USA) with electrospray ionization (ESI). The sample and standard solutions were filtered through a 0.22 µm nylon membrane prior to analysis. Each filtered sample (20 µL) was injected into the UHPLC system and separated on a UPLC ACQUITY BEH C18 column (1.7 µm, 2.1 mm × 50 mm) using an isocratic mixture of 0.1% Formic Acid: Methanol 40:60 as the mobile phase. The flow rate was 0.2 ml/min and the injected volume was 20 µl. The separation temperature was 38 °C (column temperature). The analysis was performed in ESI positive mode using daughter MS/MS function and a collision energy of 20eV. The total time for the analysis of each sample was 20 minutes. The properties of the EE were: relative density (2.50 g/cm<sup>3</sup>), pH (4.88), refractive index (1.395). While the concentration of NDGA calculated through the calibration curve equation was 12.92 ppm (r<sup>2</sup>=0.991879). The developed methodology proved to be selective, linear, precise, and accurate. This method would represent a fast alternative method for the quantification of NDGA and quality control of the species under study.

### A34

#### DESIGNS OF ORODISPERSIBLE TABLETS FROM *Larrea divaricata* Cav.

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Oropharyngeal candidiasis is a common opportunistic infection in elderly patients. *Larrea* species extracts demonstrate significant antimicrobial activity against *Candida albicans*. There is no solid herbal dosage form on the pharmaceutical market that offers a unit dose as an alternative treatment for oral candidiasis. The excipients of orodispersible tablets (ODTs) must meet some requirements such as good solubility, rapid disintegration in the mouth without leaving residue, good mouthfeel, pleasant taste, and high active pharmaceutical ingredient (API) loading capacity. They also have to be thermostable and not be affected by humidity. The lactose coprocessed excipients StarLac® 90, FlowLac® 90 and CombiLac® meet these requirements, and also offer an excellent safety profile and very good patient acceptability, including palatability. Furthermore, considering that the main cause of tablet instability is humidity, direct compression appears to be the preferred manufacturing process for ODMTs, while APIs being moisture labile. The objective of this work was to describe the design, development and evaluation of orodispersible herbal tablets of *Larrea divaricata* extract by direct compression as an alternative treatment in older adults with oral candidiasis. Three ODTs were prepared by direct compression using ethanolic extract of LD at a dose of 100 mg. Coprocessed excipients were used as diluents. Disocel® was used as a super disintegrant. TRICAFOS® 500 was used as a support. Vanilla was added to improve palatability. Physical mixtures were prepared (F1: Starlac® 90, F2: FlowLac®, F3: CombLac®), the micromeritic properties were analyzed. The tablet properties and quality parameters were evaluated according to the United States Pharmacopeia (USP-43) guidelines. The formulation that showed the best rheological properties and biopharmaceutical parameters was F1 containing ethanolic extract of LD, TRICAFOS® 500, StarLac® 90, Disocel®, Microcrystalline cellulose and magnesium stearate. Disintegration test(s): < 25, Hardness (Kp): < 7.65, Wetting test(s): < 30, Friability (%): < 0.35. The results obtained were within the acceptance range, so these LD ODT could be an alternative as antifungal herbal tablets intended for adults.

### A35

#### PROMOTION OF TOMATO GROWTH BY *Pseudomonas putida* PCI2 UNDER HYDROPONIC CONDITIONS

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Hydroponics is a production system in which the roots of plants are irrigated with a mixture of essential nutrients dissolved in water and, instead of soil, an inert mineral substrate or simply the same nutrient solution is used. Tomato cultivation (*Solanum lycopersicum* L.) is adapted to hydroponic cultivation and it is necessary that it be developed in conditions that are friendly to the environment and health. The use of microorganisms that promote the growth and health of crops constitutes a global trend in this sense. *Pseudomonas putida* PCI2 is a strain isolated from the tomato rhizosphere and on which tests were carried out regarding its beneficial interaction with crops, linked to the mobilization of nutrients, production of phytohormones and suppression of diseases. The objective of this work was to evaluate the growth-promoting capacity of *P. putida* PCI2 in tomato crops under hydroponic conditions. Tomato seeds (variety UCO16 INTA) were germinated in trays containing sterile substrate (soil:perlite 2:1) and placed in a growth chamber under controlled cycles of 16 h of light at 25°C and 8 h of darkness at 20°C. At 35 days after sowing, the seedlings were transferred to hydroponic culture containers filled with 7 liters of Hoagland's nutrient solution (100% concentration) and kept in a chamber under the same growth conditions. Subsequently, half of them were inoculated at the root level with a culture of *P. putida* PCI2 (1,106 CFU/ml) at a rate of 1 ml/plant. The hydroponic system allowed us to determine that tomato plants inoculated with *P. putida* PCI2 presented statistically significant differences compared to the uninoculated control in the parameters root length, aerial part length, dry weight of roots and aerial part, evaluated at 60 days after sowing. In in vitro assays, it was observed that this strain produced indoleacetic acid (IAA) when grown in trypticase soy (TSA) culture medium enriched with L-tryptophan. The production of IAA is postulated as a possible mechanism in promoting growth under the test conditions. These promising results allow us to infer that *P. putida* PCI2 could be used as a growth promoting biofertilizer in tomato crops.

### A36

#### VARIATION OF PHYSIOLOGICAL PARAMETERS IN THE FORMATION OF *Jatropha curcas* L. SEEDLINGS.

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*Jatropha curcas* L. (Euphorbiaceae) is native to Mexico and Central America and it is cultivated in Central America, South America, Southeast Asia, India and Africa. The interest in the cultivation of this species, suitable for production of biodiesel oil from their seeds, is due to their adaptation to marginal conditions for agriculture production. *J. curcas* is perennial plant, growing in semi-arid and arid soils, and their non-edible seeds have high oil content. The importance of their cultivation is mainly due to its ability to grow in wastelands and desert areas, in this way helping arid land revegetation and carbon dioxide capture in environments where most crop plants cannot survive. In addition, as a second-generation (non-food supply) biofuel crop, it can be considered a sustainable oil source with minimal environmental impact. The aim of the work was to determine the physiological parameters during seedling stage of *J. curcas* L. Seeds were placed in land-perlite 50:50, were kept in growth chamber at 30° C and photoperiod of 16: 8 light: dark and 60% humidity and were irrigated with distilled water three times a week. During forty days, once a week, were evaluated the followed parameters by triplicate: length of roots (RL), hypocotyl (HL) and epicotyl (EL) and fresh weight (FW) and dry weight (DW) of root, hypocotyl, epicotyl, cotyledon leaf (CL) and true leaf (TL). To obtain the fresh weight value, the organs were weighed separately and placed on the stove at 60 °C to constant weight to obtain the dry weight value. Analysis of variance (ANOVA) was applied and data were subjected to Multiple Range the Duncan, this test controls type I errors where  $\alpha = 16$  and  $MSE = 6.208333$ . Test using the software INFOSTAT-UNC. RL increased significantly from day 7, after that, there was constant until day 40. HL increased significantly until day 14 with length of 11.97cm and showed no significant differences until day 40. Growth EL started day 14 and then there were significant differences at days 21, 28 and 40, and reaching 15 cm of length. FW and DW of R, H, E, CL y TL showed significant differences at day 40. The study of seedling growth of *J. curcas* L. is important because it determines the successful establishment in its natural environment and it determines the behavior against environmental stress.



**A37**

**STUDY OF DORMANCY FACTORS IN THE GERMINATION OF *Neltuma flexuosa* 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 evaluate dormancy factors in the germination of *P. flexuosa* in a forest sector under extensive silvopastoral management of the Conlara Valley, San Luis, Argentina in order to obtain specimens suitable for restoration and/or rehabilitation of the forest in the area under study. This area consists of 200 hectares of forest under extensive livestock 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. 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 H<sub>2</sub>SO<sub>4</sub> 3'; T6: 40% NaClO by 30'; T7: concentrated HCl 7'; T8: concentrated HCl 15'; T9: Ethyl alcohol 20'; T10: Acetone 30'. 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 °C, in darkness. The germination percentage (PG) was determined, the seed with a radicle greater than 3 mm was considered germinated. 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 showed that T2 (50%) and T3 (30%) were those with the highest PG and did not have significant differences between medians. Although in the overall analysis, T3 also did not have differences between treatments with the rest of the treatments (0%), when individual contrasts were performed, it did differ from the rest. These values would indicate that to obtain a uniform germination in the field it is necessary to remove the teguments or place the seeds in boiling water prior to sowing.

**A38**

**MOBILIZATION OF TOTAL SOLUBLE CARBOHYDRATES IN POPULATIONS OF *Adesmia bicolor* (LEGUMINOSAE) UNDER TWO CONDITIONS OF CULTIVATION IN THE CENTRAL REGION OF ARGENTINA**

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The low availability of winter forages in the central area of Argentina has led to the study of native legumes, mainly those with autumn-winter growth, among which is *Adesmia bicolor*, herbaceous species, perennial and preferably winter cycle. The objective of this work was to evaluate and compare the mobilization of carbohydrates and total biomass production of five populations of *A. bicolor* originating in the province of Córdoba (Populations 1 and 2), San Luis (Populations 3 and 5) and Entre Ríos (Population 11). The trial was conducted at the National University of Río Cuarto (Córdoba). The design of the experiment was completely randomized under two growing conditions (pot and field) with four sampling times matching with the seasons. The mobilization of total soluble carbohydrates (TSC) was evaluated using the modified phenolic reaction method and the production of total biomass in an area of 0.0625 m<sup>2</sup>. The data obtained was analyzed by ANOVA and Fisher's LSD test. In pot cultivated populations, it was observed that the majority presented a winter-spring growth except for population 1 that behaved as spring-summer, although in all of them, the peak in the concentration of TSC was in the autumn sampling, population 5 reached the maximum value (0.1449 mg g<sup>-1</sup> sample). In field cultivated populations, population 1 showed autumn-winter-spring growth, populations 2 and 11 exhibited greater stability in their biomass production, showing an increase towards the autumn (3631.71 kg ha<sup>-1</sup> and 2642.8 kg ha<sup>-1</sup>, respectively), population 3 showed winter-spring growth, while population 5, yielded two growth points, one in summer and the other in winter. The TSC in these populations presented its maximum value in autumn, with population 5 reaching the highest value (0.1742 mg g<sup>-1</sup> sample). When comparing the populations cultivated in the field and in pot, in majority of samples, the highest concentration of carbohydrates and total biomass production was observed in the populations cultivated in the field. In the latter the mobilization of TSC for biomass production was more effective and immediate than in those cultivated in pots. On the other hand, the differences found are not associated with the collection environment, but with the ability to adapt to the new environment presented by each of the populations.

### A39

#### TOTAL CRUDE PROTEIN AND BIOMASS PRODUCTION IN POPULATIONS OF *Adesmia bicolor* (LEGUMINOSAE) UNDER TWO GROWING CONDITIONS IN ARGENTINA CENTRAL REGION

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Native legumes, including *Adesmia bicolor*, represent an important resource in the absence of quality forage species during the winter season in the center of the country. This species has been characterized in its form of growth, mode of reproduction and forage quality. The objective was to evaluate and compare the percentage of crude protein (% CP) and total biomass production of five populations of *A. bicolor* originated in the province of Córdoba (Populations 1 and 2), San Luis (Populations 3 and 5) and Entre Ríos (Population 11). The trial was conducted at the National University of Río Cuarto (Córdoba). The design of the experiment was completely randomized under two growing conditions (pot and field) with four samples matching with the seasons. The percentage of total Nitrogen was evaluated using the Kjeldahl method, the results obtained were multiplied by a constant value to obtain % CP. Total biomass production was estimated at 0.0625 m<sup>2</sup>. The data obtained were analyzed by ANOVA and Fisher's LSD test. In pot cultivated populations, it was observed that only in the population 11 the maximum % CP (14.82 %) coincided with the time when the crop had produced the highest total biomass (4458.65 kg ha<sup>-1</sup>). Among those cultivated in the field, only in population 11 the peak of production matched with the one of CP (3145.78 kg ha<sup>-1</sup> and 17.05 % respectively). In populations 2 and 3, although it did not coincide, at the time of increased total biomass production (4586.53 kg ha<sup>-1</sup> and 5635.12 kg ha<sup>-1</sup> respectively) % CP was also one of the highest (15.36 % and 17.58 %, respectively). Comparing the same population cultivated in the field and in the pot, it can be seen that in majority of cases, the total biomass production and % CP was higher in the field. Field-grown populations would be more efficient in the production of both total biomass and PB. On the other hand, it is observed that the differences found between the populations are not associated with the environment of origin of the same, but with the condition of cultivation.

### A40

#### PHARMACOGNOSTIC STUDIES OF *Aloysia citrodora* "CEDRÓN"

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The increasing use of traditional herbal medicines requires adequate quality controls for the safety of the people who consume them. This has led to a considerable increase in serious cases reported for adverse effects due to adulterated, altered and/or substituted drugs, which do not meet the necessary requirements to ensure their safety, effectiveness and quality. The genus *Aloysia* Ortega ex Jussieu belongs to the Verbenaceae. It comprises about 30 species, distributed in the Americas from the southern United States to Chile and Argentina. *Aloysia citrodora* Paláu, known as "cedrón, yerba luisa, yerba de la princesa, lemon verbena", is a species native to the arid and temperate zones of northwestern Argentina and southern Bolivia. Its aerial parts are widely used in traditional medicine to treat gastrointestinal disorders (digestive, antispasmodic, carminative, antidiarrheal), or used as a mild sedative, cardiogenic, febrifuge, analgesic and antiseptic and even integrates compound yerbas mate. The leaves of *A. citrodora* are codified by the Argentine Pharmacopoeia VII Ed. (FA). The objective of our work was to contribute to the characterization and the pharmacognostic quality control of the crude drug and vegetable drug preparation from the regional commerce. Aerial parts of *A. citrodora* were collected in San Luis in November 2022 and identified by classical taxonomic methodology. Documentary specimens are preserved in the UNSL Herbarium. Qualitative micrography of leaves and stems was studied. In addition, quantitative micrographic parameters of leaves were measured. Phytochemical profiles were obtained with solvents of different polarities using UHPLC-UV. The cross-sections of the leaves show a dorsiventral structure, unistrate adaxial and abaxial epidermis and the midrib presents collateral disposition. The leaf is hypostomatic and the stomata of the lower epidermis are anomocytic. Two types of trichomes are present on both sides of the foliar surfaces, non glandular cystolithic and glandular trichomes. The quantitative parameters obtained were: number of stomata (NE) and stomata index (IE) upper epidermis = 0; NE lower epidermis  $6 \pm 1.22$ ; IE ep. Inf. 6-9; palisade ratio (RE) of  $7.6 \pm 1.25$ ; number of venous islets  $4 \pm 0.81$ ; number of nerve terminals  $2.25 \pm 0.5$ . Stem anatomy showed a circular margin with 6 ribs. The extracts present spectral regions of glycoside compounds such as verbascoside, isoverbascoside and leuceptoside; polyphenols such as hydroxytyrosol, flavonoids such as quercetin, apigenin derivatives, luteolin derivatives, and in the fraction of volatile compounds such as citral, eucalyptol, limonene, among others. The results show that the analyzed samples have a very good quality control as they comply with the parameters established in the FA. This work also provides quantitative parameters (not included in FA) that help an effective quality control of commercial samples.

#### A41

### PHARMACOGNOSTIC STUDIES ABOUT *Aloysia polystachya* (Griseb.) Moldenke

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Pharmacognostic studies of medicinal plants are essential to carry out adequate quality control of vegetable drugs and medicinal herbs in order to detect possible alterations, substitutions and/or adulterations, as well as to verify the correspondence between its ethnobotanical uses and the chemical basis of its therapeutic action and/or the discovery of new pharmacologically active principles. In Argentina inhabits 11 species of *Aloysia*, which are distributed in the northern and central regions, with its southernmost limit in the province of La Pampa. One of them, *Aloysia polystachya* (Griseb.) Mold. (Verbenaceae) is native to Argentina and Bolivia, known as "burrito" or "té del burro", wild or distributed by crop in almost the whole country. In folk medicine, the aerial part is used as antispasmodic, digestive, liver protector, antihypertensive, among other popular uses. The dry or fresh plant material is dispensed in simple or in mixtures and even integrates the composition of compound "yerbas mate". Therefore, the objective of this work was to characterize this species from the macro and micromorphological and phytochemical points of view to avoid that self-consumption (self-medication) affects public health. The aerial parts were collected, preserved in acetoalcoholic formalin and macro and micrographic parameters (qualitative and quantitative), were determined. Moreover, the material for phytochemical determinations was dried, extracted with solvents of different polarity and analyzed by UPLC. The results obtained at the macro- and micromorphological level as well as the metabolites detected in extracts of different polarity allow characterizing the species. The foliar anatomy showed a unistrate epidermis, anomocytic stomata and multicellular trichomes mostly in lower epidermis. The structure is dorsiventral, the vascular bundle is collateral with angular collenchyma towards both epidermises. The stem is quadrangular and costate, the ribs are protected by caps of sclerenchyma fibers; it has an unistrate epidermis with multicellular trichomes; the cortex is mainly represented by chlorophyll parenchyma. The medullary tissue is a fundamental parenchyma formed by cells with amyloiferous contents. Quantitatively, this species is characterized by Stomata number in upper epidermis:  $2.6 \pm 1.14$ ; Stomata index in upper epidermis: 1.01-(2.77)-4.22; Palisade ratio:  $9.4 \pm 1.18$ ; Stomata number in lower epidermis:  $12.20 \pm 1.78$ ; Stomata index in lower epidermis: 15.06-(18.75)-22.22; Vein-islets:  $8.4 \pm 1.14$ ; Vein-termination:  $5.2 \pm 1.14$ . The results revealed that the extracts present similar spectral regions that the glycoside compounds like verbascoside, isoverbascoside and leuceptoside; polyphenolic compounds like hidroxytyrosol, flavonoids compounds like quercetin, volatile compounds like citral, among others. In this sense, this work may contribute to the elaboration of a pharmacopoeia monograph since this species has not yet been included in the Argentine Pharmacopoeia.

#### A42

### COMPETITION BETWEEN CADMIUM AND ZINC IN *Glycine max* (L) Merr.

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Anthropogenic contamination of soils by cadmium (Cd) is a global problem. Although cadmium is a non-essential element, it can enter plant tissues and possibly replace essential elements, causing cellular and molecular damage even at very low concentrations. Zinc (Zn) is an essential micronutrient for plants, but in high concentrations it causes alterations at a molecular, physiological and biochemical level. The objective was to evaluate the convenience of using  $Zn^{2+}$  in the phytoextraction of Cd from soils contaminated with the heavy metal. The Cd/Zn duo was studied in roots of *Glycine max* (L) Merr, evaluating modifications in the oxidative profile. Soybean roots developed for 9 days under hydroponic conditions in Hoagland nutrient solution were subjected to stress conditions with the two ions under study ( $Cd^{2+}$  and  $Zn^{2+}$ ) for an additional 7 days. We worked with  $Zn^{2+}$  concentrations between 0; 0.6 and 4.8 mM and with a constant  $CdCl_2$  concentration of 40  $\mu$ M. As indicators of oxidative damage (MDA and carbonyl groups), the content of  $H_2O_2$ , and catalase (CAT) activity, as an antioxidant enzyme, were measured. A significant increase in the content of  $H_2O_2$  and carbonyl groups were observed in the last treatments (Zn [4.8] with and without Cd) ( $p < 0.0001$  and  $p < 0.005$ ). Protein oxidation coincides with the protein values obtained, where the proteins of these treatments present a significant decrease with respect to the control ( $p < 0.0001$ ). Lipoperoxidation increased significantly in treatments without Zn with Cd, and with Zn [0.6] without and with Cd ( $p < 0.0001$ ). CAT significantly increased compared to the control in the Zn [0.6] treatment without Cd and the treatments with and without Cd Zn [4.8] ( $p < 0.005$ ,  $p < 0.0001$  and  $p < 0.0001$ ). It can be inferred that the Cd/Zn duo leads to changes in the level of antioxidant activity and damage to macromolecules, especially highest dose of Zn, which could be attributed to a radical response to the toxicity of the duo. In this study, it can be seen that the Cd/Zn duo at low doses of Zn would improve the Cd tolerance of soybean plants.



#### A43

### OXIDATIVE STRESS EFFECTS CAUSED BY THE ION PAIR CADMIO-ZINC IN *Glycine max* (L) Merr LEAVES

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Soil contamination with heavy metals has increased exponentially in recent years and has been progressively installed in different ecosystems worldwide. Cadmium (Cd) is a heavy metal that isn't essential for living beings and in very low concentrations causes alterations in physiological, biochemical, and molecular processes, causing damage at the cellular and molecular levels. Zinc (Zn) is an essential microelement for plants, however, in excessive concentrations it also causes damage and alterations. The ions of these metals share chemical characteristics that lead them to use the same transporters to enter cells, thus competing for their entry, transport, and utilization in plants. This competition generates changes in oxidative stress parameters. The objective of this study was to determine the mechanisms of stress tolerance mediated by the Cd<sup>+2</sup> and Zn<sup>+2</sup> ion pair and the damage that the oxidative stress causes in structures such as plasma membrane and proteins, evaluating the biochemical and physiological changes in leaves of *Glycine max* (L) Merr. Leaves were obtained after 12 days of plant development under hydroponic conditions in Hoagland's nutrient solution and exposed to contamination with these two ions (Cd<sup>+2</sup> and Zn<sup>+2</sup>) for a period of 7 days. The ZnCl<sub>2</sub> concentrations used were: 0, 0.6 and 4.8 mM and 40 μM CdCl<sub>2</sub>, as a constant concentration. Regarding carbonyl, H<sub>2</sub>O<sub>2</sub> and MDA content, the results showed a significant increase (p<0.01) in the last treatment [4.8] with Cd compared to the control. CAT activity decreased significantly in all treatments compared to the control (p< 0.05, 0.01 and 0.001), while in APX activity a significant increase was observed in the Zn [4.8] treatments with Cd and without Cd (p<0.001). In GR activity, a significant increase was observed in Zn [4.8] without Cd and with Cd (p <0.001) compared to the control. According to these results, we can conclude that the presence of Zn and Cd ions in the nutrient medium alters both biochemical and physiological parameters as well as antioxidant and pro-oxidant activity, mainly in treatments with high Zn concentrations, which could be considered a response of the plant to the toxicity generated by the ion pair.

#### A44

### PRESENCE OF *Fusarium* sp. IN DIFFERENT PLANTING DENSITIES AND NITROGEN FERTILIZATION IN CORN

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The management of sowing density and nitrogen fertilization are common practices in corn cultivation and aim towards better radiation capture, growth and leaf expansion to optimize productive efficiency. Changes in population density and doses of nitrogen fertilizer significantly affect the development of the crop and influence the health status of the plants. *Fusarium* is a fungus that lives in the soil or plant remains, it is saprophytic and once it is introduced into a field, it establishes itself there indefinitely. In corn it produces ear rot, which reduces the expected yield and quality of its grains. Furthermore, this pathogen, given certain environmental conditions, can produce mycotoxins that contaminate the corn grain, which has a great impact on health, animal production and the economy. The objective of this work was to evaluate the presence of *Fusarium* in vitro with three sowing densities and with three fertilization doses. The samples were obtained from a field trial carried out at the Altos de Curalicó establishment (Villa Mercedes, San Luis) during the 2022-2023 agricultural campaign with 3 planting densities (3.5, 4.1 and 5.3 pl/m<sup>2</sup>). and variable doses of a nitrogen fertilizer with 3 treatments (0, 46 and 92 kg N/ha) with 3 repetitions in a factorial design. To measure the presence of the pathogen, 5 grains per Petri dish were sown in vitro in APG medium using predetermined sterilization techniques. The plates were incubated at 25 +/- 1 °C for 7 days and then macro and microscopic observations were made of each colony in order to confirm the identity of the pathogen. The assumptions of normality and homogeneity of variances were checked and an ANOVA (α= 0.95%) and Fisher's test were performed with the counts obtained. The results indicate that there were significant differences in the densities, while the nitrogen doses tested did not show significant differences. Medium and high densities produced a lower presence of *Fusarium* with 12 and 7% incidence respectively. Although the fertilization levels did not show significant differences, a greater presence of the pathogen was observed in the treatment with the highest dose of nitrogen. Plants further apart would be susceptible to the disease and would have a greater fungal load. On the other hand, the range of N doses tested would not significantly influence the appearance of the pathogen. Likewise, it is necessary to continue these evaluations by expanding the range of established treatments, in successive agricultural campaigns.

#### A45

### RELATIONSHIP BETWEEN GRAIN YIELD AND AGRONOMIC TRAITS IN *Amaranthus* spp. UNDER CONTRASTING HYDRIC CONDITIONS

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Amaranth (*Amaranthus* spp.) is a pseudocereal with exceptional nutritional value due to its protein, essential amino acid, lipid and mineral content. This crop is an alternative for agricultural diversification because it can thrive in a wide range of soils. Also, it is characterized by a low water demand to achieve satisfactory yields. Obtaining cultivars tolerant to biotic and/or abiotic stresses is one of the fundamental objectives in plant breeding. The knowledge of the association between yield and other crop traits is useful to improve the efficiency of breeding programs. The objective of this study was to determine the correlations between grain yield and other agronomic traits in different amaranth genotypes under two water availability conditions. Five trials were carried out in the greenhouse of the Experimental Field in the National University of Río Cuarto (UNRC). A simple randomized design, during three agricultural cycles was carried out. Four of these trials involved three amaranth varieties and five advanced lines from the UNRC, and the remaining trial involved one variety and one advanced line. In each trial, two groups of pots were formed, one group was maintained at field capacity during the entire crop cycle, while the other group was subjected to water restriction at the beginning of the reproductive period. Seven traits were measured: days to panicle initiation (PI), plant height (PH), panicle length (PL), stem diameter (SD), fertility index (FI), thousand seed weight (TSW) and grain yield per plant (GY). The path analysis allowed us to determine the nature of the relationship between GY and agronomic traits for each water condition and to estimate the direct and indirect effects. The results of the path analysis revealed that GY correlated statistically significant and directly with PL and FI in the two water conditions, and indirectly with PH through PL in the absence of water restrictions and through FI in water-stressed environments. The GY was directly and indirectly correlated through PL with SD in both water conditions, and with TSW when water supply was restricted. Direct and indirect associations with GY were all positive. However, GY was not statistically significantly correlated with PI in both water conditions, nor with TSW in trials without water restriction. Therefore, the traits panicle length, fertility index and stem diameter would be important traits to determine the yield in environments with and without water stress and to select amaranth genotypes in breeding programs.

#### A46

### SELECTION OF SUPERIOR GENOTYPES OF GRAIN AMARANTHUS (*Amaranthus* spp.) UNDER CONTRASTING HYDRIC CONDITIONS

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Amaranth (*Amaranthus* spp.), a pseudocereal with high protein and lysine content in its grains, adapts to a wide variety of environmental conditions. It is therefore considered a species with the potential to become a staple crop of similar agricultural and economic importance as cereals. There is great inter- and intraspecific variability in amaranth. This variability is of great importance in genetic improvement when developing new varieties adapted to different environments. The objective of this study was to identify superior grain amaranth genotypes in yield under contrasting water conditions. Three grain amaranth varieties (Antorcha, Dorado and Candil) and five advanced lines from the National University of Río Cuarto (AMan-G1/3, H17a, H20a, H21II and H22II) were planted under greenhouse conditions in four yield trials with a simple randomized design during two agricultural cycles. Two water conditions were imposed in the trials: a.- field capacity (FC) during the entire crop cycle and b.- water restriction (WR) at the beginning of panicle and FC during the rest of the cycle. In the three experiments carried out in the first agricultural cycle, the plants of the WR treatment were subjected to a single period of water stress followed by rehydration, with different stress intensities (SI): low (0.21), moderate (0.42) and severe (0.74). In the trial carried out in the second agricultural cycle, the WR treatment was subjected to four consecutive cycles of water restriction and rehydration, with a moderate SI (0.40). Genotype main effect (G) and genotype-environment interaction (GE) analysis was used to determine the adaptability of genotypes to a given environment. The GGE biplot allowed us to distinguish superior genotypes in the different environments evaluated. The Antorcha variety showed yield superiority in environments with consecutive periods of moderate water stress and in environments without water restriction. The advanced lines H22II, H20a, H21II and the variety Dorado were superior in environments with severe and moderate water stress, while the advanced line AMan-G1/3 was superior under low stress. The Candil variety performed better in conditions without water stress and the advanced line H17a had an intermediate performance. The study made it possible to identify the genotypes with the best performance in environments with contrasting water conditions, thus allowing their recommendation to farmers and their use in future amaranth crop breeding programs.

#### A47

### PHYSIOLOGICAL AND MORPHOANATOMICAL CHARACTERISTICS OF GERMINATION PROCESS IN *Sarcocornia neei* Lag. (CHENOPODIACEAE) UNDER PREGERMINATIVE TREATMENTS

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Germination and establishment of seedlings are the most critical phases in the life cycle of plants, due to increased vulnerability to ambient stress, competence, predation and diseases. Factors more common affecting germination of plant species are light, temperature, gasses, inner inhibitors, and water. *Sarcocornia neei* Lag. is a native halophyte species, it grows in nearby salty ponds in San Luis province, Argentina. The objectives proposed were to study and characterize morphological and physiological traits and process from seeds and during germination in order to contribute to knowledge of this species. Morpho anatomical studies were conducted in seeds, during the germination process. Related to physiological studies, seeds were stratified in cold in the refrigerator during a month previous essays, and before the beginning of treatment, seeds were soaked and washed under running water for 48 hs in order to eliminate inhibitors of germination. 90 seeds were sowed in Petri dishes in embedded Whatman paper N°2 in distilled water, the essays were running for triplicate (30 seeds per Petri dishes), under light and temperature controlled. The anatomical analysis consisted of fixed seeds in FAA, ulterior dehydration, clarification and paraffin inclusion. Longitudinal cuts were performed to seeds in microtome. The results indicate that seeds are photoblastics positive, continued washing is a positive factor that favored germinative power, reaching 70% of germinative power (PG), in contrast to seeds embedded but not washed in running water (PG=34%). Related to morphological aspects it was observed that seeds contained an embryo with horseshoe form, with two cotyledons and radicle well defined, with presence of perisperm as reservoir tissue. In reference to germination process, radicles emerged between the third and fourth day after sowing, and the cotyledons emerged at day five. At day 21 the seedling with two cotyledons was observed, hypocotyl was presented and the radical system was more developed. We point out that this species presents a slow growth. We concluded that this first approach to morpho anatomical studies of *Sarcocornia neei* Lag. seeds and germination process showed common characteristics to Chenopodiaceae family, and the germination data contribute to identify some factors that could help in future projects related to establishment of the species in degraded sites in order to restoration them, and to management of this species.

#### A48

### HEAT PRIMING IN SEEDS INDUCES METABOLIC AND MORPHOLOGICAL CHANGES IN MAIZE SEEDLING

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Most of the varieties of agricultural plants use seeds to reproduce, however, unfavorable environmental conditions can modify their germination capacity. Different priming techniques: osmo, hydro, chemical, hormonal and nutrient techniques have been used to improve seed germination and crop yield. Stress is considered as a significant deviation from optimal living conditions. Plants exposed to stress undergo modifications in their metabolism to adapt to changes in their environment. The objective was to evaluate metabolic and morphological changes, determining the content of metabolites, length and width in seedlings roots in the post-germination stage of seeds with and without pre-treatments. Maize seeds (*Zea mays* L.) were subjected to pre-treatment with 40 or 50 °C for 3 and 7 d. Seeds 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. Proline (Pro), Ammonium (NH<sub>4</sub><sup>+</sup>), Ascorbic acid (ASA), Dehydroascorbic (DHA) Phenol and Sulfhydryl (SH) groups contents were determined. Seminal root length and width were measured. Our preliminary results showed a significant decreased in Pro content at 3d 40°C but an increment was observed at 7d 40°C, 3d 50°C and 7d 50°C (\*p<0,001). ASA content showed an increase at 3d 40°C, 3d 50°C and 7d 50°C (\*p<0,01). Then, DHA content was reduced at 3d 40°C. An increase of NH<sub>4</sub> was observed at 7d 40°C (\*p<0,001) and a decrease at 7d 50°C (\*p<0,001). Phenol content increases at 3d 40°C (\*p<0,001) but a significant decrease was observed at 7d 50°C (\*p<0,05). Finally, a decrease of SH groups content was showed at 3d 50°C (\*p<0,001). A decrease in root length was observed in the heat pretreatment groups respect to the control and a significant decrease (\*p<0.05) at 50 °C for 7 days. Also, an increase in root width was observed at 50 °C treatments (\*p<0,05). Our results suggest that heat priming produced changes in different routes of metabolism. Heat priming at 40°C induces antioxidant soluble defenses and N content improving morphophysiological parameters while at 50°C seems to induce higher damage. In the context of an increase in temperatures due to global warming, seeds pretreated at 40°C would activate their defense system but at higher temperatures such as 50°C the presence of damage would already be observed.

#### A49

### STATISTICAL EVALUATION OF TWO METHODS OF VIABILITY DETERMINATION IN *Physalis viscosa* L. SEEDS USING THE TETRAZOLIUM TEST

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*Physalisviscosa* L. “camambú” (Solanaceae) is a perennial herb that reproduces both by seed and by gemiferous roots, growing mainly in modified soils where it is considered a weed. It flowers and bears fruit between spring and summer. Its berries are edible both fresh and cooked, making it potentially cultivable; its berries are edible, making it a potential crop. As *Physalisperuviana* “uchuva” is grown in tropical and subtropical countries of South America and Africa to be traded for the fruit nutrition quality. In order to establish an appropriate protocol for the species under study, the aim of this work was to statistically compare different methods used to determine the viability of *Physalisviscosa* seeds using the tetrazolium technique. Ripe fruits were collected from field, selected from their optimal phytosanitary conditions. In laboratory, half of seeds were extracted from recently harvested fruits (SM) and the other half from fruits fermented for one week (M). In both treatments, SM and M, plots of 150 seeds were selected. In each plot, 50 seeds were previously soaked, 50 unsoaked seeds were cut lengthwise and 50 were selected as a control group. All were placed in the indicator solution (TZ). The Chi-square test was used to evaluate the relationship between the above methods. From the plot of 150 macerated seeds, 59.3% uncoloured and 40.7% coloured seeds were obtained. From the lot of 150 seeds without maceration, 40% undyed and 60% dyed were obtained. The seeds previously soaked, 34.8% were undyed, 31.6% dyed were obtained. The batch of seeds cut lengthwise yielded 14.6% undyed and 55.9% dyed. In the control group, 50.6% were uncoloured and 12.6% were coloured. The statistical analysis obtained in each case shows a significant p-value using  $\alpha = 0.05$  as the level of significance. (p.value=0.001165 and p.value= p-value = 4.762e-16 respectively). It was concluded that the determination of seed viability of *Physalisviscosa* L using the tetrazolium method, the appropriate protocol results from making a longitudinal slice of the seeds without prior maceration.

#### A50

### BIOCHEMICAL RESPONSES IN *Parkinsonia praecox* (RUIZ & PAV. EX HOOK.) HAWKINS PLANTS EXPOSED TO SALINITY AND DROUGHT CONDITIONS

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*Parkinsonia praecox* is a tree species native to the northwest area of the province of San Luis. In preliminary studies from our laboratory, it was shown that the growth of the root and aerial part of these plants is more affected by exposure to NaCl solutions than by irrigation suspension. However, the biochemical responses to these conditions are unknown. The objective of this study was to quantify and analyze the profile of compatible solutes involved in the biochemical responses to water deficit and excess salts in soils. The 30-day-old seedlings were exposed to different treatments: I) irrigation with NaCl saline solution II) irrigation suspension at 70% and 30% of the field capacity (FC) and III) Control irrigated with Hoagland solution 25% and at 100 % FC. In 15, 30 and 42 days after starting the treatments, samples of roots and aerial parts were collected to quantify: I) Proline (PR); II) glycine-betaine, as quaternary ammonium compounds (QACs); III) total soluble carbohydrates (CH). Results showed a progressive increase in PR, QACs and CH, in the aerial part of plants exposed to salinity and drought, being higher in those irrigated at 30% FC. In the case of roots, a significant increase in PR and QACs was observed in plants exposed to 30% FC, in relation to controls. The highest content of total soluble CH was recorded in the roots of plants exposed to saline solutions of NaCl (700 mM), indicating a higher respiratory rate, due to the demand for ATP for the incorporation of ions, due to direct contact with salinity. While, in the area part, the total CH only increased in plants at 30% FC. This response, together with the accumulation of PR and QACs, represents an efficient osmoregulation and protection mechanism in *P. praecox* to tolerate the water deficit in the soils where this species lives.

## A51

### THYME AND OREGANO ESSENTIAL OILS AS NATURAL ANTIFUNGALS FOR THE PREVENTION AND TREATMENT OF WOOD DISEASE

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The grapevine disease known as “Vine Wood Disease” (VMD), caused by ligninolytic fungi, is internationally considered to have a great impact, as it causes the death of plants. Wine production is the most important economic activity in the province of San Juan, so any pathology that alters the performance and quality of the vines has a strong economic impact. These pathologies are produced by a pool of ligninolytic fungi, which cause Eutypiosis, Esca, Petri's Disease, BAD (Dead Black Arm), Malvón Leaf. Recently, *Lasiodiplodia tehobromae* has been reported to be one of the causative agents of the irreversible destruction of the disease. We isolated ligninolytic fungi from grapevine plants in San Juan. After morphological and molecular analyses, the presence of *Lasiodiplodia tehobromae* was confirmed. In parallel, there is currently much research on the antimicrobial action of Thyme (*Thymus vulgaris*) and Oregano (*Origanum majorana*). In parallel, extractions of macerations (e.m) in dichloromethane and steam distillates to obtain the essential oils (EO) of Thyme and Oregano were obtained. A bioassay was carried out to initially determine the inhibition of the growth of *Lasiodiplodia tehobromae* and subsequently the fungicidal activity. The dilutions used in the inhibition test were 1/2, 1/4, 1/8, 1/16, 1/32 and 1/64 of the e.m and EO extracts. Three replicates of each treatment were carried out. To evaluate whether the extracts acted as a fungicide or fungistatic agent, a subsequent bioassay was performed when the absence of mycelium was detected in the growth inhibition bioassay. It was obtained that the dilutions for growth inhibition were 1/4 for the two macerates and for the Thyme EO; and 1/16 for the Oregano EO. In addition, they turned out to be fungicidal agents in the 1/2 dilution for the e.m and EOss and for the Thyme EO; and 1/4 for the Oregano EO, proving to be potential natural agents in the treatment of this disease.

## A52

### *Pyrolirion albicans*: ALKALOID GC-MS PROFILE ANALYSIS IN DIFFERENT PHENOLOGICAL MOMENTS

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Bulbous species are perennial species that exhibit active growth and flowering in spring. They survive thanks to the underground bulb, where the reserves are accumulated during senescence. The Amaryllidaceae family is widely distributed in different regions of the neotropics and temperate zones of the world. In Peru, the genus *Pyrolirion* Herb has six endemic species. The chemical composition of the alkaloid extract from *P. albicans* bulbs collected in spring, has been recently reported. The results indicated high levels of montanine and the presence of the alkaloids Galantamine (Gal), N-Demethylgalantamine, Vittatin/Crinine, Pancracine, Sternbergine, Lycorine, Hippeastrine, 2a-Hydroxyhomolycorine in similar relative proportions, while in the leaves Gal, Clidantine, Tazettine, and Lycorine, were the most abundant. The objective was to establish if the alkaloid profile (quali- and semi-quantitatively) is affected by the phenology of the species. For this comparative analysis, *P. albicans* bulbs were collected in autumn (senescence period), chopped, dried, and subsequently subjected to the alkaloids extraction. Alkaloid identification and semi-quantification by GC-MS was performed using a DB5-MS column, AMDIS 2.65 program, and private alkaloid library. A calibration curve of galantamine using codeine as the internal standard ( $y = 38.636x + 9.8269$ ;  $y = \text{mg Gal}$ ;  $x = \text{Gal area / codeine area}$ ) was applied for the quantification, with Gal ranges of 5-900 mg/mL, and 3 injections for each Gal level. The quantity expressed as ( $\mu\text{g Gal} / 100 \text{ mg dry weight}$ ). The autumn collection showed a high content of Tazettine followed by Lycorine and Sanguinine, as well as the absence of montanine. It was confirmed that, depending on the time of collection, the alkaloid profile is different. Regarding the high content of tazettine ( $32.3 \mu\text{g} / 100 \text{ mg}$ ) in the bulbs from the autumn collection, it could be explained considering that, at the time of senescence, the alkaloids from the aerial part (leaves) are stored in the bulbs. It is evident that phenology would greatly affect the composition and relative proportion of alkaloids in this species, indicating that the time of collection is decisive for the alkaloid of interest, whether it is montanine to collect in spring and whether it is tazettine to collect in autumn. Acknowledgments: O.LI-C and JEO hold CONICET scholarships. PICT 03883 ANPCYT, PIP2022-2024-0902 (CONICET). CYTED Red 223RT0140

### A53

#### COMPARATIVE ANALYSIS OF THE CHEMICAL PROFILES OF *Pyrolirion albicans* ALKALOID EXTRACT BY UPLC-ESI-MS AND GC-EI-MS

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The *Pyrolirion* Herb. genus belongs to the tribe Eustephieae, according to World Checklist of Selected Plant Families (WCSP) (2014), *Pyrolirion* Herb. consists of eight species: *P. albicans* Herb., *P. arvense* (F. Dietr.) Erhardt, Gotz & Seybold, *P. boliviensis* (Baker) Sealy, *P. cutleri* (Cardenas) Ravenna, *P. flavum* Herb., *P. huantae* Ravenna, *P. tarahuasicum* Ravenna, and *P. tubiflorum* (L'Her.) M. Roem. Most of these species are Andean, five are found in Peru, one shared with Chile, and two in Bolivia. *P. albicans* is endemic to sandy biomes between 50 and 300m in the coastal region of southern Peru, where it blooms during the fog and drizzle season. The analysis of plant extracts by GC-MS is a technique with high reproducibility and capable of detecting very low polarity compounds. UPLC-MS analysis represents a soft ionization technique that shows results that better reflects the real composition of each plant extract, and can also analyze compounds of higher polarity. Thus, both techniques are complementary in the analysis of natural products. The objective was to compare qualitatively the alkaloid composition of the *P. albicans* species by GC-MS and UPLC-MS to identify potential bioactive compounds. Dry bulbs were chopped, dried, and subsequently subjected to the alkaloids extraction. In the case of GC-MS, alkaloid identification was performed using DB5-MS column and AMDIS 2.65 program with a private library of 300 alkaloids. For UPLC-MS, Acquity BEH C18 column, 1.7 µm, 2.1 mm x 100mm and MassLynx v4.2 (ChromaLynx) program with a private library of 40 alkaloids were used. 12 alkaloids were identified by GC-MS while 6 were identified by UPLC-MS. In the GC-MS analysis, the total ion current showed Tazettine (Taz) as the main alkaloid, followed by Lycorine and Sanguinine. In the UPLC-MS analysis, the 3 alkaloids mentioned above were also observed with high abundance, showing a new signal corresponding to a tazettine-type alkaloid, suggesting that it could be Pretazettine (precursor of Taz), which does not appear in GC-MS due to the high temperature in the oven that decomposes most of the compound, transforming it into Taz. Thus, some alkaloids are preserved if analyzed by UPLC-MS, and a more real alkaloid extract profile is acquired, confirming that this method allows the detection of other potentially bioactive molecules. Pretazettine and Taz stand out in this species as alkaloids with well-known high antitumoral activity. Acknowledgments: O.LI-C and JEO hold CONICET scholarships. PICT 03883 ANPCYT, PIP2022-2024-0902 (CONICET). CYTED Red 223RT0140.

### A54

#### PRELIMINARY STUDY OF OLIVE PLANTATIONS (*Olea europaea* L.) IN PRODUCTION ESTABLISHMENTS IN THE PROVINCE OF SAN LUIS, ARGENTINA

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In the province of San Luis (Argentina), the olive tree (*Olea europaea* L.) currently reaches a total area of 150 hectares, with the largest number of establishments in the center and north of the province, where the varieties that are worked correspond to a greater extent to Coratina, Arbequina, Manzanilla, Arbosana and with a smaller surface area Frantoio and Changlot. The work consisted of collecting information on the varieties, phenology and analysis of soil and climatic data from the establishments. In surveys of the plots, the presence of tuberculosis was observed, a disease caused by the bacterium *Pseudomonas savastanoi* pv *savastanoi*, which increases the problems of alternation of production, leading many establishments to desist in control and prevention techniques. Many of the plantations have different degrees of infection of the disease, depending on varieties and productive management, reaching values of 30% to 80% of infected plants. In a plantation located in the town of Nogolí, the presence of plants affected by tuberculosis was observed, with an infection rate of about 20%. The varieties he works with are Arbosana and Arbequina, both used for oil extraction and grown in super-intensive systems. Budding begins first in Arbequina and then in Arbosana, with the middle dates of this stage being September 23 and October 20 respectively, developing flowering from October to December according to the variety. In a study period of 10 years, a 50% probability of temperatures below zero degrees was recorded in the months of September – October, reaching a minimum temperature of -2.6 °C for the area, which determines a decrease in the production of olives and their percentage of oil. As a result, the Arbequina variety, being earlier in the budding-flowering process and more susceptible to tuberculosis disease, presents a marked decrease in production, increasing the "alternation" between years, and the Arbosana variety for the study region, although also susceptible to tuberculosis, presents better productions in the face of late frost events in the study area. More field data and study of the phenology of other varieties in the area are required to increase the efficiency of the establishments. For new plantations, it is recommended to choose tuberculosis-resistant varieties such as Picual and Verdial, instead of the more sensitive ones (Arbequina and Coratina).



A55

**THE OLIVE TREE (*Olea europaea* L.), AS A POTENTIAL CROP FOR THE DEVELOPMENT OF FRUIT IN THE NORTHERN REGION OF THE PROVINCE OF SAN LUIS. ARGENTINA**

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In fruit growing, the economic development of unexploited regions requires preliminary studies of the climatic feasibility of the site for the proposed species as well as the economic feasibility of the productive approach. The Northern Region of the Province of San Luis appears as a place in the country with significant potential for growth in the fruit industry. With the intention of advancing on profitable productive approaches, the analysis of each of the intervening variables was carried out, obtaining climatic information from 2009 to 2019. The selection of olive cultivars of possible adaptation and the optimal financial evaluation for the region have been determined, which point to this crop as "one of the most suitable". The winter thermal offer of the town of Quines (2009-2019) is 425 (+/- 124) hours of cold; with an average vernalization period of 100 (+/- 19) days, and an average frequency of 12 days of devernalization period. The frost regime is 99 (+/- 35) days on average, with the most intense months being June, July and August without exceeding -4.2 °C intensity. The soils are characterized by a sandy loam texture, good drainage, adequate depth, no physical limitations, neutral pH up to 60 cm and danger of moderate wind and water erosion. In this region, the supply of irrigation water is based on the exploitation of shallow subsurface sources and the provision of the Quines and Conlara rivers. The most suitable cultivars for adaptability and commercial value in the area are: Arbequina, Coratina, Picual, Barnea, Arbosana and Manzanilla. Evaluating the implementation costs in semi-intensive projects, the operating profitability increases from the 4th year (4%) to the 8th year, stabilizing at 250%. The implementation of the Provincial Fruit and Vegetable Development Law would allow an increase of the crop planted area and quickly recover the implantation costs of the olive tree, a species of late entry into production and slow economic return. In addition, the collected information serves as a general basis for the choice of varieties according to the productive objective and the soil and climatic conditions.

**BIOTECHNOLOGY AND GENETICS**

A56

**DEVELOPMENT OF A CAPILLARY METHODOLOGY FOR THE DETERMINATION OF PHOSPHOLIPASE A2 ACTIVITY ON LOW-DENSITY LIPOPROTEIN IN PRESENCE AND ABSENCE OF ALBUMIN**

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The development of an atheromatous plaque is a complex process that begins with the oxidation of low-density lipoprotein (LDL). This triggers an inflammatory/proliferative process in which several types of cells like lymphocytes, monocytes/macrophages, smooth muscle cells and endothelial cells intervene. One of the most important enzymes that modify LDL is the Phospholipase A2 type IIA (sPLA2). This enzyme, detected in atheromatous plaques, circulates at high levels in patients with cardiovascular disease (CVD) and may be used as a marker for vascular risk. In this study, we develop a system "on capillary", using a capillary electrophoresis equipment, to determine the enzymatic activity of sPLA2 on LDL in the presence and absence of albumin. Albumin is a transport protein and a necessary participant in the removal of fatty acids and lysophosphatidylcholine from LDL oxidized by the action of sPLA2. This "on capillary" procedure was performed by partial filling technique to achieve the interaction of the enzyme with LDL in the presence and absence of albumin. The interaction process was followed by detection the products of the reactions. When albumin was not introduced into the system, the quantified product was LDL (-) or oxidized; and when albumin was part of the system, the products obtained were albumin-fatty acids and albumin-lysophosphatidylcholine. As a result we develop an on capillary methodology that allowed as, not only to separate and quantify the biomolecules, but also to study their interaction.

## A57

### SCREENING OF INVERTASE PRODUCTION FROM *Saccharomyces cerevisiae* AND ENZYMATIC ACTIVITY ANALYSIS BY THE 3,5-DINITROSALICYLIC ACID METHOD

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The enzyme known as  $\beta$ -D-fructofuranosidase, or invertase, plays a pivotal role in breaking down sucrose, resulting in a blend of its two constituent monomers: glucose and fructose. This blend is known as invert sugar and is notably sweeter than sucrose. Invert sugar finds extensive use in the food and pharmaceutical sectors. It is possible to obtain invert sugar through a chemical or enzymatic approach. The enzymatic method is significantly more efficient and yielding fewer undesired byproducts. Invertase is naturally produced by various organisms that are able to use sucrose as a carbon source. It is present in organisms such as invertebrates, vertebrates, green algae, bacteria, vegetables, yeast and fungi. In previous studies, we investigated the production of the enzyme by fungus *Aspergillus niger*, obtaining a yield of 65.17 g/l of glucose at pH 5 and 28°C working with Sabouraud Dextrose liquid medium supplemented with sucrose 20 g/l. The genus *Saccharomyces* has been shown to be a good producer of this enzyme. The aim of this work is to obtain the enzyme invertase at laboratory scale from the yeast *Saccharomyces cerevisiae* and study the influence of experimental parameters that affect the production and purification process of this enzyme. We worked with *S. cerevisiae*, analyzed the enzymatic activity of invertase using 3,5-dinitrosalicylic acid. To determine the concentration of Invertase in the samples, a glucose calibration curve was performed and absorbances of the samples were measured at 540nm. A high glucose concentration is indicative of high enzyme activity. A screening of different variables such as culture medium, time, pH and temperature was performed. A higher biomass was obtained working with Yeast glucose medium (YGM) than Potato Dextrose liquid medium (PDL) in all the cases. The highest values of glucose concentration were 49.31 and 51.55 g/l corresponding to biomass obtained in YGM supplemented with sucrose 40 g/l and 30 g/l respectively, and then suspending it in a 10 g/l sucrose solution before obtaining the enzymatic extract. The fungus was suspended in sucrose solution 10 g/l in order to increase the production of more enzyme Invertase in the presence of more substrate. These values were obtained working at pH 4.5, at a temperature of 35°C.

## A58

### EFFECT OF BACTERIAL CONSORTIA ON THE PROMOTION OF GROWTH IN OREGANO PLANTS

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The Ministry of Social Development of the Province of La Pampa has implemented programs aimed at fostering innovative primary production and productive and social value-added activities in different agro-climatic regions of the province. The possibility of finding alternative ways to inorganic fertilization would allow production to be maintained or increased, while a reserve of nutrients is formed in the soil. The objective of this work was to isolate microorganisms with plant growth-promoting properties that allow enhancing the growth of oregano plants. In the present work, phosphorus-solubilizing microorganisms in the rhizosphere of oregano were isolated. The production of indole acetic acid (IAA) and siderophores was studied. According to the results obtained, 8 bacterial isolates of the genus *Pseudomonas* were selected (identified as 2, 4, 5, 6, 7, 10, 13) and the isolate called 21, belonging to the genus *Bacillus*. Three consortia were created combining the 8 isolates. In vivo growth promotion study was carried out on oregano plants. Two isolates (10 and 6) presented IAA production with values greater than 50  $\mu$ g/ml, while the rest of the isolates ranged between values of 8 and 38  $\mu$ g/ml. It was determined that the isolates that produce the most siderophores are 10, 2 and 21, while 7 and 13 produce a smaller amount. The consortia were named I (strains 6, 4 and 7), II (strains 13, 10 and 2) and III (strains 10, 5 and 21). Finally, the plant assay allowed to observe the plant growth-promoting effects of the different consortia compared to a control treatment without inoculation. Plant growth was evaluated through the increase in leaf area. The measurements were obtained by using the imageJ® computer program. The results showed that there are statistically significant differences between the treatments inoculated with the consortia and the uninoculated control, while no significant differences are observed between the inoculated treatments. However, it can be highlighted that consortium III achieved greater growth (between 5% and 8%) compared to consortium I and II respectively. It could be inferred that the synergy produced between the bacterial isolates present in consortium III generated the difference found in growth promotion. These results allow to conclude that it is possible to use native flora to enhance the growth of horticultural species, and in this way contribute to the sustainability of the soil resource.



## A59

### CHARACTERIZATION OF PGPM ACTIVITIES OF MICROORGANISMS ISOLATED FROM ROSEMARY AND OREGANO RHIZOSPHERE

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Plant growth promotion mediated by microorganisms (PGPM) is being widely used as a sustainable way of managing crops of interest, both extensive and intensive agriculture. Aromatic and medicinal plants (AMP) are a large group of plant species used for their medicinal, flavoring or aromatic benefits. In particular, different varieties of rosemary and oregano are grown by small producers in the Province of La Pampa. The objective of this work was to isolate PGPM microorganisms from the rhizosphere of rosemary and oregano, taking the ability to solubilize inorganic phosphorus as the main selection criteria. Then, other properties such as phosphatase activity, biocontrol capacity and Minimum Inhibitory Concentration against antibiotics were evaluated on the isolates achieved. Twenty three phosphorus-solubilizing isolates were selected. In the quantification of soluble phosphorus, isolation 4 stands out, reaching values of 550 µg/ml of soluble phosphorus. Regarding phosphatase activity, it was determined that 6 isolates exhibited this property, with maximum values reached of up to 430 µg PNP (paranitrophenol)/ml.h in acidic conditions; while under alkaline conditions, the maximum was 70 µg PNP/ml.h. The bioactivity tests against pathogens (*Fusarium graminearum* and *Fusarium oxysporum*) show that the isolates called 4 and 7 showed an inhibitory effect against the phytopathogenic strains studied, considering that for *F. oxysporum* a growth inhibition of more than 20% was observed, while in *F. graminearum*, its development was inhibited only by isolation 4. On the other hand, each isolate showed a different behavior regarding the determination of the Minimum Inhibitory Concentration against antibiotics, which is useful because it allows them to be quantified individually when they are found in bacterial consortia. Of the 23 isolates, 8 were selected based on their PGPM properties, which were identified taxonomically. These studies showed that of the 7 isolates evaluated correspond to the genus *Pseudomonas*, while the remaining isolate belongs to the genus *Bacillus*. The results achieved allow us to infer that it would be possible to generate bioformulas from native strains, with activity in promoting growth and as biocontrollers of pathogens, in regional aromatic crops, as a key tool to improve the specific production system of the province. It is essential to learn to combine technologies to improve the benefits of production and preserve the agroecosystem.

## A60

### PHYSICOCHEMICAL CHARACTERIZATION OF EXTRACELLULAR POLYMERIC SUBSTANCES (EPS) OF *Bacillus atrophaeus* AND THE POTENTIAL USE IN HEAVY METAL REMOVAL

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Extracellular polymeric substances (EPS) are microbial polysaccharide. In previous work was evaluated the EPS production by *Bacillus atrophaeus* in presence of glucose or saccharose, with or without Cu(II) or Cr(VI). The results showed that production was independent of the carbon source but increased significantly with the presence of metals. The objective of this work was to physicochemical characterize of EPS produced by *B. atrophaeus* and to study its heavy metal removal capacity. EPS was produced in the absence or presence of Cu(II) or Cr(VI). The EPS solutions were dried on a glass plate for 24 h, in order to obtain a powder structure to perform both structural and thermal stability analysis using Fourier transform infrared spectroscopy (FTIR), determination of the thermal stability of the material by thermogravimetric analysis (TGA) and Differential Scanning Calorimetry (DSC). Also, Scanning Electron Microscopy - Energy Dispersive X-ray spectroscopy (SEM-EDX) analysis was performed on the EPS produced under all work conditions. Finally, to evaluate the ability EPS binding metals in aqueous systems, equilibrium dialysis experiments were carried out by placing dialysis tubing (14 kDa MWCO membrane) containing the EPS solution (67 mg/L of EPS) in closed glass flasks with 50 mL of the metal solution. The flasks were placed in an orbital shaker (150 rpm) for 24 h. Residual heavy metal concentrations were analyzed using an atomic absorption spectrophotometer (AAS). The results of the analysis with SEM-EDX and heavy metal removal with the EPS determined that only Cu(II) was adhered by EPS. The FTIR analysis demonstrated the presence of the functional groups -OH, -CH<sub>2</sub>, C=O, COO, C-O in all EPS. In EPS obtained in the Cu(II) and Cr(VI) presence, the functional groups determined were C-O-C, 1,2,4 trisubstituted pyranosic ring and monosubstituted pyranosic ring. The TGA characterization resulted in a decomposition temperature of 250°C in all EPS. With the DSC characterization the transition temperature was 57°C in the EPS obtained without the presence of metals, 71°C in the presence of Cr(VI) and 66°C in the presence of Cu(II). None presented a melting point. The characteristics of EPS of *B. atrophaeus* suggest that, it can be used as a potential copper adsorbent in bioremediation process.

## A61

### EARLY TRICEPIRO STRAINS IDENTIFICATION FOR SERVICE PURPOSE

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Variability generation in hybrid triticeae is achieved through crossings between acceptable biomass production strains. The multiple combinations obtained must be evaluated in the production environment, considering different selection purposes. Tricepiro (*X*Triticosecale Wittmack x *X*Agrotriticum Ciferri & Giacom) is a winter cereal used for its fresh forage production. Tricepiro strains present different cycle length that enable its use in agricultural systems as service crops, prior to summer crops. In order to identify early tricepiro strains for service purpose, biomass production and its distribution in time of 22 stabilized strains of tricepiro and 4 registered cultivars of triticale were measured for 4 years (2019-2022) in a randomized complete block design, with 3 replications in 7 m<sup>2</sup> experimental units. The trials were developed in the Experimental Field of the NU of Río Cuarto. Average aerial biomass in the first forage harvest at 70 days from sowing was 1.55 t.ha<sup>-1</sup> (interannual variation range: 0.42-2.83 t.ha<sup>-1</sup>), representing 59.4% of total production under defoliation (2.53 t.ha<sup>-1</sup>). The ANOVA revealed significant interaction genotype\*year, a common situation in the central subhumid area of Argentina with great interannual rainfall variation. Biplot GGE analysis of interaction allowed the identification of strains that produced most biomass in the first cut (early strains): 46SH20 in 2022, year of extreme autumn drought, produced 0.97 t. ha<sup>-1</sup> (two-fold the average of 2022 cycle). The rest of the years presented average autumn precipitations, and 88SH14 showed acceptable production (2.76 t.ha<sup>-1</sup>; VR: 1.11-4.15, representing 77.3% of total production under defoliation). The significant differences found among tricepiro strains highlight the importance of considering specific aspects in genotype selection for service purpose, since they provide high biomass production in short period of time, generating good soil coverage, protecting it from erosion and contributing with available nutrients to the next crop, with minimal water consumption.

## A62

### DESIGN OF ECONOMICAL CULTURE MEDIUM FROM WASTE OF THE CRAFT BEER MAKING BEER PROCESS IN ORDER TO MULTIPLY YEASTS

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In the brewing process, waste products such as spent yeast and low-gravity wort are generated. The spent yeasts are thrown away after the fermentation process and the low-density must is not used for fermentation because it does not have the appropriate concentration of sugars for this purpose and has the presence of tannins and silicates that are undesirable compounds for beer, besides the concentration of sugars they contain are suitable for multiplication, but not for fermentation, since they would generate beers with lower alcohol content. The aim of this study is to design economical culture media using these wastes to multiply yeast. In order to do that, two liquid culture media were designed: (A) low-density wort and (B) low-density wort with dried spent yeast (5 g/L). After harvest, the yeasts were dehydrated in an oven at 60°C for 24 hours and grounded to generate a powder. As control, a culture medium (C) YGM of known formulation was used. The concentration of sugars expressed in °Brix and the pH were calculated for each culture medium. To verify the multiplication in these culture media, native yeast biochemically characterized as *Saccharomyces cerevisiae*, was inoculated in the three-culture media at a concentration of 2.0 x 10<sup>6</sup> cel/mL, after 24hs of incubation at 27±1°C, the following parameters were measured: yeast concentration, percentages of live yeasts (viability), sugar content, and pH. The results indicated that the yeasts multiplied massively with a viability of 99% in the three designed culture media. The yeast concentration was 1.7 x 10<sup>6</sup> cel/mL in (A), 2.1 x 10<sup>6</sup> cel/mL in (B) and 1.4 x 10<sup>6</sup> cel/mL in (C); a greater multiplication was observed in the medium (A) and in (B) than in the culture medium (C). The yeasts consumed sugars was from 9.4 to 6.1 °Brix in the medium (A), from 9.4 to 5 in (B) and from 2.2 to 1.0 in (C), a decrease in pH was observed in the medium (A), from 6.3 to 4.3, in the medium (B) from 6.4 to 4.5 and in the medium (C) from 6.0 to 4.9; indicating healthy yeast growth. In conclusion, economical culture medium could be designed using waste to multiply the yeast *S. cerevisiae*, which can be used as supplies for the production of craft beer.

### A63

#### PHYCOREMEDIATION OF WASTEWATER BY *Oocystis* sp.

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Phycoremediation refers to the use of algae (including Cyanobacteria, microalgae, and macroalgae) in the industrial processes for pollutant removal or to derive products from wastewater, for example algal biomass. Microalgae used in the phycoremediation system are photosynthetic phytoplanktonic organisms, which form the first link of the aquatic food chain and represent the main component of the aquatic ecosystem. The objective of this work was to study the efficiency of phosphate and nitrate removal of municipal wastewater by native microalgae *Oocystis* sp. The samples of microalgae were collected with a phytoplankton net from local water bodies of San Luis. In order to separate algal populations, standard plating method was applied BBM (Bold's Basal Medium) and then *Oocystis* sp was grown in cultures at 25±2°C, with continuous illumination of 3000 lux for 14-16 days. The initial biomass (6 10<sup>6</sup> cells mL<sup>-1</sup>) was exposed for 10 days to four dilutions of an urban effluent (25/100 mL, 75/100 mL, 50/100 mL and 100/100 mL respectively) and a control sample. The chemical determinations of phosphates and nitrates were analyzed by Standard Methods for Examination of Water and WasteWater (APHA, 2005). Within 10 days of exposure, phosphate was almost completely removed from the wastewater effluent by algae in the 50% and 100 % dilutions while nitrate was efficiently removed at 34, 5% with respect to control. These results suggest that *Oocystis* sp could be used to remove N and P from wastewaters enhancing nutrient removal. The native species cultures have a high potential to remediate and the phycoremediation technology or integrating into the conventional systems, wherever feasible, seems to be a more economic and eco-friendly remedy.

### A64

#### SELECTION OF MICROORGANISMS RESISTANT TO THE EFFLUENT DISCHARGED IN A LANDFARMING IN THE CITY OF SAN LUIS

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Bioremediation carried out by indigenous microorganisms is one of the main mechanisms by which different contaminants can be naturally eliminated from the environment. The success of a bioremediation process depends on the intrinsic capacity of the system to trigger and maintain conditions that promote the contaminants biodegradation at high rate. The objective of this work was to study the microorganism's resistance capacity to resin effluents for their use in the bioaugmentation process. The isolated microorganisms from landfarming, in previous works, were seeded in solid Eg medium (g /L: glucose 10; K<sub>2</sub>HPO<sub>4</sub> 0.5; KH<sub>2</sub>PO<sub>4</sub> 0.5; yeast extract 1; agar 15) pH 6,0± 0,2, incubated until growth at 29°C. A central channel was made in each plate in which the effluent diluted to 50% was added. On the other hand, the inhibition effect between the different strains of bacteria and fungi was tested. And finally, a quantitative test was carried out in 10 mL of LB glu liquid medium (g/L: glucose 10, yeast extract 5, peptone 10, sodium chloride 5) at different concentrations of the resin effluent (25%, 50% and 75%) at 150 rpm to analyse the individual resistance of the microorganisms to the effluent. Culture medium and water were used as reference. From these tests, four resistant microorganisms (bacteria and fungi) were obtained, which did not show inhibition among themselves. Furthermore, growth of the microorganisms could be visualized at all effluent concentrations tested, being faster and greater at highest concentrations of effluent. The latter, shows that microorganisms use the effluent as a source of carbon and energy for its growth. The microorganisms isolated from landfarming are capable of using the effluent and therefore degrading it, a fundamental activity for bioaugmentation processes with autochthonous microorganism in the landfarming.

## HUMAN CLINICS AND ODONTOLOGY

### A65

#### BRAIN HEALTH AND ALZHEIMER'S DISEASE. KNOWLEDGE AND PERCEPTION IN SAN LUIS

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Brain Health care (BH) and the prevention of Alzheimer's Disease (AD) constitute global challenges for public health, since it is the main cause of dementia and disability in older adults. To determine: (1) the social perception of the population of San Luis about AD; (2) knowledge about BH (3) Forgetfulness, a digital Questionnaire (Google form) was voluntarily applied and with informed consent, in both sexes (N=302, 18 to 80 years old) individuals, without AD, from San Luis City. Included: EA Survey (Harvard SP School), BH Habits Questionnaire (AARP Foundation). 84.4% (n= 255) were women and 45.36% reported a university educational level or higher (n= 137). 4 age groups (G) were considered (G1) 18-30 (n=81); (G2) 31-45 (n= 110); (G3) 46-60 (n=84) and (G4)  $\geq 60$  years (n=27). All Groups maintained BH practices, with a difference observed in prevalent physical exercise habits in G1 ( $X=2.63 \pm 0.83$ ) and follow-up - medical controls in the older age groups (G2:  $2.75 \pm 0.92$ , G3  $2.63 \pm 0.88$  and G4  $2.93 \pm 0.83$ ). The following results of "normality of forgetfulness, even if they affect daily life" were observed: G1: 49.38% and G4: 59.26%. AD ranks as the second most feared diagnosis (24.17%), after Cancer (33.11%) and before stroke (22.85%). Regarding segregation, concern is observed in the older G (G1 18.52%, G2 20.91%, G3 30.95% and G4 33.3%); although greater knowledge of SC factors in young people (G1: 85.19%, G2: 75.45%, G3: 71.74% and G4: 51, 85%). (G1) obtained greater knowledge of BH, mainly due to physical exercise habits. G2, G3 and G4 stand out in medical check-ups and treatment monitoring. G1 and G4 consider "forgetfulness as normal, even if it affects their daily life." This result strengthens the need for information and awareness campaigns in relation to normal-typical aging and signs of cognitive deterioration.

## A66

### EPIDEMIOLOGY OF *Tinea capitis*: A STUDY IN SAN LUIS CITY, ARGENTINA

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*Tinea capitis* is an infection of the scalp produced by a dermatophyte fungus. It is essential to know the epidemiology of the etiological agents according to the results of mycological analyses in each region and to determine their clinical symptoms. In this work, we present the data collected by two laboratories in San Luis City. The study was descriptive, observational, and retrospective. One hundred fifty medical records of patients with mycological lesions on the scalp were reviewed, aged 0 to 18 years, from January 2020 to June 2023. The average age of patients was 10.66 years, and the male: female ratio was 3:1. Samples were analyzed using two different techniques: (a) direct microscopic examinations (DME) with 20-40% KOH and (b) cultures on Sabouraud agar with cycloheximide at 28°C for three weeks. Filamentous fungi were identified by their macroscopic and micromorphological characteristics. The most frequent clinical presentation was the non-inflammatory type. These results were similar to those obtained in studies in other regions of Argentina. DME produced positive results in 49.33% of cases (n= 74), while cultures had positive results in 46% (n= 69). Of the latter, developments of *Microsporum canis* (76.81%; n= 53), *Nannizzia gypsea* (14.49%; n= 10), and *Trichophyton mentagrophytes* (4.35%; n= 3) were found; the remaining three cultures were *Candida* spp (4.35%). *M. canis* was the most frequent fungus, followed by *N. gypsea*, suggesting that this could be the emerging etiological agent in our environment. Due to geographic variations, epidemiological surveillance is required to understand changes in the causative agents of ringworm of the head.

## A67

### DISSEMINATED CRYPTOCOCCOSIS BY *Cryptococcus deneoformans*

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Cryptococcosis is a pulmonary or generalized infection acquired by inhalation of naturally occurring encapsulated yeasts belonging to the *Cryptococcus neoformans*/*Cryptococcus gattii* species complexes. The complex includes *Cryptococcus neoformans* var. *neoformans* strains (serotype D, genotype VNIV), *C. neoformans* var. *grubii* strains (serotype A, genotype VNI or VNII), and hybrid strains (serotype A, D, or AD, genotype VNIII). *C. gattii* strains can be serotype B or C and genotypes VGI, VGII, VGIII, and VGIV. An 80-year-old man, native to Bolivia, was admitted to the COVID-19 sector of the hospital with dyspnea on moderate exertion, accompanied by cough and hemoptoic sputum of more than a year's duration, trigeminal neuralgia, and repeated epistaxis. The patient presented bilateral fibrosis and bilateral multilobar alveolar-interstitial infiltrate. Negative results were obtained for the virological panel (PCR for SARS-CoV-2, serology for HIV, Chagas disease, syphilis, chlamydia, and mycoplasma). No acid-fast bacilli were observed in serial sputum samples. After two days of incubation, development was obtained in one of the blood culture bottles, and sprouting, globose, and capsulated yeasts were observed without pseudomycelium. After 24 hours of incubation, cream-colored colonies with a wet and creamy appearance were obtained on Sabouraud glucose agar. Complementary tests were carried out, such as positive growth at 37°C, positive urease test, observation of the capsule in negative staining with India ink, and micromorphology in malt extract broth that showed globose and sprouting cells without pseudomycelium. The isolate was identified as *Cryptococcus neoformans* complex, and the culture was sent to the National Reference Laboratory for genotyping by PCR-RFLP of the URA5 gene. The isolate presented the VNIV genotype, which corresponds to *Cryptococcus neoformans* var. *neoformans*, serotype D. Here we show the clinical case of a patient whose diagnosis on admission was community-acquired

pneumonia (CAP) and the isolation in a blood culture of *Cryptococcus deneoformans* (serotype D, genotype VNIV). This isolation constitutes an important finding since the different species identified by PCR-RFLP of the URA5 gene present clinical-epidemiological, phenotypic, and susceptibility to antifungal differences. This isolation is also the first of its kind obtained from clinical samples in our hospital and contributes to the knowledge of the local epidemiology of cryptococcosis.

## A68

### IDENTIFICATION OF 2-PORE POTASSIUM CHANNELS (K2P) IN WARTY CARCINOMA OF ORAL CAVITY

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Warty carcinoma (VC) or Ackerman's tumor is considered a slow-growing neoplasm with high local aggressiveness, limited metastatic capacity, and good response to treatment, although there are cases of transformation into invasive lesions of greater malignancy. It is believed that lesions that end with anaplastic transformation are currently "hybrid or mixed" tumors composed of VC and invasive squamous cell carcinomas. Therefore, it is necessary to identify these hybrid tumors because their prognosis changes radically. However, there are no studies that indicate the expression of potassium channels in this type of lesion, as these channels are considered potential markers of tumor lesion evolution. In our previous study, we described for the first time the presence of K2P potassium channels in advanced squamous carcinomas of the oral cavity, indicating their potential as markers of sensitivity or resistance to chemotherapeutics widely used for the treatment of these carcinomas (especially taxols) in cases where surgical resection is not possible. The aim of this study was to determine the presence and distribution of K2P potassium channels in CV oral cavity samples. A retrospective study was carried out on 5 biopsy samples belonging to the bank of tumors and oral pathologies of the Anatomic Pathology laboratory of the Faculty of Dentistry of the UNCuyo, with the authorization of the bioethics committee of the Faculty of Dentistry (Acta evaluación 622 16/2/2022). Immunolabeling of K2P and beta-tubulin III channels was performed. Observation and determination of The expression levels of the immunolabeled channels were determined by confocal microscopy using an FV-1000 microscope (Olympus) at the IHEM-CONICET-Facultad Ciencias Médicas Research Center. The relative intensities of ion channels versus beta-tubulin III were measured. In warty carcinoma, the initial analysis, performed on a limited number of samples (n=5), demonstrated low expression of beta-tubulin III in the lesion per se. The low expression of beta-tubulin III indicates that the presence of cells with neoplastic potential in this type of lesion has a limited incidence. The levels of the TASK3 channel, usually elevated in solid tumors with poor prognosis, are relatively low, and they are found in the outermost cells of the lesion, while there are normal basal levels of TASK1 and TRESK, coinciding with our previous analyses performed in dysplasia and OSCCC samples. We also identified profuse innervation in the adjacent immediate reaction as a possible source of distant dissemination.

## A69

### MORPHOLOGICAL CHARACTERIZATION OF DIFFERENT VARIETIES OF *Neltuma flexuosa*, NATIVE PLANT OF SAN LUIS, ARGENTINA

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Species of the genus *Prosopis*, some currently included in the genus *Neltuma*, have pollens that can produce allergic pathologies, causing significant respiratory problems. Previous studies indicated the presence of contaminants in aerobiological samples from San Luis in pollen from *Vachellia caven* (Molina) Seigler & Ebinger, a species also native to this province, which usually forms communities in which it cohabits with *Neltuma flexuosa* (DC.) C.E. Hughes & G.P. Lewis. This species is a native tree, widely distributed in the province of San Luis (Argentina). During the pollination period, pollen concentrations increase with high temperatures (dry, sunny days) and decrease with rain or cold, a fact that is known thanks to air monitoring. The objective of this work is to identify and characterize the pollen of *N. flexuosa* in urban and rural areas of the Province of San Luis. The proposed methodology includes the collection of fresh *N. flexuosa* pollen, sample preparation using the basic glycerofuchsin dye and observation with an optical microscope at 1000X. The microscopic analysis allowed for detailed detail on the characteristics of *N. flexuosa* pollen. The description of the pollen grain of the species under study is specified below: Type of grain: monad, Shape: prolate, Polarity: apolar, Outline in polar view: elliptical to subtriangular, Outline in equatorial view: circular, Size: small, Polar diameter: 21-23µm, Equatorial diameter: 15-19 µm, Apertures: tricolporate, colps with fine, smooth margins, Grain surface: psyllate with small areoles. In the area studied in this work, allergy tests are not performed on the species of this genus. From this work, it can be concluded that the characterization of *N. flexuosa* pollen will allow monitoring of its allergenicity and its potential in the presence of environmental contaminants that interact with the surface of this type of pollen grain. In this way it will be possible to contribute to improvements in human health.



**A70**

**TECHNICAL STEPS FOR THE USE OF 6TH GENERATION SELF-ETCHING ADHESIVES**

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The advent and development of adhesive systems completely changed dental practice. Its progress and innovation, as in most dental materials, is focused on the improvement of its components and the simplification of clinical technique. Today, self-etching adhesives are consolidated as the first option in the adhesion of polymeric materials to dental tissues, because they reduce the number of clinical steps. The objective of this work is to carry out a bibliographic review in order to provide the reader with a global vision of the technical steps for the use of 6th generation self-etching adhesives taking into account different bibliographic sources. To carry out this study, a search was carried out in the electronic databases PubMed, Redalyc, Google Scholar and Scielo, from which several articles were selected and analyzed exhaustively. As a result, these studies showed that the application technique of 2-step self-etching adhesives (AdheSe, Fl\_Bond and Optibond Solo Plus Se) consists of: isolation of the operative field with rubber dam, application of Primer (rub 15" and disperse with soft air), application of the adhesive (disperse with air), then polymerize 15" and apply the sealing material, on the other hand, for 1-step self-etching adhesives (Touch&Bond, iBond and XenolIII) the procedure consists of: isolation of the operative field with rubber dam, dispense a drop of each bottle in a dappen glass, mix liquids A and B with an applicator tip. Apply the adhesive (leave on 20") and then aerate 2", photopolymerize for 10" and then place the sealing material. The 6th generation adhesives of 1 step and 2 steps give us practicality, synthesizing the number of technical steps performed, saving time and facilitating operative maneuvers.

## **MICROBIOLOGY AND IMMUNOLOGY**

**A71**

**EVALUATION OF THE ALLERGENICITY INDEX, IN THE ENVIRONMENT OF A REGIONAL HOSPITAL IN CENTRAL ARGENTINA**

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The presence of green areas favors the physical and mental health of the population. However, some plants can also cause health problems. In this sense, the management of urban green spaces is of great importance, especially in the vicinity of health centers. Many trees, and some shrubby and herbaceous plants, can cause pollen allergies. Among the herbaceous area The Chenopodiaceae-Amaranthaceae, which include plants common in disturbed areas such as quinoa, Russian thistle and morenita, implicated in summer and autumn allergies, and Poaceae such as Lolium spp. and Cynodon dactylon, which cause spring and summer allergies. In the trees and landscaping of cities, plants are used with various criteria that rarely take into account their allergenic potential. This characteristic is of great importance and, especially, when what is vegetated is an area of a hospital. In Europe, indices were developed to determine the Potential Allergenicity Value (VPA) of the species that make up a green space which, combined with a set of parameters, also makes it possible to calculate the Allergenicity Index of Urban Green Areas (IUGZA). The objective of the work was to characterize the species used in the area of the Ramón Carrillo Regional Hospital in the province of San Luis (Argentina), according to their VPA values and, to their different vegetated areas, according to the potential risk they present for the production of pollinosis, through the calculation of the potential IUGZA (IUGZAp). The area surrounding the hospital was divided into 11 areas, using Google Earth plus, and the IUGZAp was calculated for each of them, using the VPA values of each species registered and the estimation of its theoretical maximum volume. According to the results, in none of the areas does it reach the threshold value of 0.3, which indicates risks of pollinosis. The maximum IUGZAp obtained were 0.155, 0.116 and 0.114 in areas adjacent to the east, south and west of the parking lot and 0.135 in the Residence area. However, since the trees are recent and many specimens have not yet reached the reproductive stage, the index could increase. In the work, recommendations are made about the species that should be avoided and their management, in order to avoid pollinosis problems in people who come to the hospital.

A72

**EXPLORING THE EVOLUTION OF EXTENDED-SPECTRUM  $\beta$ -LACTAMASES (ESBL) IN CLINICAL ISOLATES FROM MENDOZA'S CENTRAL HOSPITAL.**

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Extended-spectrum beta-lactamases (ESBL) hydrolyzes a wide range of beta-lactam substrates, contributing to antibiotic resistance, a global concern. Understanding the evolution of ESBL is essential for developing effective antimicrobial treatment strategies. Between 2018-2019 and 2022, we collected ESBL-producing bacteria from Mendoza's Central Hospital. Our aim was to compare the prevalence of genes responsible for the ESBL phenotype using PCR technology. We compared the results obtained during the 2018-2019 period with those from the post-pandemic year 2022. In both periods, *Escherichia coli* and *Klebsiella pneumoniae* were the most prevalent enterobacteria in the samples. During 2018-2019, prevalent enterobacteria genes among hospitalized patients included *bla*OXA-1, *bla*CTX-M1, and *bla*TEM-1. We obtained preliminary results from the 2022 samples, specifically examining *bla*CTXM-1, *bla*CTXM-2, and *bla*SHV-1. We observed a significant increase ( $p < 0.001$ ) in the prevalence of *bla*CTXM-2 and *bla*SHV-1 in these bacteria. In the 2022 samples, we also detected *bla*OXA-48 from the carbapenemase family. Our results offer insights into the evolution of prevalent genes associated with the ESBL phenotype in Mendoza's hospital during 2018-2019 and 2022. This research provides valuable contributions to the limited information available in our region and may aid in designing rapid PCR-based diagnostics for appropriate antimicrobial treatment, ultimately promoting positive health outcomes for patients.

A73

**EFFECT OF  $\text{Ca}^{2+}$  AND  $\text{Mg}^{2+}$  ON THE ANTIMICROBIAL ACTIVITY OF *Lactobacillus paracasei* sp sl40 AND ITS ACTION AGAINST FOOD PATHOGENS**

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Food spoilage caused by contamination with pathogenic microorganisms is one of the most undesirable problems in food industry. In addition to modify the organoleptic characteristics of food products, these microorganisms produce toxins that can cause food intoxication. There is evidence of an increase in the antimicrobial activity of lactic acid bacteria (LAB), such as *Lactobacillus rhamnosus*, *Lactobacillus delbrueckii*, *Streptococcus thermophilus* and *Enterococcus durans* cultured in Man Rogosa Sharpe (MRS) medium, with differential concentrations of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  salts. The purpose of this work is to study antimicrobial activity of *Lactobacillus paracasei* sp sl40 against pathogenic microorganisms in presence of divalent ions. Action spectrum against pathogens or food contaminants was determined, with *Enterococcus faecalis*, *Candida albicans*, *Yersinia enterocolitica* and *Staphylococcus aureus* being inhibited and was compared with the antimicrobial activity measured in the presence of different concentrations of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . LAB strain was grown in selective medium MRS broth. Salts of  $\text{CaCl}_2$  and  $\text{MgCl}_2$  were used as ion sources. MRS medium was prepared by separately adding the salts, thereby obtaining 5, 8 and 12 mM concentrations of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . Three consecutive cultures of LAB strain were executed every 24h in 10 ml of MRS medium in order to activate antimicrobial substances production. Cell-free supernatant (CFS) was obtained using sterile 0.22  $\mu\text{m}$  syringe filters. Suspensions of each microorganism antimicrobial activity indicator were prepared from  $6 \times 10^8$  cells  $\text{ml}^{-1}$  (OD of 0.08-0.1 at 625 nm). 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 35°C, O.D. of sample ( $A_m$ ) and blank ( $A_0$ ) was measured at 700 nm. The percentage of inhibition was calculated. Each test consisted of positive controls and assays with CFS from cultures in unmodified medium and CFS from cultures in media with the three salt concentrations. Treatments were carried out with  $\text{Ca}^{2+}$  for *C. albicans* and *E. faecalis*, with  $\text{Mg}^{2+}$  for *C. albicans*, *S. aureus* and *Y. enterocolitica*. Antimicrobial activity against *C. albicans* exhibit a significant difference ( $p=0.0280$ ) between controls and treatments with unmodified and modified medium at different concentrations of  $\text{Mg}^{2+}$ . Yeast inhibition revealed no significant difference when CFS effects from cultures in MRS and MRS with  $\text{Mg}^{2+}$  were compared. Similar results were obtained when treating this microorganism with medium added with  $\text{Ca}^{2+}$ . In the case of treatments of *S. aureus* and *Y. enterocolitica* with medium added with  $\text{Mg}^{2+}$ , similar results were obtained, the difference between growth controls and treatments being very significant, however no difference was observed between treatments with CFS from unmodified media and with CFS from media modified. The *E. faecalis* inhibition assays with  $\text{Ca}^{2+}$ -modified medium, similar results were obtained, in addition lower antimicrobial activity with saline treatment was demonstrated. It is concluded that the CFS of the

LAB strain under study present a high antimicrobial activity against all the indicators tested and that it is not significantly stimulated by the addition of divalent ions to the culture medium.

#### A74

### **IN VIVO AND IN VITRO EFFECTS OF ESSENTIAL OILS FROM *Laureliopsis philippiana* ON *Leishmania amazonensis***

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Leishmaniasis, a parasitic zoonotic disease, is on the rise in South America and has earned recognition as one of the neglected diseases by the World Health Organization. In Argentina, leishmaniasis is endemic in the northeastern and northwest provinces. *Leishmania amazonensis*, one of the causative agents of this disease in our country, is notable for its capacity to induce a broad range of clinical symptoms, ranging from localized cutaneous leishmaniasis to severe forms characterized by mutilation, disability, and often poor response to treatment. Currently, the existing treatments, such as Glucantime, are unsatisfactory due to their high toxicity, cost, complex administration, and the emergence of drug-resistant strains. It is imperative to explore innovative immunotherapeutic approaches based on the search for new compounds, screening molecules purified from plant resources. Essential oils (EOs) are combinations of compounds including terpenes, that can be extracted from different parts of plants. Throughout history, EOs have been employed in traditional medicine to manage a wide range of human ailments. *Laureliopsis philippiana* is a native tree widespread in the forest areas in the south of Chile and Argentina, known for its medicinal properties. Research has established that EOs derived from *L. philippiana* exhibit antimicrobial, fungicidal and antitumoral properties. Therefore, the present work aims to evaluate the possible antiparasitic properties *in vitro* and *in vivo* of EOs from *L. philippiana* against *Leishmania amazonensis*. First, we evaluated the antiparasitic effect of several essential oils on *L. amazonensis* promastigotes. Paramela, Laurel, and Tapa significantly reduced parasite growth compared to the control group. Subsequently, we incorporated mice into our study as test subjects. Male BALB/c mice were infected in the right hind footpad with  $1 \times 10^6$  *L. amazonensis* promastigotes and treated with a formulation of Tapa+Vaselin 4%, that were administrated directly on the lesion one or three times a week. We worked with two groups: Tapa 1, which received one dose per week starting the following week after the infection, and Tapa 2, which also received one dose per week but five weeks after the infection. We analysed different parameters like the swelling of the infection site, weight of the lesion, parasite load, splenic index and determination of the humoral immune response. We observed that Tapa treatment in both groups did not induce a significant alteration in all the parameters evaluated. However, during the final week of the protocol-measurement, Tapa 2 treatment exhibited a trend toward reducing swelling. Therefore, we intend to repeat the protocol with significant modifications.

#### A75

### **ANTIPARASITIC ACTIVITY OF ABIETANE AGAINST *Leishmania amazonensis***

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The leishmaniasis are a spectrum of diseases caused by infection with protozoan pathogens of the *Leishmania* genus, with an estimated 2 million new cases per annum. *Leishmania* parasites are transmitted to a mammalian host via the bite of an infected sand fly. The clinical forms of the disease (cutaneous, mucocutaneous and visceral leishmaniasis) depend on the species of *Leishmania* involved. In Argentina, affects the northern region of the country with an incidence that has increased over the last two decades. Current treatments for leishmaniasis are unsatisfactory due to high associated toxicity, cost, complex administration and the emergence of resistant strains. Efforts have greatly increased over the last decade to identify novel compounds with anti-leishmanial properties. Thus, one strategy in the search for new compounds is the screening of molecules purified from plant sources. Terpenes appear as good candidates, because they are abundant in the plant kingdom and some of them have shown a significant activity against trypanosomatids. The terpene Abietane (HABTO), isolated from *Salvia cuspidata*, was tested against *Leishmania amazonensis*. We evaluate the effect of HABTO in an *in vitro* model and *in vivo* model of cutaneous leishmaniasis. The treatment with HABTO induced a significant increase in ROS levels in *L. amazonensis* promastigotes compared to controls. We measured ROS using the probe 2',7'-Dichlorofluorescein diacetate. Moreover, HABTO produces a significant reduction in the mitochondrial activity of promastigotes, evaluated with MTT. In promastigotes treated with HABTO there was a decrease in the inner concentration of GSH within the parasites. Male BALB/c mice were infected in the right footpad with  $1 \times 10^5$  promastigotes of *L. amazonensis* and locally treated, once a week for 4 weeks, with 1 mg/animal/day of HABTO. We observe that the treatment with the terpene



decreases footpad swelling compared to the controls. This is related to the significant decrease in parasite load, splenic index and IgG levels observed with every treatment. Moreover, the high IgG2a and low IgG1 levels of anti-Leishmania antibodies, observed after HABTO treatment, are associated with protective immunity against different Leishmania species. Although we consider it necessary to carry out more research on this terpene, our findings suggest that HABTO could be considered as possible novel candidate to be used as therapeutic agent against *L. amazonensis* and thus treat cutaneous leishmaniasis.

## A76

### ANTIBACTERIAL ACTIVITY OF *Bacillus velezensis* SL-6 AGAINST *Staphylococcus aureus*

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Several *Bacillus* species produce antimicrobial metabolites by ribosomal (as bacteriocins) and non-ribosomal synthesis (i.e., polyketides, lipopeptides and miscellaneous chemical structures), with potential use as food preservatives and human health. *Staphylococcus aureus* is a commensal and opportunistic pathogen that can cause wide spectrum of skin and invasive infections, and can also be a contaminating microorganism of food and cosmetic/pharmaceutical formulations. This study describes the production, separation and chromatographic characterization of anti-staphylococcal metabolites produced by *Bacillus velezensis* SL-6. The SL-6 strain was cultured in Synthetic Mineral Broth with orbital shaking at 200 rpm for 24 h at 30 °C. The cell-free supernatant (CFS) was obtained by centrifugation and filtration. Subsequently, the crude lipopeptides of CFS were concentrated by acid precipitation followed by methanol extraction (ME10X). On the other hand, CFS was fractionated using ultrafiltration membranes with different molecular weight cut-offs (MWCO 10 kDa and 3 kDa). Thin-layer chromatography (TLC) of ME10X was performed using chloroform-methanol-water (64:25:4 v/v/v) as the mobile phase. The bands were visualized under ultraviolet light (254 nm), by exposure with iodine vapors and sprayed with water or ninhydrin reagent to detect the lipopeptides. The bioactive bands were then revealed using TLC-contact bioautography. Antibacterial activity was tested against *S. aureus* ATCC 29213 using the agar well diffusion method. The inhibition zone diameter (mm) was measured after incubating the plates for 24 h at 37 °C and the residual antibacterial activity (RAA) was calculated as a percentage. Anti-staphylococcal activity of CFS was considered to be 100 % (23.31 mm). Hydrophilic compounds remained in the aqueous supernatant fraction after acid precipitation (RAA = 79.89 %), while this estimate for crude lipopeptides was only 4.35 %. The separation of CFS by ultrafiltration gave two bioactive fractions when the MWCO was 3 kDa (97 % and 9.9 % in permeate and retentate fractions, respectively). These results indicated that small-molecule compounds were the major active metabolites, suggesting a possible interaction between them. TLC-bioautography of ME10X showed three bioactive fractions (R<sub>f</sub> = 0.48, 0.75 and 0.92). As expected, the corresponding band of surfactin (R<sub>f</sub> = 0.75, UV-negative, iodine negative, water spray positive, and ninhydrin negative) showed anti-staphylococcal activity. Further studies are required to elucidate the structure of the remaining bands. In conclusion, the antagonism of *B. velezensis* SL-6 against *S. aureus* remained mainly in the aqueous phase, however, several bioactive compounds were extracted in methanol and surfactin was chromatographically characterized as one of them.

## A77

### ISOLATIONS OF MICROORGANISMS FROM VINEYARD SOIL WITH BIOFERTILIZERS POTENTIAL AT DIFFERENT TIMES

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Viticulture is an important agroindustrial activity that affects 19 provinces of the Argentine Republic, mainly Mendoza and San Juan. In order to ensure production and control harmful pests in vineyards, the application of chemical fertilizers and pesticides with diverse effects on agroecosystems is common. One of the solutions proposed by biotechnology to the use of agrochemicals is the use of biofertilizers, products made with live or latent microorganisms that, when added to crops, can stimulate their growth and productivity. **The objective** of this work was to isolate microorganisms from vineyards soil at two periods of development of *Vitis vinifera* plants, in order to select microorganisms with potential of biofertilizers. 40 rhizosphere soil samples were collected at two periods of development of *Vitis vinifera* plants (budbreak, October 2022 and veraison period, February 2023) from 10 vineyards (LUC, LC, AR, CA, CAICA, PE, ESP, YA, LI, CEP) in Valle of Uco and Luján of Cuyo, Mendoza. The samples were transported cold and processed in less than 48 hours in the laboratory. Serial dilutions of samples were carried out and an aliquot was inoculated in Petri dishes containing base medium with soil extract (MSE) o Sabouraud glucose medium (MSG) o medium for phosphate solubilizers (MPS). The dishes were incubated at 25 °C, 7 days. The results were expressed in CFU/g of soil (total heterotrophic microorganisms and fungi/yeast) and solubilization halo was visualized. In the budbreak, the highest counts of total heterotrophic microorganisms (MSE) were for LI (1.16 x10<sup>9</sup> CFU/g) without significant differences with YA, PE, LUC, CA and CEP, while the AR plantation presented the lowest counts (5.17x10<sup>8</sup> CFU/g). In MPS, the ESP plantation presented the lowest counts of total heterotrophic microorganisms (2.05x10<sup>8</sup> CFU/g). CFU/g soil for fungi and yeasts showed that the YA plantation presented the highest values (1.34x10<sup>8</sup> UFC/g) without significant differences with the CEP, CA, LUC and PE. Veraison season, in MSE, the highest

count was for LUC ( $2.0 \times 10^9$  CFU/g) and the lowest value was for CAICA ( $1.41 \times 10^8$  CFU/g) without significant differences with CAS, PE, YA and LI. In MPS, the CAICA plantation also presented the lowest counts of total heterotrophic microorganisms ( $9.05 \times 10^7$  CFU/g) showing significant differences with the AR, CEP and YA. CFU/g soil for fungi and yeasts showed that the LI plantation presented the lowest count values ( $1.25 \times 10^6$  UFC/g) without significant differences with CAICA, while the CEP plantation presented the highest counts ( $1.64 \times 10^7$  UFC/g). Furthermore, it was observed that the percentage of phosphate-solubilizing microorganisms ranged between less than 1% and greater than 10% in both periods. In conclusion, the soils studied showed to be an abundant source of microorganisms, mainly bacteria and yeasts, with low counts of filamentous fungi (possible phytopathogens). Therefore, phosphate-solubilizing microorganisms will be selected to continue studying their biofertilizer capacity, through nitrogen fixation assays, phytohormone production, and siderophore production.

## A78

### ANALYSIS OF SIDEROPHOR PRODUCING MICROBIOTA FROM VINEYARD SOILS IN RELATION TO THE PHENOLOGICAL CYCLE OF THE VINE

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Plants go through different stages during their life cycle, called the phenological cycle. In the case of grapevine, the stages of budbreak and veraison are important. Microorganisms produce siderophores (iron chelators) in response to the deficiency of this micronutrient; this could be a valuable property of strains that could be used as biofertilizers. The aim of this work was to analyze whether there is a relationship between the different stages of the vine phenological cycle and the siderophore-producing microbiota. 105 isolated soil samples from vineyards collected during the budding and veraison stages in two seasons were analyzed. The strains were cultured in a vitamin-free liquid medium in a rotary shaker at 120 rpm at 25 °C and then centrifuged at 10,000 rpm for 10 minutes to determine the presence of siderophores in the supernatant. Catecholates were determined by the Arnow test and hydroxamates by the ferric perchlorate test. The results were expressed semi-quantitatively according to the absorbance values obtained, and the microorganisms were classified according to the production of siderophores into the following categories: no production, weak, moderate, medium and high production. Of the total samples studied, 26 produced catecholates and 38 hydroxamates. Production of catecholates in the veraison period was mainly of moderate producers (4%) and medium producers (3%) in 2022 and weak producers (5%) and high producers (1.4%) in 2023, while at flowering stage, weak and moderate producers were observed in 2022 (1.5% each) and weak producers in 2023 (7%). Hydroxamate production of hydroxamates in veraison was mainly weak 6% and average 4% in 2022 and weak 7% in 2023; while in the flowering phase, all productions were around 2% in 2021 and mainly weak and moderate with 5% each in 2022. In conclusion, a great temporal variability was observed in the composition of the vineyard soil microbiota and its relationship with the production of siderophores. These results highlight the importance of considering multiple variables when studying the microbiota in vineyards and its influence on the grapevine cycle.

## A79

### *Yersinia enterocolitica* OUTER PROTEIN (YOPP) MODULATES INOSITOL HEXAKISPHOSPHATE (IP-6) PRODUCTION THROUGH MURINE FCγR

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*Yersinia enterocolitica* (Ye) Outer Protein (YopP) requires host factor inositol hexakisphosphate (IP6) for stabilization and activation. Previous studies in our laboratory demonstrated that YopP presents extracellular activity that influences second messenger's levels, including IP-6. However, a mechanism of action has not yet been proposed. It is known that immunoglobulin G receptor (FcγR) is coupled to the inositol phosphate pathway. In this work, we postulate FcγR as a possible target for YopP extracellular activity. Murine peritoneal macrophages (MΦ) were obtained by intraperitoneal lavage under aseptic conditions and plated at a  $1 \times 10^6$  cells/mL concentration, then purified by incubation for 2 h at 37°C in supplemented DMEM medium with 5% CO<sub>2</sub> atmosphere. Besides, MΦ were infected with Ye serotype 0:8 (pYV+, WA-314) (Ye wt) or with Ye WA-314 deficient in YopP (pYV+, WA-C pYVNalrKanr) (Ye ΔyopP) at MOI 10:1. Purified YopP extracellular binding to MΦ was studied by flow cytometry, using FITC labeled anti-YopP antibodies. Further, human IgG was used to inhibit surface YopP binding, then YopP extracellular binding to MΦ and intracellular levels of IP-6 were tested. Infection with Ye wt stimulated IP-6 production in MΦ lysates at 15 min. post infection ( $p < 0.05$ ), surprisingly, we showed a significant decrease in IP-6 production at 45 min post-infection ( $p < 0.01$ ), probably due to its consumption. On the other hand, we found that both YopP and other Yops could bind to MΦ surface ( $p \leq 0.0001$ ), and we also found that IgG improve Yops binding ( $p < 0.05$ ). However, IgG modulates the stimulatory effect of YopP on IP-6 production ( $p < 0.05$ ). Thus, in the present work we explore FcγR as a possible target for Ye to manipulate the inositol phosphate pathway. However, more studies are necessary to understand the molecular aspects of the YopP-FcγR interaction.

## A80

### APPLICATION OF MAGNETIC FIELDS IN THE DOMESTICATION OF A NATIVE YEAST OF SAN LUIS (ARGENTINA) FOR HOMEBREWING

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The domestication of microorganisms is a procedure in which it is provided with a specific environment for an adaptation to occur, generating desired characteristics. Last year the brewery sector was innovated with the use of non-conventional yeast, and due to this reason the technological scientific sector started to search for those yeasts. The desirable characteristics of new yeasts are efficient production, alcohol tolerance, the ability to carry out alcoholic fermentation, and the yield of flavor compounds that provide an organoleptic profile of beer. The objective of this study was to evaluate the sensory profile of the craft beer made with a native yeast of the province of San Luis through the application of domestication cycles using different magnetic fields. The yeast used was biochemically characterized as *Saccharomyces cerevisiae*. Previously to the domestication cycles the native yeast was inoculated in order of 108 cel/mL in base brewing wort and was applied three treatments: exposure to non-homogeneous static magnetic fields generated by a) a neodymium magnet (high intensity); b) a ceramic magnets-array (low intensity); and c) without external magnetic field (control). These were incubated at 20°C for 5 days. At the end of the cycle, a 10ml aliquot was transferred to another microfermenter with a new wort. At the start of the cycles of domestication and the end of these density and pH were measured. In this work, 5 domestication cycles were carried out. Subsequently, it was recovered and the isolation of the domesticated yeasts was later used in the production of a golden-type craft beer. Finally, the craft beers produced were tested in front of two BJCP judges and a sensory panel. Our results indicated a significant variation in the sensory profiles of beer produced by yeast treated with magnetic fields concerning control, the beer produced with yeast that was exposed to a magnets array got the major percentage of acceptance, with a 100% acceptance; while both the beers produced with yeasts treated with the neodymium magnet and the control yeasts obtained 94% of acceptance. On the other hand, concerning preference, the beer made with yeasts treated with the ceramic magnets-array presented the highest percentage of preference, with 63.16% of preference, in contrast to the beers made with the control yeasts and the yeasts treated with the neodymium magnet, which obtained 31.58% and 10.53% of preference respectively. Therefore, we conclude that a significant variation is observed in the organoleptic profile of the beers made by yeast with different magnetic treatments concerning the control.

## A81

### PRODUCTION OF *Kosakonia radicincitans* IN CULTIVATION MEDIA FORMULATED FROM CRAFT BREWER WASTE

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The bacterium *Kosakonia radicincitans* has the capacity to promote plant growth and as a biological control agent for phytopathogens, with potential application in bioinputs for agricultural production. The production model under circular economy principles reuses waste from different activities as resources. The production of a microorganism for the formulation of a bioinput economically and sustainably can apply this model to the use of waste from the food industry. The resources discarded by the craft brewery useful for the formulation of culture media are bagasse (residual grain strained from the wort), spent yeast (fermentation process completed), and low-density wort (final washing of the malted grain). Objective: quantify the growth of *K. radicincitans* in different culture media formulated from craft brewery waste. The discarded yeast and bagasse were previously dehydrated in a controlled manner and the low-density must be preserved at (4°C). Two base culture media were formulated (1. bagasse and 2. must) with three variants each: 1.a.) bagasse (70 gr./L) without additive; 1.b.) bagasse (70 gr./Lt) with yeast (5 gr/L); 1.c.) bagasse (70 gr./Lt) with commercial yeast extract (5g/L); 2.a.) must without additive; 2.b.) must with yeast (5g/L) and 2.c.) must with commercial yeast extract (5g/L). 1 ml of bacterial suspension was seeded at a concentration equivalent to 0.5 McFarland scale in Erlenmeyer containing 100 ml of each of the media studied, it was incubated at 28°C with shaking for 24 hours. 10 ml of each culture were extracted, and centrifuged at 10,000 rpm for 10 minutes; the precipitate was placed in aluminum foil capsules, and dehydrated in an oven at 45°C until constant weight. Biomass production was calculated as a difference in dry weight; two replicates were carried out in duplicate. The bagasse-based medium with yeast 1.b. showed an increase in biomass by 73.9%; regarding 1.a. and without significant differences with 1.c. Growth in the environment 1.b. produced 13.3% more biomass compared to 2.b. The results obtained showed that the medium based on dehydrated bagasse showed greater growth of the bacteria unlike the medium with low density must. The addition of dry yeast significantly improves microbial growth, compared to commercial yeast extract. The culture medium formulated from dehydrated bagasse with the addition of dry yeast has the best conditions to continue *K. radicincitans* production studies.

## A82

### ANTIFUNGAL ACTIVITY OF KEFIR AGAINST BREAD SPOILAGE FUNGI

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Food spoilage is a major issue for the food industry, leading to food waste, substantial economic losses for manufacturers and consumers. In previous work, the antimicrobial capacity of kefir, an artisanal, acidic and slightly carbonated fermented drink, has been tested, showing antibacterial activity. Therefore, the objective of the present study was to evaluate the antifungal activity of kefir against bread spoilage fungi. 21 microorganisms were isolated from kefir but none presented the desired activity, indicating that microorganisms need to grow in a consortium to produce it. For this reason, it was decided to continue working with kefir granules. Two kefir drinks were used, one with unsterilized mascabo sugar and the other with sterilized mascabo sugar (5 g%). A total of 3 g of kefir granules were inoculated in 30 mL of each medium and they were incubated to 30°C without and with an orbital shaker at 140 rpm. Samples were taken after 72h and the cell-free supernatant (CLS) was obtained by centrifugation at 10,000 x g for 10 minutes. The CLS samples were subjected to two concentration methods: water bath at 94°C for one hour and Savant speedvac for 3 h, until a final concentration of 5x by both techniques. The antifungal activity was determined by the agar diffusion technique against six filamentous fungi isolated from bread and not yet identified. The fungi were plated onto potato dextrose agar (PDA) by spreading a volume (100 µL) inoculum. Sterile mascabo sugar was used as a control. All CLS showed antifungal activity on four of the six filamentous fungi, varying the degree of inhibition according to the concentration method. The CLS kefir supernatants with sterilized mascabo sugar showed greater activity than the CLS with unsterilized sugar, showing in decreasing order of fungal inhibition: SLC Savant speedvac, SLC water bath, SLC without concentrating and static culture (without shaking). The control did not show activity. In addition, thin layer chromatography was carried out with the concentrated and unconcentrated supernatants, using BAW (butanol: acetic acid: water) as the mobile phase, and bioautography was carried out using as reporter the most sensitive fungus from the previous tests, obtaining one metabolite with fungal inhibition of Rf = 0.3, confirming the presence of an antifungal metabolite. It is concluded that cultivating kefir with agitation favors the production of the antifungal metabolite and that the application of the two methods to concentrate the SLC were effective in increasing fungal inhibition, however the high temperatures used would have a slight effect of decreasing this capacity. In future work, it is expected to advance on the nature of the metabolite.

## A83

### BOTANICAL CHARACTERIZATION AND ANTIMICROBIAL STUDY OF HYDROLATS AND OIL ESSENTIAL OF *Eucalyptus globulus* AND *Eucalyptus cinerea*

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*Eucalyptus* is a genus of trees of the family Myrtaceae. The most frequently cultivated *Eucalyptus* species in San Luis are *Eucalyptus globulus* and *Eucalyptus cinerea*, the first is the only codified by the pharmacopoeia and botanical description has been made but the determination of the micrographic parameters is missing. Essential oil (EO) of *E. globulus*, obtained from leaves, bark and fruits, has balsamic properties and it is widely used as antiseptics of the respiratory tract, expectorants and mucolytics, therefore it is administered in various pharmaceutical forms as pills, candies, infusions, syrups or in vaporizations. On the other hand, *E. cinerea*, is used as a substitute and in some cases, it is sold in local herbal store as medicinal. The objective of this work was to apply ethnobotanical and micrographic characterization to differentiate both species and study the antimicrobial activity of the EO and the hydrolats obtained from the leaves. Plant materials were obtained of San Luis. Techniques were performed by cut, paraffin embedding, staining, diaphanization and dissociation. For counting the micrographic parameters, the samples were diaphonized according to Dizeo technique. Photomicrographs were obtained with a Leitz DMRB microscope with digital camera. The reliable identification of both selected medicinal plants involved obtaining significant differences (anova  $p < .001$ ) of several parameters as: stomata number (SN), stomatal index (SI), palisade ratio (PR), nerve terminals number (NTN) and islets number (IN). The EO and hydrolats was obtained by steam distillation using the leaves and the yield was determined (mL of EO per g of leaves). Antimicrobial activity of hydrolats and EO against *Colletotrichum acutatum* (a fungal phytopathogenic), and *Escherichia coli* and *Pseudomonas aeruginosa* were evaluated by means of the diffusion well method. The fungus was plated onto potato dextrose agar (PDA) while bacteria 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 EO. With *E. globulus* a yield of 0.009 mL g<sup>-1</sup> was obtained while *E. cinerea* was half. Both EO produced bacterial inhibition but only *E. cinerea* produced fungal inhibition. None of the hydrolats gave antimicrobial activity. Each species was different both for their exomorphological, anatomical and quantitative micrographic characters. Therefore, this study contributes to the quality control of these medicinal vegetable, especially when the product is finely ground by the different morphology botanic and micrographic characters. Also, the microbial activity of the EO was different between the species and each one could be applied with different objectives, even as food additives for food preservation.

A84

**PRELIMINARY STUDY OF THE ANTICANDIDAL ACTIVITY OF ORODISPERSIBLE TABLETS FROM *Larrea divaricata* Cav.**

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An orodispersible tablets contain substances which disintegrate rapidly usually within a minutes after placed in the mouth. *Larrea* species extracts demonstrate significant antimicrobial activity against *C. albicans*. In the present study, the antifungal activity of tablets orodispersible containing *Larrea divaricata* Cav extracts (LD ODT) were assessed against *Candida*. ODTs were prepared by direct compression using ethanolic extract of LD at a dose of 100 mg. The tablet properties and quality parameters were evaluated previously according to the United States Pharmacopeia (USP-43) guidelines. The antifungal activity against *Candida* species was determined by modified plate microdilution. The yeasts were inoculated in 5 mL of liquid Muller Hinton at 37°C. The cells were adjusted to an optical density (OD) of 0.5 which corresponds to 1x10<sup>7</sup> cells/mL of *C. albicans*. Subsequently, logarithmic dilutions were made until obtaining a cell suspension of 1 per 10<sup>4</sup> cells/mL. In an apyary plate, the cell suspension of 1x10<sup>4</sup> cells/mL and the different concentrations of orodispersible compounds were placed in each well. Growth was monitored in a spectrophotometer. Readings at 580 nm were recorded every 15 minutes at a temperature of 37°C for 24 hours. Preliminary results showed a significant difference compared to the positive control (ketoconazole) and a minimum inhibitory concentration (MIC) comparable to the antifungal used as reference. Therefore, LD ODT could be a potential solid dose for the treatment of oropharyngeal fungal infections in immunocompromised patients and an alternative treatment for this recurrent pathology.

**ECOLOGY, ETHOLOGY AND BIODIVERSITY**

A85

**COMPOSITION OF DIFFERENT SPENT MUSHROOM SUBSTRATE: A SUSTAINABLE REUSE OF DIFFERENTS AGRO-INDUSTRIAL BY-PRODUCTS, CHILECITO, LA RIOJA**

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La Rioja province is characterized by an agricultural production dominated mostly by perennial crops as vine, olive and walnut. All these crops annually generate a big amount of agro-industrial by-products which are not incorporated into any industry. Utilization of wastes as a substrate for mushroom cultivation is a feasible solution to be carried out in this region. The nutritional composition of the *Pleurotus ostreatus* vary according from different substrates, such as walnut shell, olive pruning remains or vine pruning remains, where these were cultivated. Spent mushroom substrate (SMS) is a by-product of edible mushroom cultivation, and is of particular interest assess the reuse to promote the transition towards a circular economy. After mushroom harvest, SMS still holds high levels of organic matter (OM), nitrogen (N), potassium (K), and other nutrients typically required for crop growth. Previous studies have shown the benefits of using as organic fertilizer and soil conditioner. However, despite its great potential as an organic amendment, the use of SMS as a soil conditioner/fertilizer is still depend of the composition of the same. The objective of this study was to research the composition of four different types of SMS. In this experiment, spent substrates of *Pleurotus ostreatus* were evaluated: T1) pine sawdust (PS, control), T2) PS + walnut shell, T3) PS + olive pruning remains, and T4) PS + vine pruning remains. It was determined: electrical conductivity (EC), pH, OM, total nitrogen (Nt), total organic carbon (Ct), K and C:N ratio. Determination of pH for extracts of SMS was acidic at average 5.26. The results showed that the pH was lower when olive pruning residues were added to the control substrate. The SMS of T4 showed the higher EC 2.6 ms/cm, while the lower value reached to 1.07 ms/cm for T2 (with walnut shell). There wasn't significant difference in of OM content of the different substrates, and the value was at average 97.08%. The higher Nt content was 8.84 g/kg for T4 while, T1 (control) had lower nitrogen content (2.59 g/kg). The higher C:N ratio was reported with T1 (control) at value 214.9, while T4 had ratio 63.65, significantly lower (p < 0.05). The K content varies between 19.5 and 153.3 mg/100g dry matter (%), and the results showed that the K content of SMS obtained of T3 and T4 was significantly higher (p < 0.05) in relation to T1. These results showed that there is variation in composition of SMS, but overall, are suitable as a natural fertilizer and soil amender in agriculture.



## A86

### CARRION INSECT SUCCESSION IN AN ARID ENVIRONMENT FROM SAN JUAN, ARGENTINA

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Forensic entomology aims to determine *Post Mortem Interval* (PMI) or the time between the death and discovery of the body using associated arthropods with dead bodies. Diptera and Coleoptera are the most important forensic orders, arriving at decomposed bodies following a specific sequence known as ecological succession. This succession is affected by the size of carrion resources, temperature, and particular weather and biogeographical conditions of each region. This study aims to know the composition and succession pattern of forensic insects and their relationship with the various stages of decomposition states in pigs during spring in an arid location in San Juan, Argentina. This study was conducted in Médanos Grandes, Caucete, in October 2021. Three pig carcasses were used as models of vertebrate carrion decomposition and were sampled with daily base from Fresh to Remains stages. Pitfall traps, entomological nets, and hand-picking were used to collect immature and adult arthropods. A total of 24322 specimens were collected from 10 orders and 32 families. The following fly species were identified: *Chrysomya albiceps*, *Cochliomyia macellaria*, *Comptosiops fulvicrura*, *Lucilia sericata* and *Sarconesia chlorogaster* (Calliphoridae), *Fannia fusconotata*, *F. sanihue*, *F. heydenni* and *F. femoralis* (Fanniidae), *Musca domestica*, *Muscina stabulans*, *Dolichophaonia trigona* and *Ophyra aenescens* (Muscidae), *Piophilidae casei* (Piophilidae), Sarcophagidae, Anthomyiidae, and Phoridae. Coleoptera species were identified: *Necrobia rufipes* (Cleridae), *Dermestes maculatus* (Dermestidae), *Euspilotus eremita*, *E. lacordairei*, *E. lepidus*, *E. modestus*, *E. pavidus*, *E. golbachii* and *Xerosaprinus dyptichus* (Histeridae), Anthicidae, Nitidulidae, Scarabaeidae, Staphylinidae, Tenebrionidae, and Trogidae. The arrival of *Co. macellaria* and Sarcophagidae flies, followed by *F. fusconotata*, *M. domestica*, *C. fulvicrura*, *S. chlorogaster*, *M. stabulans*, and *O. aenescens* was characteristic in the Fresh stage. Furthermore, in this state, we observed Calliphoridae eggs and Sarcophagidae, and *Co. macellaria* larvae. *P. casei* flies and Staphylinidae, Trogidae, and *D. maculatus* beetles were recorded in the Bloated stage. Larvae of *Co. macellaria*, *C. fulvicrura*, *C. albiceps*, *M. domestica*, *O. aenescens* and adults of *C. albiceps* were registered in late Bloated and the early Active Decay stages. About the beetles, Anthicidae, Histeridae, Cleridae and *D. maculatus* larvae were registered. Larvae of *P. casei* were characteristic mainly in the Advanced Decay stage. In Remain stage, the absence of Calliphoridae larvae and the presence of larvae *P. casei* and *D. maculatus* were registered. Calliphoridae and Sarcophagidae flies were primary colonizers of carcasses. Histeridae, Cleridae, Staphylinidae, and Anthicidae families beetles and *C. albiceps* larvae fly showed necrophilic behavior, feeding on immature stages of flies. This research contributes to the characterization of the succession of carrion insects, recording variations between decomposition stages in the arid environment of San Juan, Argentina.

## A87

### ENVIRONMENTAL CHARACTERIZATION OF A NEW HABITAT RECORD OF *Chilina gibbosa*, A SENTINEL SPECIES

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*Chilina* genus Gray, 1828 is a group of freshwater benthic gastropod snails that includes 24 species of Argentina. Recently, *Chilina gibbosa* Sowerby, 1841 has been cited for the first time in the province of Córdoba (center of Argentina) in De los Sauces River (31°54'51.29"S; 65°6'33.08"W) far away from its known distribution (Neuquén, Río Negro). Several species of *Chilina* genus have been cataloged as sentinel species due to their high sensitivity to various pesticides, their ecological role, and the easy detection of neurotoxic signs. The aim proposed was to carry out the environmental characterization of De los Sauces River, in the province of Córdoba, where the first record of these gastropods was found. The site was visited during the wet season, where the gastropods were collected. For the ecological characterization of the site, the communities of macrophytes (qualitative sampling) and aquatic macroinvertebrates (quantitative sampling) were evaluated. Macroinvertebrates were collected with D net (multihabitat), preserved in 70% ethanol, and subsequently identified and quantified to the lowest taxonomic level. Simple metrics such as Shannon-Wiener taxa diversity index (SW), dominance index (D) and taxonomic richness (RT) were calculated. Plus the Biotic Index of San Luis Mountain (IBSSL) and the regional adaptation of BMWP (IBMWP for mountain streams) were calculated. Macroinvertebrates were also categorized into five functional feeding groups (GFA). Physicochemical parameters, evaluated according to techniques of Standard Methods, were temperature (T°), dissolved oxygen (OD), electrical conductivity (CE) and pH. In addition to *Chilina gibbosa*, other gastropod species reported were *Physella acuta* and *Pomacea canaliculata* and the invasive bivalve *Corbicula largillierii*. Macrophyte coverage presented some variations between rapids and backwater areas. Dominant macrophytes in the backwater zone were *Ceratophyllum sp.*, *Lemna sp.*, and *Azolla sp.*; following next *Roripa nasturtium-aquatium* and *Hydrocotyle sp.* On the other hand, in the rapid area only *Roripa nasturtium-aquatium* was recorded as the dominant macrophyte, and *Ceratophyllum sp.* as less abundant. The most outstanding ecological importance of macrophytes is that their abundance and diversity constitute the main source of environmental heterogeneity, constituting habitats for a large number of aquatic invertebrate species that live on them, including mollusks.



Macroinvertebrate analysis showed values of SW of 1.635 and D of 0.687, while RT was 14. According to GFA, a dominance of shredders was observed with 51.06%. The scrapers, GFA in which the mollusks are found, were represented with a low value of 9.04%. The marked abundance of shredder over the remaining GFA could be due to the abundant cover of macrophytes present in the site. These macrophytes constitute not only substrate for the organisms but also food. Based on biotic indexes, waters correspond to slightly polluted environment (IBSSL), while they are polluted waters for IBMWP. This difference could be caused because IBMWP is a more sensible index, by looking at differential tolerances at the taxonomic family level.

## A88

### **EFFECT OF *Acromyrmex lobicornis* (Formicidae: Myrmicinae) NESTS ON THE PRODUCTIVITY OF HERBACEOUS PLANTS IN THE MEDANOS OF CAUCETE, SAN JUAN, ARGENTINA**

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Ants play a key role in the structure and functioning of terrestrial ecosystems. The construction of ant nests affects the physical properties of soils, such as porosity, drainage and ventilation, as well as the amount of nutrients resulting from the accumulation of organic wastes, which is why they are considered by many authors as ecosystem engineers. The objective of this work is to evaluate the influence of *Acromyrmex lobicornis* anthills on the productivity of herbaceous plants in the large sand dunes of the Department of Caucete. The area belongs to the phytogeographic province of the monte, it is located near the national route N°141, province of San Juan, Argentina (31° 42'18.70"S, 68° 09'05.80" West). Sampling was carried out during the month of October 2019, using the transect method, 30 patches of *B. retama* that had anthills under their canopy (treatment) and 30 that did not (control) were selected for subsequent comparison. Within each site, the area was delimited with a 70 cm x 70 cm frame, opposite the location of the dump. Fresh pruning was carried out at ground level and subsequent differentiation between the vegetative and reproductive parts of each of the plants sampled. The plants were placed in labeled paper bags for transport to the laboratory. The collected samples were dried in an oven, at a temperature of 70 °C until constant weight (48 hours), to take the value of total dry aerial biomass, reproductive biomass and vegetative biomass. To detect the differences between the biomass means of both treatments, the student's T test was used with  $\alpha=0.05$ , obtaining as a result that there are differences in dry aerial biomass between sites without anthills and with their presence, the difference being in favor of the latter. In the total dry biomass, we found that there is a difference for both treatments ( $t = 0.6078$ ,  $df = 58$ ,  $p\text{-value} = 0.5457$ ,  $n=60$ ,  $n(H)=30$ ,  $n(SH)=30$ ). This same result was obtained with the vegetative biomass ( $t = 0.63365$ ,  $df = 58$ ,  $p\text{-value} = 0.5288$ ,  $n=60$ ,  $n(H)=30$ ,  $n(SH)=30$ ) and dry reproductive biomass for both treatments ( $t = 1.0343$ ,  $df = 58$ ,  $p\text{-value} = 0.3053$ ,  $n=60$ ,  $n(H)=30$ ,  $n(SH)=30$ ). It can be concluded through what was obtained in the analytical and graphic methods of the present study, that there is a positive association between ant nests and the fitness of herbaceous plants. The evidence that leafcutter ant nests positively affect plants is limited, so the present study aims to contribute and serve as a basis for further research in the mountain desert.

## A89

### **ANT DIVERSITY (HYMENOPTERA: FORMICIDAE) IN AN ANTHROPOGENIC DISTURBANCE AREA IN THE PRECORDILLERA OF SAN JUAN, ARGENTINA**

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Anthropic disturbances cause changes on arthropod communities in arid ecosystems. Ants are often used as bioindicators because they are easily collected and respond quickly to environmental changes. The abundance and richness of ants in their environment are determined by the responses of each species to various environmental characteristics. The aim of this study was to carry out a preliminary evaluation ant diversity in Tambolar hydroelectric power plant for future environmental monitoring. This project is located in the Precordillera of San Juan (Argentina), in the south-central part of the province and seeks to take advantage of the bed of the San Juan River, 18.8 km upstream from the Los Caracoles Dam. In September 2020 (in spring, the season of peak activity for ants), we set up different points in the project. Twenty-four sampling points were selected based on the criteria of the minimal anthropogenic intervention observed *in situ*. In each point was set up four pitfall traps during seven days. Fourteen species of ants of four subfamilies were collected. Ants' diversity was high (Simpson's index: 0.75 and Shannon's index: 1.58). The species with abundance significantly higher were *Acromyrmex striatus*, *Camponotus punctulatus*, *Pheidole aberrans*, *Pheidole bergi*, *Camponotus mus*, *Forelius chalybaeus* (GLM:  $p<0.05$ ). The diversity of terrestrial ant assemblages in Tambolar's project was dominated by generalist's species. Therefore, these species could be good indicators for future environmental monitoring programs. Our study represents the first inventory of ants in the Precordillera of San Juan. However, we acknowledge that this represents a preliminary exploration of ant diversity. Therefore, additional studies employing complementary sampling techniques, and conducted during different seasons of the year, such as in the summer, are warranted.

## A90

### PLANT SPECIES DIVERSITY IN URBAN GREEN SPACES IN SAN JUAN: THE VERDANT LANDSCAPE OF A DESERT CITY IN ARGENTINA.

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In extremely arid climates like the province of San Juan, creating urban green spaces with species that can survive without excessive irrigation is a challenge for forestry and landscaping. These Urban Green Spaces (UGS) play a crucial role in conserving native flora and, in many contexts, can be considered novel ecosystems as they host a mix of native and exotic species, giving rise to a unique urban biodiversity. Currently, the water requirement for maintaining UGS in San Juan poses a challenge due to the ongoing water crisis the province has been facing for about seven years. In this context, it is of great value to consider native species of arid zones in the creation and maintenance of UGS. This will ensure their sustainability by requiring little irrigation. The objective of this research was the evaluation of the diversity of urban vegetation in green spaces in the city of San Juan and the assessment of the presence of native species from arid zones. Six UGS were selected for this study: 25 de mayo, Aberastain, Bicentenario, Gertrudis Funes, Hipólito Yrigoyen, and Monseñor Severino Di Stéfano. In each of these areas, data on the plants were recorded, including their cover, species richness and abundance. Diversity indices were also used to assess their diversity: Shannon (H'), Simpson (D), and Pielou's evenness (J'). Results showed that tree cover in the studied UGS ranged from 13.14% (Bicentenario plaza) to 95.67% (Gertrudis Funes plaza). A total of 74 plant species were recorded, of which only three were native to dry areas of Argentina: *Neltuma flexuosa*, *Parkinsonia aculeata*, and *Vachellia aroma*. The number of individuals of these species was very low, ranging from 2 to 9 in total. In contrast, the UGS studied documented a total of 149 individuals of *Morus nigra* and 113 individuals of *Platanus hispanica*, both exotic species. These two species were found to be the most abundant of all the species observed. The Plaza Hipólito Yrigoyen had the highest diversity index values (H' = 2.90, D' = 0.08, J' = 0.85), whereas the Plaza Monseñor Severino Di Stéfano had the lowest diversity index values (H' = 1.58, D' = 0.38, J' = 0.61). Our findings indicate that native arid Argentine species are underrepresented in the urban green spaces of the city of San Juan. Conversely, exotic species are predominant. This situation demands the UGS manager's attention, especially considering the prevailing water crisis in the region. Effective water management necessitates the implementation of diverse strategies and practices, such as choosing drought-resistant plants and employing more efficient irrigation techniques. Our research emphasizes the importance of integrated planning and management involving multiple stakeholders, including government agencies, residents and sustainability experts. This collaborative approach is crucial in effectively tackling water scarcity challenges and fostering the development of sustainable and resilient urban environments.

## A91

### IMPACT OF DIFFERENT FIRE TEMPERATURES ON CYANOBACTERIA PRESENT IN BIOLOGICAL SOIL CRUSTS (BSCs): A CASE STUDY IN THE SIERRAS DE SAN LUIS- ARGENTINA

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Edaphic Cyanobacteria present in BSCs are deteriorated by forest fires. This deterioration depends on the reached thermal intensity. The objective of this article was to evaluate the degree of deterioration of SBCs Cyanobacteria at 5 controlled burning temperatures in the laboratory (100°-200°-300°- 400° and 500°C). Burned SBCs were sown in triplicate at the 5 temperatures in specific culture medium and weekly percentage counts were performed under an optical microscope at 40X magnification, for 9 consecutive weeks. Cyanobacteria were differentiated into Heterocysted Fixatives (HF), Non-Heterocysted Fixatives (NHF) and Non-Fixatives (NF). At 100°C, the NF species grew 33% in week 1, increased to 91% in week 2 and stabilized at 33% in week 4; NHF grew 9% in week 2, increased to 60% in week 4 and stabilized in week 6 at 22%; HF grew 9% in week 3, and increased to 65% in week 6. At 200°C, NF and NHF appeared simultaneously in week 3 with 75% and 10% respectively; NF had a maximum of 72% on week 5 and stabilized at 38% on week 6; NHF increased to 52% in week 6; HF present a 5% at week 5, rising to 10% at week 6. At 300°C, a greater thermal impact occurred on the Cyanobacteria, with the NF being the first to show growth from week 3 with a maximum growth peak of 98% and a final decrease of 35%; NHF were observed at week 5 in 2%, increased to 40% at week 5 and stabilized at 25% at week 8; The HF showed growth in week 5 with 27%, stabilizing at 40% in week 8. At 400°C, the growth of NF and NHF occurred in week 3 with a peak of 90% in the NF and a decrease to a final 14%; NHF reached 40% on week 6 and with a slight decrease at the end of 35%, and HF were recorded on week 6 with a maximum of 58%, stabilizing at 51%. At 500°C the growth occurred in week 4, the NF presented 88% and decreased to 10% in week 9, the NHF began with 24%, then, reached a maximum of 43% in week 6 and decreased to 21% at the end; HF had values of 10% rising to 75% at week 8 and decreasing to 63%. It is concluded that NF species are always the first to show growth, regardless of the reached temperature. In incomplete combustion of organic matter (100°C to 300°C) there is availability of C and N; the fixative species develop later and the appearance of HF occurs in a lower proportion. In the case of complete combustion (400 and 500°C) and scarcity/absence of N and C, the greatest thermal impact is evident.

the delay of the appearance of species. There is also a greater proportion of stable HF over time, demonstrating the need to photosynthesize and fix nitrogen under aerobic conditions in such a degraded environment.

### A92

#### ANTIMICROBIAL ACTIVITY OF *Baccharis salicifolia* AND *B. spartioides* ESSENTIAL OILS

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Essential oils (EOs) are presented as a promising antimicrobial alternative to natural products. Previously, the genus *Baccharis* has been reported as a potential insecticide against mosquitoes and flies, mainly against *A. aegypti*, *Culex sp.* and flies of the *Calliphoridae* family. Some terpenoids compounds from EO such as  $\beta$ -pinene, d-limonene, E-nerolidol and  $\alpha$ -pinene have shown significant activity in insect metabolism. Previous work developed by the authors allowed the selection of the *B. salicifolia* species *B. spartioides* that were found to have potential repellent activity against *Triatoma infestans* “vinchucas”. In order to continue investigating the properties of bioactive compounds of EOs its antimicrobial effect was evaluated. This study aimed to determine the antibacterial and antifungal properties of EOs from *Baccharis salicifolia* and *B. spartioides* aerial parts collected in San Juan, Argentina, during the flowering period 2022-2023. The EOs were hydrodistilled in a Clevenger type apparatus, according to the method recommended by the European Pharmacopoeia and characterized by GC-FID and GC/MS analysis. The antimicrobial effects were evaluated by the CLSI protocols, for yeast (2008) and bacteria (2015). The commercial standards Imipecil® and ketoconazole were used as negative controls. The chemical composition of the EO of *B. salicifolia* showed a high content of monoterpenics hydrocarbons, approximately 30%, of which 21% is represented by  $\alpha$ -pinene, the majority compound is spathulenol (14.4%), an oxygenated sesquiterpene. The EO of *B. spartioides* presented a composition dominated by the monoterpenics hydrocarbons:  $\alpha$ -phellandrene (40.5%), sabinene (22.7%) and  $\alpha$ -pinene (12.0%), which represented more than 80% of the total identified (98.0%), coincident with the literature. Regarding the antibacterial activity, *Baccharis salicifolia* EO showed activity against the Gram (+) bacteria: *Staphylococcus aureus* methicillin-resistant ATCC 43300, *S. aureus* methicillin-sensitive ATCC 25923 and Gram (-): *Escherichia coli* ATCC 25922 (MIC = 1000  $\mu$ g/ml). While the *B. spartioides* EO showed moderate activity (MIC  $\geq$  2000  $\mu$ g/ml). Regarding yeasts, *Candida albicans*, *C. parapsilosis*, clinical isolates of *C. tropicalis* C131 and *C. tropicalis* MQ1 and *Cryptococcus neoformans* provided by Hospital Público Marcial Quiroga, San Juan, Argentina, were found moderately sensitive to both EOs (MIC = 750  $\mu$ g/ml). The EOs of *B. salicifolia* and *B. spartioides* are a natural alternative to combat microbial resistance.

### A93

#### PRELIMINARY STUDY OF VULNERABILITY OF THE FREE AQUIFER TO POLLUTION IN THE LOWER BASIN OF EL MORRO (SAN LUIS-ARGENTINA)

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The expansion of agriculture in the El Morro basin has increased the use of pesticides associated mainly with direct sowing. The impacts of agricultural activities are generally associated with the use of pesticides. In the basin, corn (*Zea mays* L.) and soybean (*Glycine max* L. Merr.) crops represent 98.1% of the agricultural area. On the other hand, mixed systems develop crops of corn and soybeans for grain that represent 87.6% of the surface, they also grow forage sorghum (6.2%), corn for forage (4.4%) and alfalfa (1.9%). These systems use an average of 19 agrochemical molecules; the most frequent being: Glyphosate, 2,4 D, Picloran, Atrazine, Ammonium Sulfate, 2,4DB and Cypermethrin, where agricultural crops had the highest frequencies and application doses. To estimate the contamination vulnerability of the water table to agrochemicals, the DRASTIC indicator developed by the United States Environmental Protection Agency (EPA) was used. Data on water depth, net recharge, aquifer lithology, soil type, topography, nature of the unsaturated zone and hydraulic conductivity were used. The study was carried out in a mixed establishment, in the lower area of the basin, where alfalfa (*Medicago sativa* L) is grown corn (*Zea mays* L) and soybean (*Glycine max* L. Merr.), the depth of the water table was evaluated for one year, while data from net recharge, aquifer lithology, soil type, topography, nature of the unsaturated zone and hydraulic conductivity were obtained from different bibliographic sources. The possible range of values of the DRASTIC index is between 23-230, where the vulnerability of the aquifer could be Null: 23 to 64, Low: 64 to 105, Moderate: 105 to 146, High: 146 to 187 and Very high or Extreme: 187 to 230. In our results, a difference of 25 cm was found in the depth of the water table between the alfalfa plot (2.52 m) and the plots with corn or soybeans (2.27 m). The degree of vulnerability of the aquifer is “High” in both sites with a value of 151 and 166 values for alfalfa and corn or soybeans respectively. If we take into account the pesticides used in the basin and the vulnerability of the aquifer, the presence of agrochemical residues in the groundwater of the lower El Morro basin is likely. Subsequent studies will be aimed at validating these results with analytical determinations.

A94

**ESTIMATION OF THE ENVIRONMENTAL IMPACT AND LEACHING RISK OF AGROCHEMICALS USED IN THE EL MORRO BASIN (SAN LUIS-ARGENTINA)**

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The El Morro basin is located in the province of San Luis (Argentina), with an approximate area of 2600 km<sup>2</sup>. In this region, the increase in rainfall and the replacement of native forest with annual pastures has played an important role in reducing evapotranspiration with the consequent increase in groundwater recharge, observing increases in salinity and phreatic levels. Seventy-seven% of the surface of the El Morro basin corresponds to agricultural areas that are under the direct sowing system. In the development of crops, agrochemicals are used to control weeds, insects and diseases. The use of pesticides is considered one of the main causes of groundwater contamination by leaching in areas with agricultural production. Due to the high cost and time necessary to carry out groundwater monitoring, various methodologies have been developed based on indices and indicators, which estimate the leaching potential of pesticides and the risk of impact on groundwater. In this work, surveys were carried out on agricultural establishments (511 productive lots) covering a total area of 30,577 hectares. The Environmental Impact Coefficient (EIQ) was determined; this indicator was developed by the Integrated Pest Management Program at Cornell University that allows evaluating the environmental impact through a dimensionless numerical value. On the other hand, the danger of leaching of the main agrochemicals used was calculated through their physical-chemical characteristics: half-life of the pesticide in the soil and absorption coefficient to organic carbon, defined by the GUS index (Groundwater Ubiquity Score). From the analysis of the surveys, it was determined that 32 agrochemical molecules were used in establishments dedicated to agricultural, livestock and mixed activities. Of the 10 most used molecules, those with moderate-high EIQ are: Glyphosate, Atrazine and Sulfosate. While those that have moderate-high leaching are: 2.4-D, Picloram, Dicamba, Metolachlor, Atrazine and Sulfosate. Only three products have low EIQ values and low leaching: Flumioxazin, Cletodim and 2.4-DB. These results indicate that groundwater could be contaminated by the use of agrochemicals in agricultural establishments in the El Morro basin. The information obtained constitutes a tool that can help in decision-making and/or control for the selection of pesticides, pest management strategies associated with crops and/or production systems that minimize the risk of environmental impact.

A95

**FECAL CORTISOL METABOLITE LEVELS IN THE DRY CHACO FAT-TAILED MOUSE OPOSSUM (*Thylamys bruchi*): TOWARD THE BIOLOGICAL VALIDATION OF AN ENZYME IMMUNOASSAY**

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Recent advances in fecal cortisol metabolites (FCM) monitoring have enabled the non-invasive measurement of glucocorticoids (GCs) in small and wild species. It has been reported that GCs play a key role in senescence and mortality among semelparous marsupials in Australia. However, it is necessary to characterize this relationship in semelparous marsupials from South America. To achieve this, our study establishes the basis for the biological validation of an enzyme-immunoassay (EIA) for non-invasive monitoring of FCM in the Dry Chaco fat-tailed mouse opossum (*Thylamys bruchi*), an endemic semelparous marsupial from Argentina. From February to April 2023, ten individuals of *T. bruchi* were captured in the MaB Reserve of Ñacuñán (Santa Rosa, Mendoza, Argentina) and transferred to the bioterium (stressor event) of the Instituto Argentino de Investigaciones de Zonas Áridas (CCT Mendoza, Argentina). Fecal samples from seven of the ten individuals were collected in three periods: before the transfer (pre-transfer), 12 to 14 hours later, and 22 to 30 hours later. They were stored in a freezer at -20 °C until extraction. FCM concentration was determined by EIA using a polyclonal anti-cortisol antibody, cortisol standard, and the corresponding horseradish peroxidase conjugate (anti-cortisol R4866). Serial diluted fecal extracts were tested and showed parallel displacement with the standard curve. A peak of FCM was detected 12 to 14 hours after the transfer (233.3±97.8 ng/g feces), with FCM values marginally higher than pre-transfer's FCM values (132.7±60.6 ng/g feces; p=0.05; R statistical software) and significantly higher than the next 22 to 30 hours (114.5±91.5 ng/g feces; p=0.01; R statistical software). The transfer-induced stress resulted in a peak of cortisol, successfully detected in fecal samples using the mentioned EIA. These findings represent the biological validation of this technique for *T. bruchi*. Given that *T. bruchi* is one of the few endemic marsupials in Argentinian drylands, further investigation using this method is essential for a deeper ecological understanding of semelparity.



## A96

### ECOSYSTEM SERVICES PROVIDED BY URBAN GREEN SPACES IN AN ARID CITY FROM WESTERN ARGENTINA

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Understanding, conserving and making sustainable use of the benefits and resources provided by all ecosystems is a priority in order to live in a healthy and dignified environment. To this end, the vision of ecosystem services (ES), which refers to all the direct and indirect benefits that humans obtain from nature, is fundamental. In this sense, we understand the urban ecosystem as a key conservation link to ensure the contribution of these ES. During December 2021 and February 2022, 7 urban green spaces (EVU) -natural, semi-natural and artificial ecosystems within and around a city- were sampled in the city of San Juan, Argentina. The objective was to evaluate the different ecosystem services provided by these urban green spaces. Ecological, Microclimatic and social aspects were measured. Non-parametric statistics were used, since the data were not normally distributed. InfoStat software was used for data analysis. The results obtained suggest that the ES provided were: Regulatory Services (urban T<sup>o</sup>, noise reduction and air purification), Support Services (pollination and availability of habitats and resources for wildlife) and Cultural Services (recreation). Several proxy variables were used to estimate the ES contribution. The results obtained showed that the UGS contribute 1) regulation, 2) support and 3) cultural services: 1) as for regulation ES, the following were calculated: tree vegetation cover, substrate T<sup>o</sup> under and outside the canopy, for urban T<sup>o</sup> regulation; noise and vegetation cover, for anthropogenic noise regulation service; percentage of woody plants, morphological characteristics and foliar characteristics of the same, to estimate the air purification service. 2) There were two supporting ES. For the first one, pollination, the presence of flowering beds and the presence of registered flowering tree species were taken into account. The other support ES was the availability of habitats and resources for fauna, where the presence of the 3 plant strata and allometric trees data were estimated. 3) Finally, for the cultural ES, recreation was calculated. The proxy variables used for its estimation were: ramps for people with disabilities, lighting, drinking fountains, and sociocultural events. Each service was provided to a different extent in each study site, with urban temperature regulation being the ES with the greatest contribution.

## A97

### MODELING HABITAT SUITABILITY OF THE KENTISH PLOVER (*Charadrius alexandrinus*) IN MOLISE, ITALY

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Shorebirds, by virtue of their life history, behavior, migratory, and foraging habits, are important indicators of the integrity of coastal ecosystems. Many shorebirds are facing a global decline driven by habitat degradation and climate change. Comprehensive information on the distribution and breeding ecology of shorebird populations is crucial to understand and mitigate these threats. The Kentish Plover (*Charadrius alexandrinus*) is a small ground-nesting shorebird that breeds in sandy beaches, but information about the breeding ecology of this species in the Molise region (southern Italy) is highly limited. In this study, our aim was to analyze the relationship between environmental features and breeding habitat preferences of *Charadrius alexandrinus*, through a breeding habitat suitability modelling in a current and future scenery under climate change pressure. We collected data on Kentish Plover's nests across the entire Molise coast during several years and, from a coast photo interpretation, the following variables were extracted: Shannon Index; distance to dry sand; distance to the coastline; distance to water bodies; distance to herbaceous, shrub and arboreal vegetation; distance to artificial surfaces; distance to urban green areas; distance to agricultural areas; and distance to beach accesses. These variables were used as predictors to calibrate a species distribution model (SDM) to map the nest occurrence probability, using an ensemble modelling approach with two algorithms: Gradient Boosting Machine (GBM) and Maxent. We then generated a binary presence/absence map along the current shoreline and along a future shoreline profile as predicted for 2040 under climate change scenarios. Variables with a relative importance greater than 70% were distance to dry sand; distance to artificial surfaces; distance to the coastline; and distance to herbaceous and arboreal vegetation, indicating that these are key parameters in breeding habitat preferences of the species. Our results also showed that the Kentish Plover will reduce its suitable breeding habitat in the area by more than 20% within the next twenty years. According to our results, we point out that our modelling approach can provide inputs for conservation planning of Kentish Plover in the region. Finally, we believe that conservation of Kentish Plover breeding habitat will not only protect this species but will benefit other species, particularly those sharing similar habitats.

## A98

### **BIRDS OF THE MALVINAS ISLANDS UNIVERSITY COMPLEX**

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In urban areas, patches of remnant vegetation, whether native or exotic species, play a crucial role in providing shelter and/or food, allowing them to adapt to urbanized environments. Different sites have become highly attractive spaces for birds, with nearly 300 species having been recorded for the entire San Juan province, of which 76 have been sighted in urban environments to date. The purpose of this work is to know the richness of birds in the Islas Malvinas University Complex (CUIM). This complex is characterized by the presence of green areas, which include areas with grasses, as well as groves with native and exotic species, such as fruit trees, and also has buildings that, in many cases, serve as shelters for some species. It is important to highlight the decrease in vegetation cover in certain areas due to the cutting of trees, which can have a significant impact on the diversity of birds at the site. During the months of June-July 2023, we carried out two monthly surveys through visual records with 12x50 mm binoculars at twelve observation points with variable radius, following the visibility criterion. We also consulted literature and available sources to prepare an updated list of the bird species present at the site during that period. In total, we identified 23 species, with *Columba livia*, *Columbina picui*, *Patagioenas maculosa*, *Myiopsitta monachus*, *Furnarius rufus*, *Pitangus sulphuratus* and *Passer domesticus* being the most frequently observed. The families Columbidae (with 5 species) and Thraupidae (with 3) were represented by the highest number of species, followed by the families Tyrannidae, Furnariidae, Trochilidae and Icteridae (with 2 each). Finally, seven families were represented by a single species: Picidae, Psittacidae, Falconidae, Charadriidae, Accipitridae, Ploceidae, Fringilidae. This work provides an inventory of species present, which may contribute to the understanding of bird diversity in urban environments and lay the groundwork for future research on the avifauna at this site. It is important to highlight the need to spread the sampling over a year to obtain the seasonal variation of the avifauna. In addition, it is intended to promote the planting of native or exotic species that can serve as refuges, food sources or nesting sites, backed by an ongoing education effort that not only values the species, but also the urban environments.

## A99

### **CHARACTERIZATION OF THE PHYTOPLANKTON COMMUNITY AND ISOLATION OF NATIVE STRAINS FROM WATER BODIES IN THE PROVINCE OF SAN JUAN**

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Microalgae conform the production community of the aquatic ecosystem, which is why they are the first to respond to a change in their environment. The potential of microalgae to capture organic and inorganic pollutants has been documented in the last 30 years, but recently attention has been given to the use of microalgae for the bioremediation of emerging pollutants such as pesticides. The objective of this work was to sample different water bodies in agricultural and urban areas of San Juan to characterize phytoplankton and isolate from native strains. In order to evaluate the phytoplankton community environment, physicochemical parameters were measured in-situ from water sources located in 3 departments of San Juan province: Rawson (R), Santa Lucía (SL) and Capital (C). The sites in R and SL are agricultural areas while C corresponds to an urban lake. Sampling was carried out during the winter season. Two samples were extracted at each site, one was kept in field conditions ("ex situ") for isolation of the strains, and the other was preserved in 4% formalin for processing and taxonomic analysis. As a result, a total of 31 taxa were obtained, of which 38.7% corresponded to Bacillariophyta (diatoms) and Chlorophyta (green algae), 13% to Cyanobacteria and 9% to Charophyta. The greatest diversity of species was observed in the sample corresponding to C (urban lake), with the following species standing out for their abundance: *Chroococcus* sp., *Gloeocapsa* sp., *Crucigenia* sp. and *Staurastrum* sp. While for the samples from the agricultural areas, 11 taxa were found. The most abundant were *Pinnularia* sp. and *Oscillatoria* sp. for R and *Navicula* sp., *Nitzschia* sp. and *Stenopterobia* sp. for SL, respectively. Other native strains were isolated from agricultural areas, belonging to the genera *Scenedesmus*, *Chladophora*, *Nostoc* and *Anabaena*, which are preserved in a strainarium to be used in future studies. This study provides valuable data on the microalgae of San Juan, since by knowing their diversity and abundance, possible impacts on aquatic ecosystems can be detected early. Furthermore, due to the bioremediation capacity of these organisms, some species can be used as natural tools to mitigate water pollution.



## A100

### BIOSYNTHESIS OF SILVER NANOPARTICLES WITH GRAPE BROOM EXTRACT

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Environmentally friendly methods use living organisms or their products to obtain and stabilize nanoparticles (NPs) without producing any hazardous materials. These nanoparticles can be used in numerous industrial and medical processes due to their stability, efficiency, biocompatibility and bioactivity. Particularly, silver nanoparticles (AgNPs) have a wide variety of applications, such as in the paint and medical industries, they show antimicrobial activity against bacteria, yeasts and fungi, anti-cancer activity against human lung adenocarcinoma cell lines, among others. AgNPs can be obtained from an ecological process using plant extracts with antioxidant properties, such as those that contain polyphenols, these are capable of reducing Ag<sup>+</sup> ions in AgNPs aqueous solution. In this work, AgNPs were obtained through a biosynthetic process, from an extract of red grape stalks of the Bonarda variety, obtained with water:ethanol (70:30; v/v) at room temperature, for 48 hours. 1 mL of the extract was added to 10 mL of a 1mM AgNO<sub>3</sub> solution and the pH of the mixture was adjusted to 6. Samples were taken at 3, 10 and 20 minutes and analyzed by UV-visible spectrophotometry. The presence of AgNPs was verified by the appearance of an absorption maximum at 428 nm, due to the surface plasmon resonance. It is important to highlight that the stalk, a source of polyphenols, is a design generated by the wine industry with potential application on an industrial scale in the synthesis of AgNP

## DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY

### A101

#### DEXAMETHASONE COUNTERACTING THE EFFECT OF TUMOR NECROSIS FACTOR- $\alpha$ IN SPLEEN MACROPHAGES SECRETIONS ON RAT ESTRADIOL VALERATE-INDUCED POLYCYSTIC OVARY

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Polycystic ovarian syndrome (PCOS), described as an ovarian dysfunction, is a heterogeneous reproductive disorder with hormonal and metabolic implications. Although the major cause of this endocrinopathy is still unclear, chronic low-grade inflammation are known to be pathways involved in PCOS pathogenesis. As an inflammation-associated cytokine, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is significantly higher in the serum and follicular fluid of PCOS patients and not only is involved in local inflammation of the ovary but also induces or aggravates systemic inflammation. It has been documented that glucocorticoids, such as dexamethasone (DEX), can inhibit the induction of androgen by suppressing the adrenal glands in PCOS patients. However, DEX has much more functionality and could exert a therapeutic effect by antagonizing TNF- $\alpha$ . The aim of this work was to investigate if DEX affects the steroidogenic ability of M $\Phi$  secretions and consequently modifies the steroid release from PCO ovary, and its relationship with TNF- $\alpha$  effect. PCO condition was induced by injecting 60-day old rats with a single 2 mg estradiol valerate (Sigma, USA) dose dissolved in 0.1 mL of corn oil. After 2 months, the rats were sacrificed (N=6 per group in two different experiments). M $\Phi$  (1x10<sup>6</sup> cells) from PCO and no-PCO (control) rats were cultured for 24 h in RPMI medium in the absence or presence of 10<sup>-6</sup>M DEX. Afterward, the medium was removed, the M $\Phi$  were washed twice, and finally, the cells were cultured for an additional 24 h period. The respective culture media (M $\Phi$  secretions) were collected and used to stimulate ovaries from PCO and control rats for 3 h in metabolic bath. Also, PCO and control ovaries were incubated with TNF- $\alpha$  (15ng/mL) to test its effects on androstenedione (A2) released. The ovarian estradiol (E2) and A2 release were measured by electrochemiluminescence immunoassay (Cobas e411) and the TNF- $\alpha$  level by ELISA. The mRNA expression of TNF- $\alpha$  and P450 aromatase (P450arom) was assessed in M $\Phi$  and ovary, respectively, by RT-PCR. In PCO M $\Phi$ , the mRNA expression of TNF- $\alpha$  as well as TNF- $\alpha$  release was higher than observed in control M $\Phi$ . PCO ovaries released less A2 and E2 after stimulation with secretions of PCO M $\Phi$ +DEX, compared with PCO M $\Phi$  secretions (P<0.05). The *in vitro* added of TNF- $\alpha$  induced A2 release in PCO ovary compared to basal value (P<0.05). The mRNA expression P450arom in PCO ovaries incubated with secretions from PCO M $\Phi$ +DEX was lower than that obtained with PCO M $\Phi$  secretions (P<0.05). Our results suggest that DEX can improve not only the pro-inflammatory but also the endocrine environment of PCO. This provides a new foundation for exploring the treatment of PCOS patients with DEX.

A102

**TESTOSTERONE IS CLOSELY RELATED TO LEYDIG CELL ACTIVITY, ANDROGEN RECEPTOR DISTRIBUTION AND ENVIRONMENTAL FACTORS IN ADULT MALE LIZARDS OF *Liolaemus cuyanus* DURING THE REPRODUCTIVE CYCLE**

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Testosterone, the primary sex hormone in male lizards, is closely linked to Leydig cell (LC) activity (the cells where steroidogenesis occurs) throughout the reproductive cycle, but testosterone action is related to androgen receptors (ARs) distribution in the seminiferous epithelium. In temperate zones, environmental factors detected through the hypothalamic-pituitary-gonadal axis, down-regulate serum testosterone, resulting in a seasonal reproductive cycle. The aim of this work is to study the serum testosterone in adult male lizards of *Liolaemus cuyanus*, an oviparous species, throughout its reproductive cycle and its relationship with Leydig cell histology, the ratio of TotalLC / ActiveLC, ARs distribution in the seminiferous epithelium and the regulation by environmental factors (temperature, relative humidity and solar irradiation). Specimens (N=27) were captured (October to March) in a semi-arid zone (Valle de Matagusanos, San Juan, Argentina; -31°06'46.08''S, 68°38'03.84''W) and grouped into three relevant reproductive periods: pre-reproductive (PrR), reproductive (R) and post-reproductive (PsR). Significant differences in serum testosterone were found among these periods, highest during the R period, with a decrease in PsR. Significant positive correlation between serum testosterone and the ratio of TotalLC / ActiveLC was also observed. In PrR and R, ARs distribution was cytoplasmic and nuclear, shifting to only cytoplasmic in the PsR period. Serum testosterone has a significant positive correlation only with solar irradiation. However, solar irradiation has a significant positive correlation with temperature. These results highlight the close correspondence between serum testosterone, Leydig cell histology and activity, ARs distribution in the seminiferous epithelium and environmental factors, resulting in a synchronization that allows males of *L. cuyanus* to coordinate their reproductive cycle with the most favorable environmental conditions, probably for mating and birth of offspring.

A103

**ASSESSMENT OF REPRODUCTIVE EFFECT OF *Tessaria absinthioides* IN COMPARISON TO *Ilex paraguayensis*, *Camellia sinensis* AND ITS ASSOCIATION**

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Complementary medicine uses natural products along with standard medical treatment. It consists on the use of plant products in the form of infusions, decoction, and other presentations. The main challenge on the use of these products is to guaranty the safety used for the prevention and improve of human, animal, and environmental health. While there are indications that some natural products may be helpful, more needs to be learned about the effects of these products in the human body, and about their safety and potential interactions with medicines and other natural products. In fact, our research group previously demonstrated that *Tessaria absinthioides* (Ta) has hypolipidemic, hypoglycemic, antiatherogenic and antitumoral effects. We also showed its action on the female reproduction and pre and postnatal exposure on mice. These studies corroborate that the oral administration of Ta does not alter female reproduction, neither the development of pups during lactation. One approach of the use of Ta could be the combination with yerba mate (*I. paraguayensis*, Ip) and green tea (*C. sinensis*, Cs), two widely used infusions in our country, to obtain nutraceuticals products with health benefits. Nevertheless, up to the present there are no previous report of reproductive effects of the combination of Ta with Ip or Cs. The goal of this work was to compare the reproductive effects of Ta, Ip, Cs and its association, to discard deleterious effect on the maternal and offspring physiology. Decoctions were prepared by boiling 5 g of dry leaves in 100 mL of water (5% w/v) during 10 min and administered in the drinking water at a concentration of 1000 mg/kg/day during the entire gestation and lactation on female Wistar rats (n=6-8 per group). Reproductive performance was assessed according to the guidelines OECD (The Organization for Economic Co-operation and Development): Reproduction/Developmental Toxicity Screening Test (OECD TG 421) and Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (OECD TG 422). Our results showed that Ta did not affect fertility in comparison to controls; the administration of Ip, Cs, and their association with Ta decreased fertility in 50 % of the dams (p<0.05). The total number of implantation sites and the pups per litter were not affected by the treatments. The administration during lactation did not affect pup growth, but we observed a decrease in perinatal survival when Ip and Ta were administered in combination (p<0.05). Although the consumption of natural products is encouraged due to their health benefits, special attention must be focus on their possible effects during reproduction and pre and postnatal exposure.

#### A104

### ALTERED DISTRIBUTION OF MANNOSE 6-PHOSPHATE RECEPTORS IN RAT SPERMATOZOA DUE TO CASTRATION

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In somatic cells, both cation-independent and cation-dependent mannose 6-phosphate receptors (CI-MPR and CD-MPR) play crucial roles in delivering acid hydrolases to lysosomes. Our prior laboratory findings have demonstrated the presence of both CI-MPR and CD-MPR on the plasma membrane of epididymal rat spermatozoa. As sperm undergo maturation during their transit through the epididymal duct, MPRs exhibit dynamic changes in their distribution. Notably, their location undergoes a significant shift after the acrosomal reaction, suggesting a potential connection to the process of sperm maturation. Considering that the epididymis is a steroid hormone-dependent organ, our study aimed to evaluate the impact of testosterone deprivation on the distribution of MPRs in rat spermatozoa. Through indirect immunofluorescence, we observed a redistribution of CD-MPR and CI-MPR in spermatozoa from the epididymal caput of castrated animals when compared to control groups. This redistribution pattern closely resembles that of capacitated and acrosome-reacted spermatozoa obtained from the cauda epididymis. The shift in MPRs distribution due to androgen deprivation may indicate potential alterations in membrane composition and early sperm capacitation. These changes may be attributed to a decrease in the levels of estrogenic sulfotransferase in castrated rats (confirmed by immunoblot), which typically inhibit acrosomal proteolytic enzymes like acrosin, an essential component of sperm training and fertilization. Exploring the hormonal regulation mechanism of sperm maturation provides valuable insights into reproductive health.

#### A105

### STUDY OF THE N-ACETYLGLUCOSAMINE (GlcNAc) ROLE DURING SPERM-OVIDUCT INTERACTION IN BOVINE EPITHELIAL CELLS

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The interaction between sperm and oviductal cells is a complex process that involves various molecular mechanisms. This interaction leads to the formation of a functional sperm reservoir that serves to ensure successful fertilization. Spermatozoa can remain in the oviduct for several days by binding to epithelial cells, thus sustaining fertility by delaying capacitation. One of the key players in this process is N-acetylglucosamine (GlcNAc), a monosaccharide that is present in the oviductal epithelium and on the surface of sperm cells. In this study, we cultured primary cell lines from bovine oviductal cells (BOECs) to investigate the involvement of GlcNAc in bovine sperm-oviduct interaction. By fluorescence microscopy, we found that pre-incubation of sperm with GlcNAc before addition to BOECs resulted in a significant decrease in sperm number bound to cells. Conversely, BOECs pre-incubation with GlcNAc before addition of sperm resulted in a significant increase in sperm number bound to cells. Furthermore, pre-incubation of sperm with the lectin wheat germ agglutinin (WGA), which specifically binds to GlcNAc, resulted in a significant increase in the number of sperm bound to BOECs. In contrast, oviductal cells pre-incubation with WGA before sperm addition resulted in a decrease in the number of sperm bound to the cells. These findings suggest that GlcNAc plays a critical role in the sperm-oviduct interaction in bovine, and that lectins such as WGA may be useful tools for studying this interaction and may have potential applications in reproductive technologies. Further research is needed to elucidate the specific mechanisms by which GlcNAc and lectins such as WGA regulate this interaction.

#### A106

### INTERACTION EFFECTS BETWEEN MATERNAL HYPERTHYROIDISM AND PRENATAL STRESS ON MALE WISTAR OFFSPRING POSTNATAL DEVELOPMENT AND LOCOMOTOR ACTIVITY

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Thyroid hormones (THs) [triiodothyronine (T3) and thyroxine (T4)] are essential for various physiological functions and can cross the placenta during pregnancy to play an important role in fetal development. Hyperthyroidism (HyperT) results from excessive production of THs, which can lead to various maternal, fetal and lactational complications. Prenatal stress (PS) can alter the histoarchitecture and functionality of the offspring's brain, increasing the risk of neurodevelopmental disorders and mental illness. In addition, PS can also affect THs and corticosterone (C, stress hormone in rats) levels, which can affect reproductive health. Our aim was to determine the effects of the interaction between HyperT and PS on the development of male offspring. Pregnant Wistar rats were divided into four groups: control (euthyroid, Co), HyperT (administration of T4, 0.1 mg/kg/day dose, subcutaneous, throughout the protocol), Chronic Unpredictable Moderate Stress (CUMS), and HyperT+CUMS. CUMS was applied from gestational days 6 to 17. Male offspring were kept in standard conditions, and their developmental parameters were assessed from birth to postnatal day (PD) 42. Open Field Test (OFT) was used to analyze their exploratory and locomotor activity at two time: young (PD24) and adults (PD85). Trunk blood and adrenal glands (AG) were collected at PD110. One-way ANOVA statistical analysis was performed. These results showed that offspring exposed to HyperT+CUMS exhibited reduced size at PD1, as evidenced by lower body weight ( $p<0.01$ ) and length ( $p<0.01$ ). During postnatal development, the interaction between HyperT and CUMS resulted in incisor eruption delays ( $p<0.001$ ), the appearance of the auditory startle reflex ( $p<0.05$ ), aversion to heights ( $p<0.001$ ), and testicular descent ( $p<0.001$ ). At PD42, HyperT+CUMS offspring showed less weight gain ( $p<0.001$ ) and less length increase ( $p<0.01$ ). Regarding exploratory activity, male offspring exhibited similar behavior patterns at PD24 and PD85. Offspring CUMS increased total entries in areas of interest (center and corners) and a higher entries percentage to the center. Conversely, HyperT reduced the rate of entries and time spent in the center. The combination of HyperT+CUMS increased the time spent in the center of the box. C levels were lower in HyperT+CUMS rats at PD42 ( $p<0.01$ ) and PD110 ( $p<0.05$ ); however, the size of GAs fascicular area (site of glucocorticoid synthesis) was not affected, suggesting that the variation in C levels occurred due to its regulation rather than synthesis. Offspring exposed to prenatal thyroid imbalance and/or stress displayed distinct effects, including delays in physical development and alterations in locomotor activity. These findings are significant as they highlight how the hormonal environment during development influences offspring programming and health. Furthermore, maternal HyperT-induced hormonal imbalance during pregnancy and lactation may interact with and amplify on PS effects, potentially exacerbating the consequences aforementioned.

#### A107

### IMPACT OF ENDOGENOUS STEROIDS ON SEMEN QUALITY AND REPRODUCTIVE CAPACITY IN MEN

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The calcium channel of sperm, CatSper, is vital for male fertility. CatSper is activated by the hormone progesterone (Pg), but its pharmacological profile is not well studied. The steroid selectivity of CatSper activation has been explored using hormones to which human sperm are exposed in the male and female genital tract, with conflicting results. However, how different hormones affect the various events of sperm physiology necessary for fertilization is unknown. The aim of this study was to investigate the effect of different endogenous steroids on hyperactivation, motility, chemotaxis, and acrosomal reaction. We worked with semen samples from normal donors according to the parameters established by the WHO. Cells were retrieved using the swim-up method and capacitated for at least 3 hours. For the different methodological strategies used, capacitated sperm were incubated in the absence (control) or presence of progesterone and different steroids. We performed real-time dynamic measurements of intracellular calcium concentration, also evaluating the acrosomal reaction (AR). Furthermore, we studied the effect on chemotaxis using the sperm selection assay, and finally, using a computerized system for semen analysis, we studied all kinetic parameters of motility and hyperactivation. The results show that pregnenolone, allopregnanolone, and pregnenolone sulfate increase intracellular calcium concentration with kinetics similar to progesterone; they also agonize the effect of progesterone by stimulating the acrosomal reaction, modifying kinetic parameters, and hyperactivation. In contrast, hydrocortisone inhibited the effect of progesterone. However, when we studied chemotaxis, only allopregnanolone exhibited an agonistic effect similar to Pg.

### BIOCHEMISTRY, PHYSIOLOGY AND NEUROCHEMISTRY

#### A108

### IMMUNOHISTOCHEMICAL STUDY IN THE SUBSTANTIA NIGRA IN AN ANIMAL MODEL OF PARKINSON'S DISEASE

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The main pathological characteristic of Parkinson's disease (PD) is the progressive degeneration of dopaminergic neurons in *substantia nigra pars compacta* (SNc) of the midbrain. We performed an immunohistochemical analysis in an experimental rat model of PD. As it was assessed previously by our group, rotenone-loaded PLGA microparticles allow a slow delivery of the neurotoxin rotenone and thus a long treatment

with a single-dose subcutaneous administration. After 10 weeks of rotenone administration, the animals were euthanized by carbon dioxide inhalation (CICUA approved-protocol n°B-338). The brains were dissected and coronal tissue sections were obtained at the SNc level. Cresyl violet is able to stain Nissl substance in the cytoplasm of neurons. Thus, the neuronal density and morphology were analyzed by Nissl staining using 0,5% Cresyl violet acetate solution. After that, Tyrosine hydroxylase (TH) immunohistochemistry was performed. The number of tyrosine hydroxylase immunoreactive positive dopamine neurons (TH+) decreased in the SNc in rotenone-treated rats in comparison with the control group. Nissl staining method exhibited cells with aberrant morphology with a triangular shape and mostly exhibiting a dark staining due to the condensation of cytoplasm. The degenerated cells can be inferred based on the morphological changes: shrunken nuclei, vacuolation, and hyperchromatism. Chronic systemic exposure to rotenone in rats causes many features of PD, including nigrostriatal dopaminergic degeneration. Thus, exposure to rotenone in rats provides a valuable model for studying mechanisms of toxicant-induced dopaminergic cell injury.

### A109

#### **EFFECT OF THE ADMINISTRATION OF KETAMINE IN SUBANESTHETIC DOSES ON THE METABOLIC ACTIVITY OF THE NUCLEUS ACCUMBENS SEPTI AT 8 MINUTES AND 24 HOURS (PRELIMINARY STUDY)**

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The information available on the changes in brain metabolic activity under the effect of ketamine is very varied and contradictory. Some authors report an increase in brain metabolism and other studies report a decrease in said activity. In previous works we determined that the administration of ketamine in subanesthetic doses produces a decrease in mitochondrial metabolic activity as measured by the MTT (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) assay in spectrophotometer at 570 nm in the nucleus accumbens septi of male rats. This reduction occurs in the metabolism of mitochondrial oxide reduction. In the present study, we decided to investigate whether the decrease in nucleus accumbens septi metabolism in rats treated with ketamine occurs temporarily or permanently. The following experiment has CICUAL protocol approval (97/2017 and 128/2018). Male Holtzman strain rats weighing 240-290 g (n = 5 per dose) were used. The animals were divided into 2 groups. The first group received the drug and 8 minutes later they were sacrificed by decapitation. The nucleus accumbens septi was extracted and the MTT test was carried out. The second group was sacrificed at 24 hours and the MTT test was applied to amygdala. In each group, the doses used were administered intraperitoneally (1.25, 2.5, 5, 10, 15 and 20 mg/kg) and compared to saline control. Significance was studied using the ANOVA I test followed by the Student-Newman-Keuls post-test. In all cases a value of  $p < 0.05$  (two tails) was considered significant. A significant decrease was observed at 8 minutes with the doses of 5 mg/kg ( $p < 0.05$ ). No differences were observed with other doses. At 24 hours, no significant differences were found versus saline control. We conclude that the administration of ketamine produces a transient decrease in metabolic activity in the nucleus accumbens septi.

### A110

#### **ACUTE BEHAVIORAL EFFECTS OF D-PHENYL-ALANINE IN ANXIETY TEST**

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D-Phenyl-Alanine (DPA) has a recognized antidepressant action, and it has been shown in several clinical studies. Some patients refer a mild anxiogenic effect receiving it. Due to this reason we studied in the present experiment the possible anxiogenic effect of this drug. Male rats weighing 280-300 g from a Holtzman derived colony were used, divided in four groups. These rats were injected with saline or DPA (7.5, 15 and 30 mg/kg) 3 min. before testing. The test was the Plus-Maze test, in the conditions of our laboratory, with the classical parameters. Clear results were obtained with all doses. Time spent in the open arm was significantly decreased by all doses ( $p < 0.001$ ), and time spent in the closed arm increased ( $p < 0.001$ ). Time per entry was also reduced in a very significant manner ( $p < 0.001$  for 7.5 and 30 mg/kg,  $p < 0.01$  for 15 mg). Open arm entries were reduced by all doses ( $p < 0.001$ ), but closed arm entries were decreased only by the higher one ( $p < 0.05$ ). The extreme arrivals were decreased by all doses in the open arm ( $p < 0.001$ ) but they were not affected in the closed arm. Grooming did not show significant modifications. *Rearing* was increased by the middle dose ( $p < 0.05$ ) and decreased by the maximal dose ( $p < 0.05$ ). Expelled fecal boli were increased by the two higher doses ( $p < 0.05$ ). We may conclude that DPA has an effect compatible with an anxiogenic property.



## A111

### ESTIMATED GLOMERULAR FILTRATION USING THE SCHWARTZ EQUATION IN ADOLESCENTS WITH OVERWEIGHT AND OBESITY IN A VULNERABLE POPULATION IN PRIMARY CARE

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Obesity may increase the risk of kidney damage by diabetes mellitus, high blood pressure, and cardiovascular damage. It is a multifactorial disease that results from a number of factors including genetics, environment, diet, physical activity and a sedentary lifestyle. The aim of the study was to determine the estimated glomerular filtration rate (eGFR) in overweight and obese patients using the Schwartz formula, which uses  $k$  (0.55 in  $\leq 13$  years (a) and 0.70 in  $\geq 14$  years)  $\times$  height (cm)/serum creatinine (mg/dL). The laboratory service of Atilio Luchini Hospital, classified as level II complexity, conducted a retrospective descriptive analysis from March to June 2023. The study included 41 patients between 10 and 17 years (a) old. During adolescence, which the World Health Organization defines as the period between 10 and 19 years old, doctors requested analysis and conducted a survey on a sample of 41 people - 22 females (54%) and 19 males (46%). Consistency was maintained throughout the document by using specific terms, abbreviations, and symbols once they were introduced, and subject-specific vocabulary was used to convey precise meaning. The examination involved measuring height and weight to calculate the Body Mass Index (BMI = weight (kg) / [height (m)<sup>2</sup>]). Individuals with a BMI between 25-30 kg/m<sup>2</sup> were classified as overweight, and those with a BMI  $\geq 30$  kg/m<sup>2</sup> were classified as obese. The survey gathered information on the societal and cultural background of the participants. Creatinine levels were measured using the Jaffe kinetic method on a CM250 Wiener chemical autoanalyzer. Among the patients, 78% (n=32) consumed a diet primarily consisting of flour, whilst 90% (n=37) did not take part in extracurricular activities. In addition, 76% (n=31) of the parents had no history of chronic illnesses, and 80% (n=34) had informal employment; the remaining 20% (n=7) were unemployed. The educational qualifications of the parents are: incomplete primary education (n=14, 34%), incomplete secondary education (n=20, 49%), completed primary education (n=6, 14.5%) and completed secondary education (n=1, 2.5%). In terms of anthropometric characteristics, 63% (n=26) were classified as overweight and 37% (n=15) were classified as obese, with 7 individuals (n=7, 17%) estimated to possess a GFR. The study identified a subgroup of individuals with a Glomerular Filtration Rate (GFR) which ranged between 60-89 mL/min. In this subset, the average level of creatinine was  $0.94 \pm 0.12$  mg/dL, with a median GFR of 83. Furthermore, individuals with a GFR greater than or equal to 90 mL/min, comprising 34 (82.9%) of the study cohort, had an average creatinine level of  $0.80 \pm 0.11$  mg/dL and a median GFR of 104. In conclusion, the determination of creatinine clearance and the use of the estimation formula are essential to determine correlations. Therefore, efforts should be focused on modifying variables such as diet and physical activity to reverse the possibility of kidney damage and prevent chronic non-communicable diseases from occurring in the future.

## A112

### AN INTRACEREBROVENTRICULAR INJECTION OF AMYLOID BETA PEPTIDE MODIFIED TEMPORAL PATTERNS OF COGNITIVE FUNCTIONS, CREB, RAR $\alpha$ AND RXR $\beta$ EXPRESSION IN HIPPOCAMPUS RAT

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Alzheimer's dementia (AD) is a neurodegenerative disorder that causes memory loss and dementia in older adults. Memory and learning-related genes such as Creb (cAMP response element binding protein), Bdnf (Brain-derived neurotrophic factor) and its receptor TrkB, have been detected in the hippocampus. It is well known RARs and RXRs receptors function as transcription factors on retinoid-responsive target genes. In addition to memory loss, Alzheimer's patients have circadian rhythm dysfunction. Previously, we have demonstrated that an intracerebroventricular injection of A $\beta$ (1-42) modified the daily rhythms of cognition-related factors (Bdnf/TrkB) in the rat hippocampus. Continuing with that study, the objective of this work was to evaluate the consequences of an intracerebroventricular injection of A $\beta$  (1-42) on temporal patterns of cognitive performance, as well as on Creb, RAR $\alpha$  and RXR $\beta$  expression in the hippocampus of A $\beta$ -injected rats. Four-month-old males Holtzman rats were divided into two groups defined as: 1) control 2) A $\beta$ -injected. Rats were maintained under 12h-Light:12h-Dark conditions. The cognition ability was evaluated using object location test. Creb, RAR $\alpha$  and RXR $\beta$  transcript levels were determined by RT-PCR in hippocampus samples isolated every 6 hs. We found that an intracerebroventricular injection of A $\beta$  aggregates impaired learning and memory in rats and modified daily patterns of Creb, RAR $\alpha$  and RXR $\beta$  in the rat. Our findings showed that the increase of A $\beta$  altered temporal patterns of RAR $\alpha$  and RXR $\beta$ , and, consequently, induced alterations in daily rhythms of clock-related factors, affecting the cognitive performance of animals with Alzheimer's.



### A113

#### MILD HYPERTHYROIDISM REGULATES THE RESPONSE TO ACUTE STRESS IN ESTABLISHED LACTATION OF THE RAT

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Hyperthyroidism (HyperT) has an effect on women's reproductive health and can interfere with the establishment of lactation. This reproductive event is regulated in the CNS by neuroendocrine mechanisms present in brain areas such as the Medial Basal Hypothalamus (MBH). This region, contains a group of dopaminergic neurons responsible for regulating the secretion of prolactin (PRL). In previous studies, we found that HyperT affects the regulation of PRL secretion and circulating levels of glucocorticoids (Gs) during the transition between gestation and lactation. PRL has anxiolytic functions and its receptor is expressed in the hippocampus (HpC), an area of great interest in the regulation of the stress response. We evaluated the effect of mild HyperT on the response to acute stress by restraint in different reproductive states, focalizing in established lactation of the female rat. HyperT was induced with T<sub>4</sub> (0,1 mg/kg/day, *s.c.*) a dose that allows the maintenance of lactation. Control (Co) and HyperT female Wistar rats were bled from the tail vein during the first 5-10 and 60 minutes of restraint stress, M1 and M2 respectively. We analyzed serum PRL and corticosterone (CORT) levels on virgin rats in estrus, on day 19 of gestation (G19), on day 2 (L2) and 12 (L12) of lactation, in response to acute stress by essays RIA. To explore the neuroendocrine mechanisms of PRL release during lactation, we also determined by qPCR the expression of thyroid receptor (TR), the long isoform of the prolactin receptor (PRLr), members of the PRL signaling pathway (STAT5b, CIS and SOCS) and glucocorticoid receptor (GR) in HMB and HpC in virgin rats, G19, L2 y L12 in Co and HyperT groups. Virgin, G19, L2 and L12 Co and HyperT rats increased serum CORT release induced by restraint stress. Serum PRL levels increased in response to stress virgin Co and HyperT groups, while in G19, L2 and L12, PRL levels were not modified by stress ( $p < 0.05$ ). We also determined the effect of a previous restraint stress on the hormonal response to acute suckling, finding that stress increased CORT levels in both Co and HyperT rats and remained unchanged by suckling ( $p < 0.05$ ). Also, suckling-induced PRL secretion was partially blocked by stress only in HyperT rats. In MBH the gene expression was modulated by HyperT. We found increased levels of TR $\beta$ 1 in L12 and STAT5b, CIS, SOCS3 and GR in virgins while decreased CIS in L2 induced by HyperT. In HpC HyperT increased the gene expression of RT $\alpha$ 2, TR $\beta$ 2 and PRLr in the virgin group and STAT5b in G19, but decreased expression of RT $\beta$ 2 in G19 and PRLr in L2 ( $p < 0.05$ ). HyperT altered the expression of hormone receptors in MBH and HpC slightly affecting the hormonal release induced by suckling under acute stress conditions, causing complications in lactation and compromising litter survival.

## VETERINARY, ANIMAL ANATOMY, HISTOLOGY AND PHYSIOLOGY

### A114

#### COMPARISON OF DAILY GAIN IN PURE AND CROSS BREEDS POST WEANING LAMBS

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Córdoba province has 1.5% of the national sheep stock, is an agricultural livestock region of excellence and counts on resources to diversify animal production. 100% of sheep farming is oriented to meat production, either for self-consumption or direct marketing, being ovine production, considered in most cases a secondary activity. Election of meat breeds biotypes and crossbreeds constitute a significant technology in heavy lambs' dairy farming in other countries, allowing the use of the genetic resource available in the establishment. The aim of this work is to evaluate the daily performance increases of pure and crossbred animals in our region. 30 post-weaning lambs were evaluated during 28 days of joint fattening at the FAV, UNRC "Pozo del Carril" experimental station, located in La Aguada, at 30 km distanced from Río Cuarto city. The groups were constituted by 10 animals of each biotype, Corriedale, Hampshire Down breeds and crossbreed F1 of Karakul with Corriedale. Lambs' weight were evaluated at the beginning of the trial and every 14 days. The diet was based on graniferous oats and alfalfa hay. ANCOVA statistical analysis was performed using Infostat software. The results obtained showed statistical significant differences ( $p < 0.05$ ), where the biotype (breed or crossbred) had an influence on the daily increase and the rise obtained in fattening. Hampshire Down, the meat breed, augmented 130 g/day, Corriedale, the dual-purpose biotype obtained 60 g/day and the crossbreeds achieved 210 g/day. We concluded that there was superiority in weight gain performance in crossbred lambs compared with the other biotypes, while in relation to productive orientation of pure animals, meat breeds showed a greater gain-than double aptitude lambs.

#### A115

### COMPARATIVE HISTOCHEMICAL STUDY OF NORADRENERGIC CHROMAFFIN CELLS AND IMMUNOHISTOCHEMICAL IDENTIFICATION OF SUSTENTACULAR CELLS IN THE ADRENAL MEDULLA OF VISCACHA (*Lagostomus maximus maximus*)

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The adrenal medulla is crucial for-species survival under variable environmental conditions. In most of them, medullary parenchyma is constituted by chromaffin, ganglion and sustentacular cells. Presence and distribution of chromaffin (adrenergic and noradrenergic) and GFAP-positive sustentacular cells, show variations between species. Objectives of the present work were to identify and compare adrenal medulla population of noradrenergic cells in two species (viscacha and rat) and to study GFAP expression in medullary sustentacular cells associated with the noradrenergic cells. In order to study GFAP immunohistochemical technique was performed using the rabbit polyclonal anti-GFAP antibody (AR020-5R; BioGenex). Viscacha pineal gland was used as a positive control because the GFAP expression in interstitial cells was previously reported. Concerning the histochemical study, adrenal glands were fixed in a 5% glutaraldehyde solution for 24 h and modified Honoré technique was performed. Then, the samples were embedded in paraffin, sectioned and stained with Hematoxylin (H), Hematoxylin-Eosin (HE) and Toluidine Blue (TB). Rat adrenals were used as a positive and comparative histochemical control, because in this species the presence and distribution of noradrenergic cells were previously confirmed. The results in rat adrenal medulla demonstrated that noradrenergic cells, in the non-colored tissue, acquired a light brown or yellow hue organized in groups, like islets, between the adrenergic cells. In sections stained with H staining, noradrenergic cells showed a brown color; with HE an intense orange color and a green color with TB. On the contrary, in viscacha adrenal medulla none of the aforementioned stains revealed these cells localization, confirming noradrenergic chromaffin cells absence. Immunostaining for GFAP was negative in the gland. Our results support the hypothesis that the presence of GFAP-positive sustentacular cells subpopulation related to noradrenergic cells is species-specific. However, the biological and functional relationship between these two subpopulations of adrenomedullary cells has not yet been elucidated and will be a topic for future research.

#### A116

### ASSESSMENT OF PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) EXPRESSION IN SEMINIFEROUS TUBULES OF VISCACHA EXPOSED TO LITHIUM SALTS

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Lithium has been used for the treatment of bipolar and manic disorders. This drug has adverse effects in kidneys, thyroid gland, and gastrointestinal system. Previous studies carried out in viscacha (*Lagostomus maximus maximus*) demonstrated that Lithium salts affects the morphological organization of testicular tissue and sperm motility and viability. PCNA is a protein which plays an essential role in normal DNA replication. Thereby, it can be used as an indicator for the detection of cell division and proliferation. The decrease of PCNA expression in gonads, may indicate a reproductive dysfunction. The objective of the present work was to evaluate the effect of lithium chloride administration on the expression of PCNA in the seminiferous epithelium. Eight adult male viscachas were captured in their natural habitat during the period of maximal reproductive activity (summer to autumn) and the animals were divided into two groups: 1) Treated group received lithium chloride (Sigma) as intraperitoneal injections (1 mmol/kg b.wt./day for 35 days) while 2) the control group received sterile distilled water. Testicular samples were collected and processed for optical microscopy. For the study of PCNA, the immunohistochemical technique was performed using the mouse monoclonal anti-PCNA antibody (AM252-5M; BioGenex). Immunostaining was observed only in spermatogonia and primary spermatocytes and the relative percentage distribution of immunolabeled cells per seminiferous tubule section was determined. Relative Percentage Distribution of immunolabeled spermatogonia was 92.66% for the control group and 76.17% for the treated animals. In primary spermatocytes the Relative Percentage Distribution was similar between both animal groups (52.29% for the control group vs 50.99% in the treated group). These results showed a lower proliferative activity of germ cells, mainly spermatogonia, in viscachas under treatment with lithium chloride, which may indicate an alteration in the initial phases of spermatogenesis. Our results suggest that further studies are necessary in order to establish the degree of interference that the use of lithium salts for the treatment of mood disorders may have on reproduction.

## PHARMACOLOGY AND TOXICOLOGY

### A117

#### INHIBITORY EFFECT OF *Tripodanthus flagellaris* ON MICE GASTROINTESTINAL TRACT

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*Tripodanthus flagellaris* (Cham. & Schldt.) Tiegh. (Loranthaceae family) is popularly known as “liga blanca” or “liguilla”. It is used in folk medicine by their putative hypotensives properties, as well as abortive, emmenagogue and oxytocic. This study was aimed to investigate the effect of infusion 10% of *T. flagellaris* on small intestinal transit and castor oil induced diarrhea in mice. Infusion was prepared at 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.*, at concentrations: 0 (control group), 250 mg/kg, 500 mg/kg and 750 mg/kg, respectively. The proposed method used adult male mice with a body weight range from 25 to 30 g in a randomized manner and assigned into groups (n=6-8). All experiments were in compliance with the ANMAT N°. 6344/96 for animal care guidelines. Experimental protocols were approved by the Animal Care and Use Institutional Committee (ACUIC) of Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. Small intestinal transit: in control mice the charcoal meal traversed 69.14±3.69% of the total length of the small intestinal transit. Infusion at 250 mg/kg delayed gastrointestinal motility 51.28±3.84% (p<0.05), at 500 mg/kg: 50.72±2.86% (p<0.05) and 750 mg/kg: 36.53±5.84% (p<0.001), respectively. Pretreatment of mice with verapamil did not influence per se small intestinal transit but antagonized significantly the inhibitory effect of infusion at concentrations 750 mg/kg: 57.73±4.87%. On the contrary, the effect of infusion was not influenced by atropine (40.89±5.11%), cyproheptadine (33.77±8.31%), yohimbine (37.69±7.02%) or propranolol (50.09±3.14%). It is suggested that calcium channels mediate, at least in part, the effect of infusion in intestinal motility. All values were expressed as the mean ± SEM. Student's *t*-test was performed to evaluate the statistical differences between the control and the experimental samples. Castor oil induced diarrhea: three hours after castor oil administration all control mice produced copious diarrhea. Treatment with infusion of *T. flagellaris* at 250 mg/kg (p<0.05), 500 mg/kg (p<0.01) and 750 mg/kg (p<0.01) prevented diarrhea, respectively. Chi-square test was used. Thus, the antidiarrheal activity of *T. flagellaris* is possibly related to its inhibitory action against gastrointestinal motility. Phytochemical screening indicated the presence of flavonoids (quercetin, catechin, epicatechin and proanthocyanidins) among other compounds. These constituents may be responsible for the antidiarrheal activity.

### A118

#### EFFECT OF *Parasenegalia visco* IN SEROTONIN-INDUCED PAW EDEMA IN RATS

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*Acacia* is a genus that exhibits a wide range of bioactive agents with notable pharmacological properties, being effective as anti-inflammatory, antioxidant, antidiarrheal, antidiabetic, anticancer, antiviral, liver protectors, etc. Thus, the literature shows the phytochemical impact of the genus *Acacia* in medicine. In previous studies, we demonstrated the anti-inflammatory activity in chronic inflammation and gastric antiulcer activity of the ethanolic extract of *Acacia visco* leaves, called *Parasenegalia visco* (Lorentz ex Griseb.) Seigler & Ebinger since 2017. The objective of this study was to evaluate the effect of the ethanolic extract of *P visco* leaves in the model of acute inflammation, serotonin-induced paw edema in rats. We worked with female Wistar rats (175-200g); they were fasted for 24 h, with water *ad libitum* and divided into four groups of six animals each. Control group was administered 1 mL saline orally (v.o.), experimental groups (1 and 2), received 300 mg/kg of dry ethanolic extract of *P. visco* leaves (*PvEtL*) dissolved in saline orally (v.o.), and intraperitoneal route (i.p.), respectively. Diphenhydramine (DPH), 60 mg/kg was administered (i.p.) to the reference group. One hour later, all groups were injected in the subplantar region of the left hind paw with 0.1 mL/rat of 0.01% serotonin. Paw edema by difference between the left and right paw using a UGO BASILE plethysmometer at 30, 60 and 120 minutes was determined. The percentage of inhibition for each group with respect to the control group was calculated. Statistical analysis: ANOVA two-way, test with Dunnet post-test. Group 2 treated with *PvEtL* (i.p.) showed anti-inflammatory effect with an inhibition of 59, 66 and 54%, while (DPH) showed 43, 53 and 40% both at 30, 60 and 120 minutes, respectively. *PvEtL* (v.o.) was not significant activity compared to control. In conclusion, *P. visco* leaves extract (i.p.) showed a significant inhibition of acute inflammation compared to the control throughout the experiment with values similar to (DPH). Previous phytochemical studies demonstrated presence of Lupeol,  $\alpha$ -amyrin and  $\beta$ -amyrin isolated from *P. visco* leaves, they could be contributing to the anti-inflammatory effect in the acute inflammation induced with serotonin.

## A119

### HYPOGLYCEMIC ACTIVITY OF *Prosopanche americana* IN WISTAR RATS

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Diabetes mellitus is a health problem in the current world and it is characterized by a metabolic disorder that produces hyperglycemia resulting from defects in cellular insulin function, secretion, which affects millions of people every year. In recent years, interest has increased in using natural products for pharmacological purposes, as a form of complementary or replacement therapy. *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, 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 VI Ed Argentine National Pharmacopoeia and then lyophilized to preserve it. The objective of this study was to determine the effect of the *P. americana* rhizome lyophilized aqueous extract (PALE) on hyperglycemia induced by dexamethasone. The proposed method used male rats Wistar with a body weight range from 170 to 190 g in a randomized manner and assigned into groups (n=6-8). The experiment was in compliance with the ANMAT N° 6344/96 for animal care guidelines. Experimental protocols were approved by Animal Care and Use Institutional Committee (ACUIC) of Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. Diabetes was induced in rats by dexamethasone (10 mg/kg, b. wt., s. c.) for 11 consecutive days to all group animals (except control group). After confirmation of diabetes, the animals were divided into 5 groups: diabetic control treated with dexamethasone only, standard control treated with metformin (40 mg/kg, b. wt., p.o.) and three groups received dexamethasone plus PALE at graded doses of 125, 250 and 500 mg/kg, b. wt. p.o. for 21 days. There were a significant alteration in the serum glucose, total proteins and serum lipid profile parameters in dexamethasone-treated animals when compared to the normal control rats. The results indicated that the rhizome of PALE has shown the hypoglycemic effect at doses of 125 mg/kg:  $1.05 \pm 0.03$  g/L ( $p < 0.001$ ), 250 mg/kg:  $1.28 \pm 0.10$  g/L ( $p < 0.01$ ) y 500 mg/kg:  $1.08 \pm 0.06$  g/L ( $p < 0.001$ ) and treated with metformin rats  $1.35 \pm 0.06$  g/L when compared to the diabetic control rats ( $1.90 \pm 0.11$  g/L). The extract increased in the serum total protein levels (125 mg/kg:  $5.89 \pm 0.08$  g/dL, 250 mg/kg:  $5.73 \pm 0.19$  g/dL y 500 mg/kg:  $5.92 \pm 0.11$  g/dL,  $p < 0.001$ ) when compared to group dexamethasone treated ( $4.27 \pm 0.13$  g/dL). PALE increased serum concentration of HDL and decreased valours of LDL and triglyceride when compared with diabetic rats. The results indicated that the lyophilized extract of *Prosopanche americana* (R. Br.) Baillon has shown hypoglycemic activity.

## A120

### IN VIVO ANTIMETASTATIC EFFECT OF *Tessaria absinthioides* AND ITS COMBINATION WITH CHEMOTHERAPEUTIC DRUGS IN A BREAST CANCER XENOGRAFT MODEL

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In Argentina, according to the Global Cancer Observatory, breast cancer was the most prevalent in 2020, representing 16.8% of the new cases and being the most frequent in women (32.1%), with the highest mortality (19.95%). This reveals the need for new treatments with improved effectiveness and reduced side effects. Biotechnological strategies such as the application of plant-derived products with beneficial properties are being studied. Our previous results demonstrated the antimetastatic potential of the aqueous extract of *T. absinthioides* (AETa) in a murine melanoma model, showing its capability of inhibiting *in vitro* the metastatic processes of cell adhesion and migration and a significant reduction *in vivo* of lung metastasis. These results promote the study of AETa as a potential metastasis inhibitor in triple negative breast cancer, a highly metastatic cancer with poor prognosis. This work aims to investigate the antimetastatic effect of AETa and its combination with the chemotherapeutic agents carboplatin (CBP) and paclitaxel (PTX) in a breast cancer xenograft model. AETa was prepared by boiling 5 g of dry leaves in 100 mL of water (5% w/v) for 10 min. MDA-MB-231 cells ( $8 \times 10^5$  cells) were injected into the tail vein of 8-week-old female NOD scid gamma mice, that were randomly divided into 6 groups of 5 animals: control, AETa, CBP, PTX, AETa+CBP and AETa+PTX. AET was orally administered in drinking water at 15.6 mg/animal/day, whereas CBP and PTX were injected intraperitoneally at a single dose of 30 mg/kg and 5 mg/kg, respectively. Mice were sacrificed at week 10. Lungs were collected and the metastatic pulmonary nodules were counted using a binocular loupe. Results were expressed as a percentage of the control group. AETa significantly reduced the number of metastatic nodules by 22.2% vs the control group, showing a similar effect to PTX (19.8%) while CBP did not show a significant decrease (7.6%). The combination of AET with CBP reduced the amount of metastatic tumors by 37.5%, whereas AETa+PTX produced a decrease of 20.5% (ANOVA followed by Tukey's multiple comparisons test;  $p < 0.05$ ). This demonstrates that AETa reduces the metastatic potential of MDA-MB-231 cells with much greater effectiveness than CBP and in a comparable way to PTX. Besides, AET combined with CBP shows an increased antimetastatic activity than the individual treatments, suggesting a synergistic combination. In conclusion, this work represents a

valuable evidence of the antimetastatic potential of *T. absinthioides*, promoting its use in the development of new therapeutic strategies in the oncology field.

### A121

#### CYTOTOXIC EFFECT OF AQUEOUS DECOCTIONS OF *Tessaria absinthioides*, *Ilex paraguariensis*, *Camellia sinensis* AND ITS COMBINATIONS ON A MURINE MELANOMA CELL LINE

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Complementary medicine is being increasingly used in Occidental countries. This term refers to medical products and practices that are not part of standard medical care and it includes the use of natural products such as plant derivatives. Its most widespread practice consists in infusions, decoctions, pills and capsules of plant products. It has been previously demonstrated that aqueous decoctions of *T. absinthioides* (Ta) exhibit antitumoral and antimetastatic effects on murine melanoma. Besides, it was shown that phenolic compounds of Ta have a highly intestinal absorption rate by ex vivo intestinal permeability assay, with an absorption >90% at 120 min. These backgrounds promote the use of Ta in combination with yerba mate (*I. paraguariensis*, Ip) and green tea (*C. sinensis*, Cs), two widely diffused infusions in our country, to obtain nutraceuticals products with health benefits. The aim of this work was to study the cytotoxic activity of aqueous decoctions of Ta, Ip, Cs and its combinations at different fractions on murine melanoma B16F0 cell line. Decoctions were prepared by boiling 5 g of dry leaves in 100 mL of water (5% w/v) during 10 min. In order to quantify cell proliferation, the MTT assay was performed in a dose-response experimental design. Cells were treated for 72 h and then, the median effective dose (ED50) and combination index (CI) of the treatments were calculated by Compu Syn Software. CI values < 1 indicate synergism, CI = 1, additivism and CI > 1, antagonism. Ta, Ip and Cs decoctions inhibited B16F0 cell proliferation in a dose-dependent manner, with ED50 values of 1391.4, 315.1 and 655.8 µg/mL, respectively. The combination of Ta with Ip in 1:1, 2:1 and 4:1 ratios resulted in CI values of 1.99, 1.55 and 0.89 respectively, showing a synergistic effect only when they were merged in 4:1 ratio. This suggests that a major proportion of Ta than Ip benefits the interaction. In contrast, Ta+Cs combination in 1:1, 2:1 and 4:1 ratios, with CI values of 0.43, 0.86 and 0.93 respectively, exhibited a pronounced synergism when both decoctions were combined 1 to 1. These results indicate that Ta and Cs should be merged in equal proportions or using more quantity of Cs than Ta. The present work demonstrates that Ip and Cs aqueous decoctions inhibit murine melanoma cell proliferation and that their cytotoxic effect might be potentiated by the incorporation of Ta, promoting the *in vivo* study of Ta influence in the bioavailability of Ip and Cs. Finally, these results encourage the production of nutraceutical infusions of *I. paraguariensis* and *C. sinensis* with *T. absinthioides*.

### A122

#### PREPARATION AND CHARACTERIZATION OF POLYMERIC NANOPARTICLES AS A POTENTIAL TREATMENT FOR LUNG CANCER

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While mortality due to lung cancer continues to increase, advances in nanotechnology tend to focus on improving the delivery of drugs to tumour tissues and making diagnosis more effective in advanced stages. In the past decade, the importance of polymeric drug delivery systems in oncology has grown tremendously. Particularly, efforts were directed to drug release via nanoparticles (NPs), to enhance therapeutic effectiveness and reducing the adverse effects associated with the high doses used. The nanometric size of NPs means that they can interact better with biological systems. In this context, combined anti-cancer therapy promises to generate synergistic antitumor effects to maximize the treatment and overcome resistance. However, although NPs for biomedical applications can show clear benefits in the field of oncology, there are various important aspects to consider such as their cellular uptake, toxicity and immunogenicity. Furthermore, a complete study of the NPs biocompatibility in the body is necessary. The aims of this work were to design and characterize polymeric NPs for transporting combinations of cytotoxic drugs, and also to study their biocompatibility. NPs were obtained by electrospraying, nanoprecipitation and evaporation of solvent from emulsion. The polymers used were polycaprocate (PCL), polylactic acid (PLA), poly (lactic-coglycolic) acid (PLGA) and the PCL/PEG diblock. The samples obtained were characterized by scanning electron microscopy (SEM) and dynamic light scattering (DLS). The biocompatibility was studied by evaluating its ability to produce haemolysis, activate the coagulation cascade or activate the humoral (via complement) and cellular (producing IL-2) immune system. The electrospray methods with both PCL and PLA did not yield NPs but rather microparticles. Nanoprecipitation methods generated PCL/PEG NPs whose most important parameters in terms of size and surface charge were as expected. Particularly, they presented a bimodal distribution but with an acceptable polydispersity index, and thus they were satisfactory. With the biocompatibility study, it was demonstrated that even at concentrations higher than those that could be used in an *in vivo* treatment, the PCL/PEGNPs did not produce considerable haemolysis or affected the coagulation process. Also, they were not immunogenic since they did not activate complement by the alternative route or stimulated the production of IL-2 by blood lymphocytes,



indicating that the NPs are biocompatible and can be loaded with cytotoxic drugs. More pre-formulation and effectiveness studies are required to consider the PCL/PEG NPs as candidates for their use in the treatment of lung cancer.

## A123

### ABILITY OF *Lactobacillus* POSTBIOTICS TO COMBAT VIRULENCE FACTORS OF *Staphylococcus aureus*

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*Staphylococcus aureus* is a commensal bacterium that inhabits around 20% of the world's population, has a gram-positive morphotintorial characteristic and tends to cluster. Its risk arises from its nature as an opportunistic pathogen, which can cause infections in skin, soft tissues, blood vessels and internal organs. Crowding is associated with biofilm formation, a virulence mechanism capable of offering protection against external conditions such as unfavorable temperature and pH and lack of nutrients. The aim of this study was to evaluate the ability of postbiotics from 4 strains of Lactobacilli (*L. acidophilus*, *L. delbrueckii*, *L. johnsonii*, *L. plantarum*) to combat biofilm formation, virulence factor of *S. aureus*, bacteria capable of inhibiting or delaying the process healing of skin wounds, with consequent infection thereof. For antibiofilm activity, postbiotics were incubated pure and at minimal inhibitory concentrations (1/4 for all strains), using three approaches, namely, Precoated, which consists of administering and incubating the postbiotic, after which the compound is removed and the pathogen is added followed by new incubation, Co-incubated, which consists of simultaneous addition and incubation of the postbiotic and the pathogen, and Pre-formed, in which the pathogen is initially incubated and then the postbiotic is inserted. Analyzes were carried out by staining the remaining biofilm with Crystal Violet dye (0.5%) and reading in a spectrophotometer at 540 nm. The results obtained in the different approaches tested revealed the ability of the extracts to satisfactorily inhibit biofilm formation, especially *L. johnsonii* and *L. plantarum*, which obtained the best results in two strategies. Postbiotics were able to reduce, to different degrees, the formation of *S. aureus* biofilm. For the Co-incubated strategy, the best results were found for *L. johnsonii* (MIC) and *L. delbrueckii* (pure), for the Pre-formed strategy, *L. plantarum* in both concentrations and, for the Pre-coated approach, *L. johnsonii* showed the best results. The study of postbiotics is essential for the advancement of new treatments and adjuvant therapies, therefore, more research must be carried out to understand all the actions of these compounds.

## A124

### EVALUATION OF DIFFERENT LACTOBACILLUS LINES BIOTICS FOR COLLAGEN HYDROGELS SUPPLEMENTATION

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Hydrogels are generally polymeric biomaterials with a three-dimensional structure with high affinity for water. One of the most used materials for making hydrogels is collagen, given its high biocompatibility arising from its natural abundance in the human body, especially in the skin and cartilage. In order to increase the properties and benefits of this material, to be used in skin wounds, supplementation with certain compounds can bring added value to hydrogels. Among the possible supplements, probiotics (live microorganisms that, when administered in adequate concentrations, can confer benefits to the host's health), paraprobiotics (inactivated fractions of probiotics), and postbiotics (bioactive functional compounds produced by probiotics from metabolic processes). Considering the benefits associated with these compounds, such as the ability to increase healing in skin wounds and the ability to compete with pathogenic bacteria, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and to inhibit virulence mechanisms, administration associated with hydrogels could be beneficial for skin lesions. The aim of this study was to study factors associated with the functionality of biotics from 4 strains of lactobacilli (*L. acidophilus*, *L. delbrueckii*, *L. johnsonii*, *L. plantarum*) for application in hydrogels. All probiotic fractions showed the ability to reduce pathogen aggregation. The *L. delbrueckii* strain showed greater co-aggregation value against *S. aureus*, in the same way that *L. plantarum* showed greater co-aggregation against *P. aeruginosa*. For the other fractions, the postbiotics, were able to inhibit the growth of pathogenic bacteria while the paraprobiotic fraction did not show any degree of inhibition. The antioxidant activities found for postbiotics and for paraprobiotics showed similar results to those found for commercial vitamin C. All probiotics showed resistance to clindamycin, erythromycin, gentamicin and streptomycin; *L. acidophilus* and *L. johnsonii*, chloramphenicol and tetracycline; only *L. rhamnosus* was resistant to vancomycin and all were sensitive to ampicillin. None of the strains degraded mucin, and only *L. plantarum* promoted hemolysis, which was partial ( $\alpha$ -hemolysis). The study of probiotic bacteria and their different fractions is essential for the advancement of new treatments and adjuvant therapies and more studies must be carried out to determine their different mechanisms of action, as well as the advantages of using microorganisms or their fractions in



association with hydrogels to be used in the future in living beings. The results obtained so far allow us to infer that probiotics were able to reduce the aggregation of pathogens and the postbiotic fraction of all strains studied, at higher concentrations they were able to inhibit the growth of the pathogens analyzed. All studied fractions of *Lactobacillus* showed a potent antioxidant activity, especially the postbiotics, with activity considerably greater than that of one of the commercial brands that were analyzed.

## A125

### EFFECTS OF *Cannabis sativa* L. STEAM DISTILLATION OVER PHYTOCANNABINOIDS DECARBOXYLATION

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Phytocannabinoids (PCs) decarboxylation from *Cannabis sativa* L. to obtain the bioactive compounds THC and CBD is performed by exposing plant material to high temperatures (100-180°C). Steam distillation to obtain essential oils (EOs) and hidrosols is a process in which plant material is exposed to water steam temperatures. In this work, we studied THCA and CBDA decarboxylation rates of steam distilled cannabis flowers. EOs from dried flowers of *Cannabis sativa* were obtained by steam distillation during 40 minutes. After distillation, plant material was recovered and dried at room temperature and light protected during 1 week. PCs from distilled and non-distilled plant material were obtained by alcohol extraction, posterior vacuum evaporation at 45 °C and redissolution in sesame oil. In addition, plant material distilled and redissolutions of cannabis extracts in sesame oils were submitted to decarboxylation at 120°C during 45 minutes in order to compare with decarboxylation by steam distillation. Quantification of PCs Δ9-THC, CBD, CBN, THCA and CBDA were made in alcohol extracts from distilled and non-distilled plant material and extracts redissolved in sesame oils by HPLC-MS. Plant material had a EOs yield of 0.87 % and original plant material contained a ratio of THCA/CBDA=419 in alcohol extracts. THCA/Δ9-THC ratio in this alcoholic extract was 13.44 but in steam distilled plant material THCA/Δ9-THC decrease to 0.07 (94% decarboxylation). Distilled and decarboxylated plant material decreased further to 0.01 (99% decarboxylation). In sesame oil, steam distillation produced 95-99% THCA decarboxylation but in non-distilled plant material submitted to decarboxylation (120°C, 45 minutes) reached 36%. Results show that a steam distillation process is an effective means to achieve decarboxylation in which essential oils and hidrosols are obtained as added-value products.

## A126

### POTENTIAL NATRIURETIC EFFECT OF *Jungia polita* IN RATS

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*Jungia polita* Griseb. (Asteraceae-Multisicaceae) is popularly known as “zarzaparrilla” or “viña”. This shrub species is used in argentine folk medicine as diuretic, anti-sclerotic, hypotensive, and also for skin affections; antihyperlipidemic, bradycardic and depurative. Infusion (10%) of the aerial parts was prepared, separated by filtration and the aqueous extract was concentrated and lyophilized to preserve it. Orally administration of *Jungia polita* up to 2g/kg produced no mortality and visible signs of delayed toxicity 14 days post-treatment. This study was designed to determine the natriuretic activity of *Jungia polita* lyophilized extract (JPLE). The test was performed as described by Lipschitz *et al.* The experiments were approved by the local Committee CICUA (Protocol F-405/22). Wistar rats (150-180 g) were employed. The animals, randomly assigned into groups (n=6-8), were deprived of food for 18 hours prior to starting the experiments and had free access to water. The test groups were administered with different doses of JPLE (250 or 500 mg/kg, orally). Reference group received Furosemide (10 mg/kg, intraperitoneal). Control group received only the vehicle (50 ml/kg, orally). Immediately after administration, rats were paired and placed in metabolism cages. At the end of the experiments, the animals were euthanized by inhalation of carbon dioxide. Urinary volumetric excretion (UVE), urine chemical parameters, urine Na<sup>+</sup> and urine K<sup>+</sup> were measured in 3-hour diuresis. All values were expressed as the mean ± SEM. Graph Pad Prism was used for the statistical analysis and p values less than 0.05 were considered statistically significant. Student's *t*-test was performed to evaluate the differences between the control and the experimental samples. Lot treated with JPLE showed natriuretic activity between 250 mg/kg doses (urine Na<sup>+</sup> (mEq/L): 17.13±5.52 vs. control: 9.01± 2.65; p<0.05) and 500 mg/kg doses (urine Na<sup>+</sup> (mEq/L): 14.91±4.67 vs. control: 9.01±2.65; p<0.05). The urinary K<sup>+</sup> showed significant differences for the lot treated with 500 mg/kg JPLE [urine K<sup>+</sup> (mEq/L): 66.79±9.25 vs. control: 50.30±10.23; p<0.05]. Non-significant differences were observed with 250 mg/kg JPLE [urine K<sup>+</sup> (mEq/L): 51.09±24.45 vs. control: 50.30± 10.23; p>0.05]. The lot treated with JPLE (500 mg/kg) showed diuretic activity between 45 min (UVE: 20.06±8.04 vs. control: 4.84± 1.69; p<0.01) and 180 min (UVE: 82.95±8.92 vs. control: 52.74±6.03; p<0.001). Urine samples presented normal chemical parameters in all the cases: urinary density and pH were similar to controls. The data reported in this work indicate that the infusion of *J. polita* showed diuretic activity (0.59), compared to furosemide, a loop diuretic potent. Further investigations are necessary prior to their recommendation for use as natriuretic.

A127

**CADMIUM EFFECTS ON FOOD INTAKE AND GROWTH OF *Rhinella arenarum* TADPOLES**

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Aquatic environments are daily contaminated with heavy metals such as cadmium (Cd) from atmospheric depositions, geologic processes or through anthropogenic activities from various sources, mainly industrial and agricultural activities. Cd is a nonessential, highly toxic metal with high persistence, low rate of excretion and a robust bioaccumulation of many aquatic species. Unfortunately, Cd has been detected in water and sediments from the Embalse La Florida, an artificial lake of San Luis province, Argentina. This metal was also detected in adult anuran amphibians collected from the shores of the Embalse. Amphibian life cycle consists of a gelatinous egg, deposited in the water, an aquatic larval stage, and a terrestrial adult form following metamorphosis. 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. However, relatively few studies have experimentally examined the effects of aquatic reservoirs Cd contamination on *Rhinella arenarum* (sapo argentino) physiology. This study aimed to evaluate whether sub lethal concentrations of Cd may affects physiological aspects such alimentation and growth of *R. arenarum* tadpole under toxicological habitat. We performed an alimentation assay in order to standardize and determine the daily-consumed amount of food in each tadpoles group. In anurans, tadpoles are fishlike when they hatch. Anuran larvae are microphagous and thus feed largely on alga and blended plants. We collected amphibian larval stages (IV-V according to Martin et al.) of *R. arenarum* in the month of November in San Luis city. Fifteen specimens were exposed in triplicate to different concentrations (0.5 and 0.25 mg/L) of Cd for a period of 15 days. We used equal amount of blanched spinach and removed the left over every day and feed it again with a new piece of blended spinach. We did a qualitative and quantitative analysis of the amount of spinach that each group consumed in 24hs at the 7<sup>th</sup> and 14<sup>th</sup> day of experiment. After a week, we observed that Cd increases the amount of aliment ingested. The quantification of the remnants confirms that observation. Interestingly, this effect ceased after two weeks treatment. Interestingly, we found that the weight of Cd treated tadpoles were significant superior than controls at the end of the experiment. We also established that both concentrations of Cd significant increases the length and the size of the bodies of *R. arenarum*, and these alterations are in a dose dependent manner. In summary, the findings of the present study clearly demonstrate that Cd affects the fitness of *R. arenarum* tadpoles, which may translate into future negative population consequences. The relative sensitivity of tadpoles to Cd as compared to the adult forms reiterate the fact that the aquatic ecosystems are more vulnerable to Cd contamination and should be protected.

**NUTRITION AND HEALTH**

A128

**EFFECT OF DIETARY SUPPLEMENTATION WITH DEHYDRATED OLIVE OIL WASTE ON THE FATTY ACID PROFILE OF RABBIT MEAT**

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The olive industry finds significant growth in the La Rioja province where olive oil is produced in massive quantities, in a short time. The olive oil processing chain generates a significant quantity of wastes generally known as olive by-products. This industry production also has an undesirable aspect by the environmental effects associated with accumulation of by-products generated. The use of by-products as part of the rabbit diet can be a very effective way of developing a more sustainable production system. Besides, compared to other meat sources, rabbit meat has a low amount of fat and cholesterol and a high content of polyunsaturated fatty acids (PUFA), which makes it a natural functional food. Considering that the olive oil by-products are a low-cost way of improving the fatty acids profile of milk fat, the aim of this study was to evaluate the effect of the incorporation in the diet of rabbit different levels of dehydrate olive oil waste on fatty acid profile of meat. Thirty-two rabbits of the French Hyplus hybrid breed, weaned at 25 days of age, housed in individual cages, were used. The rabbits were randomly distributed at a rate of 8 animals per treatment, a completely randomized block experimental design (blocking based on weight) with 4 treatments and eight (8) repetitions was obtained. The treatments were: T1= 100 % commercial balanced feed (BC); T2= 97,5% BC + 2,5% dehydrate olive oil waste (DOW); T3= 95% BC + 5% DOW y T4= 90% BC + 10% DOW. Experimental diets were offered ad libitum during

10 weeks until the slaughter. After dissection, fresh rabbit meat was obtained for analysis. The *longissimus dorsi* muscle were excised from the two sides of carcasses and subsequently, was used for fatty acids profile evaluation by gas chromatography. The average amount of each fatty acid was used to calculate the sum of the total saturated (SFA), total monounsaturated (MUFA) and total polyunsaturated (PUFA) fatty acids. The inclusion of DOW in the diet not vary significantly ( $p < 0.05$ ) the fatty acid composition. The concentration of PUFA improving when 2.5% of dehydrate olive oil wast was including in the diet, but this difference was not significative ( $T_2 = 36.92\%$  vs  $T_1 = 31.66\%$ ). In this sense, the increase in PUFA observed in  $T_2$  it's mostly due to higher of C18:2 n-6 (linoleic acid) and C18:3 n-3 (linolenic acid) contents. Results suggest that DOW did not negatively affect the fatty acids profile of rabbit meat. Further research is needed to identify the optimum conditions necessary to obtain enrichment of both linoleic and linolenic acid in the rabbit meat results at lower costs.

## A129

### INFLUENCE OF SOLUBLE FIBER COMBINATION IN THERMAL PROPERTIES OF SUGAR-FREE AND FAT-REDUCED WHITE CHOCOLATE

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Fiber presents different technological properties that can improve the characteristics of food products for diabetic people. The fiber addition improves the textural and sensory characteristics and shelf lifetime of food products due to their capacity as fat mimetics. Besides, consuming fiber-enriched food prevents diseases like obesity and diabetes. The structure of chocolate consists of a fat matrix with a polycrystalline system with sugar and milk particles homogeneously distributed. The fat crystallization and the morphology of the crystals determine the chocolate's ability to form three-dimensional networks and the rheology properties. This behavior depends on fat polymorphism, which determines the product properties and shelf-life. Cocoa butter presents different polymorphic forms with characteristic fusion and crystallization temperatures. This work determined the thermic properties of sugar-free and fat-reduced white chocolate developed against a control sample, to evaluate the mimetic fat properties of the fibers used as a sugar and fat replacements. Therefore, the white chocolate thermic properties formulated with soluble fiber combinations (Inulin and Maltodextrin) as fat replacers were studied. The 35% of fat was replaced by: i) 100% with inulin (100%I), ii) 100% with maltodextrin (100%M), iii) 50% with inulin and 50% with maltodextrin (50%I+50%M). Free-sugar and fat-reduced white chocolate samples were analyzed against a control sample (C) without a fat replacement with a study of Differential Scanning Calorimetry (DSC). The crystallization behavior was studied. The graphics shows the sample crystallization polymorphism identifying three peaks that correspond with three polymorphic forms: Polymorphism I (⊙), polymorphism II (⊘), polymorphism III (⊙<sup>+</sup>). The onset temperature ( $T_o$ ) and peak temperature ( $T_p$ ) of crystallization were determined. The results show that fat replacement by 100%M (⊙ =  $-12.18 \pm 0.12$  °C; ⊘ =  $-2.81 \pm 0.08$  °C; ⊙<sup>+</sup> =  $12.42 \pm 0.18$  °C) and 100%I (⊙ =  $-11.35 \pm 0.41$  °C; ⊘ =  $-2.84 \pm 0.16$  °C; ⊙<sup>+</sup> =  $12.17 \pm 0.02$  °C) generated a statistically significant reduction of  $T_o$  when compared to the control sample (⊙ =  $-10.87 \pm 0.55$  °C; ⊘ =  $-1.08 \pm 0.28$  °C; ⊙<sup>+</sup> =  $13.29 \pm 0.09$  °C), ( $P < 0.05$ ). A similar behavior was observed for  $T_p$  when compared the samples 100%M (⊙ =  $-18.33 \pm 0.56$  °C; ⊘ =  $-5.42 \pm 0.29$  °C; ⊙<sup>+</sup> =  $4.9 \pm 0.23$  °C) and 100%I (⊙ =  $-18.55 \pm 0.24$  °C; ⊘ =  $-5.42 \pm 0.43$  °C; ⊙<sup>+</sup> =  $4.88 \pm 0.43$  °C) with the control sample (⊙ =  $-17.58 \pm 0.64$  °C; ⊘ =  $-3.69 \pm 0.15$  °C; ⊙<sup>+</sup> =  $7.07 \pm 0.21$  °C), ( $P < 0.05$ ). A synergic effect was observed in the sample with the combination of inulin and maltodextrin (50%I+50%M) obtaining a significant increase in the temperatures  $T_o$  (⊙ =  $-10.07 \pm 0.12$  °C; ⊘ =  $-0.89 \pm 0.20$  °C; ⊙<sup>+</sup> =  $12.91 \pm 0.23$  °C) and  $T_p$  (⊙ =  $-17.31 \pm 0.02$  °C; ⊘ =  $-3.41 \pm 0.66$  °C; ⊙<sup>+</sup> =  $6.16 \pm 0.46$  °C) with respect the samples 100%M and 100% I, this sample did not present a significant difference with the control sample ( $P > 0.05$ ). Therefore, it was possible to mimic the thermic properties of the crystallization process of cacao butter in a matrix of free-sugar fat-reduced white chocolate from the combination of the soluble fiber's inulin and maltodextrin.

## A130

### *Citrullus lanatus* SUBSP. *lanatus*. ANTIOXIDANT AN ANTINUTRIENT ACTIVITY

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The concept of food and nutrition is currently changing. In fact, besides their nutritional and sensory properties, the role of certain foods as health protective agents is being recognized. Although the composition of foods indicates their nutritional value, this information is not nutritionally sufficient, since the nutrients are not fully available to the body after ingestion. Plant materials, as fruits contain bioactive compounds such as antinutrients and antioxidants, which have some influence in diet and human health.

Antioxidants in fruits and vegetables is related with a reduced risk of certain chronic disorders such as coronary disease and some forms of cancer. This is attributed to the fact that these foods provide an optimum combination of natural antioxidants such as polyphenols, flavonoids and antioxidant micronutrients: vitamin C, vitamin E, selenium, zinc, copper, iron and manganese among others. These components collaborate with defense system against free radicals and exert their protective activity through of different mechanisms. A large number of beneficial components are currently being reported for fruit and vegetable foods, and their consumption is being valued and recommended. Antinutrients may act in preventing the availability of certain nutrients, such as minerals and proteins, having a negative effect such as harmful to the organism, altering digestion, absorption and metabolism. They can be naturally present in raw and inadequately processed foods, like nitrates, phytates, trypsin inhibitors, oxalic acid, saponins and others.

*Citrullus lanatus* commonly known as sandilleja, wild watermelon, chayote, cayota or citron, is a creeping plant native from Africa and is an important weed in the central region of Argentina. It has a large fruit, with an approximate size of between 20 to 50 cm long and a 10 to 20 cm diameter. Immature fruit rind is dark green alternated with light green spots, but mature fruit rind completely turns pale yellow. The flesh of the sandilleja is white yellowish, with olive green seeds. This work aims to determine antioxidants and some antinutrients constituents from whole *Citrullus lanatus* fruit, in order to deepen knowledge this wild fruit from San Luis range, promoting his inclusion in the Argentine diet, verifying the nutraceutical potential being a possible innocuous vegetal source as far as contributing to wild fruits revaluation.

We carried out the study of bioactive compounds such as vitamin C, total polyphenols, flavonoids. In addition, were determined the mineral micronutrients content associated with antioxidant defense such as Mn, Zn, Cu and Se using Official food analysis methods, as well as other methodologies previously optimized and validated for this type of matrices, which include UV Visible, UV spectrophotometry techniques, and Atomic Absorption Spectroscopy, among others. Antinutrients constituents such as nitrates, saponins by foam index (FI) were determined by Cataldo, WHO/PHARM/92559 (1992) respectively. Ripe fruits of *Citrullus lanatus* were collected in the capital of San Luis province. Immediately, these whole fruits were washed, samples were dried, labeled and stored for determinations. All analysis were carried out in triplicate. The fruit analyzed showed: 319 mg ácido gálico /100g of total polyphenols, 56 mg QE/ 100 g of flavonoids, 90 mg /100 g of vitamin C, respectively. They also have an important amount of Mn (8,2), Cu (6,3), Zn (3,6) and Se (8,7) mg/100g, which was the greater value. Quantities obtained allow to wind up that this fruit has higher antioxidant metal values than most conventional fruits. Consequently, these fruits can be considered a very good source of bioactive components making their antioxidant composition comparable with other fruits considered as good antioxidants. Nitrate concentration was 0.71 mg/100g and saponins < 100 foam index (FI). These contents were within the non-critical values and was within the range for edible fruits. Compounds considered toxic, such as alkaloids and cyanogenic glycosides, were not detected. This work serves as a foundation information on which *Citrullus lanatus* safe raw material could be used in the development of new products in nutraceutical industry, permitting no commercial underutilized fruits revaluation in our country.

### A131

#### PROTEIN EXTRACTION OPTIMIZATION FROM HAIRLESS CANARY SEED (CDC MARIA)

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Food products demand is increasing worldwide due to the exponential population rise. Furthermore, covering the nutritional needs of a growing global population and their protein intake represents a challenge for food technology. Therefore, the development of ingredients innovative sources is essential to cover this demand. The hairless canary seed (CDC María) has a high protein content of approximately 20% (w/w) and Argentina is one of these seeds largest producers worldwide. Hairless canary seed has proteins with a relatively complete amino acid profile containing essential amino acids like tryptophan, cysteine and phenylalanine. Current trends in extraction technologies have been focused on finding alternatives to traditional procedures and minimizing the use of strong acids, bases, and solvents. This study aimed to investigate protein extraction from hairless canary seeds (CDC María) using safe compounds with green and environmental-friendly technologies. Therefore, the seeds were disinfected with a 3% (v/v) hydrogen peroxide solution for 30 minutes. Subsequently, disinfected seeds were rinsed and immersed in sterile water at  $23 \pm 1$  °C for 12 hours to soften the cell walls and facilitate the enzyme extraction process through milling. Afterward, seeds were ground with various buffer solutions at different concentrations: sodium bicarbonate, sodium citrate and potassium citrate at 0.05 M, 0.1 M, 0.15 M and 0.2 M. A 1M citric acid solution was used to adjust the buffer system pH and was mixed at room temperature during 15 min. The Kjeldahl method was used to measure protein content for each tested buffer solution and was expressed as the protein extraction percentage to the total content in the seeds. The Statistical analysis was performed with Graph Pad In Stat software. Results obtained showed that the concentration increase of sodium citrate buffer from 0.05 M to 0.2 M statically improved the protein extraction from  $62.8 \pm 2.9$  % to  $87.9 \pm 4.1$  % concerning the total protein seed content ( $P < 0.01$ ). Buffer solutions of potassium citrate at pH 7.5 presented a similar behavior, reaching a maximum value of  $75.3 \pm 3.8$  % for 0.15 and 0.2 M concentrations. Buffer concentration decreasing of sodium bicarbonate allowed the highest extraction values, obtaining  $90.2 \pm 3.6$  % and  $100 \pm 3.9$  % concerning the total protein seed content for 0.05M and 0.1 M, respectively. No studies have been found that use a system conformed by the sodium bicarbonate buffer at pH 7.5 adjusted with a citric acid solution as a protein extraction medium in seeds. Using potassium citrate buffer at pH 7.5 allowed a high protein extraction percentage from hairless canary seeds, avoiding the harmful consequences of dangerous chemical residuals. The proteins extracted can be used to develop gluten-free food formulations.

### A132

#### POTENTIAL ANTIOXIDANT ACTIVITY OF AMARYLLIDACEAE SPECIES FROM PERU

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Plants produce secondary metabolites as defense compounds to protect themselves against abiotic (e.g., UV irradiation and desiccation) and biotic factors (herbivory and phytopathogenic microbial infections), as attractants for pollinators and seed-dispersing animals, as well as agents in plant-plant competition and plant-microbe symbiosis. The Amaryllidoideae subfamily of the Amaryllidaceae belongs to the order Asparagales, and consists of 14 tribes and about 70 genera. This family is widely distributed in different regions of the neotropics and temperate areas around the world. In Peru, 15 genera and 68 species belonging to the Amaryllidoideae have been reported in different types of forest, ranging from wet montane to dry, as well as the sandy biomes of the Pacific coastal region, with the greatest diversity in the south. The aim of this work was to determine the potential antioxidant of five species from Peru, through quantification of total phenolic and flavonoid content. In autumn 2023, bulbs of *Hieronymiella* spp., *Pyrolirion albicans* from Moquegua (Peru), and *E. coccinea*, *E. darwinii*, *E. kawidei* from the localities Cusco and Apurimac (Peru), were collected. From the dried bulbs, the methanolic extracts (MeOHE) were extracted by maceration at room temperature during 48 h, and concentrated under reduced pressure, and the percentage of yield (w/w) in terms of dry starting material was calculated. The total phenolic content (TFC) was determined by the Folin-Ciocalteu technique using a calibration curve with gallic acid, and the total flavonoid (FC) content was estimated by the aluminium chloride method with quercetin as standard both according to Luna *et al.* (2013). The yield values were *Hieronymiella* spp 7.01%, *P. albicans* 0.52%, *E. coccinea* 11.16%, *E. darwinii* 7.05%, and *E. kawidei* 2.51%. In *E. coccinea* and *E. darwinii*, CFTs were  $11.44 \pm 1.08$  and  $5.62 \pm 0.87$  mg EAG/100 g dry bulb) respectively, and were significantly different from *E. kawidei* ( $2.69 \pm 0.87$ ). *E. coccinea* and *P. albicans* showed the highest content of FC ( $2.02 \pm 0.02$  mg and  $1.75 \pm 0.71$  mg EQ/100 g of dry bulb, respectively). This is the first report regarding other metabolites such as phenolics and flavonoids, showing these compounds importance as responsible of the potential antioxidant properties in Amaryllidaceae species. Acknowledgement: Llalla-Cordova O. and Ortiz J.E. hold CONICET scholarships. PICT03883 ANPCYT, PIP2022-2024-0902 (CONICET)

### A133

#### BIOACTIVE COMPOUNDS OF *Eustephia aff. coccinea* FROM CUSCO, PERU

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A large number of modern medicinal drugs are obtained from native plants as a source of bioactive molecules. The Amaryllidaceae family is a large group of 1100 species divided among 75 genera, which are used for floricultural-ornamental and medicinal purposes. Their chemical composition has attracted research interest due to their wide range of biological and pharmacological activities. In Peru, 15 genera and 68 species belonging to the Amaryllidoideae have been reported in different types of forest, ranging from wet montane to dry, as well as the sandy biomes of the Pacific coastal region, with the greatest diversity in the south. *Eustephia aff. coccinea* is bulbous plant of the Amaryllidoideae subfamily belong to the Amaryllidaceae family and is used to treat inflammation, bacterial and fungal infections. The aim of this study was to identify the bioactive compounds present in the *E. aff. coccinea* alkaloid extract by UPLC-MSMS and GC-MS analysis. This species was collected in the localities Tinta en Canchis, Cusco (Peru). Dry bulbs were chopped, dried, and subsequently subjected to the alkaloids extraction. In the case of GC-MS, alkaloid identification was performed using DB5-MS column and AMDIS 2.65 program with a private library of 300 alkaloids. For UPLC-MS, Acquity BEH C18 column, 1.7  $\mu$ m, 2.1 mm x 100 mm and MassLynx v4.2 (ChromaLynx) program with a private library of 40 alkaloids were used. The proportion of each alkaloid present in the extract was established according to the total ion current (TIC) showing sternbergine (34.3%) and haemanthamine (11.7%) as the most abundant identified alkaloids. Vittatine, 11,12-dehydroanhydrolycorine, tazettine, pseudolycorine, and hippeastrine were also identified with relative proportions of 0.5, 0.5, 3.3, 1, and 0.8% of the TIC. It is known that haemanthamine displays significant *in vitro* cytotoxic activity against several types of cancer cell lines, including MoltT-4, HepG2, HeLa, MCF7, CEM, K562, A549, Caco-2, HT-29, A2780, SW1573, and T47-D, whereas pseudolycorine showed antiviral effects on several RNA and DNA-containing viruses. To the best of our knowledge, there are no biological activities reported for sternbergine but, considering the structure similarities with pseudolycorine, it is probable that this alkaloid also exerts antiviral activity. Finally, *E. aff. coccinea* represents a valuable source of bioactive alkaloids with cytotoxic and antiviral activities. Acknowledgments: O.LI-C and JEO hold CONICET scholarships. PICT 03883 ANPCYT, PIP2022-2024-0902 (CONICET). CYTED Red 223RT0140.

### A134

#### IMPACT OF PHYTASE-RICH DIETARY SUPPLEMENT ON FEMORAL MORPHOLOGICAL FEATURES

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Phytases are enzymes that have the ability to hydrolyse phytates. Phytases are present in fungi, yeasts, and bacteria that hydrolyses phytic acid by releasing phosphate ions in solution. Phytates are the primary form of phosphorus storage, but they can also hinder the absorption of minerals, including humans and other monogastric, such as birds, pigs, and rodents. These active enzymes are used as additives to improve the

bioavailability of essential minerals in feeds, thereby releasing phosphorus and other minerals that would otherwise be inaccessible for absorption. It has been shown that a phytase supplement can influence the microscopic structure of bone, promoting greater mineralization and stronger bone architecture. In addition, the femur is the longest and strongest bone in the human body and its morphology can be influenced by various factors, including diet and nutritional supplements. Some studies show that phytase is an enzyme that improves the absorption of phosphorus in the gut, and its supplementation in the diet may have effects on femur morphology. This study aimed to evaluate the effect of phytase supplementation in Wistar rats through general morphological and biochemical characteristics and structural changes of the femur. Three generations of Wistar rats were used for this purpose (F<sub>0</sub>, F<sub>1</sub> and F<sub>2</sub>). Each generation with an N= 15 (males and females), separated into four groups: control females (HC) and control males (MC) fed normal diet (DN) and phytase females (HF) and phytase males (MF) fed DN+phytase. The extraction of the enzyme from yeasts (*Saccharomyces cerevisiae*) was performed at the end of the fermentation process in brewing. ANOVA analysis was performed. Female (229.6 g +/-12.05 g vs 209+/-17.09) and male (399.5 +/-6.025 g vs 366+/-6.57) F<sub>0</sub> rats fed phytase had lower body weight (p<0.05) compared to control rats. When determining phosphate in urine, F<sub>1</sub> rats treated with phytase had significantly higher phosphate value (5.23+/- 0.1 mg/L than control 4.79 +/- 0.1 mg/L, p<0.05). No significant differences were found in blood glucose, and in general the haematological parameters did not show significant differences. F<sub>3</sub> 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<sub>3</sub> 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). In summary, dietary supplement rich in phytase can improve the metabolism of phosphorus in bone, by breaking down phytates, and consequently, increase bone mineral density, however phytase fed rats showed a decline in their body weights.

### A135

#### **RECOVERY AND PROCESSING OF CARROT LEAVES AND STEMS TO REDUCE THE BITTER TASTE FOR ITS USE AS A FOOD INGREDIENT**

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Byproducts from vegetable processing, like leaves and stems, represent a negative environmental impact and a nutrient-rich biomass loss. Recent studies show that vegetable leaves like carrots present 21% of proteins and 25% of fiber in dried matter. However, the carrot leaves and stems have anti-nutrient compounds and bitter tastes that limit their use. Therefore, carrot leaves and stems recovery and processing is of great interest for a food ingredient extraction with a high nutritional quality, adding an aggregate value to a product that is usually wasted. In this work, the fresh carrot leaves were stored and refrigerated (4.5 °C). The leaves and stems were cutted. An hydrothermic pretreatment was performed using a combination of salts and carbohydrates to reduce and/or eliminate bitter tastes and inactivate anti-nutrient compounds. Therefore, blanching in boiling water was applied in a 5 to 40 min range with combinations of sodium chloride, calcium citrate, magnesium citrate and sucrose. The leaves and stems were strained to eliminate the water excess and dehydrated in a camera with hot and dried convective air at 65 °C. A sensory study was done to evaluate the bitter taste with a discrimination test where the treated samples were compared to a control sample without pretreatment. Statistical analysis was performed with Graph Pad In Stat software. The samples processed with combinations of sucrose at 3.5% (w/v) and potassium chloride (0.2%, w/v), magnesium citrate (0.1%, p/v) and calcium citrate (0.35%, p/v) during a blanching time of 15 min presented organoleptic characteristics improved. Discrimination studies detecting a statically significant reduction of the bitter taste compared to the control sample (P<0,001). This sample was dehydrated and grounded to obtain a powder and was stored in a hermetically sealed container. Subsequently, the utilization of this powder for a food product formulated to people who suffer desnutrition, was assessed. A high-protein powder sauce that can be accompanied with rice (risotto) was developed. This product was formulated with a combination of different vegetables and animal proteins: carrot leaves and stems powder without a bitter taste, bovine plasma, and cheese whey, among others. This item for consumption was analyzed by a sensory study, obtaining a high global acceptability of 90% from the evaluation panel. Therefore, in this work, it was possible to apply technology development to take advantage of wasted vegetable parts, like carrot leaves and stems, in order to obtain a food ingredient with suitable sensorial properties for their use in different food products development.

### A136

#### **DEVELOPMENT OF FAT-REDUCED MINCED MEAT FREE OF CHEMICAL ADDITIVES, SUITABLE FOR CELIAC INDIVIDUALS**

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Commercial minced meat presents a high-fat content and is considered an ultra-processed food with a low nutritional value and with excess of additives. Therefore, consuming high quantities of this product can generate health issues like cardiovascular disease, obesity and malnutrition.



In this work the development of a fat-reduced minced meat suitable for celiac people free of chemical additives was assayed. The preservative method employed for producing minced meat is sterilization at 125°C for 60 min. This process generated thermic stress that produces product liquid separation. In minced meat, the separation of fat and water after sterilization is avoided using a high quantity of chemistry additives. Therefore, the technological challenge was developing a free chemistry additive product without liquid separation after finishing the sterilization process. This work has studied the influence of incorporation of the following ingredients over liquid separation: i) rice flour at 0.05 % (w/w) and 0.15% (w/w), (high carbohydrate content, 80% w/w); ii) soy flour at 4 % (w/w) and 6 % (w/w), (high protein content, 42% w/w); iii) oat bran at 4 % (w/w), (high fiber content, 15% w/w). The capacity of these ingredients to bind the product water and avoid their separation after the thermic process was evaluated. The emulsion stability was determined as follows: 5 g of each sterilized sample was placed in a centrifuge tube and centrifuged at 3000 r/min for 30 min and the release exudate was measured. Emulsion stability, defined as total fluid release, was expressed as g/100 g of initial sample weight. The statistical analysis was performed with Graph Pad In Stat software. The results show that small increases in rice flour generated product destabilization with a significant increase in the liquid separation after the sterilization process (20 g/100 g of product,  $P<0.05$ ). Soy flour in the concentration range studied allowed avoid the visible liquid separation. This fact may be due to the protein denaturalization at high temperatures, which allowed obtaining gels with a high water binding capacity and stable structures at higher temperatures. However, at a concentration of 6% (w/w) soy flour, the product hardening was produced. The separation of liquid was reduced using a low concentration of soy flour (4%, w/w, 2.5 g/100 g of product,  $P<0.001$ ) without generating the product hardening. Nevertheless, adding soy flour at 4% (w/w) caused unpleasant flavors and aromas in the product. Using oat bran at 4% (w/w) allowed obtaining minced meat without liquid separation, an adequate texture, and a pleasant flavor and aroma. These results may be related to the high soluble fiber content in the oat bran, which could absorb high water quantities. Furthermore, previous studies demonstrated that this behavior could be related to a synergist effect between the proteins contained in the product and the fiber that generated an improved water-binding capacity of the protein gel. Therefore, this work developed fat-reduced minced meat suitable for celiac people free of chemical additives, without liquid separation, using minimally processed ingredients.

### A137

## CRYOPRESERVATION OF *Spirogyra* spp. OF SAN LUIS (ARGENTINA), USING DIFFERENT CARBOHYDRATES AS CRYOPROTECTIVE AGENTS

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Chlorophytes, a group of photosynthetic green algae, are non-toxic and dominant in hypertrophic shallow lakes and rivers. The algae conservation in food manufacturing by freezing the stock cultures that are used as starter cultures is essential to maintain the integrity of the plasma membrane and thus normal cell viability and functionality. Cryoprotective agents can improve the viability of frozen starter cultures by interacting with the lipid bilayer, preventing membrane destabilization by dehydration and ice crystal formation. This study aimed to investigate different molecular weight carbohydrates (fructose, sucrose and inulin) as cryoprotective agents for *Spirogyra* spp., a group of filamentous green algae belonging to the division Chlorophyta, at concentrations of 5%, 12% and 20% (w/v). The samples were prepared with a concentration of algae at 10% (w/v) in combination with the different cryoprotective agents in hermetically sealed containers of 120 ml. The samples were frozen and stored at -20 °C for 20 days and later were thawed by exposure to 4.5°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 plasma membrane contraction within 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 GraphPad InStat 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 not occupied inside the cell wall (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 50.12±5.08%. The samples with glucose at 12 and 20% (w/v) and sucrose at 12% (w/v) generated a contraction percentage or a loss of the spatial conformation statically inferior compared to the control sample ( $P<0.01$ ): 12% (w/v) glucose = 37.61±5.73%; 20% (w/v) glucose = 29.23±6.14% y 12% (w/v) sucrose = 38.26±4.79 %. Therefore, the cryoprotectants glucose and sucrose interact with the polar head groups of the lipid bilayer, maintaining suitable spacing between the lipids and preserving in a higher proportion the cell-membrane structural integrity. Inulin, a high molecular carbohydrate, produced a cell membrane contraction similar to the control sample (50.19±5.20 %), ( $P>0.05$ ). Hence, the ability of cryoprotectants to prevent the loss of the membrane structural integrity depends on their molecular weight and capacity to surround the membrane and interact with the polar groups of the lipid bilayer during the freezing process. Therefore, low-molecular-weight carbohydrates like fructose and sucrose allowed preserved the membrane-cell structural integrity.