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ASSOCIATION TUNISIENNE DES SCIENCES BIOLOGIQUES
29EME CONGRES INTERNATIONAL DE L'ATSB. 26-29 MARS 2018. SOUSSE. TUNISIE

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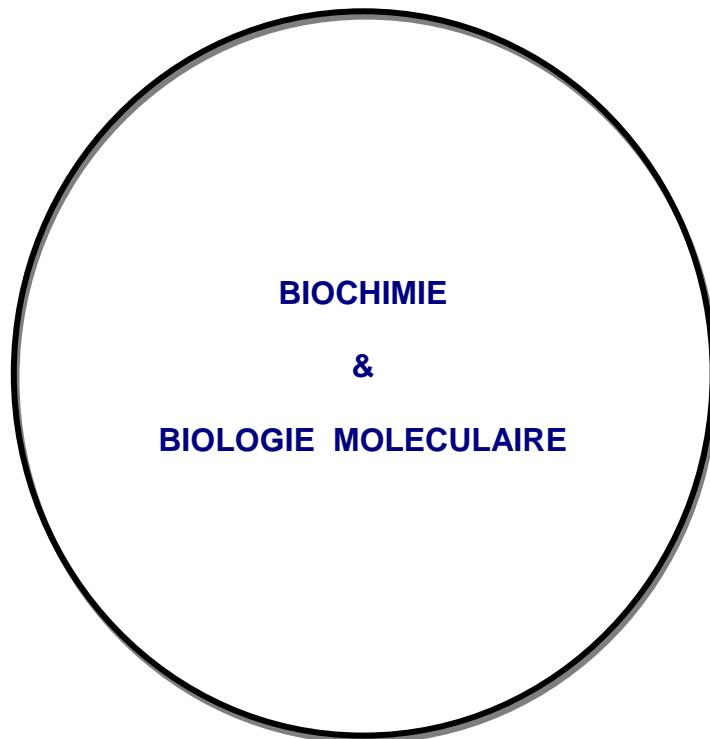


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**C. ORALE N°:1.****THE 6-AMINOCHOLESTANOL INHIBITS THE LEISHMANIA DEAD-BOX HELICASE EIF4A (LEIF) BY TARGETING THE RNA BINDING SITE****ABDELKRIM YOSSER ZINA^{1,2,3}, EMNA HARIGUA^{1,4}, JOSETTE BANROQUES², ARNAUD BLONDEL⁴, MICHAEL NILGES⁴, IKRAM GUIZANI¹ AND N. KYLE TANNER²**

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LeIF, the Leishmania eukaryotic initiation factor eIF4A, constitutes a potential drug target. The eIF4A proteins belong to the DEAD-box family of RNA helicases, which are ATP-dependent RNA-binding proteins and RNA-dependent ATPases. DEAD-box proteins contain a commonly-shared core structure consisting of two linked RecA-like domains that contain 11 conserved motifs that are involved in ligand binding and in enzymatic activities. When compared to the mammalian protein, LeIF presents extensive sequence divergence in its NH2 terminal domain and presents significant differences in its affinity for ATP and RNA. We previously used LeIF to identify novel leishmanicidal molecules by virtual and biochemical screenings targeting this protein. Among different compounds retained, one (6-aminocholestanol) was selected for further investigation. It is an effective inhibitor of the ATPase activity of translation-initiation factor eIF4A from mouse (eIF4AI) and the parasite Leishmania (LeIF) with different kinetic behavior. This molecule has potent selective effect on Leishmania viability with little toxicity towards host cells. Interestingly similar analogues inhibiting ATPase activity also affect Leishmania viability and infectivity while the inactive compounds do not have such effects. Here, we further characterize the effects of 6-aminocholestanol on LeIF enzymatic activities. The compound inhibits both the ATPase and helicase activities by perturbing RNA binding. We undertook kinetic analyses and found that the Leishmania LeIF protein binds 6-aminocholestanol with a higher apparent affinity than for ATP, although additional binding site(s) are probably involved. Competition experiments with the individual RecA-like domains indicate that the primary binding site is on RecA-like domain 1, probably within a cavity that we identified by molecular modeling of LeIF involving conserved RNA-binding motifs. We have extended comparisons between LeIF and eIF4AIMus, to assess their sensitivity to rocaglamide, a known molecule that targets eIF4A and interferes with the RNA binding. A different sensitivity was observed, the compound being more potent on the mouse protein. So, although both proteins are sensitive to these different compounds, we found important differences in the compounds effects on their enzymatic activities. In conclusion, 6-aminocholestanol interferes with RNA binding and presents significant differential effect on RNA helicase activity of LeIF. Our results also indicate that it is in principle possible to selectively target a conserved protein in relation to significant sequence divergence and functional properties. This is desirable for the selective treatment of parasitic infections such as Leishmaniasis.

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Keywords: 6-aminocholestanol, inhibitor, LeIF, ATPase, RNA binding site

C. ORALE N°:2.**EVALUATION DE LA NARINGINE COMME AGENT ANTI-CANCEREUX DANS LE TRAITEMENT DES TUMEURS CEREBRALES****AROUI SONIA¹, ANNIE-CLAIRE MEUNIER², ABDERRAOUFKENANI¹**

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Parmi les composés pharmacologiques inhibant la prolifération de cellules tumorales sans effets secondaires, une faible proportion présente un potentiel prometteur d'utilisation comme agent anti-cancéreux. En effet, plusieurs études récentes réalisées majoritairement *in vitro* ont montré que la naringine, la composante active du pamplemousse, présentait des propriétés chimio-préventives et anti-tumorales intéressantes. Nous avons donc évalué la naringine comme agent anti-cancéreux dans le traitement des gliomes *in vitro* et *in vivo*. Le potentiel d'utilisation de la naringine a d'abord été estimé sur une lignée de glioblastome en culture (U87) par des essais de prolifération. Nous avons pu insidérieurement déterminer la concentration qui inhibait 50% de la prolifération cellulaire (IC50), soit 40 µM pour ce composé. En plus, une inhibition de la migration, de l'invasion et de la tubulogénèse a été observée sur cette lignée de cellules cérébrales ayant été traitée avec la naringine. D'autant plus, une inhibition de l'activation des métalloprotéases de la matrice, des enzymes importantes pour la croissance tumorale, a été mesurée. L'efficacité anti-cancéreuse de la naringine a ensuite été démontrée *in vivo* en traitant des souris athymiques ayant subi des implantations sous-cutanées et intracrâniennes de U87. L'impact du traitement (60 mg/kg/jour) sur la croissance tumorale (taille des tumeurs) et la survie des animaux a été estimé. Les résultats ont montré que la naringine exerce un effet anti-cancéreux sur les tumeurs sous-cutanées, provoquant un ralentissement significatif de la croissance tumorale. De plus, un accroissement du temps de survie moyen a été observé chez les souris traitées ayant subi une implantation intracrânienne de cellules cancéreuses. Cette action anti-tumorale de la naringine confirmerait l'effet anti-angiogénique observé *in vitro*. De plus, le dosage du contenu en hémoglobine dans les tumeurs, qui permet de quantifier le niveau de prolifération des vaisseaux sanguins, a montré que les tumeurs sous-cutanées des souris ayant reçu de la naringine avaient une concentration d'hémoglobine moindre que les tumeurs provenant des souris non-traitées. Ces résultats suggèrent que la naringine possède des propriétés anti-cancéreuses intéressantes sur les glioblastomes et peut être considéré comme un agent dans la prévention et le traitement des tumeurs cérébrales.

Mots clés: naringine, gliome, anti-tumorale, anti-angiogénique



C. ORALE N°:3.

THE MAJOR RISK FACTORS FOR FETAL MACROSOMIA IN TUNISIA

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Key words: Macrosomia, maternal overweight, maternal age, diabetes, glycemia, male fetuses.

Fetal macrosomia corresponds to the delivery of a newborn whose weight is 4 kg or more. In Tunisia, its frequency is estimated to 11.29 % in the Center of Maternity and Neonatology of Tunis, service A (CMNT). The aim of our study is to determine the relationship between Tunisian mother's characteristics and macrosomic births. Our study involved 40 mothers of macrosomes and 40 term women pregnant of normal infants. Our survey revealed that overweight of mothers and their advanced age (over than 35 years) increase the probability of the birth of a macrosome, more common in male fetuses with a sex ratio of ¾.

Our study has also demonstrated that the presence of macrosomes (46.42%) in a family could be a ground that favours the outcome of new macrosomes. In another stage of our study, diverse biological analysis were performed for all selected patient, such as the glycemia value on the day of delivery. We have observed that blood glucose levels of macrosomes are significantly higher (5.31 ± 1.26 mmol/L, P-value = 0.01) than those of controls (4.72 ± 0.89 mmol/L). We have also found that previous family history of diabetes increases about 50% the prevalence of newborn macrosomes.

Thus, maternal characteristic such us overweight, advanced age, hyperglycemia are major risk factors in close relationship with fetal macrosomia.

C. ORALE N°:4.

METABOLIC SYNDROME AND CARDIOVASCULAR DISEASE IN HEMODIALYSIS PATIENTS

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Background: Cardiovascular complications were the major cause of morbidity and mortality in hemodialysis patients (HD). It was closely related with metabolic syndrome (MS) in general population. This study aimed to determine prevalence of MS in HD patients and to evaluate the relationship between this prevalence and cardiovascular disease (CVD) in HD patients.

Methods: A total of 200 participants were included in this study, 100 patients receiving regular hemodialysis and 100 healthy controls. Clinical data, lipid profile and atherogenic indices (cholesterol/HDL, triglycerides/HDL) were measured.

Results: Prevalence of MS in HD patients was 46%. Cardiovascular diseases present 43%. We found statistic significant difference between level of lipid profile and atherogenic index between the two groups of participants. For patients with MS and with CVD, a significant increased level was found between cholesterol (TC), triglycerides (TG) and atherogenic index. Patients with MS have an increased prevalence for CVD compared to patients without MS.

Conclusion: We showed a significant association between MS and CVD. The lipid profile and atherogenic index in HD patients were increased with presence of SM.

Mots clés : metabolic syndrome, cardiovascular disease, hemodialysis

**C. ORALE N°:5.****EFFECTS OF COLLAGENASE-1 (MMP-1) AND GELATINASE A (MMP-2) IN TUNISIAN OBESE SUBJECTS****BOUMIZA SOUMAYA^{1,2}, CHAHED K.², NOREL X.³, TABKA Z.², JACOB M.P.³***1 Université de Carthage, Faculty of Sciences of Bizerte, Departement of biology, .**2 Faculty of Medicine of Sousse, Department of Physiology and Functional Exploration, UR 12ES06,**3 INSERM U1148, CHU X. Bichat, 46 rue H. Huchard, F-75018 Paris, France.*

AIM: Our aim was to evaluate matrix metalloproteinases (MMP)-1, MMP-2, TIMP tissue inhibitor metalloproteinases)-1 and TIMP-2 in a group of obese and non-obese subjects.

METHODS: The plasma concentrations of MMP-1, MMP-2, TIMP-1 and TIMP-2 were determined by using specific ELISA kits in 185 obese subjects (BMI ≥ 30 Kg/m²), and 227 non-obese participants (18.5 \leq BMI < 25 Kg/m²). Allelic discrimination between variants MMP-2(-735C/T) and MMP-1 (519A/G) was performed by PCR-RFLP.

RESULTS : A significant increase was found in plasma concentration of MMP-1 in obese subjects versus non-obese subjects ($p < 0.05$). In contrast, a significant decrease of MMP-2 was found among obese. In addition, a positive correlation was showed between anthropometric parameters and MMP-1 [BMI ($r=0.24$, $p=0.002$); WC ($r=0.28$, $p<0.001$)]. In similar manner, a positive correlation was found between the index of activity of MMP-1 (MMP-1/TIMP-1 ratio) and WC ($r=0.18$, $p=0.01$) in obese group. In contrast, a negative correlation was observed between anthropometric parameters, MMP-2 and net index of activity (MMP-2/TIMP-2 ratio) [MMP-2 (BMI ($r=-0.24$, $p=0.002$); WC ($r=-0.23$, $p=0.004$)); MMP-2/TIMP-2 (BMI ($r=-0.19$, $p=0.01$); WC ($r=-0.19$, $p=0.02$))]. Alterations of MMP-1 and MMP-2 pathways in obesity could be related to functional SNPs in MMP-1 and MMP-2 genes. Regarding the MMP-2(-735C/T) SNP, individuals carrying the (CT/TT) genotype showed increased BMI, WC, and WHtR values when compared to CC genotype carriers (BMI (31.61 kg/m² \pm 0.88 versus 28.96 kg/m² \pm 0.56, $P=0.01$); WC (106 cm \pm 2.23 versus 97.78 cm \pm 1.58, $P=0.004$); WHtR (0.64 \pm 0.01 versus 0.58 \pm 0.01, $P=0.005$).

CONCLUSION : The expression and the activity of MMP-1 and MMP-2 revealed reversible effects on Tunisian obese subjects.

Keywords: Obesity, MMP-1, MMP-2.

C. ORALE N°:6.**A NEW PHENOLIC GLYCOSIDE INHIBITS AMYLOID- β AGGREGATION, REMODELS AMYLOID FIBRILS AND REDUCES CYTOTOXICITY****DHOUAFLI ZOHRA^A, HICHEM BEN JANNET^B, BORHANE MAHJOUR^C, STEFANIA RIGACCI^D, MONICA BUCCIANTE^E, JEROME GUILLARD^E, RIADH KSOURI^A, MOUFIDA SAIDANI TOUNSI^A, MASSIMO STEFANI^D AND EL AKREM HAYOUNI^A***(a) Laboratoire des Plantes Aromatiques et Médicinales (LPAM), Centre de Biotechnologie de Borj- Cédria, Hammam-Lif, Tunisie.**(b) Laboratoire de Chimie Hétérocyclique, Produits Naturels et Réactivité (LR11ES39), Equipe : Chimie Médicinale et Produits Naturels, Faculté des Sciences de Monastir, Université de Monastir, Tunisie.**(c) Laboratoire de chimie, Institut supérieur agronomique de Chott Meriem, BP 47, Sousse 4042, Tunisie.**(d) Departments of Clinical and Experimental Biomedical Sciences, Viale Morgagni, 50, 50134 Florence, Italy.**(e) Institut de Chimie des Milieux et Matériaux de Poitiers, IC2MP UMR CNRS 7285, 4 rue Michel Brunet, TSA 51106, 86073 Poitiers Cedex 9, France E-mail : zohra.dhouafl@gmail.com*

The aggregation and deposition of misfolded β -amyloid peptide ($A\beta_{42}$) is a consistent pathological hallmark of Alzheimer Disease (AD). Mounting pieces of evidence indicate that the auto-assembly of $A\beta_{42}$ into toxic oligomers and fibrillar aggregates is a multi-steps process that causes an increase of ROS level, disruption of calcium homeostasis and altered membrane integrity leading to neuronal death. Oxidation is thought to promote $A\beta_{42}$ aggregation, so the use of natural dietary antioxidants that can interfere or inhibit $A\beta$ aggregation and its cytotoxicity is a promising approach. In this study, a new natural phenolic glycoside isolated from *Lawsonia inermis* leaves was utilized to investigate its effects on aggregation, fibrils growth, fibrils disaggregation and cytotoxicity induced by $A\beta_{42}$ aggregates. We used a panel of different spectroscopic, cell viability and imaging techniques to provide a detailed description of the $A\beta_{42}$ structural modifications arising in the presence of the inhibitor. It was found that the compound hinders and delays the aggregation of $A\beta_{42}$ by inhibiting its conformational change and its polymerization during the early stage of the aggregation process. Moreover, these polyphenol showed a strong inhibition of the increase in intracellular levels of ROS and Ca^{2+} by reducing the interaction of $A\beta_{42}$ aggregates with plasma membrane. It was also found to remodel the preformed $A\beta_{42}$ fibrils to non-toxic off-pathways aggregates. These results suggested that the new molecule acts as neuroprotective and therapeutic factors against toxic $A\beta_{42}$ oligomers and it's of great potential as neuroprotective agent that could be a good candidate for future drug of AD.

Keywords: Alzheimer's disease, $A\beta_{42}$ aggregation, phenolic glycoside, neuroprotection, amyloidogenesis.



C. ORALE N°:7.

THE METAL ION INDUCED SELF-ASSEMBLY OF M42 AMINOPEPTIDASES

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The M42 aminopeptidases are dinuclear metallopeptidases able to self-assemble into a tetrahedron-shaped structure with 12 subunits. Their quaternary structure consists in the association of six dimers to form the functionally active dodecamer. In *Pyrococcus horikoshii*, both dimers and dodecamers co-exist *in vivo*, suggesting a regulated self-assembly. The self-assembly, however, is ill described due to the lack of dimer structure. The two catalytic divalent metal ions could play an important role in the oligomeric state transition.

To unravel the role of the metal ions in the self-assembly, we have studied TmPep1050, a M42 aminopeptidase from *Thermotoga maritima*. This enzyme could be a model to understand the balance between dimers and dodecamers. To understand TmPep1050 oligomeric state transition, enzymatic assays, native mass spectrometry, thermal shift assay, and X-ray crystallography were used to characterize the wild-type enzyme. In addition, several catalytic residues were mutated and the subsequent mutants were biochemically and structurally characterized. TmPep1050 self-assembles into a tetrahedron-shaped structure with 12 subunits. TmPep1050 is a cobalt-dependent aminopeptidase displaying broad substrate specificity. Regarding the thermostability, TmPep1050 half-life is about 20 days at 75°C. The loss of cobalt ions, however, led to a dramatic decrease of thermostability and to the disassembly of dodecamers into dimers. The structure of TmPep1050 dimer showed the catalytic site is strongly impaired due to metal ion absence. Notwithstanding, the disassembly is reversible: dimer-to-dodecamer transition was characterized by native mass spectrometry. TmPep1050 cobalt ion binding was studied even further - through fluorescent cobalt ion quantification assay - revealing its binding kinetic. The structural characterization of mutants supports the hypothesis of a cobalt-driven self-assembly. Indeed the catalytic site must be correctly folded, via metal ion binding, to allow dodecamerization.

Keywords: M42 aminopeptidase, metalloenzyme, oligomeric state transition, thermostability.

C. ORALE N°:8.

ANTI-INFLAMMATORY AND ANTI-CANCER EFFECTS OF TERPENES FROM OILY FRACTIONS OF TEUCRIUM ALOPECURUS, BLOCKER OF IKBA KINASE, THROUGH DOWN REGULATION OF NFKB ACTIVATION, POTENTIATION OF APOPTOSIS AND SUPPRESSION OF NF-KB-REGULATED GENE EXPRESSION

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In the present study, the chemical composition, anticancer and nuclear factor- κ B (NF- κ B) inhibitory effects of *Teucrium alopecurus* leaf essential oil was investigated. The analysis of *Teucrium alopecurus* (TA-1) with Gas Chromatography–Mass Spectrometry (GC/MS) showed that α -Bisabolol, (+)-epi-Bicyclosquiphellandrene and α -Cadinol, were found in relatively high amounts (16.16%, 15.40% and 8.52%, respectively). Cell viability was determined by 3-(4–5-dimethylthiazol-2-yl) 2–5-diphenyl-tetrazolium (MTT) assay. Cell cycle and apoptosis assay were determined by flow cytometry. TA-1 functions as an anticancer agent by triggering apoptosis potentiated by chemotherapeutic agents and TNF in human myeloid leukemia cells (KBM5) through a mechanism involving poly(ADP-ribose) polymerase (PARP) cleavage and initiator and effector caspases activation. Moreover, electrophoretic mobility shift assay (EMSA) revealed that TA-1 downregulated nuclear localization of NF- κ B and its phosphorylation induced by TNF- α and this, allows the suppression of the degradation and phosphorylation of I κ B and the inhibition of the phosphorylation of p65 phosphorylation and the p50-p65 heterodimer nuclear translocation, causing attenuation of NF- κ B-regulated antiapoptotic (Survivin, Bcl-2, c-IAP1/2, Bcl-xL, Mcl-1, and cFLIP), invasion (ICAM1), metastasis (MMP-9), and angiogenesis (VEGF) gene expression in KBM5; and finally reporter gene expression. Furthermore, treatment with essential oil and TNF- α suppressed the NF- κ B DNA binding activity. Finally, the activation of nuclear factor- κ B induced by different plasmids (TNFR1, TRADD, TRAF2, NIK, TAK1/TAB1, and IKK β) was inhibited following treatment with TA-1. Overall, TA-1 inhibits NF- κ B activation and further growth and proliferation of cancer cells.

**C. ORALE N°:9.****ENHANCEMENT OF SOLUBILITY, PURIFICATION AND INCLUSION-BODIES-REFOLDING OF AN ACTIVE PECTIN LYASE FROM *PENICILLIUM OCCITANIS* EXPRESSED IN *ESCHERICHIA COLI*.****HADJ SASSI AZZA, HELA TRIGUI-LAHIANI, SALMA ABDELJALIL, ALI GARGOURI***Laboratoire de Biotechnologie Moleculaire des Eucaryotes, Centre de Biotechnologie de Sfax*

Pectin lyase (pnl) is the only pectinase able to hydrolyze directly the highly methylated pectin without liberating the toxic methanol and without disturbing ester content responsible for specific aroma of juices. The cDNA of *Penicillium occitanis*pnl (mature form) was cloned into *pET-21a* as expression vector and over-expressed into *Esherichia coli*. Most of recombinant pnl was expressed as inclusion bodies. Pnl activity was confirmed by colorimetric assay. To enhance the solubility yield of the expressed pnl, the effects of induction temperature, host strain and expression level were optimized. Maximal production of functional pnl was obtained after induction by 0.4 mM IPTG at 30 °C and 150 rpm for 16h. Interestingly, the use of Origami host strain, having an oxidized cytoplasm favoring disulfide bonds formation required for the active conformation of the enzyme, has significantly improved the yield of the soluble active form of recombinant pnl. This pnl was successfully purified through a single step purification using His-Trap affinity column chromatography. This work is the first to report pnlexpression into Origami strain. Alternatively, the inclusion bodies were isolated, denatured by high concentration of urea and gradually refolded by successive dialysis, leading to their transformation into soluble and active form.

Keywords:Pectin lyase,Heterologous expression,Inclusion bodies.

C. ORALE N°:10.**DRUG REPURPOSING TOWARDS THE IDENTIFICATION OF NOVEL ANTI-LEISHMANIA MOLECULES TARGETING THE METHYLTHIOADENOSINE PHOSPHORYLASE****HARIGUA-SOUAI EMNA¹, ZEINEB MOUSSA¹, MOURAD BARHOUMI¹, IKRAM GUIZANI¹**⁽¹⁾ *Laboratory of Molecular Epidemiology and experimental parasitology (LR16IPT04) – Institut Pasteur de Tunis*

Leishmaniasis are vector-borne diseases caused by kinetoplastidae of the genus *Leishmania*. They constitute major public health problems worldwide. The current drugs are toxic, costly and in most cases inefficient. Thus, developing new anti-*Leishmania* drugs is a research priority. Nevertheless, drug discovery is a highly cost-intensive process. In this context we conducted a structure-based drug discovery approach based on drug repurposing, which relies on discovering new applications for drugs that are already approved and available in the market. We focused on the *Leishmania infantum* Methylthioadenosine phosphorylase (LiMTAP), a homotrimeric enzyme that plays a crucial role in three metabolic pathways, namely the purine and methionine salvage pathways and the polyamine pathway.

We modeled the 3D structure of this protein using multiple methods. The model with the highest quality was retained and used to develop the trimeric structure model of the protein. In fact, the quaternary structure is crucial to define the active site (AS) of LiMTAP, which is embedded between two adjacent monomers. We then performed the docking of methylthioadenosine (MTA), the natural substrate, and a sulfate ion as a co-factor on the defined AS. Interactions that are characteristic of the MTA-MTAPs were obtained, along with interactions specific to LiMTAP as compared to the mammalian orthologue. Then, we performed a virtual screening of the FDA- approved drugs collection against the AS of LiMTAP. We selected the most promising molecules as follows: (i) their belonging to the anti-infectious class, (ii) having binding energies better than the MTA and (iii) presenting high chemical similarity to MTA, as the efficacy of transition-state inhibitors was proven on MTAP proteins in human and *Trypanosoma brucei*, a related kinetoplastid parasite. Three molecules were identified as potential anti-LiMTAP effectors with leishmanicidal profile.

To conclude, through a cost-effective computational approach we could predict three novel transition-state inhibitors of LiMTAP enzyme with potential anti-*Leishmania* effects. We will further confirm their effects on the enzymatic activity of the recombinant protein and on the viability of the promastigote form of *Leishmania* parasites.

Key words: Drug repurposing, Molecular modeling, 3D structure, *Leishmania infantum*, MTAP.

This work received financial support from the ministry of higher education and research (LR16IPT04).



C. ORALE N°:11.

IDENTIFICATION AND PURIFICATION ESSAY OF A GERMINATING ALMOND (*PRUNUS AMYGDALUS* MILL.) SEEDLINGS ALPHA AMYLASE

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In Tunisia, the almond tree (*Prunus amygdalus* Mill.) (Family: *Rosaceae*, genus: *Prunus*) is of great economic importance. Few studies have concerned its germination. In order to better understand the almond metabolism and its germination, we chose the study of an almond alpha-amylase during the *sensu stricto* germination phase.

In a first step, we confirmed the detection of alpha-amylase by a colorimetric method using 3,5-dinitrosalicylic acid (DNS). This technique has been optimized to characterize the enzyme. We also searched by SDS PAGE for the protein band corresponding to the enzyme from the S10 extract active fractions. We observed the presence of protein bands around 65 and 45 kDa. We observed the presence of protein bands around 65 and 45 kDa.

In another approach we used a comparative modeling by bioinformatics. After an extensive literature review we found that there is no almond alpha-amylase sequence in the databases using the Phyre 2 server (Protein Homology / analogY Recognition Engine) and from the Pistachian alpha-amylase (*Prunus persica*), a species of the same family as the almond tree. We observed the presence of a folding beta / alpha whose conservation was found between two phylogenetically different species: *Prunus Persica* and also *Hordeum vulgare*, a species of the family *Poaceae*. This result, which suggests the probable presence of beta / alpha folding in almond α -amylase, remains to be confirmed by further studies both at the level of the purification of the enzyme and its characterization.

Key words: Alpha-amylase, *Prunus Amygdalus* Mill., germination, PAGE-SDS, folding beta/alpha, *Prunus Persica*.

C. ORALE N°:12.

RELATIONSHIP BETWEEN METHYLGLYOXAL AND DISEASE ACTIVITY IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: The contribution of methylglyoxal (MGO) in the presence of rheumatoid arthritis (RA) is still unknown. It is the most powerful precursor to the formation of advanced glycation end products. We investigated whether serum MGO was related to the presence of disease activity in RA.

Methods: 80 patients with RA and 30 control subjects were included in a cross-sectional study. The severity of RA was assessed using the disease activity score for 28 joints (DAS28). Serum MGO was measured by ELISA Kit.

Results: Serum MGO levels were significantly higher in patients with RA vs control subjects ($P < 0.001$) and were increased in RA patients with higher disease activity vs RA patients with moderate disease activity ($P = 0.019$). A multiple logistic regression analysis demonstrated that MGO were independently associated with the presence of activity disease in RA (OR= 1.17, 95% CI: 1.02-1.31, $P = 0.01$).

Conclusion: Serum MGO level is related to disease activity of RA. Serum MGO may be a reliable marker for the diagnosis of RA.

Keywords: methylglyoxal, rheumatoid arthritis



C. ORALE N°:13.

EVALUATION DE L'EFFET NEPHRO-PROTECTEUR DU DECOCTE DU GINGEMBRE CHEZ DES RATS WISTAR CONTRE LES ALTERATIONS BIOCHIMIQUES ET HISTOLOGIQUES INDUITS PAR UN INSECTICIDE ORGANOPHOSPHORE.

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Les plantes médicinales sont couramment utilisées à coté des médicaments synthétiques contre divers symptômes et pathologies, et ceci grâce à leurs vertus thérapeutiques prouvés et de ses moindre effets secondaires. En effet de nos jours, l'exposition continue à des polluants environnementaux devient inévitable d'où l'intérêt d'incorporer des antioxydants naturels dans l'alimentation. Dans ce contexte, ce travail vise à prouver l'effet protecteur d'un remède traditionnel : le décocté du gingembre vis à vis l'exposition à un pesticide organophosphoré : le diméthoate. Nous nous sommes penchés, au cours de ce travail, sur le fonctionnement rénal chez des rats *Wistar*, et la capacité de l'extrait aqueux du gingembre à s'opposer aux altérations provoquées par le diméthoate, est recherchée.

Plusieurs paramètres biochimiques liés aux fonctionnement dont la créatinine, l'urée et l'acide urique sont comparés entre les groupes intoxiqués recevant ou non le gingembre, Aussi, et en vue de prouver l'action antioxydante de notre extrait aqueux, le stress oxydatif est évalué en dosant la catalase, la superoxyde dismutase et la glutathion peroxydase. De plus, un examen détaillé des coupes histologiques rénales a été effectuée. Les paramètres du stress oxydatif étudiés ont été modifiés chez les rats intoxiqués par le pesticide par comparaison à des rats témoins. Ces changements ont été au moins partiellement restaurés par le gingembre. De même, l'observation des coupes histologiques a permis de révéler plusieurs altérations néphrologiques qui ont parues être atténuées grâce au gingembre. Une corrélation entre l'altération de l'histoarchitecture rénale et le déséquilibre du stress oxydatif peut s'interpréter tandisque le traitement par le gingembre seul n'a pas révélé de modifications des paramètres étudiés en comparaison avec ceux des rats témoins. L'effet antioxydant et néphroprotecteur de l'extrait aqueux du rhizome de gingembre est ainsi prouvé. Et ce travail ne fait que confirmer que la racine du gingembre est une plante médicinale intéressante. Ce qui nous amène à proposer d'inclure les antioxydants naturels dont le gingembre dans l'alimentation pour une santé meilleure.

Mots clés : Gingembre, Diméthoate, reins, stress oxydatif, histologie.

C. ORALE N°:14.

EFFICIENT BIOCONVERSION OF MACROALGAL BIOMASS USING A SPECIFIC THERMOSTABLE GH 12 ENDOGLUCANASE FROM *ASPERGILLUS TERREUS*

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An efficient cellulase producer and thermostable fungus was isolated and identified as *Aspergillus terreus* using morphological and ITS sequence analysis. Depending on the carbon source used, *Aspergillus terreus* secreted two isoforms of endoglucanase in presence of AVICEL cellulose and filter paper. In the other hand, it released a single isoform when *Ulva lactuca* was assayed as substrate as shown by zymogram analysis. The crude extract showed a maximal endoglucanase activity on the 9th day (773.6 U/g DW), a maximal xylanase activity on the 11th day (264 U/g DW) whereas the β -glucosidase activity reached its maximum on the 13th day (294.4 U/g DW). The single endoglucanase released in the presence of algae was purified and analyzed using LC MS/MS that revealed a GH 12 superfamily endoglucanase. The enzyme showed a maximal activity at 60°C and pH 5. Its activity was increased by 24% in presence of Ca²⁺ ions while it was inhibited by AgNO₃ and NaClO.

Mots clés : *Aspergillus terreus*, endoglucanase, purification, characterization, algae.



C. ORALE N°:15.

VARIATIONS IN TUNISIAN *MEDICAGO MINIMA* PHENOLIC AND FLAVONE CONTENTS BETWEEN DIFFERENT PLANT ORGANS AND THEIR ANTIOXIDANT ACTIVITIES

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Medicago minima constitutes a legume, encountered in the arid and semi-arid environment in Tunisia. This species is virtually unknown while its exploration can further improve its interests, especially in foods, feeds and agriculture. The purpose of this research was to investigate the phenolic content of the various parts of twelve Tunisian populations and their antioxidant potential. Total phenolic compounds were extracted by maceration of dried plant material in MeOH (80%) and their antioxidant activity was determined in percentage of reduction of 1, 1-diphenyl-2-picrylhydrazyl by (DPPH) radical scavenging assay. The total phenolic and the flavone contents were determined using respectively Folin-Ciocalteu colorimetric method and Aluminum chloride colorimetric method for all organs studied (leaves, stems, roots, seeds). The results were expressed in mg of Gallic acid equivalence per g of dry matter for the total phenolic contents and mg of quercetin per g of DM for the flavones content. Then, the entire biochemical markers were used in a statistical analysis. The obtained results indicate that the highest levels of total phenolic content were respectively 16.65 and 15.97 mg EAG/g DM in leaves and seeds extracts. The same result was observed with the flavone contents assay. However, seeds and roots extract highlighted higher antioxidant activity with respectively 86.9% and 68.29%. In both cases, seed extracts show a significant correlation between the total phenols or flavonoid contents and antioxidant activity estimated. Nevertheless, no significant correlation was observed with root extract that may suggest the presence of other compounds responsible for their important antioxidant capacity. Moreover, the large variability exhibited by biochemical markers in all populations and organs highlighted the opportunity to select a suitable plant material to exploit for further improvement in agro-economic system.

C. ORALE N°:16.

BACTERIES LACTIQUES: PROPRIETES PROBIOTIQUES ET TECHNOLOGIQUES

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Cent souches de bactéries lactiques isolées à partir de lait cru bovin, provenant de différentes fermes, ont été soumises au stress régnant dans le tractus gastro-intestinal *in vitro*. Douze souches étaient capables de tolérer la pepsine au pH 2, la pancréatine et les sels biliaires (0,3%). Par la suite, ces bactéries ont été identifiées en utilisant le séquençage du gène de l'ARNr 16S. Huit souches étaient identifiées *Lactobacillus plantarum* et quatre souches étaient identifiées *Lactobacillus fermentum*. Ces bactéries étudiées sont considérées comme sécuritaires « GRAS », en raison notamment de la non dégradation de la mucine et de l'absence des activités α et β -hémolytiques. Toutes les souches ont une activité antibactérienne contre *Staphylococcus aureus*, *Listeria monocytogenes* et *Salmonella thyphimurium*. Cependant, seulement six souches ont inhibé *Escherchia coli*. Toutes celles-ci ont montré une capacité d'auto-agrégation et / ou d'hydrophobicité. Elles ont été également caractérisées en ce qui concerne leurs propriétés technologiques. Une acidification importante et de faibles capacités protéolytiques et lipolytiques ont été détectées pour toutes les souches. En outre, ces bactéries probiotiques étaient capables de produire des exopolysaccharides et de se développer à des températures non optimales de 45 °C, + 4 °C et - 20 °C. Ces bactéries peuvent être davantage utilisées pour la fabrication d'aliments fonctionnels et confirmer leur pertinence en tant que cultures probiotiques.

Key words: Lait cru bovin, *Lactobacillus plantarum*, *Lactobacillus fermentum*, Probiotique, Aliments fonctionnels.

**C. ORALE N°:17.****GENETIC, IMMUNOLOGIC AND PHENOTYPIC FEATURES OF TUNISIAN PATIENTS WITH LIMB GIRDLE MUSCULAR DYSTROPHY TYPE 2C****REKIK SABRINE, SALMA SAKKA, MARIEM DAMMAK, CHOKRI MHIRI***Research Laboratory in Neurogenetics, Parkinson's Disease and Cerebrovascular Diseases*

Sarcoglycanopathies are a group of autosomal recessive limb girdle muscular dystrophies (LGMD2C-2F) characterized by a progressive weakness of pelvic and shoulder girdle muscles. However, γ -sarcoglycanopathy (LGMD2C) is the most frequent form of LGMD in North African populations. Genetic features: LGMD2C caused by mutations in gene encoding the γ -sarcoglycan protein (SGCG), expressed exclusively in skeletal and cardiac muscle. About 90% of all LGMD2C cases in Tunisia carry 525delT exon 6 founder effect mutation. The homozygous deletion generates a truncated SGCG without EGF-like domain.

Immunologic features: 15 patients belonging to 9 Tunisian families were prospectively evaluated from the neurology department of CHU Habib Bourguiba of Sfax. Immunostaining of muscle biopsy, by immunohistochemistry and Western blot, of the 15 patients revealed different patterns of sarcoglycans expression. A complete absence of γ -sarcoglycan expression with a relatively preserved expression staining of α , β and δ sarcoglycans was found in 11 patients. Two patients showed a severe reduction of α and γ -sarcoglycans and two patients of β , γ and δ -sarcoglycans. However, usually mutation in one of the sarcoglycan genes causes the absence of the expression of the corresponding subunit and a secondary reduction that can vary from partial deficiency to total absence of the other three glycoproteins.

Phenotypic characteristics: Neurological examination of the 15 patients showed the same clinical features of severe limb girdle muscular dystrophy with onset during the first decade, progressive course of muscle weakness in four limbs with moderate calves hypertrophy and elevated CPK values. Muscle biopsy showed typical myopathic dystrophic features. In addition, a cardiomyopathy and scoliosis were observed respectively in 6 and 8 patients.

Conclusion: All of the patients have a similar clinical manifestation, but additional symptoms such as cardiomyopathy and scoliosis can occur in some cases. Also, screening of c.525delT mutation in LGMD2C patients is required to confirm the diagnosis.

Keywords : LGMD2C, γ -sarcoglycan, 525delT

C. ORALE N°:18.**CLONING AND BIOCHEMICAL CHARACTERIZATION OF THE PURIFIED ELASTASE FROM PSEUDOMONAS AERUGINOSA****RIGANE BOURMECHE EMNA^A, RAPHAEL DUTOIT^B, SIGRID FLAHAUT^B, KARIMA SRIH-BELGHITH^A***(a) Faculty of Sciences of Sfax – University of Sfax-Tunisia**(b) Institut of microbiological research Jean-Marie-Wiame, Belgium*

Elastase is an extracellular enzyme of *Pseudomonas aeruginosa* and belongs to the thermolysin-like family. The lasB gene encoding RBSP was cloned, sequenced, and expressed in *Escherichia coli* under the control of the T7 promoter. It was chromatographically purified using ion chromatography Resource 15Q column. The LC/ESI/MS/MS analysis revealed that the purified enzyme (called RBSP) was a monomer with a molecular mass of 33112 Da. It showed optimal activity at pH 8 and 45 °C.

The stability of the purified recombinant enzyme (rRBSP) were determined by the thermal shift-assay. The use of dyes to monitor thermal denaturation of proteins with sensitive fluorescence detection enables rapid and inexpensive determination of protein stability using real-time PCR instruments. By screening a wide range of inhibitors (EDTA, EGTA, Phenanthroline...) and metal ions (Ca^{2+} , Zn^{2+} and Co^{2+}) in a 96-well format in the presence of SYPRO Orange dye, the thermal shift assay easily identifies conditions that significantly enhance the stability of recombinant enzyme. This assay shows that the stability of the purified enzyme is affected with the ethylenediamine Tetra-Acetic (EDTA), ethylene glycol-bis (aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) and by 1,10-phenanthroline, proving that it belongs to metalloprotease family. This alkaline protease could be considered a potential candidate for application as the depilating enzyme.

Keywords : *Pseudomonas aeruginosa*, Elastase, thermal-shift assay, metal ions, inhibitors



C. ORALE N°:19.

LA COMPOSITION CHIMIQUE ET L'ACTIVITE INSECTICIDE DE L'EXTRAIT ORGANIQUE DE LA PLANTE *MENTHA SPICATA* AUTOCHTONE A LA REGION ARIDE BISKRA

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L'objectif de ce travail est déterminer la composition chimique et l'effet insecticide des concentrations 20, 40, 80, 120 µl de l'extrait organique de la plante *Mentha spicata* autochtone à la région aride de Biskra, sur un des ravageurs les plus persistants à la culture de la fève, à savoir *Aphis fabae Scopoli* (Hemiptera: Aphididae). Les résultats obtenus montrent que cette extrait organique se compose de 63 molécules bioactifs. Ces composants ont prouvé une activité insecticide relativement efficace à légards de l'*Aphis fabae*, leur CL50 et CL90 est relativement faible de 3,18 et 10,96µl/ml respectivement et l'analyse de variance a donné un résultat hautement significatif. Cet extrait peut être considérée comme une source de composés bioactifs contre les ravageurs et pourquoi pas un biopesticide après avoir faire des tests moléculaires approfondis et dans le terrain.

Mots clés : *Aphis fabae*, extrait organique, Biopesticide, *Mentha spicata*, Biskra.

C. ORALE N°:20.

DIFFERENTIAL TOXICITY OF ANTIFUNGAL PROTEIN ACAFP AGAINST MUTANTS OF *FUSARIUMOXYSPORUM*

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AcAFP peptide is a small, basic and cysteine-rich antifungal peptide secreted by a local strain of *Aspergillus clavatus* (VR1). The peptide showed its ability to prevent growth of various human- and plant-pathogenic filamentous fungi. It was assayed for toxicity against *Fusariumoxysporum* wild-type strain and mutants in genes involved in: cell signaling ($\Delta pacC$, $pacC^c$, $\Delta con7$), chitin cell-wall biogenesis ($\Delta chsV$, $\Delta chsVb$, $\Delta chs7$) and β 1-3 glucan cell-wall biogenesis ($\Delta gnet2/5$, Δgas). The mutants were classified into two groups according to their sensitivity to AcAFP: $\Delta chsV$, $\Delta chsvb$, $\Delta con7$ and $pacC^c$ were significantly more sensitive to AcAFP than the wild-type, and $\Delta pacC$, $\Delta chs7$, $\Delta gnet2/5$ and Δgas , which were more resistant. Western blot analysis revealed increased binding of AcAFP to sensitive mutants, but also to two resistant mutants: $\Delta pacC$ and $\Delta gnet2/5$, indicating that differential binding to the surface of these strains may not be a key determinant for sensitivity. This study shows that mutations in single genes have significant effects on the sensitivity and binding of fungal strains to the antifungal protein AcAFP. Pointing the molecular bases underlying these differences should provide further insight into the antifungal mode of action of AcAFP.

Keywords: Antifungal peptide. *Fusariumoxysporum*. Cell wall. Mode of action



C. ORALE N°:21.

EFFECTS OF LAMB INTAKE OF ROSEMARY DISTILLATION RESIDUES ON MEAT'S ALPHA-TOCOPHEROL CONTENT AND OXIDATIVE STABILITY

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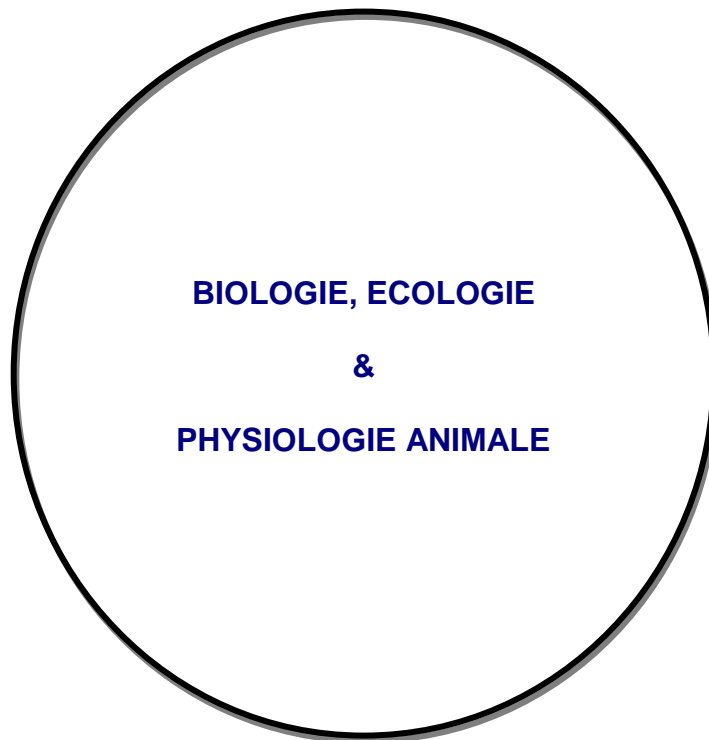
Nowadays consumers are becoming more health-conscious and are searching meat products naturally produced that contains natural antioxidant than synthetic ones. For that, the effects substitution of oat-hay by pellets of rosemary distillation residues (RR) with two different protein proportions on meat alpha-tocopherol content and lipid oxidation was studied on Barbarine lambs (23.7 ± 4.4 kg.). The lambs were divided into three groups each with seven lambs depending on their body weight. They were assigned to one of three treatments (control, RR60 and RR87). The control group (C) was offered 600 g of oat hay, RR87 and RR60 received 600g of pellets containing 87 and 60% of RR, respectively. All animals were supplemented by 600 g of concentrate. After 77 days of fattening, lambs were slaughtered to study the meat quality parameters. Meat α -tocopherol concentration was significantly ($P < 0.001$) affected by the RR intake and it was 4 times higher for RR87 and RR60 (6.64 and 7.77 $\mu\text{g/g DM}$, respectively) than the control group (1.59 $\mu\text{g/g DM}$). Moreover, lipid oxidation was strongly reduced with RR diets ($P = 0.001$) during the storage. Both groups receiving RR showed similar TBARS values that did not exceed 1.5 mg of MDA/kg meat across 9 days of storage, while the highest value (3.13 mg of MDA/kg meat) was recorded for C group. As conclusion, the rosemary residues intake improved lamb's meat quality; it increased the vitamin E content which consequently enhanced the oxidative stability of Barbarine lamb meat. Furthermore, the RR87 without soya bean resulted in the same performance and meat quality as RR60 containing soya bean which was imported and expensive. So the RR87 should be used given its nutritive and economist efficiency.

Key words: rosemary residues, lamb' meat, alpha-tocopherol, lipid oxidation



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C. ORALE N°:22.

EFFET DE LA SUPPLEMENTATION EN ENZYMES FIBROLYTIQUES EXOGENES SUR L'UTILISATION DIGESTIVE ET LA FERMENTATION RUMINALE *IN VITRO* DES COPRODUITS DES PALMIERS

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Dans les oasis tunisiennes, les éleveurs font souvent recours à diverses stratégies afin d'assurer les besoins alimentaires de leur bétail. En effet, ils utilisent les coproduits des palmiers, notamment les rebuts des dattes (RD), les noyaux des dattes (ND), les feuilles des palmiers (FP) et les hampes des palmiers (HP) pour garantir des réserves alimentaires. L'objectif de ce travail consiste à étudier l'effet d'une supplémentation en enzymes fibrolytiques exogènes (SEFE) sur l'utilisation digestive et la fermentation ruminale *in vitro* de ces coproduits. De ce fait, la technique de production de gaz *in vitro* a été utilisée pour déterminer les différents paramètres fermentaires et la dégradabilité des coproduits. Un complexe enzymatique, extrait à partir de la fermentation d'*A. niger*, d'*A. tubingensis*, d'*A. oryzae*, d'*A. sojae* et de *N. intermedia*, a été utilisé suivant 3 doses: d1= 0.5, d2= 1 et d3= 2 mg /g MS. L'aliment témoin ne reçoit aucun traitement. A part les différents paramètres caractérisant la cinétique de production gaz, la digestibilité de la matière organique (dMO), la teneur en énergie métabolisable (EM) et la production d'acides gras volatils (AGV) ont été aussi déterminées.

Pour les ND, la SEFE et suivant la dose faible d1, a amélioré d'une manière significative ($p < 0.05$) la production potentielle de gaz qui passe de 234.1 à 268.7 ml/ g MS sans modifier la vitesse de fermentation. Avec cette même dose, la dMO, l'EM et l'AGV ont tendance à s'améliorer ($p < 0.1$). Par rapport à l'aliment témoin, ces paramètres passent respectivement de 38.0 à 39.8%, de 6.1 à 6.3 MJ/ kg MS et de 0.57 à 0.61 mmol/ 200 mg MS. Avec des doses plus élevées (d2 et d3), ces paramètres restent identiques à ceux de l'aliment témoin. Pour les 3 autres coproduits étudiés, la SEFE n'exerce aucun effet significatif sur tous les paramètres étudiés et quelle que soit la dose utilisée. Par rapport à l'aliment témoin, la dMO passe de 60.6 à 60.7%, de 33.8 à 32.2% et de 29.9 à 29.2% respectivement pour les RD, les FP et les HP

C. ORALE N°:23.

EFFECT OF THREE TUNISIAN FODDER RESOURCES ON THE EXSHEATHMENT PROCESS OF HAEMONCHUS CONTORTUS INFECTIVE STAGE

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Parasitic infections with *Haemonchus contortus* (*H. contortus*) remain a major problem in grazing small ruminants. The aim of this study was to assess the *in vitro* anthelmintic activity of acetic extract of oat hay, commercial concentrate, and *Hedysarum carnosum* Desf. (*H. carnosum*) by a larval exsheathment assay (LEA) on the infective larvae of *H. contortus*.

The chemical composition of the collected samples was determined. The Folin-Ciocalteu method was used to quantify the concentrations of total polyphenols (TP) and total tannins (TT) in the resource samples. In addition, the condensed tannins (CT) of each sample were determined by the butanol-HCl method and the biological activity (BA) of tannins was quantified using the Radial Diffusion method. The LEA was used at different concentrations (1200, 600, 300, 150 µg of acetic extract/ml of purified buffer solution (PBS)). To confirm the role of tannins in the anthelmintic effects of extracts, polyvinylpyrrolidone (PVPP) was used as deactivating chemical tannins. Besides, the effective concentration for 50 % inhibition (EC50) was calculated with the PoloPLUS 2002-2003.

Crude protein was relatively high for commercial concentrate (14.01 %Dry matter (DM)) and *H. carnosum* (13.05 %DM). The highest concentration of neutral detergent fibre (NDF) was recorded for the oat hay. *H. carnosum* showed the highest content of TP, TT CT, and BA. These *in vitro* assays had highlighted the effect of fodder resources ($P < 0.0001$), concentration of extract ($P < 0.0001$) and their interaction ($P < 0.0001$) on the kinetics of exsheathment of the L3 of *H. contortus*. After the addition of PVPP, the restoration of L3 exsheathment to values similar to those of control indicates that tannins are compounds involved in anthelmintic effects. The lowest value of EC50 was obtained for *H. carnosum* (180.04 µg/ml).and the highest value was recorded for oat hay (>1200 µg/ml). Indeed, *in vivo* tests are required to confirm these results.

Keywords: *Haemonchus contortus*, Tunisian fodder resources, larval exsheathment assay, condensed tannins.



C. ORALE N°:24.

CHEMICAL CONTROL OF THE TOMATO LEAFMINER *TUTA ABSOLUTA* (MEYRICK) (LEPIDOPTERA: GELECHIIDAE) IN TUNISIAN TOMATO OPEN FIELD CROP

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Abstract: The tomato leafminer *Tuta absoluta* is considered a serious pest of tomato cultivation worldwide. Chemical control was considered as the main management tool used against this pest. In this view, two trials were carried out to evaluate the effectiveness of seven insecticides in Takelsa tomato main crop season (1 ha). For the first trial, two actives ingredients (chlorfenapyr (40 cc/hl) and the association of chlorantraniliprole and abamectin (60 cc/hl)) were tested on 23/06/2011. However, five insecticides were tested on 27/06/2011 for the second trial including: *Bacillus thuringiensis* (250 g/hl), azadirachtin (150 cc/hl), *Verticilliumlecani* (100 cc/hl), cyromazin (30g/hl) and indoxacarb (50cc/hl). Each insecticide was tested on 100 tomato plants and four repetitions were considered for each treatment. 400 tomato plants were left for the untreated control. Our results indicated that all tested insecticides were efficient in reducing the infestation level caused by *T. absoluta*. Furthermore, a significant difference was found between the tested insecticides and the control untreated ($P < 0.05$). This work highlights the efficacy of some insecticides in controlling *T. absoluta*, however, alternatives methods should be tested to avoid the possible development of insecticides resistance.

Key words: *T. absoluta*, insecticides, tomato main crop season

C. ORALE N°:25.

STRUCTURAL AND MORPHOMETRIC STUDY OF FOLLICULOGENESIS IN A DESERT RODENT *PSAMMOMYSOBESUS* (CRETZSCHMAR, 1828)

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The sand rat, *Psammomysobesu* (Cretschmar, 1828), a diurnal species, is part of the desert wildlife, living in the region of Beni Abbes, situated at North-West of the Algerian Sahara. It exhibits seasonal sexual activity with a long active period, which extends from autumn to early spring followed by a short rest period, from late spring until early summer.

The aim of this study is to describe the ovary during the active period by a structural and morphometric analysis of the different follicles present in order to establish the kinetics of the follicular growth in this species. Histological results indicate that the structural evolution of folliculogenesis demonstrates a continuous cyclicality of ovarian activity. The morphometric and statistical analysis of follicular and oocyte diameter at different stages during basal growth did not show significant differences. This suggests that basal follicular growth appears to be gonadotropic independent except for pre-antral follicles growth that may be due to fluctuations in gonadotropic hormone levels. This study revealed a biphasic follicular growth during the reproduction cycle, the first one appears in the luteal phase and leads to atresia, the second appearing is at the end of the luteal phase, at luteolysis, and leads to ovulation.

Key words: Sand rat, oestrian cycle, folliculogenesis, morphometry, reproduction season.



C. ORALE N°:26.

LES DEGATS CAUSES PAR LES RONGEURS DANS LES CHAMPS D'ORGE AU SUD EST DE LA TUNISIE

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Les rongeurs, en particulier sous la famille des muridés, peuvent causer de graves dommages aux cultures céréalières de toutes sortes, y compris l'orge. La présente étude a été menée pour observer le type de dommages causés par les rongeurs (Rodentia: Muridae) dans l'orge conjointement avec leurs densités comparatives tout au long de la phase de croissance de la culture, dès la présence de premières feuilles jusqu'au stade des grains mûrs. Plusieurs espèces de rongeurs ont endommagées le blé, mais des variations de leur prédominance ont été détectées. Parmi les ravageurs piégés et identifiés, *Merione libycus* était l'espèce dominante avec une abondance relative de (46,34%), suivie de *Psammomys obesus* (17,07%), *Gerbillus henleyi* et *Gerbillus nanus* (12,19%), *Gerbillus pyramidium* (9,75%) et *Gerbillus tarabuli* qui était la moins abondante parmi les rongeurs piégés (2,43%). Ces différents rongeurs ont commencé à attaquer et endommager la récolte d'orge dès l'apparition des graines et ont continué jusqu'au moment de leur maturation. Cette information sur les habitudes alimentaires de certains rongeurs constitue une base pour la gestion de leurs populations et pour réduire les dommages

C. ORALE N°:27.

DIAGNOSTIC ÉCOLOGIQUE DES SITES « RAMSAR » EN TUNISIE : CAS DU PARC NATIONAL ICHKEUL

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Résumé. Au niveau des zones humides tunisiennes, notamment les sites RAMSAR, les suivies ornithologiques que nous avons réalisés révèlent une richesse spécifique totale qui dépasse 70 espèces et une densité annuelle moyenne de 8490 oiseaux aquatiques/jour/an. Une concentration hivernale des oiseaux herbivores, a été également notée. A titre d'exemple, l'Ichkeul qui a gardé ses potentialités de milieu important pour l'estivage des oiseaux à régime mixte, il se montre en régression pour ce qui concerne les espèces hivernantes-herbivores, de passage-herbivores et nidificatrices. En effet, la construction de trois barrages, les conditions climatiques contraignantes, la mauvaise gestion de l'écluse Tinja et le développement des terres agricoles au détriment des milieux naturels ont entraîné un doublement de la salinité, une diminution de la hauteur d'eau, une exondation des marais et une diminution de la productivité végétative. Dès lors, nous avons constaté que la densité annuelle du peuplement des oiseaux a chuté d'environ 40 % en comparaison avec les données antérieures. Cette diminution concerne particulièrement les populations hivernantes et de passage avec des coefficients de variation de la densité respectifs de - 54,88 % et - 22,37 %. En revanche, le nombre total d'espèces fréquentant le PNI s'avère stable et les variations annuelles sont dues aux espèces sporadiques dont la présence ou l'absence sont probablement liées à des facteurs exogènes.

Mots-clés : Sites Ramsar – Ichkeul – oiseaux aquatiques - Tunisie.



C. ORALE N°:28.

DIRECT INTERACTION BETWEEN *TRICHOGRAMMA CACOECIAE* (HYMENOPTERA: TRICHOGRAMMATIDAE) AND *ASCOGASTER QUADRIDENTATA* (HYMENOPTERA: BRACONIDAE), TWO PARASITOIDS OF THE PEST CODLING MOTH *CYDIA POMONELLA* (LEPIDOPTERA: TORTRICIDAE).

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Understanding the role of intraguild interactions may contribute to an effective pest management strategy when multiple natural enemies may be necessary to control a given pest. In our study, we explored interactions between two natural enemies of the codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae), namely the egg-larval parasitoid *Ascogaster quadridentata* Wesmael (Hymenoptera: Braconidae) and the egg parasitoid *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae).

In our approach we focused on the question whether potential negative interactions between the egg parasitoid *T. cacoeciae* and the egg-larval parasitoid *A. quadridentata* competing for the same host resource exist, which may facilitate or hinder *C. pomonella* biological control in the case of *Trichogramma* mass-releases and natural control provided by endemic *A. quadridentata*. Therefore we conducted many experiments such as direct confrontation tests between adults. Single females of both species were either introduced together, or the second species was introduced when the first species started parasitizing the single provided *C. pomonella* egg (fresh or one-day old eggs). Afterwards, the parasitoid females were left together over night in an incubator at controlled conditions. Ten replicates were undertaken for each experiment.

In experiments one and two, where both parasitoids were introduced together or where *A. quadridentata* was allowed to encounter eggs first, less than 50 % of the eggs were successfully parasitized by *T. cacoeciae* afterwards. In contrast, in the third experiment, where *T. cacoeciae* was allowed to start the parasitization, the success of *T. cacoeciae* was higher (on average 75 %). The proportions of the events in the three experiments varied significantly. These experiments show that although inferior in extrinsic competition, *Trichogramma cacoeciae* tends to win in intrinsic competition as the eggs parasitized by both parasitoids evolved solely in viable *T. cacoeciae* adults.

Key words: Biological control; Direct interaction; *Ascogaster quadridentata*; *Trichogramma cacoeciae*.

C. ORALE N°:29.

REPRODUCTIVE CYCLE OF THE PEARL OYSTER, *PINCTADA RADIATA* (MOLLUSCA: PTERIDAE), IN ZARAT SITE (GULF OF GABÈS, TUNISIA)

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The gametogenic cycle of the pearl oyster, *Pinctada radiata*, from the region of Zarat (Gulf of Gabès) in Tunisia was investigated monthly during a one year cycle. Histological analysis of the gonads was used to portray their Gametogenic activity. *P. radiata* exhibited a clearly defined annual reproductive cycle. This species had two spawning periods a year: the first occurred in June and August for both sexes and the second one was observed index shows that the main periods of gametogenesis and spawning around the year were from September to December for males and from September to November (except October) for female. Two types of hermaphroditism emerged after the ripeness of gonads. Sex ratio showed a clear relationship with the size: males dominated among smaller individuals (shell height SH < 65 mm), while females were predominant in larger size-classes (SH > 65 mm). The condition index proved to be a strong indication of the gonad cycle as it illustrates the reserve accumulation during gametogenesis, maximum maturity as well as gamete emission. Maturity is roughly synchronous between sexes. The beginning of reproduction seems to be controlled by sea surface temperature rather than salinity.



C. ORALE N°:30.

DIFFERENCIATION DE L'ORIGINE GEOGRAPHIQUE D'AGNEAU DE RACE LOCALE TUNISIENNE PAR L'OUTIL DU RATIO ISOTOPE STABLE

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L'efficacité de spectrométrie de masse à ratio isotopique (SM-RI) dans le traçage du système de production d'agneau a été examinée pour quatre régions du Nord Ouest Tunisien: Ain Draham et Fernana, caractérisés par le pâturage ligneux et Amdoun et Joumine, caractérisés par le pâturage herbacé. L'objectif de la traçabilité de l'alimentation n'est pas seulement de mettre en évidence des différences significatives entre modes d'alimentation, mais bien de les discriminer. Cette expérimentation a été conduite sur des agneaux de race locale âgés de 3 à 5 mois. Des échantillons de muscle Longissimus dorsi ont été prélevés sur huit agneaux de chaque système de production pour l'évaluation de ratio d'isotope stable des cinq principaux bio-éléments. Les résultats ont montré que la variabilité des RI entre sites est supérieure à celle entre les types de pâturage. Ainsi, les valeurs de $\delta^{13}C$ de protéines ont montré des différences statistiquement significatives entre les sites. Le soufre a permis de discriminer parfaitement le type d'agneau qui découle des différents systèmes. Les deux systèmes de pâturage ligneux (Ain Draham et Fernana) sont les plus proches mais ils sont aussi les plus proches de la mer. La forte teneur en S34 semble être un signal "côtier" de ce qu'on appelle "sea-spray". Le 'sulfate Sea-spray' est déposé sous forme d'aérosol sur les pâturages et les cultures, en quantités décroissantes avec la distance de la mer. Des résultats similaires ont été obtenus dans une étude sur les types d'agneau Italiens des îles de la Méditerranée, la Sicile et la Sardaigne (5 - 10 km de la mer). Après traitement des données Tunisiennes et Italiennes avec une analyse discriminante « Partial Least Squares » (PLS), le profil de RI a permis de la réaffectation correcte des de types d'agneaux Tunisiens. Cependant, les différences entre eux n'étaient pas suffisamment larges et systématiques pour être validées par un ensemble externe d'échantillons incluant 10 types d'agneau Italiens. A échelle géographique plus large la signature discriminante de la zone Amdoun, du reste du nord-ouest de la Tunisie et de l'Italie, semble être réalisable.

Mots clés : viande d'agneau, Nord Ouest tunisien, isotopes, traçabilité.

C. ORALE N°:31.

EPIDEMIOLOGY OF COLORECTAL CANCER IN EASTERN ALGERIA BETWEEN 2006 AND 2016

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Introduction: Colorectal cancer (CRC) is a public health problem because of its frequency and severity. In Algeria, it ranks 2nd among the most common cancers. Objective: Our aims is to carry out an epidemiological study on colorectal cancer in the eastern region of Algeria. Methodology: A descriptive survey was conducted in Anti-Cancer Center (CAC) of Batna and the Bouguerra Boulaares Bekaria public hospital (EPH) in Tebessa. From the register of oncology services, we collected data from 8286 cases of cancer diagnosed between 2006 and 2016: year of admission, age, sex, wilaya and localization of cancer were collected. Results: We noted 1015 patients with colorectal cancer. The age was between 15 and 92 years, with an average age of 56.01 ± 14.23 years. Women are the most affected by CRC (516 women vs 499 men, $p = 0.000$) with a sex ratio of 0.96. The CRC ranks second (12.25%) after breast cancer (2416 cases, ie 29.16% of the total). He is in first place (15.24%) in men followed by cancers of the upper aero-digestive tract (14.12%) and lung cancer in third position (12.82%). In women, it was ranked 2nd with a prevalence of (10.26%) after breast cancer (47.09%). We found that the CRC experienced a huge increase in the last decade in eastern Algeria, especially in 2015 when it recorded its maximum prevalence (14.86%). The CRC has a much larger impact on the 50 to 59 age group for both sexes. The predominance in this study was male for rectal cancer, but female for colon cancer, and both combined ($p = 0.034$). The wilayas most affected are: Biskra, Khenchela and Tébessa, but it was more frequent in Batna. Conclusion: Colorectal cancer has continued to increase in recent years. We believe that care for patients at risk is to be considered in our country and around the world.

Key words: Epidemiology, cancer, colorectal, Eastern Algerian.



C. ORALE N°:32.

PHYSICO-CHEMICAL CHARACTERISTICS OF FARM AND EXOTIC EGGS CONSUMED IN ALGERIA

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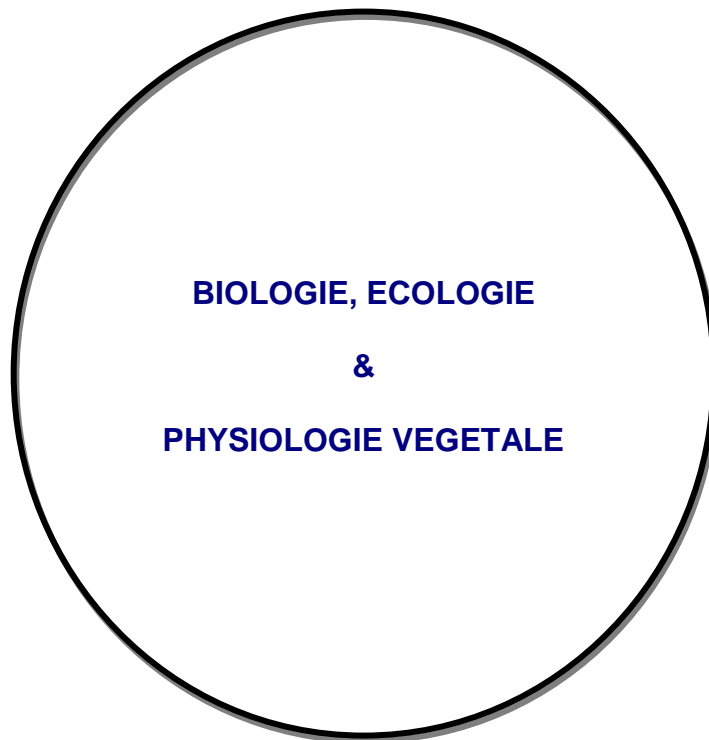
Nowadays poultry farming contributes significantly to food security. The chicken egg is an important food for humans and is considered as a perfect natural food. It contains the nine essential amino acids that the human body can not synthesize, and is an easily renewable source of proteins, lipids, minerals and vitamins. The new consumer trends in organic products prompted us to carry out this study whose objective was to compare exotic eggs and farm eggs in terms of composition and conformation consumed in the region of Chlef in Algeria; thus a total of 100 eggs (50 eggs of each type) were used. The results showed that the weight of the exotics and farms was (80.2g against 61.8g), the weight of the eggshell was (8.78g vs 7.53g), the weight of the albumen was (33.7g vs 30.69 g), the percentage of albumen was (37.22% vs 32.91%), the egg yolk weight was (33.7g vs 30.69g), the yellow percentage was (49.80% vs 53.87%), Height (0.8cm vs 1.2cm), Haugh unit (57.92 vs 44.98) and shape index (93 vs 75). The average pH of the albumen varied very significantly ($P < 0.01$) between farm eggs and exotic eggs, no significant difference ($P > 0.05$) was revealed in terms of total protein content and ash, unlike the lipid content that was highly significantly different ($P < 0.001$) between the two types of eggs. This study allowed us to build a preliminary database on the physical and chemical characteristics of farm and exotic eggs in the region of Chlef in Algeria, a microbiological quality assessment would provide additional qualitative data on both types of eggs.

Key words: farm eggs, exotic eggs, weight, protein, fat.



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C. ORALE N°:33.

EFFECTS OF DEFICIT IRRIGATION ON AGRO-PHYSIOLOGICAL PARAMETERS OF PISTACHIO (*PISTACIA VERA* L.) VARIETIES

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The experiment orchard was located in the Agriculture Regional Research Center (CRRA, SidiBouzid) in west central Tunisia. Ten-years-old pistachio trees cv. Mateur on *P.atlantica* root stocks were studied. Three water treatments were applied; T₀ (100% ET_c), T₁ (50% ET_c during stages I and II of fruit development followed by full irrigation 100 % Etc during stage III and T₂ (50% ET_c). Agronomical traits such as production, trunk cross sectional area, yield efficiency, fruit weight and growth parameters were evaluated. Physiological parameters such as photosynthetic parameters and midday leaf water potential were measured. Results showed that water relations in pistachio trees change over the growing season. Significant differences were found in the daily pattern of leaf water potential in all the phenological stages considered, while only in the last one the net photosynthesis was affected by water stress. Moderate levels of water stress during the last measurement date reduced the maximum values of g_s and A_n. Under moderate water stress conditions, transpiration efficiency was maximized due to the maintenance of the photosynthetic rate. These results provide physiological evidence of increased resistance to drought in this species during stage II of fruit development.

Key words: Pistachio, Mateur, regulated deficit irrigation

C. ORALE N°:34.

EFFECT OF SALT STRESS ON MOLECULAR AND OSMOTIC RESPONSE OF POTATO: INVOLVEMENT OF THE PHYSICAL METHOD OF SALINE WATER TREATMENT

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Salt stress is hampering plant water status and osmotic adjustment in arid and semi-arid regions due to the severity of climatic conditions and salinisation of scarce water resources. To understand mechanisms that are involved in potato sensing and signaling under salinity-osmotic stress and the implication of the magnetization of saline irrigation water, a field experiment was therefore conducted to assess the malicious effects of salinity on potato. Two saline treatments were induced after potato emergence (non electromagnetized saline water (NESW) : 6g NaCl/l and electromagnetized saline water (ESW) : 6gNaCl/l) vs control (C) : 2g NaCl/l. Potato proved to be significantly ($p \leq 0.05$) affected by salt stress (plant water status, proline metabolism, expression of ion transporters and productivity). Electromagnetized saline water reduces the harmful effects of osmotic stress in potato yield through the better osmotic adjustment achieved by an increase in water potential, osmotic potential, turgor potential, membrane stability index, as well as the less accumulation of proline and the expression of ion transporter genes. Conclusively, salt tolerance potential is dependent on the salinity of the irrigation water as both saline treatments exhibited different performance towards the studied attributes.



C. ORALE N°:35.

ASSESSMENT OF TOLERANCE TO SEVERE WATER STRESS OF OLIVE SEEDLINGS AND PRELIMINARY SELECTION OF DROUGHT-TOLERANT GENOTYPES

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Climate change has increased the frequency and the severity of drought in the world. This situation affects the olive tree productivity (*Olea europaea* L.), one of the most important crop in the Mediterranean basin and well adapted to prolonged drought periods. Therefore, new approaches including the selection of drought-tolerant genotypes have been considered to develop sustainable agriculture in dry lands. In this respect, one-year-old olive seedlings (*Olea europaea* L. cvs. Chemlali, Chetoui and Zarrazi) were subjected to severe water stress under greenhouse conditions during two months followed by a prolonged period of rehydration. Plant water status and gas exchange parameters, as well as total chlorophyll level were assessed. Results indicated that leaf water potential and gas exchange parameters were significantly affected by withholding irrigation. Moreover, total chlorophyll level was significantly decreased after two months of severe water stress, with the exception of Zarrazi. Photosynthesis limitation was attributed to stomata closure and CO₂ mesophyll conductance decrease. Our study indicated that leaf water potential, net photosynthesis rate and total chlorophyll level could be good markers to evaluate drought tolerance in olive seedlings under severe water stress conditions. Moreover, rehydration period is a better method to determine the effect of water stress in olive seedlings. Non recovery of the photosynthetic gas exchange parameters and growth was observed after a prolonged period of rehydration.

C. ORALE N°:36.

VARIABILITY IN SALT TOLERANCE OF SOLANUM LYCOPERSICUM

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Salinity is a significant constraint for crop quality and production, affecting wheat growth, yield and quality in the major wheat growing regions of the world. To counter this, it is necessary to develop tolerant varieties through selection and breeding techniques. The present study was conducted to evaluate salt tolerance in tomato varieties (*Solanum lycopersicum* L.) (Saada, Nemador, Atala, Templar, Elba, Perfect Peel) based on several physiological and biochemical traits. Seedlings were exposed to salinity in the form of NaCl (150 mM) for 21 days. The results showed that at seedling stage 150 mM NaCl decreased chlorophyll content, leaf length, number of leaves per plant, shoot length and shoot fresh and dry weights. Reduction in growth in the presence of salt was associated with K⁺ and Ca²⁺ absorption. Whereas Na⁺ and Cl⁻ uptake and transport increased by the presence of NaCl but Na⁺ accumulation varied among the varieties. In the other hand, a significant difference in organic osmolyte content was noted under the effect of NaCl. The plantlets also accumulated a high content of soluble sugars and proline which varied with varieties. In conclusion, salt tolerance of tomato varied with varieties. Templar seem the most salt tolerant varieties, while Elbashed showed the least salt tolerance.

Key-words: variety, salinity, tolerance, organic osmolyte, mineral nutrition.



C. ORALE N°:37.

CALCIUM ET CITRATE MODULENT L'HOMÉOSTASIE REDOX CONTRE LES DOMMAGES OXYDATIFS INDUITS PAR LE CUIVRE CHEZ LE PETIT POIS

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L'effet protecteur de l'application d'un compétiteur ionique (calcium ; 10 mM) et d'un chélateur organique (citrate ; 100 µM) sur la toxicité de 200 µM Cu au niveau des radicules des germinations de petit pois (*Pisum sativum* L.) est présenté dans ce travail. L'exposition des graines au cuivre en excès entraîne des changements dans le statut redox cellulaire des jeunes germinations, notamment Cu stimule la teneur en groupements thiols (-SH) et les activités des enzymes des systèmes redox (Trx/NTR), (Fdx/FNR) :thiorédoxine, ferrédoxine, NADP-thiorédoxine réductase et NADP-ferrédoxine réductase. Cependant, Ca et citrate annihilent cet effet. Une approche protéomique (1D) ayant été significativement suggestive quant aux changements relatifs aux thiols des protéines, ce qui a permis de conclure que Ca et citrate sont capables de protéger le petit pois contre les dommages oxydatifs induits par Cu, probablement par une modulation des systèmes thiorédoxine/ferrédoxine et, par conséquent, l'évitement de l'oxydation des protéines.

Mots clés : Cuivre, Effecteur exogène, Homéostasie redox, Petit pois, Stress oxydant

C. ORALE N°:38.

DIVERSITE ET AFFINITES ECOLOGIQUES DE LA FLORE BRYOLOGIQUE DE LA KROUMIRIE

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Les travaux réalisés sur la flore bryologique de Tunisie sont anciens, et sa richesse, sa répartition et son écologie sont très peu connues. Afin de contribuer à pallier cette lacune, nous avons mené des prospections en mai 2018, dans 18 sites de la Kroumirie. Tous les échantillons des Anthocérophytes et des Marchantiophytes ont été identifiés, et les données recueillies ont subi des analyses numériques. Les résultats obtenus ont permis, d'une part, de mettre à jour la liste des Anthocérophytes et des Marchantiophytes de la Kroumirie sur la base de la littérature et de nouvelles collectes. 35 taxons ont été identifiés, dont 3 sont nouveaux pour la Tunisie (*Fossombronia caespitiformis* subsp. *multispira*, *Lejeunea lamacerina* et *Riccia huebeneriana*) et 2 pour la Kroumirie (*Riccia crinita* et *R. gougetiana* var. *armatissima*). D'autre part, ce travail a permis d'améliorer les connaissances sur l'écologie de la flore hépatologique. En effet, l'analyse des affinités écologiques de cette flore a révélé une dominance de la forme terricole (89%), une nette préférence pour l'ombre (74%) et une prédilection pour les habitats présentant une ambiance humide (71%). Enfin, une AFC et une CHA ont permis de distinguer 7 groupes écologiques différents : un groupe hygrophile thermophile mésophotophile sur substrat minéral (2 espèces), un groupe mésoxérophile à tendance sciaphile sur substrat organique (7 espèces), un groupe mésohygrophile à tendance sciaphile sur substrat minéral (6 espèces), un groupe mésohygrophile très sciaphile sur substrat minéral (3 espèces), un groupe mésohygrophile à tendance photophile sur substrat minéral (7 espèces), un groupe hygrophile très photophile sur substrat minéral (3 espèces), et un groupe hygrophile photophile sur substrat organique (4 espèces). Ce travail constitue une première base pour l'étude de la flore bryophytique de la Kroumirie. Il souligne cependant l'insuffisance de la connaissance de ces végétaux en Tunisie, et ouvre de larges perspectives de recherche dans le but d'établir une liste complète actualisée des bryophytes tunisiennes, de mieux comprendre leur écologie, d'analyser leur signification biogéographique, et de définir leurs enjeux de conservation, afin de proposer des mesures conservatoires.



C. ORALE N°:39.

EFFECT OF MONO-AMMONIUM PHOSPHATE UPSTREAM AND DOWNSTREAM OF THE PURIFICATION ON GROWTH AND FRUIT YIELD OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL)

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MAP fertilizer production causes serious environmental problems, such as heavy metal pollution, radioactivity pollution, and phosphogypsum abandon. Thus, with a view to reduce the indiscriminate use of chemical fertilizers an effective approach is needed to eliminate impurities. In the present work, we are interested in eliminating impurities from industrial MAP and to study its effect in field grown tomato. The aim of this work is to evaluate the effect of purified MAP and unpurified MAP on growth parameters and yield of tomatoes.

An in vivo study of purified and unpurified MAP was performed on tomato for three different concentrations (0.5g/l, 1g/l, and 2g/l). The effect of MAP is tested on tomato growth parameters and fruit yield.

Growth parameters and yield of tomatoes were affected by MAP and varied among different treatments. All these treatments showed increased trends compared to untreated control. The effect of these treatment on leaf number, plant height and stem diameter depend on the used concentration and the type (purified/unpurified) of MAP as well as on the number of fruit (green, red and total). The lowest fruit numbers were obtained by plants treated by unpurified MAP, compared to untreated plants, and plant treated by purified MAP, respectively. Similarly, fruit weight significantly improved when plants were treated by purified MAP, compared to untreated plants and plants treated by unpurified MAP. Integrating 1g/L of purified MAP gave the best yield (176.66 g/plant). The purified MAP affected intensively chlorophyll a and b, compared to untreated control.

The concentration 1 g/l for purified monoammonium phosphate showed the most vigorous and mature plants.

Keywords: *Lycopersicon esculentum*, MAP, Purification, Growth, Fruit yield

C. ORALE N°:40.

L'EFFET DE L'INTERACTION COMBINEE AMMONIUM/NITRATE ET NaCl SUR LES PARAMETRES PHOTOSYNTHETIQUES DE LA NIGELLE (*NIGELLA SATIVA*).

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La salinisation des sols est l'une des contraintes majeurs qui limite le rendement des terres cultivées et menace le couvert végétal dans les régions arides et semi- arides. Ces sols, sont aussi caractérisés par une très faible disponibilité en azote minéral. L'objectif de notre travail est donc d'étudier l'effet combiné de ces deux contraintes sur la nigelle (*Nigella sativa*), une plante annuelle dicotylédone appartenant à la famille des Renonculacées. Elle est connue pour ses usages culinaires et médicinaux. Le traitement appliqué, consiste à utiliser quatre rapports $\text{NO}_3^-/\text{NH}_4^+$ (en mM), selon les proportions suivantes: 2.5/0; 1.25/1.25; 0.75/1.75 et 0/2.5 additionné ou non de 60 mM NaCl.

Pour cela, les graines ont été mises à germer dans des boîtes de Pétri. A l'émergence de la racine, les plantules, âgées de 12 jours, ont été transférées dans un milieu hydroponique (solution de Hoagland, carencé en azote, 0,8 mM) durant trois semaines, dans des conditions contrôlées. A l'âge de 33 jours, le traitement nitrate/ammonium a été appliqué, pendant 14 jours. La récolte des plantes a été réalisée. Des mesures de croissance des plantes et des paramètres photosynthétiques ont été effectuées.

Les résultats obtenus ont montré que la présence du chlorure de sodium a diminué la croissance des plantes cultivées sur milieu strictement nitrique et mixte. Cette perturbation se traduit par une diminution variable des paramètres des échanges gazeux photosynthétiques (conductance stomatique, assimilation photosynthétique et transpiration) et de la teneur en chlorophylles. Par contre, les plantes cultivées sur milieu strictement ammoniacal ont montré un meilleur comportement face à la contrainte saline, se traduisant par une meilleure assimilation photosynthétique.

Mots clés : Nigelle, NaCl, ammonium, nitrate, photosynthèse.



C. ORALE N°:41.

MOLECULAR CHARACTERIZATION OF AN AP2/ERF TRANSCRIPTION FACTOR ISOLATED FROM A TUNISIAN VARIETY OF DURUM WHEAT

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In Tunisia, durum wheat yield is too low to guarantee food security. This is mainly due to abiotic stresses. Drought and salinity stress tolerance is very complex and involves many protective and regulatory proteins such as transcription factors. Therefore, we studied the ERF (ethylene responsive factor) transcription family which represents one of the most important families of transcription factors in plants. One novel gene, *TdSHN1*, was isolated from a Tunisian durum wheat variety. To investigate the implication of *TdSHN1* in stress tolerance, its expression profile, following abiotic stress treatments such as, salinity, drought, cold and hormones, was determined. In addition, the subcellular localisation of TdSHN1 protein was verified using a translational fusion with the GFP protein and confocal microscopy. Finally, the binding to its specific cis-elements and its transactivation capacities were assessed, by agroinfiltration technique, using synthetic promoters, the GUS reporter gene, and an effector construct. The ability of TdSHN1 to confer stress tolerance was tested in yeast system.

Keywords: abiotic stress, transcription factor, durum wheat

C. ORALE N°:42.

TEMPERATURE EFFECTS ON TUBER PRODUCTION, CARBOHYDRATE ACCUMULATION AND PARTITIONING IN POTATO (*SOLANUM TUBEROSUM* L.)

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The aim of this work was to assess responses of mid-early and mid-late potato cultivars to different temperature regimes during subsequent stages of potato growth. Experiments were carried out with Spunta (mid-early) and Bellini (mid-late) cultivars. The impact of high temperature (25/22°C day/night), low temperature (18/16°C day/night) and intermediate temperature (20/18°C day/night) was tested for different growth stages. Data were obtained for photosynthesis, carbohydrates in leaves, stems and tubers as well as production parameters. Enzyme activities were determined for sucrose-phosphate synthase in leaves, acid invertase in stems and acid and neutral invertases in tubers. Gene expression levels were analyzed for AGPase, *StInv6* and *StSUS4*.

A detailed correlation analysis revealed a strong impact of the expression level of sugar metabolizing enzymes in leaves on the final tuber outcome.

Whereas total tuber yield increases with temperature, the number of tubers per plant was highest under low temperature conditions. Our data suggest an important role of the temperature on the length of the different growth stages.

Keywords: *Solanum tuberosum*, Temperature, Carbohydrate partitioning, Enzyme activity, Gene expression, Yield



C. ORALE N°:43.

SPENT COFFEE GROUND A PROMISING SOIL AMENDMENT: EFFECT ON SOIL CHARACTERISTICS AND PLANT PHYSIOLOGY

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The sustainability of production is a major challenge to agriculture. This is especially based on the organic soil fertility which mainly depends on its richness in organic matter. To improve the fertility there is recourse to the use of organic amendments, among other household waste. Hence this work, aims to valorize spent Coffee grounds as an organic amendment for some plants. This will improve soil fertility, productivity and sustainable waste management. The preliminary study involved two components namely the impact of the addition of spent ground coffee on soil physicochemical and biological characteristics and its impact on the germination capacity and the physiological behavior of some plants especially broadbean

We were then able to show an increase in organic carbon content directly related to the increase of the dose of spent ground coffee. In addition, the soil respiration monitoring in soil spent ground coffee mixtures showed the presence of bacterial communities resistant to phenolic compounds. The tests of germination and growth showed that the germination rate broadbean is more important when using spent coffee ground as amendment. In addition we showed amelioration in photosynthetic rate and stomatal conductance.

These results suggest the effectiveness of the coffee grounds as an amendment. These studies must be pushed further by studying the effect of coffee grounds on plant growth and therefore test its effect on a larger scale by studying its effect on other plants like olive trees.

Keywords: spent ground coffee, soil, germination, broadbean, physiological parameters

C. ORALE N°:44.

STUDY OF THE EFFECT OF WATER STRESS ON THE BEHAVIOR OF THE LEAVES AND FRUITS OF TOMATO PLOVDIV

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In nature, plants are often exposed to a multitude of environmental constraints that severely limit crop productivity. Water deficit is one of the factors that most affects agricultural production. The objective of this work is to evaluate the effect of water stress on growth, development and certain metabolic parameters (soluble sugars, organic acid, starch and vitamin C) of the cultivated tomato (*Solanum lycopersicum* cv. Plovdiv). The study was carried out during the winter of 2014 in a greenhouse near Avignon (France). The plants were divided into two lots (one control and one stressed). The results obtained showed that the height growth of the different organs (leaves, fruits) is reduced under water stress conditions. Similarly, we observed that water potential was lower in stressed plants as compared to the controls. Water stress induced a significant accumulation of some organic and osmoprotective solutes such as sugars soluble sugars, as well as an increase of fruit antioxidants such as vitamin C.

Key words: Tomatoes, water stress, fruit quality, irrigation management



C. ORALE N°:45.

PROTEOMIC COMPARISON OF THE ALEURONE LAYER IN *TRITICUM AESTIVUM* AND *TRITICUM MONOCOCCUM* WHEAT VARIETIES

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Abstract: The aleurone layer (AL) is an inner tissue of the wheat grain. It contains micronutrients, vitamins, antioxidants and fibre, and can greatly increase the nutritional quality of flour if it is not removed from the kernel with the bran. The AL of mature kernels of three varieties of the two major cultivated wheat species *T. aestivum* (genome A, B and D) and *T. monococcum* (genome A) were manually dissected and analyzed using two-dimensional gel-based proteomics. In *T. monococcum* although composed of only genome A, the maximum number of Coomassie stained AL spots was close to the number found in the bread wheat varieties (1320 and 1258, respectively). Inter-variety variation in spots was higher in the three *T. monococcum* varieties (103 spots) than in the three *T. aestivum* varieties (79 spots). Comparison of the two species revealed that only 88 spots differed significantly either in abundance or presence/absence. The B and D genome did not drastically modify the AL proteome, as demonstrated by the fact that 93% of the spots present in *T. Monococcum* AL spots were also present in *T. aestivum*. Proteins which differed within and between species were identified using MALDI-TOF and LC-MS/MS Mass Spectrometry. Among the 182 spots that differed, 115 were identified, 53 differed between the two species and 44 (83%) were globulin (Glo) storage proteins. The remarkable environmental stability of the AL proteome previously observed in *T. durum* and *T. aestivum* species was confirmed in the variety *T. monococcum* DV92, grown for two consecutive years in field conditions. Only 15 proteins (out of 1320 AL spots) exhibited significant quantitative variations.

Keywords: Aleurone layer, globulins, metabolic pathway, wheat species.

C. ORALE N°:46.

FOLIAR SILICON SPRAYS REDUCE THE DEPRESSIVE EFFECT OF SALT STRESS IN TOMATO PLANTS (*SOLANUM LYCOPERSICUM*)

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Abstract

Silicon (Si) is an excellent growth promoting agent. It has been reported that it increases plant growth and stimulates productivity in various crop plants, under different stressed conditions such as water deficit and salinity. The purpose of this study is to evaluate the effect of exogenous Silicon supplementation on cultured tomatoes salinity tolerance. In fact, tomato seeds (*Solanum lycopersicum*, Var 'Marmande') were germinated in Petri dishes. A hydroponic system is adopted for seedling culture. Culture was carried out for 21 days under controlled conditions of temperature and humidity, in absence/presence of NaCl (100 mM) and Silicon (4 mM). Our study is based on physiological (biomass production, chlorophyll content, mineral nutrition) and biochemical parameters (soluble sugars, proline, total proteins and antioxidant activities). Our results showed that foliar silicon spraying improves tomato seedlings growth. It was attributed to the improvement of green pigment (Total chlorophyll), proline and proteins contents and antioxidant enzyme activities enhancement (CAT, APX).

Key-words: Salinity, *Solanum lycopersicum*, Silicon, Tolerance.



C. ORALE N°:47.

EVALUATION OF THE MYCORRHIZAL INFECTIVITY OF *RETAMA MONOSPERMA* RHIZOSPHERIC SOILS IN MOROCCO.

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Legumes represent an important and varied family of angiosperms. The genus *Retama* belong to this endemic family of the Mediterranean basin, is distributed in different bioclimatic stages, from humid to arid and it characterizes the dune ecosystems, scrubland and desert. In Morocco it's a eu-monosperma subspecies, *Webbii* variety that is prevalent on the Atlantic coast from Tangier to Sous.

Retama monosperma, object of our study, is particularly developed on the coastal road linking Safi to Souira kedima, along the Marrakech Safi road and along the road (A7) that connects Marrakech to Casa. It has the property of establishing symbiotic mycorrhizal associations favoring the biofertilization of saline and poor soils in which they thrive.

The objective of this study is to evaluate the impact of *Retama monosperma* on the biological and chemical soil fertility in the Marrakech-Safi region through the evaluation of the mycorrhizal soil potential and the study of the richness and diversity from the community of arbuscular mycorrhizal fungi. The results obtained showed a higher mycorrhizal potential in *Retama monosperma* Rhizospheric soils, 829.30 infectious propagules / 100 g of the soil were highlighted by the most probable number method, as for the non rhizospheric soils contained only 14,71 infectious propagules / 100 g of soil. The isolation of the spores revealed the presence of 4 mycorrhizal fungi morphotypes with different arbuscules according to their predominant color.

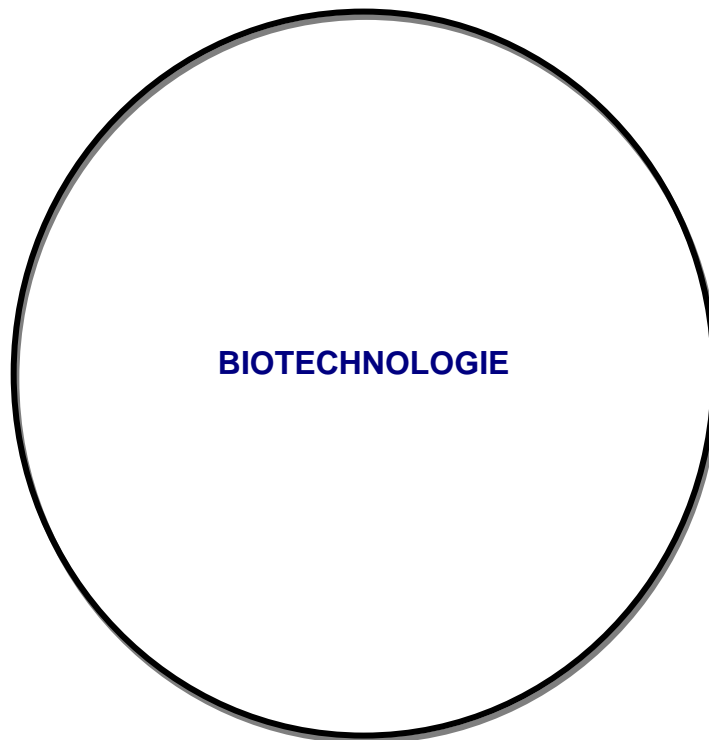
The rhizosphere soil of *Retama monosperma*, forms endomycorrhizal arbuscules symbioses with a colonization rate of important root systems, which favor the soils biofertilization and the fight against desertification.

Keywords: *Retama monosperma*, mycorrhizal potential, Arbuscular mycorrhizae, Morocco.



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C. ORALE N°:48.

IN SILICO IDENTIFICATION OF KNOWN OSMOTIC STRESS RESPONSE GENES FROM MEDICAGO TRUNCATULA IN FABA BEAN (VICIA FABA L.)

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In Tunisia, faba bean (*Vicia faba* L.) is the major grain legumes cultivated. Abiotic stresses, leads to a significant reduction in faba bean plant growth, productivity and yield. The present study considered transcriptional profiles and protein expression analyses from leaf and/or root tissues under two abiotic stress conditions (salinity and drought) as well as following exogenous abscisic acid treatment, at different time points (0, 3, 6, 12, 24 and 48 h) of stress exposure in two faba bean genotypes, Giza 3 (salt and drought sensitive), and Bachar (drought and salt tolerant). A total of 25 genes revealing high homology with known genes involved in drought and salt stress response in *Medicago truncatula* were identified in the faba bean through an *in silico* approach. Relative water content (RWC) level, malondialdehyde (MDA), hydrogen peroxide (H₂O₂), proline and soluble sugars content were determined. The expression profiles of these identified genes in response to NaCl, PEG-6000, and abscisic acid (ABA) were assessed using real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Gene expression profile revealed considerable differences between Giza 3 and Bachar faba bean genotypes, as the expression in the latter was higher even at the constitutive level, whereas it was inducible only by corresponding stress signals in Giza 3. Whether in roots or leaves, heat map data showed that differential transcript abundance in the both genotypes, correlating the observation with transcript profiling. The generality of stress responses at the transcriptional level was therefore time dependent. Overall, the transcriptome analysis undertaken in the present study indicated that genes/proteins conferring tolerance, belonging to different functional classes, were overrepresented, thus providing novel insight into the functional basis of multiple stress tolerance in faba bean species. These results could help to improve understanding of the mechanism of faba bean osmotic stress response and tolerance.

Key Words: Gene expression, Faba bean, In silico analysis, Osmotic stress, RT-PCR

C. ORALE N°:49.

PROBIOTIC PROPERTIES OF ENTEROCOCCUS FAECALIS OB14 AND OB15 ISOLATED FROM TUNISIAN TRADITIONAL FERMENTED DAIRY PRODUCTS

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Lactic acid bacteria (LAB) strains OB14 and OB15 were isolated from tunisian traditional fermented dairy products, cheese and Ricotta, respectively, and evaluated for functional properties and probiotic characteristics. They were identified as *Enterococcus faecalis* by phenotypic (colony morphology, Gram staining and catalase assay), and molecular assays (species-specific PCR), and by the spectrometric MALDI (matrix assisted laser desorption-ionization) biotyper system. *E. faecalis* OB14 and OB15 were compared to *E. faecalis* Symbioflor 1 clone DSM 16431, as probiotic reference and found to be tolerant to acidity and bile salt. The results also showed that the tunisian isolates adhere to Caco-2/TC7 intestinal cells and were not cytotoxic. These bacteria strengthened the intestinal barrier through the increase of the transepithelial electrical resistance (TER), and *E. faecalis* OB14 was able to stimulate the secretion of the IL-10 anti-inflammatory cytokine. Finally, the two new isolates were tested for susceptibility to ampicillin, vancomycin, gentamicin and erythromycin, and the results showed the same pattern as *E. faecalis* Symbioflor 1 clone DSM 16431. In conclusion, *E. faecalis* OB14 and OB15 may be seen as reliable new probiotic candidates for further in vivo validation and use in the food industry.

Keywords : *Enterococcus faecalis*, probiotic, adhesion, cytotoxicity, transepithelial resistance, antibiotic susceptibility.



C. ORALE N°:50.

ANTI-INFLAMMATORY EFFECTS OF ESSENTIAL OILS FROM *ROSMARINUS OFFICINALIS* AND *POPULUS ALBA* ON EXPERIMENTAL MODELS OF ACUTE AND CHRONIC INFLAMMATION IN RATS

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Objective / Purpose: Essential oils (EOs) distilled from aromatic plants have a wide range of uses because of their rich pharmacological activities including, anti-inflammatory, antitumor, antimicrobial, antioxidant, antidiabetic and hepatoprotective. The present research aimed to investigate in-vivo anti-inflammatory effects of EOs from *Rosmarinus officinalis* and *Populus alba* as biomarker levels in well-defined acute and chronic inflammation models. Material and Methods: The anti-inflammatory activity of EOs of *R. officinalis* and *P. alba* was carried out using two models, one of them is acute inflammation that induction by dextran 1% at the rat paw¹ and another is chronic inflammation that induction by monoiodoacetic solution². To evaluate the anti-inflammatory properties of essential oils, a measure of paw thickness was carried out with the calculation of percent inhibition. Thus, monitoring of some biomarkers will be paramount. Results / Discussion: The results indicate the absence of severe clinical signs or dead in rats during the observation period. Therefore, the essential oils of *R. officinalis* and *P. alba* are devoid of acute toxicity in rats. For treatment with the essential oil of *R. officinalis*, *P. alba* and Diclofenac®, the results showed a significant reduction for responses induced by dextran. Treatments produced reductions in inflammation ranging from 2.19 to 15.15%. Treatment with essential oils showed a recovery of values of biochemical markers during the experimental period. Conclusion: Consequently, the EOs of *R. officinalis* and *P. alba* demonstrate anti-inflammatory activity on both acute and chronic inflammation models, thus they could be used for pharmacological purposes as anti-inflammatory agents.

Keywords: Anti-inflammatory, Essential oils, Experimental model, *Rosmarinus officinalis*, *Populus alba*.

C. ORALE N°:51.

EFFECTS OF PROBIOTIC STRAINS, *LACTOBACILLUS PLANTARUM* TN8 AND *PEDIOCOCCUS ACIDILACTICI*, ON MICROBIOLOGICAL AND PHYSICO-CHEMICAL CHARACTERISTICS OF BEEF SAUSAGES

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Abstract : This study was undertaken to evaluate the effects of a combination of probiotic strains namely, *Lactobacillus plantarum* TN8 and *Pediococcus acidilactici* MA 18/5M and dietary fiber on microbiological and physico-chemical characteristics of sausages stored at 4°C up to 12 days. *L. plantarum* TN8 might be considered a potential candidate for application as probiotic agent for food additive in the meat industry. Microbial counts revealed a decrease in *enterobacteriaceae* of inoculated sausages, which reached values below 2 log CFU/g in the stored product. Sausages reformulated recorded good textural attributes and improved sensory analysis. At the end of storage period, sausages with different probiotic strains decreased the b* color parameter and cooking loss when compared with control samples. Sensorial and color parameters could be used for constructing regression models to predict overall acceptability. On the other hand, the free fatty acid profile was not significantly affected by probiotic strains. Therefore, endogenous lipases contributed to the release of free fatty acids. Overall, the inclusion of two probiotic strains and fiber improved the hygienic quality and sensorial features of sausages.

Keywords: beef sausage; probiotic strains; dietary fiber; microbial counts; physico-chemical analysis.



C. ORALE N°:52.

SACCHARIFICATION DES MACROALGUES VERTES PAR DE NOUVELLES CELLULASES THERMOSTABLES PRODUITES PAR UNE SOUCHE SPECIFIQUE D'ASPERGILLUS FUMIGATUS

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Mots clés : Macroalgues, cellulases, saccharification, optimisation, Aspergillus

Résumé : Les algues marines représentent une biomasse abondante disponible sur les côtes Tunisiennes, souffrant du problème d'eutrophisation, mais elle reste très peu valorisée. Trouver une approche efficace pour le traitement de ces déchets algaux et leur bioconversion énergétique pourrait être une bonne solution de bioremédiation des marées vertes. Nous nous intéressons dans ce contexte à la saccharification enzymatique de cette biomasse algale via la production de nouvelles cellulases spécifiques thermostables. Un champignon cellulolytique a été isolé à partir de macroalgues vertes et identifié, par des observations microscopiques et analyse moléculaire, comme *Aspergillus fumigatus* SL1. Cette souche, cultivée sur les macroalgues *Ulva* sp. comme seule source de carbone, a permis la production de cellulases spécifiques (activités spécifiques de 30 et 33 U / mg de protéines pour l'endoglucanase et la β -glucosidase, respectivement) différentes des cellulases commerciales comme montré par le zymogramme. L'application du cocktail enzymatique à base de cellulases produit pour l'hydrolyse d'*Ulva* prétraité par la soude, révélé comme meilleur prétraitement pour la déstructuration des algues, a donné un rendement de saccharification de 58%. Ce taux de saccharification a été optimisé en utilisant la méthodologie de surface de réponse (plan d'expérience central composite). Une augmentation de 36% a été obtenue dans les conditions optimisées (CMCase 13 U, substrat 4% et agitation à 135 rpm), ce qui correspond bien aux prédictions du modèle. *A.fumigatus* SL1 peut être un champignon prometteur pour le traitement des déchets d'algues et la production de bioéthanol à partir de cette biomasse.

C. ORALE N°:53.

STUDY OF HEAVY METALS AND ANTIBIOTICS MULTI-RESISTANT BACTERIAL STRAINS ISOLATED FROM MARCHICA LAGOON IN MOROCCO

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The pollution of the environment with toxic heavy metals is spreading throughout the world along with industrial progress. Removal of heavy metals from wastewater needs advance chemical technology and is more expensive too. The cheaper alternative for this is the bioremediation using heavy metals resistant microorganisms.

In the present investigation the strains, isolated from Marchica lagoon in Morocco from water and soil samples, was studied for resistant to heavy metals and antibiotics. Metal-resistant bacteria were selected and minimal inhibitory concentrations of heavy metals (MICs) for each isolate against Cd, Cu, Ag, Pb and Hg was evaluated by comparing results obtained by two tests of toxicity in solid and liquid media.

the experimental results indicated that the MIC determined in solid media were high compared to the MIC values in the liquid cultures. The strains exhibit high minimal inhibitory concentrations for heavy metals and for the same metal, the strains presented different resistance levels at the measurement. Our isolated strains showed a resistance to all tested antibiotics, and more resistance was observed to Ampicillin and amoxicillin.

Keywords: Marchica lagoon, bioremediation, Heavy metals resistant, Strains, MIC, Antibiotics resistance.



C. ORALE N°:54.

OPTIMIZATION OF GIBBERELIC ACID PRODUCTION BY THE ENDOPHYTIC *FUSARIUM OXYSPORUM* USING PLACKETT–BURMAN AND TAGUCHI METHODS, AND EVALUATION OF ITS EFFICACY ON THE TOMATO GROWTH IN SALINITY STRESS.

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Abstract: Four endophytic fungi were isolated and screened for the production of gibberellic acid (GA3) using low cost substrates under solid state fermentation (SSF). The strain which present the highest phytohormone production on submerged and solid state fermentation was identified as *Fusarium oxysporum*. Successive optimization strategies, including Plackett–Burman and Taguchi's (L25) orthogonal array designs, were used to optimize the GA3 production. Using the optimized conditions, the GA3 yield (7.14 g/Kgs) reached the maximum yield derived from the Taguchi method, which increased about 2.62-folds compared to the preliminary condition. (NH₄)₂SO₄ used as an ammonium source had a negative effect on the production of GA3 under SSF. In order to constrain various abiotic stresses affect the agricultural productivity, some strategies have been employed, such as exogenous application of GA3 (as a plant growth regulator), to decrease the bad effect of salinity and enhance growth performance. This study was carried out to investigate the improving role of GA3 on adverse effect of salinity in tomato plants (*Solanum lycopersicum* L.). The interactive effects of different concentrations of GA3 (10⁻⁵, 10⁻⁶ and 10⁻⁷ M) and NaCl (50 and 100 mM) were examined on certain morphological and physiological parameters of tomato plants. Plant growth parameters were affected by salinity. The combined application of GA3 and NaCl improves growth related parameters, total chlorophylls, soluble sugars and starch, proline and ions contents. Consequently, this exogenous application plays an important role to develop salt tolerance in tomato plants.

Keywords: Gibberellic acid, optimization, *Fusarium oxysporum*, Plackett–Burman design, Taguchi Method, salt stress, plant growth

C. ORALE N°:55.

SALICYLIC ACID AND HYDROGEN PEROXIDE IMPROVE GROWTH OF *LENS CULINARIS* UNDER SALT STRESS

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Salinity is one of the major and increasing problems in agriculture. Many methods were used to alleviate the salt effects on plant growth. Salicylic acid and hydrogen peroxide were, two signal molecules, well used to ameliorate tolerance of plants to different biotic and abiotic stress. The aim of this work is to evaluate the effects of Salicylic acid and hydrogen peroxide on growth of *Lens culinaris* under normal (0mM NaCl) and saline (75 mM NaCl) conditions to determine their usefulness to alleviate salt stress. Salicylic acid (SA) and hydrogen peroxyde (H₂O₂) were applied in two ways; pretreatment of seeds and exogenous application. Their effects were investigated through physiological parameters like dry weight, photosynthetic parameters, photosynthetic pigments, mineral nutrition, proline content, H₂O₂ content, MDA content and some antioxidant enzymes. Salinity prevented growth of *Lens culinaris* by decreasing dry weight of shoots and roots. This decrease was due to reducing photosynthesis and photosynthetic pigments content. The mineral nutrition was perturbed by increasing the levels of Na⁺, Cl⁻ and decreasing levels of K⁺ and Ca²⁺. Increasing salt in the medium of culture heightened proline, MDA, H₂O₂ levels and stimulated some antioxidant enzymes (SOD and GPOX activities). All these effects of salt were more or less alleviated by SA or H₂O₂. Then, seed's priming and exogenous application by the two molecules improved the net assimilation of photosynthesis, transpiration and stomatal conductance. A rise in chlorophyll and carotenoid levels were observed. Also, they inhibited absorption of Na⁺ and Cl⁻ ions and induced accumulation of K⁺ and Ca²⁺. SA and H₂O₂ reduced the accumulation of MDA, proline and H₂O₂ in shoots and roots. While, the SOD and GPOX activities were even more induced by SA and H₂O₂ in tissues of stressed plants. The degree of improvement of all these parameters depends on the molecule used (SA or H₂O₂), the way of application and the organ studied. Salicylic acid and hydrogen peroxide may indirectly attenuate salt stress through a general anti stress response of plants, which probably includes the regulation of mineral nutrition, the photosynthesis and the antioxidant system. This suggests that SA and H₂O₂ could be used as potential molecules to improve plant growth under salt stress.

Keywords: *Lens culinaris*, Antioxidant, Nutrition, Photosynthesis, Salt



C. ORALE N°:56.

EFFECT OF A-AMYLASE SOURCE ON ENZYMATIC ACTIVITY IN SORGHUM STARCH HYDROLYSIS

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Sorghum (*Sorghum bicolor* (L.) Moench) exhibit unique starches properties. These properties influence the digestibility of starch in the presence of α -amylases and can be a good raw material for glucose production. The aim of this research is to determine the susceptibility of amylase from different sources: α -amylases from human salivary, α -amylases from fungus (*Aspergillus oryzae*) and α -amylases from bacteria (*Bacillus subtilis*) and show how the source of amylase affects the activities in starch hydrolysis.

Starch sorghum was isolated and purified in the laboratory from white and pigmented seeds of cultivars from Algeria (In Salah). The enzymatic hydrolysis reactions were done in batch bioreactors and the reducing sugars obtained were determined quantitatively and qualitatively using HPAEC-PAD. The kinetic studies of enzymatic catalysis had allowed to identify of optimum operating conditions and to calculate enzymatic activities.

The results showed a significant influence of amylase source on the kind of maltooligosaccharides. The fungal α -amylases is more efficient than bacterial α -amylases.

C. ORALE N°:57.

PRODUCTION AND CHARACTERIZATION OF A BIOSURFACTANT PRODUCED BY PSEUDOMONAS STUTZERI 'STRAIN PYRKP' ISOLATED FROM HYDROCARBON CONTAMINATED SEAWATER

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Abstract: A hydrocarbonoclastic marine bacterial strain PYRKP, isolated as a pyrene degrader (200 mg/l) and affiliated to *Pseudomonas stutzeri*, showed a significant potential to produce biosurfactant reducing the surface tension to 29.5 mN/m, after 3 days of incubation at 37 °C, in the presence of glycerol (3%, v/v), as a substrate. The biosurfactant, designated Bios-PYRKP, retained its properties during exposure to various temperatures (from 30 to 55 °C), salinities (from 0 to 200 g/l NaCl), and pH values (from 6.6 to 9.7). The Fourier Transform Infrared (FTIR) spectroscopy revealed that the chemical structure of this biosurfactant belonged to lipopeptide class. The critical micelle concentration (CMC) of Bios-PYRKP was about 0.25 g/l. Furthermore, biosurfactant produced by strain PYRKP showed an interesting healing activity, on a wound, in a rat model. It increased significantly the percentage of wound closure when compared to the untreated and CICAFLORA (a reference pharmaceutical product)-treated groups, using two different concentrations (5 and 10 mg/l) of Bios-PYRKP. In addition, Bios-PYRKP showed interesting anti-adhesive and anti-biofilm activities being able to inhibit biofilm adhesion and formation of five pathogenic microorganisms.

Keywords: Biosurfactant, Dermal wound healing, anti-adhesive activity, anti-biofilm activity.



C. ORALE N°:58.

ETUDE FONCTIONNELLE D'UN FLAVOCYTOCHROME B558 RECOMBINANT EN SYSTEME IN VITRO

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Résumé :

Le flavocytochrome b558 (Cytb558), hétérodimère de deux protéines membranaires gp91phox et p22phox, correspond à la partie catalytique de la NADPH-oxydase des phagocytes. Ce complexe joue un rôle crucial dans le système immunitaire inné en générant l'anion superoxyde, le précurseur d'espèces réactives toxiques de l'oxygène. Un manque de données structurales sur le Cytb558 est dû à la production insuffisante de protéines pures. La capacité de gp91^{phox} à produire du superoxyde en l'absence de son partenaire p22^{phox} est peu étudiée. Nous avons réussi pour la première fois à générer une forme active de gp91^{phox} bovin dans la levure *Pichia pastoris* capable de réaliser l'activité NADPH oxydase entière en l'absence de p22^{phox}. Ensuite, et dans le but d'optimiser son niveau de production dans *Pichia pastoris*, nous avons exploré l'impact de l'emplacement de His-tag sur les extrémités de la protéine 22phox.

Une production stable de p22phox mature a été observée au niveau de la membrane plasmique des cellules qui expriment seulement p22 avec le tag His en N-ter mais qui était associée à une diminution de la biomasse de levure (limitation membranaire). Nos données soutiennent l'idée que *Pichia pastoris* présente les critères d'un système puissant pour la production du Cytb558 avec une introduction optimale de la protéine de fusion pour une meilleure production du complexe membranaire de la NADPH-oxydase.

(Projet financé par l'Agence Nationale de la Recherche ; ANR)

Mots clés : Flavocytochrome b₅₅₈, NADPH oxydase, *Pichia pastoris*, Ingénierie des protéines.

C. ORALE N°:59.

NEW ALKALINE PROTEASES FROM *BACILLUS INVICTAE* AH1: BIOCHEMICAL CHARACTERIZATION AND EFFECT OF STORAGE TIME ON THE SPRAY-DRIED ENZYMES

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Alkaline proteases have been recently reported as promising candidates for a wide range of biotechnological bioprocesses, especially in detergency. In this sense, the present study reports, firstly, on the biochemical characterization of stable alkaline proteases from a newly isolated *Bacillus invictae* AH1. Considering their promising properties under alkaline conditions, *B. invictae* AH1 crude enzymes could be potentially used in detergents. As a second purpose of this work, the enhancement of proteases stability during storage was investigated. Thus, microencapsulating process of proteases, using spray drying technology, was used to improve their use in industrial processes and commercial products. In fact, in order to protect enzymes against the high temperature and pressures applied during the spray-drying, *B. invictae* AH1 crude enzymes were treated in the presence of glucose, PEG (polyethylene glycol) and CMC (carboxymethylcellulose), as drying adjuvants, at different concentrations (0.5, 1, 2 and 3%). The results showed a decrease proteolytic activity in the absence of additives (-24%). However, only CMC at 0.5% as used as drying adjuvant promoted the best retention of enzymatic activity (116%), compared with control. More interestingly, the CMC (0.5%) was the best polymer used to protect the proteolytic activity which remains stable after 90 days of storage at 25 °C. To better understand the biochemical findings, the micro-particulates (conjugated or not) produced by spray drying of *B. invictae* AH1 crude enzymes were analyzed by scanning electronic microscopy.

In conclusion, the results revealed that the proteases from *B. invictae* AH1 were highly stable and active at high temperature and alkaline pH, maintained their activity during spray-drying and remained stable during long-term storage; which encourage their future use in several industrial applications.



C. ORALE N°:60.

TOWARDS INCREASED BIOAVAILABILITY AND ENSURE PRESERVATION OF VITAMINS IN FENUGREEK AND CAROB SEED BY THE INSTANT CONTROLLED PRESSURE DROP PROCESS (DIC PROCESS)

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Fenugreek and carob seed were germinated in the dark for 4 days and 15 days respectively. The two species are rich in vitamins A, B1, B3, B8. Germination promotes the increase in their concentrations. To ensure preservation and increased bioavailability of the vitamins, it is necessary to apply a DIC of 30s to 0.4mPa making it possible to support the cellular expansion and the decontamination of germinated seeds. Vitamin A content in germinated carob seed increased by 82,54%, which is not the case for fenugreek. A reduction of vitamin A content is the order of 4 µg per 100 g of the dry matter. DIC increase vitamin B1, B3 and B8 in germinated fenugreek seeds. A slight decrease of B vitamins in germinated carob seed after treatment with DIC has been noticed. This loss can be explained by the low bioavailability caused mainly by the botanical structures of the seeds.

Keywords: fenugreek; carob; seed; germination; preservation; bioavailability; vitamin; Instant Controlled pressure Drop (DIC).

C. ORALE N°:61.

PIGMENTS OF FUSARIUMVERTICILLIOIDES AND THEIR TOXICITY ON TUMOR CELL LINES

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The demand for natural colors is increasing day by day due to harmful effects of some synthetic dyes. Production of pigments by filamentous fungi is gaining interest owing to their use as food pigments, in cosmetics and textiles, and because of the important biological properties of these compounds such as antioxidant, antimicrobial and anticancer activity. In contrast to other natural pigments, they have enormous advantages including rapid fungal growth, easy processing, and independence of weather conditions.

We studied the production of pigments from a strain named E4-2, isolated from soil and identified as *Fusariumverticillioides*. It has the ability to produce different pigments depending on environmental factors such as carbon sources, nitrogen sources, pH, temperature...). Glycerol, maltose and saccharose as carbon sources and sodium nitrate, ammonium nitrate, urea and yeast extract as nitrogen ones impacted the production of red pigment. Intra and extracellular pigments were extracted using ethanol as solvent and fractionated by thin layer chromatography. These extracts were tested on various cell lines using MTT assay. Intracellular extract showed a significant inhibitory effect on four cancer cell lines, Hela cervix carcinoma, MCF7 breast cancer, HepG2 human hepatocellular carcinoma and HT29 colon carcinoma with IC50 values 118 µg/ml, 78 µg/ml, 183 µg/ml and >1000 µg/ml, respectively. Extracellular extracts had the most potent effect against three cancer cell lines, with IC50 value 42 µg/ml on MCF7, 84 µg/ml on HepG2 and 466 µg/ml on HT29. The Thin-layer chromatography showed that the obtained extracts are heterogeneous and contain a mixture of several colors, red, yellow and purple. The comparison between the IC50s of the most active fractions showed that the single violet fraction is the most toxic in both intracellular and extracellular extracts.

Keywords: pigment extraction, cancer cell lines, MTT assay, toxicity

**C. ORALE N°:62.****CARACTERISATION DES MICROALGUES D'INTERET NUTRITIONNEL ET APPLICATION DANS LA FORMULATION D'UN NOUVEAU PRODUIT : « RICOTTA ENRICHIE AUX MICROALGUES »****KHEMIRI SHEYMA, NADIA BERREJEB, ISSAM SMAALI, NADIA KHELIFI***Laboratoire d'ingénierie des protéines et des molécules bioactives, Université de Carthage, 8 INSAT- BP 676, Centre urbain nord, 1080 Cedex Tunisia*

Les microalgues sont utilisées dans plusieurs secteurs industriels nécessitant ainsi, une bonne connaissance de leur composition biochimique. Notre étude a porté sur l'évaluation de l'effet de quatre milieux de culture sur la composition biochimique de deux espèces de microalgues d'intérêt nutritionnel : *Chlorellavulgaris* (*Ch1*), *Nanochlorepisgaditana* (*N2*), ainsi qu'un échantillon de *Spiruline* commerciale de Gatrana. Les résultats montrent que les espèces contiennent entre 6 et 38,9 % de lipides et que le milieu f/2 (pauvre en N et P) a marqué le meilleur rendement. De même, les taux les plus élevés en sucres totaux pour *Ch1* et *N2*, sont obtenus dans le même milieu (34% ; 44% respectivement). Pour le dosage des composés phénoliques *Ch1* a présenté le meilleur taux 45,52 mg EAG/gMS, Pareillement pour la teneur en caroténoïdes *Chlorellavulgaris* (*Ch1*) a présenté le taux le plus élevée (2,45 mg/g ME) dans le milieu BG11. Dans ce même milieu les extraits méthanoliques de *N2* et *Ch1* ont montrés des pouvoirs d'inhibition intéressants contre le radical libre DPPH. Le test d'évaluation préliminaire du nouveau produit « Ricottalgue » a donné des résultats encourageant.

Mots clés : microalgues, Lipides, sucres, Composés phénoliques, Caroténoïdes, Activité antioxydante, Ricottalgue

C. ORALE N°:63.**CHARACTERIZATION AND KINETIC STUDY OF AN IMMOBILIZED B-AMYLASE EXTRACTED FROM PERGULARIA TOMENTOSA****LAHMAR IMEN^{1,4*}, GRETA RADEVA², DESSISLAVA MARINKOVA¹, MAYA VELITCHKOVA³, HAFEDH BELGHITH⁵, FERJANI BEN ABDALLAH⁴, LYUBOV YOTOVA¹, KARIMA BELGHITH⁶***1 Department of Biotechnology, University of Chemical Technology and Metallurgy, Sofia, Bulgaria**2 Department of Physical Chemistry, University of Chemical Technology and Metallurgy, Sofia, Bulgaria**3 Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria**4 Laboratory of Plant Biodiversity and Dynamics of Ecosystems in Arid Environment, Faculty of Sciences, Sfax, Tunisia**5 Laboratory of Molecular Biotechnology of Eukaryotes, Center of Biotechnology, Sfax, Tunisia**6 Laboratory of Plant Biotechnology Applied to Crop Improvement, Faculty of Sciences, Sfax, Tunisia*

β -amylase (EC 3.2.1.2) defined as an exo-amylase, hydrolyses α -1,4 glycosidic linkages of polyglucan chains at the non-reducing end to produce maltose (4-O- α -D-Glucopyranosyl- β -D-glucose) during starch degradation. *Pergularia tomentosa*, a wild plant growing in Mediterranean areas, was reported in previous study as a rich source in polyphenol oxidases, tyrosinase, lipase, liopxygenase, L-asparaginase and rennet.

In the present work, β -amylase extracted from *Pergularia tomentosa* was characterized by 1.98 U/mg protein of enzyme activity and a molecular weight of 24 KDa. The enzyme was immobilized onto Titanium dioxide-based hybrid materials composed of an organic polymer of cellulose acetate butyrate and a copolymer of acrylonitrile and acrylamide. Immobilized β -amylase was highly active at pH 7.0, 70°C and thermal-stable at 60°C. The kinetic relations in function of the time-temperature were investigated in the course of the β -amylase hydrolysis of starch by the means of the conversion degree α . It was found that the Modified Prout-Tompkins topochemical equation well describes the kinetic process of the immobilized enzyme. The rate depended on the size and the changing contact surface. The power factor χ decreased with temperature increasing, while the dependence of the apparent rate constant k increased. The decrease of the current rate v of the process, 7.91% at 70 °C, was more pronounced at 60 and 70°C and was due to the change in the number and the accessibility of active centers on the surface.

The factor $\alpha^{\frac{\chi-1}{\chi}}$ decreased with α and takes into account the inactivation effect on the enzyme treatment, especially after the 90 min of the process, when the rate slowed significantly. In addition to the increase of the pre-exponential factor A, the activation energy E increased from 4.71 to 14.35kJ/mol.

Key words: β -amylase, *Pergularia tomentosa*, immobilization, Modified Prout-Tompkins topochemical equation, conversion degree.



C. ORALE N°:64.

ISOLATION AND CHARACTERIZATION OF ENTOMOPATHOGENIC FUNGI FROM TUNISIEN SOIL

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Background and aim: Entomopathogenic fungi are soil-dwelling microorganisms that infect host insects, and some have been developed as commercially available biopesticides. Knowledge of the composition and distribution of native entomopathogenic fungal species are essential to evaluate the potential of biological control in a given ecosystem. Diversity and abundance of entomopathogenic fungi depend on many factors such as vegetation, presence of host and abiotic conditions. The aim of this work is to select indigenous isolates of entomopathogenic fungi useful as biocontrol agents in their original habitat.

Methods: In this study, entomopathogenic fungi were isolated from soil using selective media. Fungal isolates have been identified morphologically and molecularly. The thermotolerance of these isolates and their pathogenicity against *Aphis gossypii* (Hemiptera: Aphididae), *Dacus frontalis* (Diptera: Tephritidae) and *Euphyllura olivina* (Hemiptera: Psyllidae) were investigated.

Results: Entomopathogenic fungi were isolated from 88% of soil samples. The most frequently species was *Isaria* sp. (74%), followed by *Beauveria* sp. (40%) and *Metarhizium* sp. (34%). The assays identified a number of *Beauveria* and *Metarhizium* isolates with virulence equal to or greater than that of the commercial strains against *Aphis gossypii*, *Dacus frontalis* and *Euphyllura olivina*. Isolates that have a broad spectrum of action and others that are specific for an insect species were selected. High variability in conidial thermotolerance was found among the *Beauveria* sp. and *Metarhizium* sp. isolates after exposure to 45°C. Six *Metarhizium* sp. isolates that have the ability to germinate after a heat treatment at 45°C for 8 hours were selected. However, only two isolates of *Beauveria* sp. demonstrated significant greater thermotolerance were selected.

Conclusion: Based on their thermotolerance and their highly toxicity, some of the examined fungal isolates may have potential as biological control agents.

Keywords: Entomopathogenic fungal, Biopesticides, Pathogenicity, Thermotolerance.

C. ORALE N°:65.

CHARACTERIZATION AND BIOLOGICAL CONTROL OF CLEMENTINE TRUNK AND BRANCH CANKER CAUSED BY *PHYTOPHTHORA CITROPHTHORA* IN TUNISIA.

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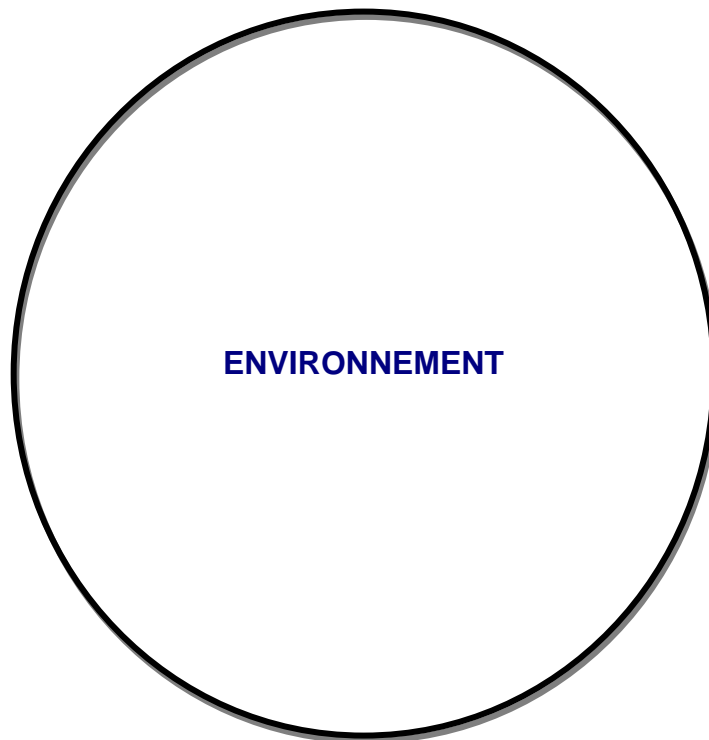
Clementine production has been expanding continuously in the last few years to reach the second place of citrus production in Tunisia right after the Maltese and the third place of citrus exports (GIFruits, 2018). This progress comes after the introduction of new varieties such as 'Marisol' and 'Hernandina' via the FAO's program in 1996 from Corsica, France. Since 2015, in the Cap Bon peninsula, a new devastating disease was observed in clementine orchard by farm advisors. This disease is characterized by trunk and branch cankers and gum exudation leading to death of the trees. This new syndrome was reported in Spain in 2008 (Alvarez, 2008) and in South Africa in 2010 (Schutte, 2010), characterized by gum exudation, browning of the crust, cracking and canker. In our study, the sampling campaign of spring/autumn 2016 in the northern region of Tunisia leads to a collection of 35 isolates of *Phytophthora* spp. Isolated from characteristic symptoms of trunk gummosis and branch canker. All the isolates were identified by amplifying and sequencing the region DNAr ITS1/ITS4. In this sampling campaign we have reported a new disease causing trunk and branch canker in Clementine and we have found that the predominant species was *Phytophthora citrophthora*. In order to develop a solution based on biological control using antagonistic bacteria, a collection of rhizospheric bacteria was tested by dual culture toward the pathogen. These in vitro tests allowed us to select 5 highly antagonistic bacteria belonging to the genus *Bacillus* and *Pseudomonas* identified by amplifying and sequencing the region DNAr 16S. All of these strains have the ability to produce cellulase, protease, Phosphatase and siderophore. The strain S31 *Paenibacillus polymyxa* had the ability to produce the phytohormone acid 3 acetic indoles "Auxine". The greenhouse trials demonstrated that the strain M-316 was the most effective to suppress the branch canker on Clementine mandarin with 50% reduction in the lesion length.

Key words: trunk gummosis, branch canker, *Phytophthora*, biological control, *Bacillus*



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C. ORALE N°:66.

THE ENVIRONMENT IN ALL ITS FORMS

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Of a masculine gender, derived from the verb environner meaning action to surround, the environment is defined as the set of biotic elements of abiotics that surround an individual or a species and some of which contribute to provide for its needs.

Designated under the term "environnement" in French since 1265 in the sense of "circuit, contour", polysemous it must be said, the environment today encompasses both natural environments, the impacts of man on this entity, than the actions taken to reduce them.

Historically, its purpose is to retrospectively study the state of this environment at different times, from the pre-19th century to the present day, and its interactions with human activities, the realization of its existence. 'being developed by wave and in different ways according to the times, the regions and the human cultures.

Ubiquitous, the environment has "squatted" art in multiple components, to see today this science used with out moderation to address the awareness of the public in defense of this environment.

As for the environmental sciences, it is not enough to say that science has experienced a tremendous development in the last century, some disciplines, such as ecology, having been specially dedicated to it

Until then, environmental agencies or observatories have emerged in many countries, such as the United Nations Environment Program (UNEP). Without transition, bring to light the sinister impacts of man on the environment. Thus, having become largely majority at the end of the 20th century, the idea of a degradation of the environment of the Earth by the effect of the pollution, effect taking the form of a global ecological crisis, the hand of the Man at this level no longer to demonstrate.

Also, to measure this degradation, we can, not without shame, quote several indicators that are apparent pollution, namely traces of compounds synthesized by humans in natural environments such as soil, air and water. We will talk about the quality of water and air, without concealing the scarcity of natural sources and the loss of biodiversity, demography and economic development being scapegoats for excellent. We will also talk about ecological or environmental disasters, a vocabulary that has become familiar in these times.

In conclusion the observation of the environmental degradation being consumed, it is imperative to deploy, all-out, all that is obligation to everyone to reduce at best the degree of nuisance of this undeniable social evil, preservation of the survival of humanity requires

C. ORALE N°:67.

BIOCONTROL OF WHEAT FUSARIUM CROWN AND ROOT ROT BY TRICHODERMA STRAINS

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Fourteen strains of *Trichoderma* spp. were isolated from Algerian desert soils and assessed for their antagonistic activity against *Fusarium* crown and root rot of wheat. *Trichoderma* spp. efficiency was carried out by in vitro and in vivo based bioassay against three species of the pathogen agent: *F. culmorum*, *F. graminearum* and *F. verticillioides*. By in vitro based bioassay (dual culture) results obtained showed high decrease of pathogen colony growth compared to the control with all *Trichoderma* spp. isolates. The highest percentages of diameter colony reduction were obtained with *T. harzianum* Thr.4 causing a growth reduction of 70.68%, 67.05 and 70.57 % against *F. culmorum*, *F. graminearum* and *F. verticillioides* respectively. Once more all *Trichoderma* spp. isolates were able to overgrow and sporulate above *F. Culmorum* colony but no overgrowth was observed with *F. graminearum* and *F. verticillioides*. The seed treatment by *Trichoderma* spp. isolates before sowing in a soil already infested by the pathogen led to a significant decrease of disease severity compared to the untreated control. The highest disease index decrease (>70%) was obtained with the two isolates of *T. harzianum* (Thr.4 and Thr.10) and *T. viride* Tv.6 against the three fungal pathogens. Lytic enzymes production by *Trichoderma* spp. isolates was tested in liquid cultures containing fungal cell walls of each pathogen as sole carbon source. Higher levels of protease and chitinase activities were induced by hyphal cell walls of *F. graminearum* than cell walls of *F. verticillioides* and *F. culmorum*.

Keywords: *Trichoderma* spp., biological control, *Fusarium* spp., wheat, cell wall degrading enzymes.

**C. ORALE N°:68.****BIOCHEMICAL PROPERTIES OF NOVEL DYE-DECOLORIZING HEME PEROXIDASES FROM THE WHITE-ROT FUNGI FOR LIGNIN BIODEGRADATION****JAOUADI BASSEM^{1,*}, H REKIK¹, N ZARAÏ JAOUADI¹, K BOUACEM², A BOUANANE-DARENFED², BILAL ZENAT³, SIDALI KOURDALI³, M EL HATTAB⁴, S BEJAR¹, R ANNANE³ & A BADIS^{3,4}**

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Synopsis: At present lignocellulose is a major raw material for forestry, pulp and paper industry and the emerging second generation biofuel production. Among cellulose and hemicellulose, lignin is a major component of lignocellulosic biomass and largely responsible for its strength and renewable source of green energy. Inside the Northern coniferous forest belt the importance of lignin utilization is stressed in wood-based biorefineries due to high amounts of lignin in softwoods. Because of its recalcitrance, lignin complicates the utilization of biomass polysaccharides in biorefineries and increases the energy consumption in mechanical pulping. In nature one group of organisms, the basidiomycetous fungi are able to effectively degrade lignin by employing a family of lignin degrading enzymes. Fungal attack on lignin is attributed to certain secreted nonspecific oxidoreductases, which produce low molecular weight mediators able to intrude recalcitrant biopolymers. In the other hand, humic acids are the most explored group of humic substances. During the disinfection process, those compounds react with chlorine and produce intermediary products called THM, which poses several harmful effects on human health and the environment. In fact, there is a large body of literature showing that a number of naturally-occurring phenolic monomers can undergo oxidative coupling reactions catalyzed by oxidative enzymes (laccases and peroxidases), to produce humic substances-like. Microbial peroxidases have often been reported to constitute a resourceful class of enzymes with promising industrial applications. The biochemical and biochemical characterization of the pure peroxidases were investigated through physico-chemical determination as well as spectroscopy analysis. Accordingly, this study reports, for the first time, the purification and biochemical characterization of a novel three peroxidases MnP TP55 from *Trametes pubescens* strain i8; and LiP BA45 and MnP BA30 from *Bjerkandera adusta* strain CX-9 and 4 novel humic acid peroxidases (HaP1-4) from decolorizing actinomycetes strains isolated from Algerian and Tunisian sites. We also explore their promising potential for their useful tool for lignin biodegradation, and other environmental applications. Overall, those properties make MnP TP55, LiP BA45, and MnP BA30 as well as HaP1-4 potential candidates for future application in lignin and humic acid biodegradation.

C. ORALE N°:69.**METABOLIC DISTURBANCE AND DETERMINATION OF GST/GSH BIOMARKERS ACTIVITY IN BIOINDICATOR PLANT****KHALDI FADILA^(1,2), NEDJOUJ GRARA⁽³⁾, NAILA MAIZI⁽⁴⁾**

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The air pollution by emissions of gas, dust, odors a nuisance forms to which the opinion is, rightly, the most sensitive. To protect air quality, it is necessary to know the nature of pollutants namely dosing and treatment. This action is fundamental because it is that it relies on the development strategies of reductions in the amount of pollutants released into the atmosphere. Research applied to air pollution using lichens as bioindicators have multiplied. Initial estimates of air pollution, by such plants were initially concerned acid pollution. Then came the first work on the accumulation of radionuclides and heavy metals by lichens. The problem is whether lichens, already likely to detect a fluoride pollution or an acid cleanup can attest to the reduction of NOx abatement. The phenomenon of exsorption already demonstrated in favor of the retransplantation of samples in their original, unpolluted, suggests that lichens respond rapidly to an air pollution control. The objective of complex Annaba is part of the promotion of Algerian agriculture represents an indispensable tool for the country's independence in terms of food self-sufficiency. This plant is located on the coast east of the city of Annaba. Therefore, it contributes to the relatively large levels of dust, NOx and NH3. It is interesting to use of indicator plants in order to estimate the impact of these pollutants on the environment. Study refers to bio estimate of overall pollution after an incineration plant with lichens. Bioaccumulation potential of lichens appeared to us a sensible approach to assess the impact of plant fertilizers on the environment.

The experimental study also that the use of antioxidant enzymes: glutathione-S-transferase (GST), glutathione reductase (GR) as biomarkers of air pollution has been shown using the species *Ramalinafarinacea* (lichens) transplanted at 5 sites. Dosages of these biomarkers confirmed the disruption of lichens by the air pollutant (NOx) emitted by the industrial complex. The photosynthetic and respiratory metabolism following fluctuations at different sites.



C. ORALE N°:70.

SECRETOME ANALYSIS OF A NOVEL FUNGAL STRAIN *C. GLOBOSUM* IMA1KJ472923 USED FOR INDUSTRIAL TEXTILE WASTEWATER BIOREMEDIATION

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Abstract: The present work aims to analyze the secretome of a novel isolated fungal strain *C. globosum* IMA1 KJ472923, which can grow efficiently on industrial textile effluent (ITE) by secreting a diversified pool of enzymes. Enzymatic assays, under optimized culture conditions, showed LiP, MnP and laccase activities of 832 UL-1, 250 UL-1 and 83.4 UL-1, respectively. They also showed a considerable proteolytic and cellulolytic enzymes activity of 643 U/mL-1, 1.1 U/mL-1 and 0.25 U/mL-1 for proteases, FPases and CMCases, respectively. So, the identified proteins were associated to the bioremediation of the ITE by biological degradation mechanism. To confirm the capacities of this isolate for enzymes production, a proteomic analysis by nano-LCMS/MS of the secretome was investigated at the end of culture in batch systems. Several potential enzymes and their functional classification have been shown, such as ligninolytic enzymes (22%), cellulases (20%), proteases (7%), hypothetical proteins (30%) and other proteins (21%). This approach clearly showed that complex colored effluents media, which is rich in dyes, can induce the secretion of a broad set of extracellular enzymes. Therefore, biochemical assays growth behavior and nano-LC/MS/MS analysis of secretome for the new fungus strain revealed its indigo-dyes degradation potential when it has been cultivated on colored effluents.

Keywords: *C. globosum* IMA1, Textile effluent, Decolourization, Biodegradation, Secretome, Nano/LC/MS/MS.

C. ORALE N°:71.

EFFET DE DIFFERENTES ORIGINES ET CONDITIONS CLIMATIQUES SUR LES COMPOSES PHYTOCHIMIQUES DE L'OLIVIER, *OLEA EUROPAEA* L.

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En Tunisie, la filière oléicole constitue une composante principale des secteurs agricole et agroalimentaire. Cette filière a fait preuve d'une croissance socio-économique importante, ce qui offre à la Tunisie une position de choix à l'échelle mondiale. Cependant, ce pays méditerranéen est gravement touché par le réchauffement climatique. Chétoui est une variété d'olive principale, caractérisant le nord tunisien. Pour s'adapter aux conditions climatiques difficiles du centre et surtout du sud du pays, indiquées par des sécheresses fréquentes et prolongées, cette variété doit adopter un système d'adaptation adéquat. Souvent, le déficit hydrique est associé à des niveaux accrus d'espèces réactives de l'oxygène causant le stress oxydatif en outre imposé. Ainsi, Chétoui, cultivé au centre et au sud, synthétise de nombreux composés antioxydants intéressants qui sont analysés et identifiés comme étant des indicateurs de stress. L'activité antioxydante des extraits méthanoliques foliaires de cette variété est ainsi testée vis-à-vis des deux radicaux DPPH et ABTS^{•+}. Alors que les antioxydants impliqués sont identifiés en utilisant des analyses chromatographiques par GC-FID et GC-MS. Testés à une concentration de 1 mg/ml vis-à-vis du DPPH, les extraits méthanoliques exercent une activité très importante au nord (91,4%) et au centre (90,7%) mais moins au sud (73,8%). Cette activité est plus faible vis-à-vis d'ABTS^{•+}, mais reste plus importante dans le sud. Les phénols totaux, les flavonoïdes les tannins, les diphénols, les sitostérols, la chlorophylle a et b et les caroténoïdes sont quantifiés en tant qu'antioxydants. Les phénols totaux de Chetoui sont maximaux au centre, de même pour les tannins et les caroténoïdes. Leurs teneurs sont moindres au sud mais restent plus significatifs que ceux du nord. Chétoui maintient aussi un taux de diphénol plus important au centre, mais ce dernier diminue significativement au sud. De même, le sitostérol, la chlorophylle a et b diminuent significativement au sud. En revanche, Chétoui montre un taux plus élevé de flavonoïde et de saponine au sud. En outre, plusieurs composés sont identifiés, à des proportions importantes chez Chétoui du sud et sont connus par leur activité antioxydante puissante, soient 2(3H)-5-méthylfuranone, 4-vinyl-2-méthoxy-phénol, acide hexadécanoïque, 2-méthyl-3-oxime-1-cyclohexen-3-on; acide trans-o-coumaric et vomifoliol.

Mots clés: Olivier, Chetoui, Antioxydants, Conditions Climatiques.



C. ORALE N°:72.

IMPROVEMENT OF BIOHYDROGEN PRODUCTION FROM HYPERTHERMOPHILIC CO-DIGESTION OF ORGANIC WASTES (FRUIT –VEGETABLE AND FISH WASTES) BY *THERMOTOGA MARITIMA* IN MINIMUM MEDIUM

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Abstract: Today, the world is suffering from serious threat of climate change which was started by the degradation of the atmosphere's protective ozone layer caused by greenhouse gas emissions. Hence, Renewable energy sources have attracted significant increase of interest in recent years thanks to their aptitude to reduce dependency on fossil fuels and their clean and environmentally approaches. Among various alternatives, hydrogen seems to be the most attractive clean future energy vector which emits only water when burned and has various renewable feedstock sources. The main goal of this work is to produce hydrogen by hyperthermophilic co-digestion of organic wastes collected from the group market of Tunis in a minimum medium based on natural seawater. *Thermotoga maritima* is the halophilic bacterium used in continuously stirred tank reactor (CSTR) under controlled parameters (pH, Temperature, agitation). We demonstrate the ability of *T. maritima* to produce H₂ in a minimum medium composed only of fruit and vegetable wastes, seawater, nitrogen and sulfur sources. Total H₂ production and the maximum H₂ productivity are 109 mM and 7.3 mmol.h⁻¹.L⁻¹, respectively. To improve biohydrogen production with more simplification of the culture medium, co-digestion seems to be a suitable technology. It combine different organic substrates (fruit –vegetable and fish wastes) to satisfy the requirement of *T. maritima* growth and to increase process performance. It was observed that treated fish wastes additions enhanced total H₂ production and the maximum H₂ productivity by 62% and 27%, respectively. Increasing the amount of carbohydrate concentrations (equivalent to 50 to 123 mM) with treated fish wastes addition have improved total H₂ production and the maximum H₂ productivity which reached 412 mM and 44 mmol.h⁻¹.L⁻¹, respectively. Removing the nitrogen and sulfur sources from the medium did not affect the amount of biohydrogen produced through the addition of treated fish wastes. They provided nitrogen and sulfur content necessary for *T. maritima* growth.

Keywords: Biohydrogen, bioenergy, fruit and vegetable wastes, fish wastes, *Thermotoga maritima*, CSTR reactor, natural seawater, hyperthermophilic co-digestion



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GENETIQUE & IMMUNOLOGIE



C. ORALE N°:73.

STUDY OF THE RESISTANCE OF THE LOCAL COW POPULATION TO ABORTIONS DUE TO *NEOSPORA CANINUM*.

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In Algeria, the local cattle breed is characterized by a low level of productivity explained primarily by genetic, nutritional, health and climatic constraints. Among all these factors, abortion is a real economic problem for farmers. Thus, improving the fertility remains a priority objective to optimize reproductive potential and thus producing cattle. Data from the literature indicate that *Neospora caninum* is a major cause of abortion in cattle worldwide including Algeria.

The objective of this study was to determine the seroprevalence of *Neospora caninum* antibodies in 176 pregnant cows of local breeds from 60 different farms in the region of Jijel, Algeria. Serum samples were tested for the presence of *Neospora caninum* antibodies using an ELISA assay. Overall seroprevalence of *Neospora caninum* antibodies was 85%. The case-control study did not reveal an association between seropositivity to *N. caninum* and abortions OR= 0, 3 (IC 95% 0, 03 -2, 86) p=0, 3. The risk factors analysis indicated that parity, the presence of abortion, age, stage of pregnancy were not significantly associated with seroprevalence. The results obtained suggest that the local cattle breed is resistant to abortions due to *Neospora caninum*, despite a high exposure to the parasite. Our study on the evolution of the serological status of 23 pregnant cows (on heat synchronized) shows the following: 8 out of 23 cows tested were seropositive to *Neospora caninum* throughout gestation calved calves seropositive and clinically healthy and 15 These were found seronegative cows calved calves seronegative without clinical signs. A cow aborted seronegative 5th and a half months gestation fetus 4. The results obtained suggest that the local cattle breed is resistant to abortions due to *Neospora caninum*, despite a high exposure to the parasite.

Keywords: cattle, local breed, pregnancy, abortion, serology, *Neospora caninum*.

C. ORALE N°:74.

EVOLUTIONARY AND DEMOGRAPHIC HISTORY AMONG *AVENA STRIGOSA* ACCESSIONS BASED ON THE NUCLEOTIDE SEQUENCES OF THE CHLOROPLAST DNA BARCODE *TRNH-PSBA*.

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Abstract: Black oat (*Avena strigosa*) is a small coarse cereal used all over the world for food and feed. Despite an important cereal, the area under cultivation of oat has been continuously decreasing during the past few decades. However, recent demands for human consumption in oat have been gradually increased, particularly owing to its nutritional benefits. In order to establish a genetic fingerprint and examine the intraspecific polymorphism of an introduced collection of *Avena strigosa*, the intergenic spacer *trnH-psbA* was examined since these are the most variable regions in the chloroplast angiosperm genomes. After DNA amplification of the targeted regions, sequencing proves an important genetic diversity first revealed at the level of the nucleotide composition and secondly by haplotypic diversity and nucleotide diversity tests. Results showed that the sequences size vary from 518 bp to 534 bp. The recorded GC and AT content is 33.1% and 66.9%, respectively. The relatively high values of the content in AT bases and the ratio of transitions / transversions was 0.533 explained the high proportion of transversions. In addition, a dendrogram was established showing robust genetic relationships between the studied accessions.

Key words: *Avena strigosa*, chloroplast DNA, genetic polymorphism, trnH-psbA.



C. ORALE N°:75.

MOLECULAR POLYMORPHISM STUDY OF EIGHT SPECIES OF CITRUS L. GENUS IN TUNISIA USING IRAP MARKERS

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Due to their ubiquity and high ability to transpose, retrotransposons appear to be very powerful molecular markers. In this study, Polymorphism of Amplified Inter-retrotransposon Regions (IRAP) markers was used to study genetic diversity in 29 varieties belonging to 8 Citrus L. species in Tunisia. Five IRAP primers were chosen during this study. A total of 86 amplicons were generated of which 98.75% are polymorphic. The average polymorphic information content (0.918) and the resolving power (45.31) reflect the high reproducibility of the IRAP primers used as well as their efficiency in generating molecular polymorphism study. The Shannon index (0.458), the Nei index (0.296) and the gene differentiation coefficient (0.511) highlight the presence of genetic variability at the inter-specific level existing within the Citrus genus. The gene flow (0.478) shows a weak gene exchange between the 8 species studied. Molecular analysis of variance shows that 92% of the total genetic diversity is found within the species studied while only 8% of this diversity is detected among the eight Citrus species. In addition, the Neighbor-Joining dendrogram, principal coordinate analysis and Bayesian analysis based on IRAP markers also confirmed that intraspecific variability was higher than inter-specific diversity and the presence of an overlap between the study species. Our results confirm the efficacy of IRAP markers in the detection of polymorphism, the characterization and the analysis of the genetic diversity of Citrus species.

Key words: Citrus L., IRAP, Neighbor-Joining, principal coordinate analysis, Bayesian analysis, inter and intra-specific genetic diversity, Tunisia.

C. ORALE N°:76.

PARKINSON'S DISEASE IN TUNISIA: HOW WE ARE GENETICALLY DISTINCT FROM OTHER POPULATIONS

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Background: In Tunisia, the prevalence of Parkinson's disease (PD) is notably higher than other countries and the high rate of consanguinity and low rates of migration increase the risk to develop its genetic forms. In this study we investigated genetic and clinical characteristics of PD in Tunisia.

Methods: A total of 250 patients were recruited. All patients were screened for the LRRK2 G2019S mutation using Taqman assay and non-carriers were screened for other genes involved in PD using NGS technology. Founder event and ethnic origin for two frequent mutations were investigated using a genotype-based analysis of bi-allelic SNP markers.

Results: Among the 250 Tunisian patients, men predominance (61%, $p=0.005$) was observed. Genetic analysis showed that LRRK2 mutations are the major cause of PD in Tunisia, particularly the G2019S mutation responsible for 42.8% of the cases. This mutation was found to be the result of a single nucleotide change occurring in a common ancestor of all carriers in Tunisia. Ethnic origin analyses showed that all G2019S male carriers had a Berber origin carrying the sub-clade E1B1B1B (M81), a lineage which appeared in North Africa, approximately 5,600 years ago. Mutations in PINK1 were responsible for 4% of cases including the Q456X mutation resulting from a founder event among Berber Tunisians. PARK2 mutations only accounted for 0.8% of all cases and included a new triplication of exons 5-7 associated with atypical severe motor symptoms, fast progression and not responsive to levodopa. We also found a new mutation (D180G) in the GBA gene which was responsible for 1.2% of the cases and two related cases with compound heterozygous mutations in SYNJ1 (L406* and L1406fs*42).

Conclusion: Our findings showed that the Tunisian population is distinct from other populations, as it is striking that more than 50% of all cases have a genetic origin. In fact, we confirmed the very high frequency of LRRK2 G2019S mutation and evidenced the relatively frequent PINK1 Q456X mutation the latter in the Tunisian Berber population.



C. ORALE N°:77.

MUTAGENESIS INDUCTION FOR BIOMASS, LIPID AND CAROTENOIDS PIGMENT PRODUCTION OF THE MARINE YEAST *RHODOTORULA MUCILOGINOSA* RMTUN15

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Yeasts of the genera *Rhodotorula* are able to synthesize different pigments of high economic value and represent a biotechnologically interesting group of yeasts. Therefore, the aim of this work was to generate a mutant strain of *Rhodotorula muciliginosa* RmTun15 that could accumulate and produce a high amount of lipid and carotenoids using Ethyl Methyl Sulfonate (EMS) as a mutagen agent. The wild strains were isolated from fresh seawater in La Goulette, Tunisia. Following exposure to EMS during various time (T15, T30, T60, T45 min.), the growing mutants were selected and compared to wild strains for biomass, lipid productivities (BP, LP respectively) and the carotenoids content.

Among *R. muciliginosa* mutants, RmTm15-T15min showed the maximum BP (0,319 mg/ml/h) and LP (0,113 mg/ml/h) as compared to wild strain (0,119 and 0,073 mg/ml/h respectively). EMS mutagenesis also induced a significant change of fatty acids composition in mutant strains in relation to the time of exposure. Mutated strains lipid profiles were richer in fatty acid with 18 and 16 carbon atoms.

HPLC and TLC analysis exhibited that the carotenoids fraction increases significantly over the time of exposure to EMS (T15, T30 and T45) compared to the wild-type control strain. This fraction consists mostly of β -carotene, astaxanthin, fucoxanthin and torulene.

Key words: Lipid, Biomass, Carotenoids, *Rhodotorula*, Mutants, EMS

C. ORALE N°:78.

S-GENOTYPE PROFILES OF TUNISIAN APRICOT (*PRUNUS ARMENIACA* L.) GERMPLASM

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In new apricot plantations, self-incompatibility is one of the most important problems. In order to solve this issue, molecular techniques are used to determine self-incompatibility in apricot accessions. In this study, the S-genotypes of 74 Tunisian apricot accessions were determined by polymerase chain reaction (PCR) amplification of the second intron of the S_RNase locus. In total, seven different alleles (S₂, S₆, S₇, S₁₁, S₁₂, S₁₉ and S₈/S_C) were revealed through the considered accessions. The allele S₇ was the most frequent S-allele that occurred in 31 genotypes. Related to the geographical regions of apricot accessions in Tunisia (North, Center, South and Oasis), results revealed that the frequency of the S-alleles was different. However, no differences were highlighted regarding to the mode of propagation of the Tunisian apricot material (grafted and seed propagation). Among the 74 studied accessions, 14 heterozygous genotypes and 7 homozygous genotypes were obtained and many of them were classified into previously established cross-incompatibility groups. The Tunisian apricot material shared four alleles of self-incompatibility (S₂, S₆, S₇ and S₁₁) with Moroccan apricot material suggesting that the apricot material of Morocco and Tunisia enclosed the same genetic pool.

Keywords: *Prunus armeniaca* L., Self-(in)compatibility, S-alleles, Tunisia

**C. ORALE N°:79.****DEUX NOUVELLES MUTATIONS DU GENE BEST1 RESPONSABLES DE DEUX FORMES DIFFERENTES DE DYSTROPHIE MACULAIRE DANS DEUX FAMILLES TUNISIENNES****CHIBANI ZOHRA¹, IMEN ABID², NEILA BELGHITH³, JAMEL FEKI², MOUNIRA HMANI-AIFA¹***1 laboratoire de génétique moléculaire et fonctionnelle, faculté des sciences de Sfax**2 service d'ophtalmologie CHU Habib Bourguiba- Sfax**3 service de génétique humaine-CHU Hedi chaker Sfax*

Les dystrophies maculaires héréditaires sont des affections dégénératives de la région centrale de la rétine (macula) associant des anomalies primaires de la rétine sensorielle, de l'épithélium pigmentaire et des remaniements de nature variable (dépôts pigmentaires, lipofuscine, oedèmes...). Ces affections sont rares et se caractérisent par une hétérogénéité phénotypique ainsi qu'une hétérogénéité génétique. L'étude épidémiologique des maladies héréditaires de l'œil nous a permis d'identifier deux familles tunisiennes atteintes de dystrophies maculaires. Des examens ophtalmiques complets comprenant la tomographie en cohérence optique (OCT), l'électrorétinographie (ERG), l'électrooculographie (EOG) et l'autofluorescence (AF) ont permis de diagnostiquer deux garçons de 10 ans atteints de bestrophinopathie autosomique récessive (ARB) et une jeune fille de 8 ans atteinte de dystrophie maculaire vitelliforme (BVMD). Le séquençage par la méthode de Sanger de tous les exons du gène BEST1 chez ces trois patients ainsi que les membres disponibles des deux familles a permis d'identifier deux nouvelles mutations : une mutation faux-sens C91A (p.Leu31Met) au niveau de l'exon 2 chez la famille atteinte de l'ARB et une mutation non sens C1550G (p.S517X) a été détectée au niveau l'exon 10 de ce même gène chez la patiente atteinte de BVMD.

En conclusion, diverses mutations du gène BEST1 ont été rapportées et qui sont responsables de plusieurs pathologies oculaires. Mais en Tunisie, à notre connaissance, c'est la première fois qu'on identifie des mutations au niveau de ce gène. Dans cette étude, nous avons identifié deux nouvelles mutations du gène BEST1 qui ségrégent dans deux familles différentes atteintes de différentes formes de dystrophies maculaires.

C. ORALE N°:80.**ASSOCIATION OF HNF1A GENE VARIANTS WITH METABOLIC SYNDROME: A CASE/CONTROL STUDY IN THE TUNISIAN POPULATION****DALLALI HAMZA^{1,3}, MERIEM HECHMI^{1,3}, SAHAR ELOUEJ^{1,4}, HAIFA JMEL^{1,3}, YOSSRA BEN HALIMA^{1,2}, MAJDI NAGARA^{1,4}, MARIEM CHARGUI^{1,2}, INES KAMOUN⁵, ZINET TURKI⁵, ABDELMAJID ABID^{1,5}, SONIA BAHRI⁶, AFAF BAHLOUS⁶, RAMON GOMIS⁷, ABDELHAMID BARAKET⁸, FLORIN GRIGORESCU⁹, CHRISTOPHE NORMAND⁹, HENDA JAMOUSSE^{1,5}, SONIA ABDELHAK^{1,2}, RYM KEFI^{1,2}**

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Background and aim:Metabolic syndrome (MetS) is characterized by the clustering of risk factors for heart disease and type 2 diabetes (T2D). These risk factors include central obesity, hyperglycemia, hypertension, and altered lipid profile. Recent genome wide association studies (GWAS) have identified several genetic loci associated with MetS and/or its components including the hepatocyte nuclear factor 1 alpha (HNF1A) gene. Variants in this gene were mainly associated with lipoproteins levels and T2D. However, the association of HNF1A variants with MetS in the Middle East and North Africa region is largely unknown. This study aims to examine their association with MetS and its components in the Tunisian population. Population study and methods:A total of 594 Tunisian individuals (295 cases/299 controls) were genotyped for two variants (rs1169288 and rs2464196) located in the HNF1A gene using KASPar technology. Statistical association analyses were performed with the R software. Results:Our results showed no association between HNF1A variants and MetS in our studied Tunisian population. However, a significant association was observed between the variant rs2464196 and both waist circumference and high density lipoproteins (HDL).The investigation of the interaction gene/sex highlighted a significant association between the variant rs1169288 and high cholesterol levels after Bonferroni correction only in the group of women. Conclusion:Our findings exclude the implication of HNF1A gene variants (rs1169288 and rs2464196) in the susceptibility to MetS in our studied Tunisian population but they emphasize the role of these variants in the metabolism of lipids with gender-specific differences. The genotyping of a third variant located in HNF1A gene will allow us to perform a haplotypic analysis.

Key words:HNF1A; Metabolic syndrome; Genetic association.

**C. ORALE N°:81.****LACK OF ASSOCIATION OF LEPTIN GENE VARIANTS IN TUNISIAN ARAB WOMEN WITH POLYCYSTIC OVARY SYNDROME****DALLEL MERIEM¹, ZAINEB DOUMA¹, SAMEH SARRAY², DHAFER BEN LETAIFA³, TOUHAMI MAHJOUB¹, WASSIM ALMAWI²***1 Laboratory of Human Genome and Multifactorial Diseases (LR12ES07), Faculty of Pharmacy, University of Monastir**2 Faculty of Sciences, El Manar University**3 Faculty of medicine, Sousse, Tunisia.*

Polycystic ovary syndrome (PCOS) is a complex endocrine-metabolic disorder of reproductive age women. High prevalence of metabolic syndrome such as insulin resistance, dyslipidemia and in particular hirsutism and obesity, has been detected early in life with PCOS women compared to controls. Obesity was associated with a serial health concerns of PCOS and is a multifactorial nature. Several factors are reported to precipitate obesity, these include hyperandrogenemia, hyperinsulin with a subsequent insulin resistance and all tends of secretion of adipokines. In particular, leptin (Lep) which is closely associated with obesity and the serum leptin concentrations are correlated with high BMI either in normal and PCOS women. We therefore examined whether the Leptin SNPs rs10487506, rs7799039, rs2167270, rs12706832, rs10954173 are associated with PCOS in Tunisian Arab population. We conducted a case-control study comprising 320 Tunisian women with PCOS diagnosed according to the Rotterdam criteria, and 447 healthy controls. SNPs rs10487506, rs7799039, rs2167270, rs12706832, rs10954173 were genotyped by polymerase chain reaction (PCR).

Results: The outcome was that the Minor allele frequencies of the five leptin variants tested were similar between women with PCOS and control women ($P > 0.05$), even before correcting for multiple testing. Lack of differences of heterozygous frequencies (1/2) among of rs10487506, rs7799039, rs2167270, rs12706832 and rs10954173 genotype carriers, were seen between PCOS cases versus controls. None of the tested Leptin variants were associated with PCOS features (BMI, HOMA-IR, total testosterone, and SHBG..). Haploview analysis revealed limited linkage disequilibrium between the tested Leptin variants. Taking common AAGGG haplotype as reference (OR=1.00), [rs7799039, rs2167270, rs12706832, rs10954173] 6-locus haplotypes analysis demonstrated lack of association of any of the Leptin haplotypes with PCOS, even before correcting for multiple testing.

To conclude we demonstrated lack of association of Leptin SNPs rs7799039, rs2167270, rs12706832, rs10954173 tested for the first time with PCOS.

Keywords: polycystic ovary syndrome, leptin variants, SNPs, Haplotypes

C. ORALE N°:82.**GENETIC DIVERSITY AND MOLECULAR POLYMORPHISM IN TUNISIAN POPULATIONS OF CORK OAK (*QUERCUS SUBER* L.) BASED ON CDDP MOLECULAR MARKERS****ELHABASSI ASMA¹, AMEL SALHI HANNACHI¹, HELA SAKKA^{1,2*}***1 : Laboratoire de Génétique Moléculaire, Immunologie et Biotechnologie. Faculté des Sciences de Tunis, Université de Tunis El Manar, 2092 El Manar, Tunis, Tunisie.**2 : Faculté des Sciences de Bizerte, 7021 Zarzouna, Tunisie***: e-mail: hela_sakka@yahoo.fr*

The Mediterranean countries, and particularly Tunisia, possess a rich and diversified forest heritage that is highly adapted to eco-geographical conditions. Our interest is focused on the study of forest species very widespread in Kroumirie and Mogods forests specially cork oak (*Quercus suber* L.). This species is threatened by biotic and abiotic stresses and by intense genetic erosion; hence, this forest essence deserves to be preserved. The study of genetic diversity and molecular polymorphism in 6 Tunisian cork oak populations was undertaken by CDDP markers (Polymorphism of conserved regions derived from DNA). The use of 5 CDDP primers have generated of 193 polymorphic amplicons with an optimal rate of polymorphism (PBP = 100%). The average of the Resolving Power (Rp) and Polymorphism Information Content (PIC) are 7,167 and 0,962 respectively, testifying the powerful of the used primers and the high resolution of the CDDP technique in term of showing of polymorphism. The AMOVA analysis shows that the intra-population genetic diversity is 39%, while the inter-population variability recorded between the six cork oak populations is 61%. In addition, the Nei diversity index ($h = 0.097$), the Shannon index ($I = 0.14$), the genetic differentiation coefficient ($Gst = 0.645$) and the gene flow index ($Nm = 0.275$) confirmed the results obtained by AMOVA analysis and explained the strong divergence observed between the studied populations. The NJ tree shows the existence of three groups: the first one contained of Northeastern populations and the two others are constituted by Northwestern populations. The topology of the NJ dendrogram showed that obtained groupings are distributed according to their geographical origin. The results of the multivariate analyzes (ACP and PCoA) are in agreement with the tree NJ.

Keywords : *Quercus suber* L., genetic diversity, CDDP molecular markers, genetic relationships

**C. ORALE N°:83.****GENETIC ASSOCIATION BASED ON SRAP MARKERS AND AGRO-MORPHOLOGICAL TRAITS IN CORONILLASCORPIOIDES GERMPLEASMS USING BULK DNA TEMPLATES****HAKIM LAMIA, SDOUGADORRA, KABTNISOUHIR, TRIFI-FARAH NEILA, MARGHALI SONIA***Laboratory of Molecular Genetics, Immunology & Biotechnology, Faculty of Sciences, University of Tunis El Manar, Campus Universitaire El Manar, 2092 Tunis, Tunisia.*

Thanks to their pastoral and food use, Angiosperm family plants and especially *Coronillascorpioides* contribute to the development of the Economic and the Agriculture in Tunisia. The chemical compounds secreted by *Coronillascorpioides*, such as Psoralen, have several pastoral uses and medicinal effects. Rather than the biochemical studies of *Coronillascorpioides*, this paper adopted a different approach which focuses on the genome analysis of this species targeting the coding part of the genome. Sequence Related Amplified Polymorphism (SRAP) markers are used to associate specific molecular markers to some interested agronomic parameters. In fact, association studies give the opportunities to identify genetic variation that is highly correlated with the expression of interested agronomic parameters such as the digestibility of the dry matter. Thus, SRAP markers were used, through bulk templates, to characterize ten populations of *Coronillascorpioides* collected from different regions in Tunisia. Among fifteen SRAP primer combinations, some of them have generated specific markers to each population. The genetic association was assessed through Principal Component Analysis (PCA) and cluster analysis by using the Unweighted Pair Group Method Arithmetic mean method (UPGMA). Moreover, thanks to the electrophoretic profiles and the resulting of the binary matrix, some markers, such as the L2 and F6 loci which are respectively specific to the most biomass-producing population and the most digestible population, has been identified. We emphasize the powerful approach to identify specific markers that can be correlated with agronomic parameters of interest.

Key words: SRAP markers, Bulk DNA, *Coronillascorpioides*, Fabaceae.

C. ORALE N°:84.**GENETIC DIVERSITY OF SUNFLOWER BROOMRAPE POPULATIONS FROM TUNISIA****JEBRI MALEK^{1,*}, BEN K. MEKKI^{1,2}, FAKHFAKH HATEM^{1,2}, BEGOÑA PÉREZ-VICH³, LEONARDO VELASCO³***1 Laboratory of Molecular Genetics, Immunology and Biotechnology, Faculty of Science of Tunis, University of Tunis El-Manar, Tunisia.**2 Faculty of Sciences of Bizerte, Zarzouna, University of Carthage Tunisia**3 Institute for Sustainable Agriculture (IAS-CSIC), Finca Alameda del Obispo, Avda. Menéndez Pida s/n, 14004 Córdoba, Spain***: Jabri.malek@gmail.com*

Sunflower broomrape (*Orobanche cumana* Wallr.) was detected for the first time in sunflower fields of Tunisia in 2010 in the Béja region. No information is thus far available on the genetic diversity of populations parasitizing sunflower in that area. Our objective was to study molecular characterization using SSR Markers of sunflower broomrape populations from Tunisia. Plant tissues from nine populations were collected from different areas of Beja region. Cluster analysis of inter-population relatedness suggested that Tunisian populations were more related to Eastern Europe populations than to Spanish Populations, with Beja northern populations clustering separately from the rest of Tunisian populations. Analysis at the intra-population revealed the existence of two gene pools (GP1 and GP2) that were present in all populations at different frequencies. We hypothesized that GP1 was probably an initial introduction of a race E population while GP2 is most likely a later introduction of a race G population, introduced initially in Béja North area but spreading nowadays to other areas in Tunisia. The risk of a generalized expansion of race G in this sunflower cultivation area is discussed.

Key words: *Orobanche cumana*, SSR markers, SNP markers, Tunisia.



C. ORALE N°:85.

DISTRIBUTION AND DOMAIN ORGANIZATION OF THE LECTIN SUPERFAMILY IN CHICKPEA

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One of the most important direct defense responses in plants against the attack by pathogens is the production of proteins with resistance activities. One particular class of entomotoxic proteins present in many plant species is the group of carbohydrate-binding proteins known as lectins, they are divided into 12 families. During the last decade a lot of progress was made in the study of a few lectins that are involved in a range of important processes such as plant defense and stress signalisation. In Tunisia, the chickpea "*Cicer arietinum*" is the most cultivated legume, after the bean. However, cultures of this legume are subject to several biotic and abiotic constraints. In this context, we were interested in chickpea lectins. In a first part, an *in silico* search for lectin superfamily in the chickpea genome CDC frontier was realised (BLASTn and BLASTp). The results obtained revealed the presence of 172 putative lectin sequences belonging to 12 families with an abundance of lectin B (52 sequences) and lectin of legumes (30 sequences). Then, a structural and functional annotation were performed by bioinformatic software (CDD/SPARCLE of NCBI and Blast2GO). Domain architecture analysis revealed that most of these lectin sequences contain multiple domains, where lectin domains are linked with other domains, such as creating chimeric lectin sequences encoding proteins with dual activities. Subsequently, the phylogenetic relationships between identified lectins in chickpea and known lectins of certain species namely *Medicago truncatula*, *Glycine max*, *Phaseolus vulgaris* and *Arabidopsis thaliana* were created and showed that most lectin sequences sharing the same domain architecture were evolved together. In a second part, lectins from four selected families were searched by PCR using *Cicer arietinum* specific primers identified *in silico*. The amplification was performed using genomic DNA of the Tunisian variety "Nayer". The resulting PCR products were subsequently purified and cloned into the pGEM cloning vector. The recombinant clones obtained were sequenced by the universal primer T7. The analysis of the sequences obtained allowed us to validate the presence of two families of lectins on the four sought. Our study provides a general overview of chickpea lectin domains and gives insight into the evolutionary relationships and functional divergence of plant lectins that will help to understand their implications in plant defence.

Keywords: Lectins, domains architectures, chickpea genome

C. ORALE N°:86.

THE STRUCTURE, ANATOMY AND MORPHOLOGY OF ARGAN FLOWERS FROM TWO SITES IN TUNISIA

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Argan (*Argania spinosa* (L.) Skeels) flower buds and flowers was observed in 2 different sites in Tunisia. Flowering for the majority of trees normally began in April, a second one starts in November, but not all trees flowered during a season or two, although some trees flowered every season which shows the extremes of the large variability between argan trees. In both cases flowering precocity and frequency was different in each tree. The study was aimed to determine the floral morpho-anatomy and to observe the stages of flowering. Information about the argan flowers are so limited, this is the first study that shows the anatomy of *Argania spinosa* flowers. For this we used the paraffin method for flowers and floral buds collected from 30 trees located in 2 different sites in Tunisia (Ariana, Korbous). Longitudinal and transversal cuts were performed to get a clearer idea about the composition of argan flower and the localization of each organ. Different stages of flowering were also observed by optical microscopy. This study represents the first comprehensive morphological study of *Argania spinosa* flowers.

Keywords floral morpho-anatomy, Paraffin method; *Argania spinosa*; flower buds, flowers;



C. ORALE N°:87.

CLINICAL, MOLECULAR AND COMPUTATIONAL ANALYSIS IN TWO CASES WITH MITOCHONDRIAL ENCEPHALOMYOPATHY ASSOCIATED WITH *SUCLG1* MUTATION IN A CONSANGUINEOUS FAMILY

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Deficiency of the mitochondrial enzyme succinyl COA ligase (SUCL) is associated with encephalomyopathic mtDNA depletion syndrome and methylmalonic aciduria. This disorder is caused by mutations in both SUCL subunits genes: *SUCLG1* (α subunit) and *SUCLA2* (β subunit). We report here, two Tunisian patients belonging to a consanguineous family with mitochondrial encephalomyopathy, hearing loss, lactic acidosis, hypotonia, psychomotor retardation and methylmalonic aciduria. Mutational analysis of *SUCLG1* gene showed, for the first time, the presence of c.41T>C in the exon 1 at homozygous state. In-silico analysis revealed that this mutation substitutes a conserved methionine residue to a threonine at position 14 (p.M14T) located at the *SUCLG1* protein mitochondrial targeting sequence. Moreover, these analysis predicted that this mutation alter stability structure and mitochondrial translocation of the protein. In Addition, a decrease in mtDNA copy number was revealed by real time PCR in the peripheral blood leukocytes in the two patients compared with controls

Keywords: *SUCLG1*; mtDNA copy number, succinyl COA ligase

C. ORALE N°:88.

A CNV MAP OF THE TUNISIAN POPULATION

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Copy Number Variation (CNV) is a type of genetic variation defined as DNA segments of more than 1kb presenting copy-number differences when comparing two or more genomes. CNVs are generated by one-single or a combination of multiple genomic rearrangements such as deletion, insertion, duplication and unbalanced translocation. They can impact on gene expression and phenotypes by altering gene dosage, interrupting coding sequences and influencing neighboring gene regulation. Therefore, CNVs have been reported as associated with several complex traits including neurological diseases such as Autism and Schizophrenia. Recent studies focusing on CNVs in some ethnic groups such as Koreans, Chinese and Europeans had highlighted population-specific enrichment of CNVs. Such data are not available for populations from North Africa as Tunisia. In this work, we aimed to study CNV from 102 Tunisian healthy individuals genotyped with the Affymetrix 6.0 array. PennCNV software was used to call 3964 CNVs with an average of 39 segments per individual. The CNV mean length was 96.7 kb. Deletions represented 66.5 % of the called CNVs with an average length of 65.98 kb which was less than mean length duplication (173 kb). These CNVs have been clustered in 751 CNVRs (CNV regions) with a mean length of 104 kb and covering 78 Mb of the genome. Sixty percent of these CNVRs have a frequency of less than 1%. Three percent of these CNVRs are not found in the DGV database. About 34% of CNVRs overlapped with 234 RefSeq genes. Gene Ontology annotation revealed that genes overlapping CNVRs are significantly enriched in « calcium ion binding » molecular function (0.4%; p-value=7 10⁻⁴), involved in biological processes as « cellular adhesion » (7 %; p-value=1.45 10⁻⁴) and « nervous system development » (19%; p-value=1.45 10⁻⁵). Over-representation of cellular components are « synapse » (9.5 %; p-value=6.47 10⁻³) and « integral component of plasma membrane » (13.2%; p-value=3.6 10⁻²). Our preliminary results suggest the need of a complete characterization of the CNVs in the Tunisian population and the map that we build will serve as a useful resource for further medical studies.



C. ORALE N°:89.

CARACTERISATION DE L'EXPRESSION DES MICRO-ARN DANS LE CANCER DE LA PROSTATE EN TUNISIE

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Le cancer de la prostate (PCa) occupe en Tunisie la troisième place de l'ensemble des cancers chez l'homme. Ce cancer est le siège de plusieurs altérations génétiques et épigénétiques. Ces dernières peuvent être des modifications chimiques de l'ADN, des histones ou des perturbations de l'expression des micro-ARN (miARNs). Les miARNs sont des marqueurs stables, efficaces ayant une importance dans le diagnostic et la thérapie de plusieurs types de cancers y compris le PCa. La dérégulation de l'expression de micro-ARNs pourrait moduler de façon très sophistiquée les voies de signalisation cellulaires. miR-1260a et -1274a ont été décrits comme des oncomiRs dans différentes pathologies comme le cancer du sein. Ces miRs sont impliqués dans différentes voies de signalisation dans la cancérogenèse notons l'apoptose, PI3k-AKT et ayant comme cibles des gènes suppresseurs de la Tumeur p53, PDCD4 etc... Dans ce présent travail, nous avons examiné par RT-qPCR l'expression des miR-1260a et 1274a au niveau des blocs de paraffine de 105 échantillons. Les prélèvements sont repartis comme suit : 71 tumeurs d'adénocarcinome prostatique pris au diagnostic, 15 blocs de paraffine prélevés après échappement à l'hormonothérapie (CRPC) et 19 échantillons de contrôles (des sujets ayant une hypertrophie bénigne de la prostate (HPB)).

Nos résultats montrent une surexpression des miR-1260a et 1274a par rapport aux contrôles ($p = 0.0001$). La comparaison du niveau d'expression de deux miRs chez les deux groupes des patients (PCa pris au diagnostic et CRPC), montre que la surexpression de miR-1260a et 1274a atteint ces valeurs maximales chez les patients résistants mais sans être statistiquement significative ($p = 0.401$ et 0.272 respectivement). Par ailleurs, aucune association significative n'a été trouvée entre le niveau d'expression de ces marqueurs et les paramètres cliniques des patients atteints de PCa. L'ensemble de ces résultats suggèrent que ces 2 micro-ARNs sont impliqués dans les mécanismes d'initiation du cancer de la prostate tout en restant surexprimés chez les patients résistants au traitement.

C. ORALE N°:90.

ASSOCIATION OF FGF2 VARIANT WITH PROLIFERATIVE DIABETIC RETINOPATHY INDEPENDENT FROM FGF1 AND METABOLIC SYNDROME IN TUNISIAN POPULATION

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Angiogenic cytokines have been demonstrated as influential in the progression diabetic retinopathy (DR) since some previous studies demonstrated their elevated levels in the ocular fluid compared with normal conditions. Fibroblast growth factors (FGFs) are molecules with angiogenic properties and putatively involved in ocular neovascularization. Since neovascularization has been implied in proliferative retinopathy, defining genetic markers to be used for prediction of the follow-up of DR progression is an important goal in clinical genetics. This study was aimed to evaluate the genetic association of single nucleotide polymorphism (SNP) of FGF2 (Chr4q28.1) compared to FGF1 (Chr5q31.3) and other clinical conditions with the severity DR in Tunisian population. Subjects and Methods. A retrospective case-control study was performed in patients with type 2 diabetes ($n = 781$) among whom there were patients ($n = 299$) without retinopathy (DWR), patients ($n = 155$) with non-proliferative DR (NPDR) and patients ($n = 326$) with proliferative DR (PDR). Genotyping of leader SNPs in FGF1 and FGF2 was performed by PCR-RFLP while the association was tested by logistic regression. Metabolic syndrome (MetS) was defined by ATP III criteria and prediction of transcriptional activity was tested in Haploreg. Results. In PDR patients compared to NPDR, higher prevalence was found for hyperglycemia (85 versus 53.6%), obesity (75.0 versus 61.5%) and MetS (63.5 versus 40.2%). The BMI was not different from other T2D but significant higher than non-diabetic controls (28.2 ± 0.3 versus 25.0 ± 0.17 , $P < 0.0001$). SNP rs2922979 (C/G) of FGF2 had a MAF between 0.35 and 0.44 in controls and 0.3-0.35 in T2D, concordant to CEU population (0.30) and lower than Sub-Saharan Africans (0.82). Compared to other diabetics, rs2922979 was associated with PDR with $P < 0.03$, OR 2.295% CI [1.1-4.7] with a gene dosage effect. Interestingly, main effect was observed in the absence of MetS ($P < 0.05$, OR 3.195% CI [1.1-8.5]). This was explained by the fact that MetS had an independent effect on PDR ($P < 0.0001$, OR 2.4 95% CI [1.6-3.6]). The prevalence of GG was not significantly different between NPDR and other T2D without ocular complications. The effect of homozygous GG was also independent from rs34011 of FGF1, since homozygous GG was associated with $P < 0.005$, OR 3.195% CI [1.4-7.2] in conditional testing. In conclusion. Although metabolic abnormalities or metabolic syndrome by itself contribute to more severe T2D with DR, influential effect of FGF2 variant in PDR appeared to be independent from FGF1 or other components of MetS. Sincere rs2922979 and its proxy rs308403 are predicted to contain binding sites numerous proteins including NFkB in circulating white cells, these data are in favor of a rather vascular mechanism independent from insulin resistance in PDR progression.

**C. ORALE N°:91.****THE SIGNIFICANCE OF LET-7C AND MIR-143 EXPRESSION IN BLADDER CANCER IN TUNISIA****SETTI BOUBAKER NOUHA¹, AYMONE GURTNER^{2*}, R. SAID^{1*}, LUCIA CICHILLITTI⁵, H. AYED², A BLEL³, O KARRAY², A SAADI², M GHARBI², SRAMMEH ROMMENI³, M CHEBIL², GIULIA PIAGGIO⁴, S OUERHANI¹.**

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Urinary bladder cancer is the seventh most common malignancy in the world and the second in Tunisian male patients. Around 70% of the patients present non-muscle-invasive cancers (NMIBC) at first diagnosis; they are treated with transurethral resection (TURBT) followed by adjuvant intravesical therapy (BCG therapy). However, about 50%–70% of them will relapse and roughly 10%–20% will progress to muscle-invasive cancers (MIBC). A set of genetic and epigenetic alterations have been identified in bladder tumorigenesis. Recently MicroRNAs (miRNAs) have been reported to play important roles in the initiation and progression of BC. In the present study, we aimed to investigate the association between let-7c and miR-143 expression levels and BC prognosis. The expression levels of let-7c and miR-143 were measured using quantitative Real-time polymerase chain reaction (qRT-PCR) analysis in 90 cases with different stages and grades. The association between let-7c and miR-143 expression and epidemiological and clinicopathological factors was analyzed. Our data indicated that let-7c and miR-143 were lowly expressed in BC compared with non-malignant tissues ($p < 0.05$). The correlation between let-7c and miR-143 expression levels and epidemiological parameters (alcohol consumption, exposure to professional risk factors and tobacco status) doesn't report any statistical significant association. However, we found that the miR-143 expression level was positively correlated to the intensity of tobacco consumption (PY) ($p = 0.04$). In the other hand, the expression of the two miR's was studied in association to clinical parameters. Our results indicated that the miR-143 has a different expression profiles across the stages ($p = 0.01$). Moreover, miR-143 expression was associated to tumor multifocality ($p = 0.04$). Finally, we have found that let-7c and miR-143 expression levels were not associated to progression ($p = 0.07$ and $p = 0.11$) and early recurrence after BCG therapy ($p = 0.18$ and $p = 0.13$). We conclude that that let-7c and miR-143 were lowly expressed in BC. These down regulations allow to discriminate tumors from non-tumoral samples and could be associated to bladder cancer initiation by deregulating directly or indirectly pathways like the RAS_MAPK or PI3K-AKT. However these biomarkers were not associated to BC prognosis.

C. ORALE N°:92.**EXPLORATION CYTOGENETIQUE ET MOLECULAIRE D'UN CAS DE DYSMORPHIE ASSOCIEE A UN RETARD MENTAL****TOUHAMI RAHMA¹, HAJER FODDHA¹, NADIA LEBAN¹, SARRA DIMASSI², DORRA HMIDA², ALI SAAD², DAMIEN SANLAVILLE, JEMNI BEN CHIBANI¹, AMEL HAJ KHELIL¹.**

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Dans les populations humaines, les dysmorphies associées à un retard mental de type sévère représentent des handicaps très lourds à gérer aussi bien au niveau familial que national. La connaissance de la cause biologique de l' handicap chez un patient constitue, dans certains cas, un soulagement pour les parents et permet un conseil génétique, voire un diagnostic prénatal.

Notre travail a porté sur une patiente atteinte de dysmorphie faciale, d'un retard mental sévère associé à des troubles moteurs et comportementaux évoquant un Syndrome d'Angelman ou de Kleeftstra.

Nous avons entrepris d'explorer cette patiente sur les plans cytogénétique et moléculaire à la recherche d'éventuelles aberrations de nombre ou de structure des chromosomes, de petites délétions ou de mutations géniques. Tout d'abord, un caryotype standard a été réalisé suivi d'une FISH (Fluorescence in Situ Hybridization) puis d'une CGH (Comparative Genome Hybridization). Ces explorations ont été complétées par une analyse moléculaire au niveau génique basée sur la PCR et le séquençage automatique d'une région du gène ABO. En effet, une donnée sur le groupe sanguin de cette patiente, comparé à ceux de ses parents, est en faveur d'une délétion au niveau de ce gène.

Les résultats du caryotype standard, de la FISH et de la CGH se sont révélés négatifs pour les aberrations de nombre et de grandes délétions, éloignant ainsi les hypothèses des syndromes d'Angelman, de Kleeftstra ou d'autres syndromes dus à des aberrations de structure des chromosomes. Par contre, le séquençage d'une région du gène ABO est en faveur de la présence d'une délétion, à l'état hétérozygote, au niveau de ce gène, qui n'aurait pas été détectée par les autres techniques à cause de la limite de leur résolution.

Nous envisageons de compléter ce travail par des méthodes de PCR à grande échelle pour confirmer cette délétion et pour identifier les limites de la région délétée.



C. ORALE N°:93.

ETUDE DE L'IMMUNOSCORE COMME MARQUEUR PRONOSTIQUE ET PREDICTIF DANS LA PRISE EN CHARGE DES CANCERS COLORECTAUX

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Le pronostic du cancer colorectal est défini à partir du score TNM qui permet d'évaluer la progression tumorale et de choisir le traitement adjuvant approprié. Cependant, malgré la puissance pronostique de ce système, le devenir clinique des patients est très variable pour un même stade TNM. De ce fait, l'étude des cellules lymphocytaires, infiltrant la tumeur, notamment CD3 et CD8, a permis d'adapter un score immunitaire dénommé «immunoscore» qui pourrait améliorer davantage l'appréciation pronostique et la prise en charge thérapeutique des patients. Notre objectif était d'apprécier la valeur pronostique et prédictive de l'immunoscore et de les corrélérer avec les paramètres clinicopathologiques spécifiques à notre population. Nous portons sur une étude rétrospective de 106 patients atteints de cancer colorectal sporadique avec un minimum de 5 ans de recul permettant d'évaluer la survie. La quantification de CD3 et CD8 a été réalisée moyennant un logiciel dédié à l'étude de l'immunoscore sur des lames immunohistochimiques scannées. Des études uni-variées et multi-variées ont été réalisées en utilisant deux logiciels de statistiques SPSS v19.0 et R. Contrairement aux résultats obtenus avec la classification TNM en analyse multi-variée ($P=0.057$), l'immunoscore a révélé une forte association significative avec la survie globale ($P<0.00011$), et avec la survie sans récurrence ($P<0.0008$). Il y a fort à parier qu'une meilleure compréhension des paramètres immunitaires dans le microenvironnement tumoral recto-colique serait indispensable pour instaurer un traitement anti-tumoral efficace. A travers cette étude, nous avons constaté que l'immunoscore pourrait être un facteur pronostique et prédictif à la réponse au traitement contrairement à la stadification TNM.

C. ORALE N°:94.

MARINER LIKE ELEMENT ANNOTATION AND DYNAMICS IN THE GENOME OF *BEMISIA TABACI*

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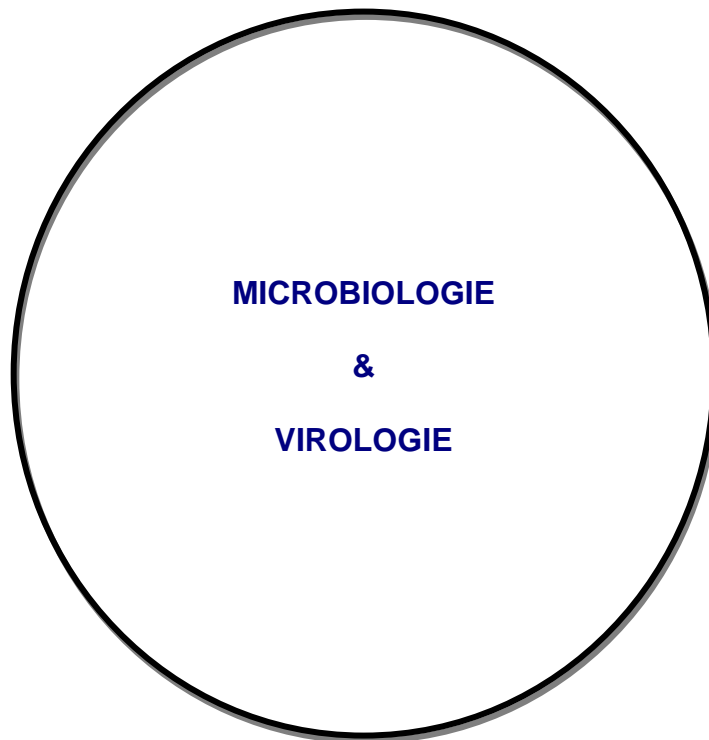
Transposable elements are mobile genetic units that occupy a large fraction of many eukaryotic genomes and have the ability to induce genome evolution in diverse ways of almost all organisms. In *Bemisia tabaci*, transposable elements were found to occupy almost the half of the genome with 40,29 percent. In this study, we focus on the annotation of Mariner Like Elements (MLE) in *Bemisia tabaci* genome and emphasize how this family of elements is distinguished from other types of mobile elements in terms of their structure, amplification and dynamics. We provide the first research on the diversity of MLE subfamily based on both in silico and in vitro identification. The in silico analysis using numerous programs allowed us to identify 546 non redundant MLE sequences that are clustered into 12 consensus and 15 singles. The phylogenetic analysis showed that most of the identified MLEs (506) belongs to the irritans subfamily and the remaining elements to the mauritiana subfamily. Data analysis showed that irritans elements are very heterogeneous and are subdivided into 9 consensus and 11 singles. Whereas mauritiana elements are homogeneous and subdivided into 3 consensus and 4 singles. Moreover, copies structure analysis revealed the presence of two potentially active elements within the irritans and mauritiana subfamilies with a complete ORF, 2 TIRs and 2 UTRs. The presence of the active elements was verified in vitro by PCR with primers designated from the MLEs identified in silico. The PCR products were purified and cloned into the pGEM cloning vector and subjected to sequencing. Sequence analysis confirmed the presence of irritans elements in the *Bemisia tabaci* genome; nevertheless, the mauritiana elements could not be detected experimentally by the primers defined in silico. This could be explained by mutations in the primers hybridization regions.

Keywords: Transposable elements, MLEs, evolutionary dynamics, *Bemisia tabaci*.



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C. ORALE N°:95.

ROLE OF AN ANCIENT BACTERIAL SIGNALING PATHWAY IN PLANT IMMUNITY

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The nucleotide guanosine tetraphosphate (ppGpp) accumulates in bacteria in response to stress. ppGpp slows down proliferation by acting on replication, transcription and translation and can be required for survival during stress exposure. The level of ppGpp is modulated by the enzymes RSH (for RelA /

SpoT homologues according to the enzymes in *E. coli*). During the 2000s, RSH enzymes were also discovered in plant chloroplasts. However, the role of ppGpp in the functioning of the chloroplast and the response to stress in plants remains unknown. By studying different pathosystems (bacterial and viral) we were able to demonstrate and verify that RSH enzymes are involved in the regulation of plant-pathogen interactions and that a variation in the levels of ppGpp appears to affect pathogen resistance. Our results support recent research showing that chloroplast function can play a decisive role during growth, development, and plant immunity.

Mots clé:

ppGpp - RSH enzymes- pathogen – chloroplast

C. ORALE N°:96.

PREVALENCE OF EXTENDED- SPECTRUM B-LACTAMASES PRODUCING ENTEROBACTERIACEAE IN SHEEP MEAT MARKETED AT TEBESSA- ALGERIA

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Extended- spectrum β -lactamase (ESBL)-producing enterobacteriaceae, now pose a major public health problem, in the worldwide, because of the difficulty of treatment of infections due to these multiresistant bacteria. Several studies have been carried out on the detection of these multidrug bacteria in different environments: especially in the hospital environment and even in the natural environment (sewage) and food products particularly meats. The aim of this study is to evaluate the frequency of sheep meat contamination marketed in Tébessa, by ESBL-producing enterobacteriaceae. The sampling of sheep meat was carried in aseptic conditions. Isolation, identification and antibiotic susceptibility testing were performed according to standard methods. ESBL detection was performed according to the synergy test and double disk test. From six meat samples, 40 enterobacteriaceae strains have been identified. The results showed a relative diversity of genera and species. The most dominant species belongs to the genus *Klebsiella* presented mainly by the species *Klebsiella ornithinolytica*, followed by *Serratia* and *Hafnia*. The most marked resistance is observed with regard to antibiotics: Ticarcilina 92.5%, Cefoxitin 62.85%, Aztreonam and Amoxiciline 52.5%, Amoxiciline / Clavulanic acid, Tetracycline and Trimethoprim-sulfametoxazole 47.5%. The results of the ESBL detection tests allowed to screen 23 strains ESBL-producing, that is to say 57.5% among the total enterobacteria, with a predominance of the species *Serratia odorifera* (39.13%) and *Klebsiella ornithinolytica* (26.09%). The presence of ESBL-producing enterobacteriaceae in sheep meat may be related to the use of beta-lactams in veterinary medicine. This study suggests the detection of ESBL-producing enterobacteriaceae in sheep meat, prompting the need for close monitoring of the spread of these resistant bacteria to different levels.

Keywords: Enterobacteriaceae, Extended- spectrum β -lactamase, sheep meat, antibiotic resistance. Key words: E

**C. ORALE N°:97.****POTENTIAL OF AN ENDOPHYTIC BACTERIA IN TOMATO GROWTH PROMOTION AND PROTECTION AGAINST VERTICILLIUM WILT DISEASE****DHOUB-FEKI HANEN, TOUNSI SLIM***[Centre de Biotechnologie de SfaxP B 1177, 3018, Sfax, Tunisie.]*

[Vascular pathogens, including *Verticilliumdahliae*, are generally devastating disease for which no effectiveness of control measures exist. Given its endogenous behaviors, the application of endophyticbacteria can be able to limit the disease. The main objective of this study is to evaluate, in vitro and in vivo the efficacy of a novel endophytic strain of *Bacillus amyloliquefaciens*, named C2, which is isolated from sterilized tomato crown, in tomato protection against *V. dahliae*. It has been selected based on its potent in vitro antifungal activity against *V. dahliae*, and itsability to produce several antifungal and growth promoting metabolites. The treatment of tomato plant with C2 at 108 CFU/mL permit to reduce disease by 83,18%, compared to V30-inoculated untreated control. A significant enhancement of growth parameters was recorded on tomato plants inoculated or not with the pathogen. The rhizospheric competence and endophytic colonization were demonstrated at rhizosphere and in the different internal tissues of tomato (root, crown, stem, leaves, flower and fruit), respectively, for a period of 50 days. The aptitude of this strain to protect and promote the growth of tomato seemed to be the fruit of interaction of multiples mechanism: rhizospheric competence, potential endophytic colonization and ability to produce siderophore, lytic enzymes, lipopeptides, polyketides and dipeptide. *B. amyloliquefaciens*C2 has a great potential to be commercialized as a biocontrol agent against vascular fungal pathogens.]

Keywords:*Bacillus amyloliquefaciens*, *Verticillium dahliae*, Disease suppression, Tomato growth, Endophytic colonisation.

C. ORALE N°:98.**PRODUCTION AND CHARACTERIZATION OF LICHENYSIN BIOSURFACTANTS FROM A MARINE *BACILLUS LICHENIFORMIS* STRAIN PYR2****HENTATI DORRA *, NEILA MAKHLOUFI, ASMA MAHMOUDI, SAMI SAYADI, MOHAMED CHAMKHA***Laboratory of Environmental Bioprocesses, Centre of Biotechnology of Sfax, University of Sfax, PO Box 1177, 3018 Sfax, Tunisia.***Email contact : dorra_90@live.com ; mohamed.chamkha@cbs.rnrt.tn*

Abstract: An aerobic marine *Bacillus licheniformis* strain PYR2 was isolated after enrichment on pyrene, a persistent and toxic polycyclic aromatic hydrocarbon (PAH). Strain PYR2 was able to degrade pyrene (100 mg l⁻¹), used as the sole source of carbon and energy, in basal liquid medium, in the presence of 30 g l⁻¹ NaCl and at 37 °C. In addition, the isolate PYR2 showed a remarkable capacity to use a wide range of hydrocarbons as the sole carbon and energy sources (hexadecane, naphthalene, fluoranthene, diesel fuel, crude oil, etc.). Besides, strain PYR2 showed its capacity to produce an efficient surface active agent BS-PYR2 on several substrates and in particular the residual frying oil, which is an alternative, cheap and renewable carbon source, thus minimizing the high cost of producing surfactants. MALDI-TOF MS/MS analyses confirmed the presence of lipopeptides identified as members of lichenysin series. The critical micellar concentration of the purified lipopeptides produced by strain PYR2 was 25 mg/l and, at this concentration, the surface tension of the water was reduced from 72 to 27 mN/m. Furthermore, the crude lipopeptides showed an interest stability against a broad range of pH (2.1-12), temperature (4-121 °C) and salinity (0-200 g/l NaCl). The application of BS-PYR2 in oil recovery from soil contaminated by hydrocarbons (used motor oil) showed that it was more effective on the hydrocarbon-remobilization than some tested synthetic surfactants. Moreover, BS-PYR2 showed an interesting healing activity, on the wound site in a rat model. They increased significantly the percentage of wound closure when compared to the untreated and CICAFLORA- (a reference pharmaceutical product) treated groups, using two different concentrations (5 and 10 mg.l⁻¹) of BS-PYR2.

These results highlight the applicability of the biosurfactants produced by the new marine *Bacillus licheniformis* strain PYR2 in different fields, especially in environmental remediation processes.

Keywords: Biodegradation; Pyrene; *Bacillus licheniformis*, Lichenysin; Remobilization; Dermal wound healing



C. ORALE N°:99.

SYNERGISTIC INTERACTION OF ETHYL ACETATE FRACTION OF *EMEX SPINOSA* AND COMMERCIAL ANTIBIOTICS AGAINST PATHOGENIC BACTERIA

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[Strain-resistant bacteria infections have become difficult to handle. Thus, combining medicinal plants with synthetic drugs against such bacteria might be an urgent need. In this study, the *Emexspinosa* (*E. spinosa*) aerial parts in inhibiting pathogenic bacteria as well as their interaction with commercial antibiotics (Ampicillin, Kanamycin, Chloramphenicol, and Streptomycin) were investigated. The ethyl acetate fraction of this plant (EtOAc-F), containing high concentration of phenolics, was shown to be the most active against the tested bacteria, thanks to its antibacterial compounds richness. It was therefore selected for its synergistic interaction with antibiotics using the checkerboard method. The MICs of this fraction varied between 0.3125 and 5 mg/ml, whereas those of the antibiotics ranged between 1.125 µg/ml and 16 mg/ml. The achieved results showed different types of interaction (synergistic and additive) between the EtOAc-F and antibiotics with FIC values ranging between 0.03125 and 2 respectively for both of them. These findings also suggest the possible use of EtOAc-F in combination with some antibiotics, mainly kanamycin and streptomycin, to enhance the effect of antibiotics in order to overcome bacterial resistance.]

Keywords: *Emexspinosa*; checkerboard assay; synergistic effect; resistant bacteria.

C. ORALE N°:100.

COMPOSITION CHIMIQUE ET ACTIVITE ANTIBACTERIENNE DE L'HUILE ESSENTIELLE D'ORIGAN (*ORIGANUM COMPACTUM*) ET CELLE DE LA CORIANDRE (*CORIANDRUM SATIVUM*) CONTRE 40 SOUCHES AVIAIRES D'*E. COLI*

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En médecine vétérinaire et notamment en aviculture, les antibiorésistances constituent un réel problème de santé publique, en effet, l'usage anarchique des antibiotiques, soit à titre thérapeutique, soit à titre prophylactique, a entraîné le développement de bactéries qui présentent, de plus en plus, des profils de résistance alarmants vis-à-vis de la plupart des antimicrobiens qui sont utilisés non seulement en médecine vétérinaire, mais aussi en médecine humaine. En Algérie, le secteur avicole est l'un des secteurs les plus prospères et la viande blanche est la viande la plus consommée, du coup, toute sélection d'antibiorésistance va se répercuter inévitablement sur la santé du consommateur Algérien. Suite à cette constatation, notre approche a été de réfléchir à de nouvelles substances pouvant avoir le même effet antibactérien que celui des antibiotiques, permettant ainsi d'éliminer le problème des mortalités et des morbidités rencontrés lors de l'élevage, tout en ayant l'avantage d'éviter la sélection de nouvelles bactéries résistantes, voire multi résistantes à ces antimicrobiens et c'est ainsi que notre choix s'est porté sur l'utilisation de substances aux vertus ancestrales, à savoir, les plantes aromatiques et plus précisément leurs huiles essentielles. Par conséquent, après avoir isolé 40 souches pathogènes d'*E. Coli* aviaires (24 provenant du poulet de chair et 16 isolées de la dinde) et identifié leur profil de résistance aux antimicrobiens, nous les avons soumises à l'action de deux huiles essentielles: celle de l'Origan (*Origanum compactum*) et celle de la Coriandre (*Coriandrum sativum*) et ceci afin de déterminer leur effet antibactérien supposé. Notre travail nous a permis, suite à des analyses par Chromatographie en Phase Gazeuse couplée à la Spectrométrie de Masse (GC-MS), d'identifier les principaux composants chimiques des deux huiles essentielles suscitées et de démontrer que la comparaison entre ces deux huiles essentielles a permis de conclure que l'huile essentielle d'Origan (*Origanum compactum*), de par sa CMI et ses zones d'inhibition, a eu une activité antibactérienne plus importante que l'huile essentielle de la Coriandre (*Coriandrum sativum*).

Mots clés : Antibiorésistance, *E. coli* aviaires, Huiles essentielles, *Origanum vulgare*, *Coriandrum compactum*. Effet antibactérien



C. ORALE N°:101.

CIRCULATION DU VIRUS WEST NILE CHEZ L'AVIFAUNE SAUVAGE EN ALGERIE.

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Résumé : Le virus West nile (WNV) est un *Flavivirus* qui appartient au séro groupe de l'encéphalite japonaise, maintenu dans la nature dans un cycle enzootique qui fait intervenir les oiseaux comme des amplificateurs et les moustiques ornithophiles comme vecteur. Il est endémique dans le bassin méditerranéen, depuis 1999 le virus est émergé même dans le nouveau monde. En Algérie et depuis l'épidémie de 1994, la situation épidémiologique reste dans le domaine de l'inconnu. L'objectif de cette enquête transversale à visé descriptive est de détecter une éventuelle circulation du WNV chez l'avifaune sauvage dans la région de Kabylie. 165 sérums ont été prélevés chez deux espèces d'oiseaux sauvages à savoir *Turdus philomelos* (la grive) espèce migratrice et *Passer domesticus* (moineau) espèce pré-domestique. Les sérums ont été analysés d'abord par la technique ELISA de type compétition comme première screening, dans un second lieu, par le test de séroneutralisation pour confirmation. 13 échantillons ont fourni un résultat positif à l'ELISA, soit une séroprévalence de 8,4% (13/154) [IC à 95% : 4,1-12,7%] et 13 sérums ont été confirmés par la neutralisation, soit une prévalence de 7,8% (13/165) [IC 95% : 3,8-11,8%] avec une séropositivité de 10,8% [IC à 95% : 7,6-14%] pour la grive et de 4% [IC 95% : 2-6]. Aucune association statistiquement significative n'est observée entre les facteurs espèce, sexe, le site de capture et la séroprévalence.

Mots clés : Virus West nile, Avifaune sauvage, sérologie, prévalence, Kabylie.

C. ORALE N°:102.

BIOLOGICAL ACTIVITIES OF SOME ALGERIAN OLIVE OIL SAMPLES

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The olive oil is a veritable source of natural molecules has antimicrobial and antioxidant activities, in this context our study aims to evaluate the antimicrobial and antioxidant properties of some Algerian olive oil samples collected from the areas of: skikda, Blida, Mascara, Sidi Belabesse, Boumerdes, Ghelizene, Tlemcen, Ain Defla, Bedjiaï, Chlef, Jijel, Tissemsiltand Tizi Ouzou

Found results showed that the total polyphenols content in olive oil samples is significantly variable between the thirteen samples where we recorded that it is superior than 200eqggallic acid/g in Skikda's and Tissemsilt's oils, inversely to Ghelizene's and Tizi ouzou's oils which didn't exceed 80eqggallic acid/g; The antioxidant activity of Blida's, Boumerdes's and Ain Defla's oils has exceeded 75%. The statistical study shows that there is no correlation between the polyphenol content and the antioxidant activity in our samples.

The behavior of the bacterial strains to the phenolic extracts varied between the extreme sensitive, the sensitive and resistant without correlation between the content of oils in polyphenols and the antibacterial activities.

Keywords: Algerian, olive oil, antioxidant activity, antibacterial activity



C. ORALE N°:103.

LUPINUS MICRANTHUS IS NODULATED IN TUNISIA BY A NEW SYMBIOVAR "LUPINAE" WITHIN THE GENERA BRADYRHIZOBIUM

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A polyphasic approach analysis of two strains (LmiH4 and LmiM2) isolated from root nodules of *Lupinus micranthus* in Tunisia and described by Msaddak et al. (2017) are carried out. The phylogenetic analysis of the concatenated *rrs*, *recA* and *glnII* genes showed that the strain LmiH4 has 100% concatenated genes sequence identity with the type strain *Bradyrhizobium retamae* Ro19^T. Similarly, the strain LmiM2 shared 100% concatenated genes sequence identity with the species *B. valentinum* LmjM3^T. The analysis of the *nodC* gene showed that these strains are phylogenetically divergent to that formed by the *Bradyrhizobium* symbiovars defined to date, and represent a new symbiovar. By host range, plant infection analysis revealed that the two strains showed a large host range and diversity on symbiotic specificities. Based on the *nodC* phylogeny and nodulation phenotype on different host legumes, our result report that the two *Lupinus micranthus* strains belong to a new symbiovar which we propose the name *lupinae* within the species *Bradyrhizobium valentinum* (type strain LmiM2) and *B. retamae* (type strain LmiH4).

Keywords: *Bradyrhizobium*, *Lupinus micranthus*, Phylogeny, new symbiovar

C. ORALE N°:104.

CHARACTERIZATION AND BIOLOGICAL CONTROL OF CITRUS BLAST AND CITRUS BLACK PIT CAUSED BY PSEUDOMONAS SYRINGAE IN TUNISIA

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Citrus are of paramount importance in the agricultural sector, and are among the strategic products. Despite the use of several chemicals, including copper products, citrus fruits continue to face some biotic problems such as bacterial infections. The citrus blast and citrus black pit caused by the phytopathogen *Pseudomonas syringae*, have become more important in recent years. Many studies have been concentrated to screen antagonists bacterial with potent activity and reduce the damage caused by this bio-aggressor. The present work takes place within this research. The bacteria used were isolated with the pathogen *P.syringae* from symptomatic samples of citrus fruits and compared in vitro with Luria broth by the double layer method. The antagonists strains, confirmed effective in vitro, were injected alone on fruits and leaves to eliminate pathogens, and mixed with *P.syringae* to ensure their effects in vivo by the difference in necrosis diameters between Ps and (P.s + antagonist). The selected bacteria were subsequently identified by phenotypic identification: microscopic and biochemical, and molecular identification by amplification of citrate synthase and rpoD rDNA.



C. ORALE N°:105.

STUDY OF THE SEROPREVALENCE OF ANAPLASMA SPP. IN GOAT FARMS OF THE NORTH-EAST REGION OF ALGERIA.

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Anaplasmosis is rickettsiosis caused by an obligate intracellular Gram-negative bacterium of the family Anaplasmataceae; it is a vector disease transmitted by ticks. It is considered an emerging zoonotic infection with significant economic and epidemiological impact worldwide. In Algeria, some studies have been described with regard to bovine and canine anaplasmosis, but no studies have been conducted to investigate the possible circulation of *Anaplasma* spp. in goats. Therefore, it seemed interesting to carry out an epidemiological investigation on the erythrocyte pathogens of *Anaplasma*. The present work has given as main objective to make an original contribution to the knowledge of infections by *Anaplasma* spp. in goats in the northeastern region of Algeria (wilaya of Guelma and wilaya of El-taref), as well as the associated risk factors. Thus, 182 goat blood samples were taken between March and September 2016 and analyzed by ELISA. Information on the individual characteristics of the animals as well as on rearing practices was collected.

Overall seroprevalence by cELISA for *Anaplasma* spp. was 78.02%. The analysis of the risk factors, likely to influence the prevalence of the pathogen studied, showed the influence of the state of pregnancy ($P = 0.002$), of the season ($P = 0.015$), climatic conditions ($p = 0.000$) and rearing type ($p = 0.000$).

Key words: *Anaplasma* spp., C-ELISA, seroprevalence, risk factors, goats, North-East of Algeria.

C. ORALE N°:106.

BIOLOGICAL DIVERSITY AND PATHOGENICITY OF COLLETOTRICHUM GLOEOSPORIOIDES, CAUSAL AGENT OF CITRUS ANTHRACNOSE IN TUNISIA

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Anthracnose caused by *Colletotrichum gloeosporioides* is one of the most important postharvest diseases in Citrus. In the present work we studied the biological diversity and the pathogenicity of *C. gloeosporioides* associated with anthracnose symptoms on Citrus in Tunisia. The study was performed on a total of 54 monosporic isolates obtained from a survey conducted in 3 different governorates over two years. In vitro biological characterization highlighted a significant difference between the isolates for mycelial growth and sporulation rate after 7 days of incubation at 25°C on PDA medium. Diversity in macroscopic features of *C. gloeosporioides* cultures was also observed, which allowed a classification of the isolates into 8 groups with a morphologically different appearance. Overall, the biological diversity could not be explained neither by the host species, the sampling region, nor the year of sampling. In vivo pathogenicity tests realized on detached leaves of 6 Citrus species with 10 representative isolates of *C. gloeosporioides* population in Tunisia revealed the presence of 3 groups of isolates according to their virulence spectrum. A follow-up was conducted during 20 days after inoculation and *C. gloeosporioides* was re-isolated from symptomatic fruits and Koch's postulates were fulfilled. Knowledge on biological diversity and virulence spectrum of *C. gloeosporioides* population, generated in this study will enable the development of improved diagnostic approaches and disease management strategies.

Key words: *Colletotrichum gloeosporioides*, Citrus, biological diversity, pathogenicity test



C. ORALE N°:107.

SEQUENCE ANALYSIS OF POTATO VIRUS Y (PVY) STRAINS FROM TUNISIA.

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A survey of Potato virus Y (PVY) was conducted in cultivated fields from nine Tunisian regions between 2007 to 2013. A total of three hundred potato samples were collected and analyzed by enzyme-linked immunosorbent assay (ELISA). Among them, 80 Tunisian PVY isolates (PVY-TN) were subjected to systematic biological, serological using PVYO-Cand PVYN specific monoclonal antibodies. Furthermore, molecular typing of HC-Pro/P3 region was realized by immunocapture reverse-transcription polymerase chain reaction. PVY was detected in all surveyed regions, and the most common strain identified was the recombinant PVYNTN variant (67%) of which 9 were sequenced to assess the genetic diversity and phylogenetic relationship of PVY-TN against other worldwide PVY isolates. Moreover, sequencing analysis performed for nine Tunisian isolates belonging to different geographical regions revealed for the first time the presence of a new recombinant strain group of PVY, similar to PVYN:O or N-W variants.

Key words: PVY, potato, sequencing, recombinant isolates

C. ORALE N°:108.

CHARACTERIZATION OF THE MELON NECROTIC SPOT VIRUS STRAINS IN TUNISIA

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Melon necrotic spot virus (MNSV), belonging to the *Carmovirus* genus of the *Tombusviridae* family, is an endemic virus in greenhouse and open field crops of melon (*Cucumis melo*), cucumber (*Cucumis sativus*) and watermelon (*Citrullus lanatus*). Even the host range of MNSV is limited exclusively to plant species of Cucurbitaceae family, this virus has been reported in several countries in America, Africa, Asia and Europe. Symptoms in melon consist of stem necrosis at the crown level and, less frequently, of small necrotic spots that sometimes abscise to leave holes on leaves and fruits. Wilting and plant death have also been observed. Experimentally, this virus can be transmitted by mechanical inoculation. Despite of this, MNSV is also transmitted by both Chytrid fungus (*Olpidium bornovanus*) and seeds. Resistance of melon (*Cucumis melo* L.) to MNSV is inherited as a single recessive gene, denoted *nsv*. No MNSV isolates described to date, except the MNSV-264 strain, are able to overcome such resistance. To the aim of identification and further molecular characterization of Tunisian MNSV isolates, 30 samples of cucurbit plant hosts were collected from the main agricultural growing areas in Tunisia. Total RNA was extracted from samples and then analyzed by Northern Blot using several MNSV-CP probes. We were able to detect 12 MNSV isolates among which four were found to belong to MNSV-264 strain and eight correspond to MNSV-Mα5. To explore the potential contribution of some viral regions in symptom expression, the 3'UTR region of different strains were sequenced and compared to each other as well as to well characterized MNSV ubiquitous strains. Coat protein genes were also used in a viral system expression to explore whether this region is involved or not in MNSV pathogenicity.

Key word: MNSV, 3'UTR region, CP gene, Northern blot, Sequencing, viral expression



C. ORALE N°:109.

ÉVALUATION *IN VITRO* DU POTENTIEL PROBIOTIQUE DE *PEDIOCOCCUS PENTOSACEUS* MZF16, ISOLE D'UN PRODUIT CARNE ARTISANAL « OSSBAN SECHE »

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Le « Ossban séché », un produit carné artisanal, représente une niche écologique originale pour l'étude de sa microflore lactique. Le screening d'une trentaine d'échantillons couvrant quasiment tout le territoire de la Tunisie a permis d'isoler une centaine de bactéries lactiques. Les tests physiologiques et biochimiques ont permis de garder quelques isolats pour des études ultérieures. Ainsi, l'isolat MZF16 a été sélectionné pour son fort pouvoir inhibiteur contre *Listeria monocytogenes*, *Enterococcus faecalis* ATCC 29212 et *Listeria innocua* HPB13. L'identification par MALDI-TOF a montré qu'il s'agit d'une souche de *Pediococcus pentosaceus*, ce résultat a été confirmé par le séquençage du gène de l'ARNr 16S. L'étude *in vitro* du potentiel probiotique de MZF16 a montré une très bonne résistance à l'acidité et aux sels biliaires. Cette souche est résistante à la gentamicine mais présente une bonne sensibilité à quatre antibiotiques, et une absence de cytotoxicité sur des cellules intestinales Caco-2/TC7. Malgré un taux élevé d'auto-agrégation (89%). MZF16 est faiblement adhésive sur les cellules Caco-2/TC7, mais elle permet de renforcer légèrement la barrière intestinale. Ainsi, dans ce travail, nous avons étudié pour la première fois l'activité probiotique d'une souche isolée d'un biotope original « Ossban séché ». Les résultats obtenus montrent que la souche MZF16 satisfait aux exigences principales d'un probiotique et possède une bonne activité anti-*Listeria*. Cette souche pourrait donc être un excellent candidat pour des travaux complémentaires dans le but de valider son utilisation en tant que probiotique dans les produits carnés.

Mots clés : Bactérie lactique, *Pediococcus pentosaceus*, produit carné artisanal, Ossban séché, potentiel probiotique.



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**TOXICOLOGIE,
&
PHARMACOLOGIE**

**C. ORALE N°:110.****ANALGESIC, BEHAVIORAL EFFECTS AND CHRONIC TOXICITY OF THE NEW 3,5-DIAMINOPYRAZOLE AND THE THIOCYANOACETAMIDE****BEN ALI RIDHA^A, AMAL BEN OTHMAN^A, KHOULOU BOKRI^B, SAMIRA MAGHRAOUT^C, ADEL HAJRI^P, AZAIEZ BEN AKACHA^B, CHADLI DZIRI^A, MICHÈLE VÉRONIQUE EL MAY^{A,E}**

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The synthesis of pyrazole derivatives remains of a great interest owing to their wide applications in agrochemical and pharmaceutical industries due to their herbicidal, insecticidal, analgesic, antipyretic, anti-inflammatory, anticancer, antituberculosis, antihypertensive, antifungal, antidepressant and antimicrobial properties. Thioamide includes the major drugs for the treatment of thyrotoxicosis and hyperthyroidism by inhibiting the enzyme thyroid peroxidase in the thyroid. Thioamide derivatives, such as thiocyanacetamide, were used in the chemical synthesis of 3,5-diaminopyrazole. This study aimed to explore the analgesic, antioxidant, behavioral and toxicological effects of 3,5-diaminopyrazole and thiocyanacetamide. Caffeine was used as reference drug. Twenty male Wistar rats were divided into 4 groups, tested and the effects were known after oral treatment with an efficient dose (10 mg/kg/day) for 30 days.

The preliminary bioassays indicated that both compounds at this dose have strong antioxidant capacities and present highly analgesic effects. The behavioral study showed an activation of the rat memory by thiocyanacetamide. This molecule caused a phobia state to open areas in the elevated plus maze and specifically agoraphobia in the open field with a lack in the development of the exploratory capacity. 3,5-Diaminopyrazole caused memory troubles in rats that forgot the pathway to the exit from the maze, and induced an anxiety state revealed by immobility in closed arms of the elevated plus maze. All these observations were compared to the treatment by the known analgesic, caffeine, which increased the state of vigilance of the rats and developed their exploratory capacity. The chronic treatment with the three investigated compounds showed no sign of toxicity with the absence of effect on the body and organ weights, blood count, kidney and liver function and histology. 3,5-Diaminopyrazole and thiocyanacetamide have potent antioxidant and analgesic activities that are higher than caffeine with a safety profile. The chronic treatment by thiocyanacetamide activated the memory and caused an emotional state of agoraphobia.

Thus, the present study warrants further investigations involving these novel molecules for a possible development of new strong analgesic and antioxidant drugs which have an effect on the memory capacity.

Keywords: Analgesic, Antioxidant, Behavioral effect, Safety profile, 3,5-diaminopyrazole, thiocyanacetamide

C. ORALE N°:111.**IPECAC INFUSION: ASSESSING POLYPHENOLS BIOACCESSIBILITY, BIOAVAILABILITY AND ANTIOXIDANT POTENTIAL USING AN IN VITRO DIGESTION SYSTEM AND A CACO2 CELL MODEL.****BEN HLEL TAKOUA^{A,B}, THAYS BORGES^C, ASCENCION RUEDA^C, ISSAM SMAALI^A, ISABEL SEIQUER^C AND MOHAMED NEJIB MARZOUKI^A**

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Carapichea ipecacuanha root better known as ipecac has been consumed as an infusion or a syrup for a long time for several purposes. In this report, we investigated for the first time the polyphenols content and antioxidant activity of ipecac infusion before and after digestion using an in vitro oral and gastrointestinal digestion system in vitro and Caco2 cell model in order to study the bioavailability of its total phenolic content after ingestion. In addition, we checked the often neglected insoluble residual fractions for any antioxidant activity and ultimately, we evaluated the ability of ipecac bioaccessible fraction to inhibit reactive oxygen species generation at a cellular level. Results revealed that ipecac polyphenols increases in content and activity after digestion and they are highly bioavailable after intestinal absorption (75.25%). Polyphenols were also present in the residual parts which indicate a possible local activity. The ability of the bioaccessible fraction to protect Caco2 cells against ROS generation suggest that ipecac infusion could represent a promising source of bioavailable polyphenols to be exploited in functional food field.

Mots clés : *Carapichea ipecacuanha*, digestion, bioaccessibility, Bioavailability, Caco-2 cells



C. ORALE N°:112.

SYNTHESE ET ETUDE DE L'ACTIVITE ANTIBACTERIENNE DE NOUVEAUX DERIVES THIAZOLIQUES

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Depuis leur apparition, Les antibiotiques sont restés le moyen privilégié de lutte contre les infections bactériennes. Si la découverte et l'utilisation des antibiotiques ont été à l'origine des plus grands succès de la médecine, aujourd'hui, l'émergence et la diffusion des bactéries multi-résistantes dans les populations humaines sont devenues des problèmes de santé publique très préoccupants.

Les hétérocycles soufrés, oxygénés et azotés naturels et synthétiques, connus pour leurs diverses et importantes activités biologiques. Parmi les différents hétérocycles aromatiques, les thiazoles occupent une position de premier plan dans le processus de découverte du médicament. Les dérivés thiazoles ont été utilisés comme médicaments vitaux dans le traitement de diverses pathologies. Ils ont été signalés pour posséder des activités biologiques importantes et diverses telles que antimicrobienne, analgésique, anti-inflammatoire, antioxydante et anticancéreuse.

Dans ce contexte et dans l'optique de rechercher de nouvelles molécules d'intérêt pharmacologique, nous avons opté pour la synthèse et la valorisation biologique de nouveaux dérivés thiazoliques. Tenant compte des résultats obtenus par les trois méthodes étudiées : dilution en milieu liquide, diffusion des disques sur un milieu gélosé solide et la méthode de des stries parallèles, les tests microbiologiques in vitro des composés synthétisés sur des souches bactériennes d'intérêt médical (*Escherichia coli*, *Pseudomonas aeruginosa* et *Staphylococcus aureus*) ont permis de mettre en évidence une bonne activité antibactérienne et s'avèrent comme de bons intermédiaires thérapeutiques.

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C. ORALE N°:113.

SHELF LIFE OF COOKED COLD SMOKED MEDITERRANEAN MUSSELS (*MYTILUS GALLOPROVINCIALIS*): EVALUATION OF THE SENSORY, MICROBIOLOGICAL, CHEMICAL AND BIOCHEMICAL ATTRIBUTES

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Abstract

The effect of cold smoking on the quality and shelf-life of cooked mussels *Mytilus galloprovincialis* was studied during 15 days of refrigerated storage (4°C). The analysis concerned microbiological (*Escherichia coli* and *Salmonella* spp), sensory (odour, colour, taste, texture and general acceptability), physicochemical (pH and water activity), biochemical (moisture, ash, total proteins, total lipids and total carbohydrates) and quality indices (TVB-N, TMA-N, TBA, biogenic amines) of cooked mussels, cold smoked and vacuum-packed products. Microbiological analysis revealed a total absence of *E.coli* and *Salmonella* spp in all samples. The TVB-N, TBA and histamine levels remained under the thresholds of acceptability (25 mg / 100g; 1 mg MDA / kg and 100 mg/Kg, respectively) during 12th days of storage in both batches. Principal component analysis (PCA) showed that cold smoking procedure has improved the sensory attributes of the product. Sensory evaluation and TMA-N's data indicated a shelf life of 9 days for both products.

Keywords: *Mytilus galloprovincialis*, cold smoking, cooking, vacuum packaging, refrigerated storage



C. ORALE N°:114.

EFFET DE TRAITEMENT THERMIQUE SUR L'ACTIVITE ANTI-OXYDANTE ET ANTI-TUMORALE DE MESQUITOL VIS-A-VIS DES CELLULES DE GLIOBLASTOME U87

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Résumé : Le traitement thermique peut affecter la structure chimique des flavonoïdes engendrant des variations de leurs potentiels biologiques. Le mesquitol, un flavan-3-ol, connu pour son potentiel antioxydant, anti-radicalaire et anticancéreux. Dans ce contexte, la présente étude vise à suivre l'effet de la chaleur sur le mesquitol traité à 130°C dans un bain d'huile pendant 120 min et de le comparer avec celle de la molécule native, afin d'estimer son potentiel antioxydant enzymatique (catalase, SOD et GST), non enzymatique (ABTS et OH.) et son pouvoir anticancéreux au niveau des cellules du glioblastome U87. Les résultats obtenus montrent que le traitement thermique réduit l'activité anti-radicalaire et antioxydante enzymatique de mesquitol de façon significative. De plus, le mesquitol montre un effet antimétastatique vis-à-vis de la prolifération, d'adhésion et d'invasion des cellules U87, tandis qu'après traitement thermique, elle présente des pourcentages d'inhibition moins importants pour les mêmes activités. De même, le mesquitol montre un effet oxydant via la génération des radicaux libres d'oxygène au niveau des cellules cancéreuses U87. Aussi que, cette réduction d'activité de mesquitol traité thermiquement n'est pas spécifique des cellules de glioblastome seulement puisqu'on a enregistré aussi une baisse de l'inhibition de l'angiogenèse des cellules endothéliales (HMEC-1). Et comme conclusion, il est prouvé qu'il est plus préférable de consommer certains aliments crus.

Mots clés : Mesquitol, traitement thermique, glioblastome, adhésion, invasion, angiogenèse, potentiel anti-oxydant.

C. ORALE N°:115.

PROTECTIVE EFFECT OF POMEGRANATE BARK EXTRACT AGAINST ALUMINUM-INDUCED COGNITIVE IMPAIRMENTS.

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Al is highly neurotoxic metal. The aim of this study is to evaluate the risk of aluminum chronic toxicity by using neurobehavioural and histological approaches as well as evaluate the neuroprotective effect of pomegranate bark. The use of the behavioral (Open field, elevated plus-maze) and memory (Morris water maze) tests have shown that administration of aluminum chloride (500mg / kg) orally to mice for 90 days caused neurological disturbances; locomotor hyperactivity, a decrease on learning and memory lost ($P < 0.05$) and anxiety ($P > 0.05$). On the other hand an increase in histological alterations in the cerebral cortex, hippocampus have been detected by haematoxylin & eosin staining. These neurological and histological alterations were restored by daily supplementation of methanolic extract of Punica granatum bark (500mg / kg). This extract showed anxiolytic effect as well as improved learning, memory and locomotion ($P > 0.05$) attributed to the high content of bioactive molecules (polyphenols, flavonoids, Tannins). These beneficial effects show that the grenade possess a neuroprotective effect.

Keywords: Pomegranate bark, mice, behavioral tests, Memory tests, Neurotoxicity, Histopathology.



C. ORALE N°:116.

TOTAL POLYPHENOL, FLAVONOID, AND PROANTHOCYANIDINE CONTENTS AND IN VITRO ANTIOXIDANT, ANTIMICROBIAL, AND ANTI-ENZYMATIC ACTIVITIES OF INULA GRAVEOLENS EXTRACTS COLLECTED FROM CHEBBA (TUNISIA) SALT MARSH COAST

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Abstract:In the context of screening the biological activities of the flora, living in Chebba salt marsh coast under strong salinity condition, this work was aimed to investigate the polyphenolic contents of *Inula graveolens* aerial part extracts and their antioxidant, antimicrobial, anticholinesterase and antityrosinase activities that could lead to the finding of more effective agents for the treatment and the management of several infections and diseases. To understand, in one hand the effect of salinity stress on polyphenol production, and in other hand, the contribution of *I. graveolens* polyphenolic compounds to these biological activities, the determination of total polyphenols (TPP), flavonoids (TF), and proanthocyanidines (TPC) contents was performed, and the correlation between their amounts and the different biological activities was established. The results of this study recorded important quantities of polyphenols, especially in acetone extract, that reflected strong antiradical activities, and revealed that the polyphenolic compound content differences could be determinant for the medicinal properties of *I. graveolens* especially for scavenging power and antiacetylcholinesterase activity. However, there were not an important correlation between polyphenol amounts and the antimicrobial activities, yet the activities against some strains seem to be correlated to TPP content. Tyrosinase inhibitory power, instead, seem to be not correlated to the polyphenol amounts.

Keywords: *Inula graveolens*, polyphenolic contents, antioxidants, antimicrobials, anti-acetylcholinesterase, anti-tyrosinase.

C. ORALE N°:117.

IN VIVO STUDY OF THE ANTIPARKINSONIAN EFFECT OF QUERCUS SUBER (EXPERIMENTAL STUDY IN MICE)

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Parkinson's disease is characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta in the basal ganglia of the central nervous system. This results in an engine malfunction including idiopathic three main symptoms: posture disorders, bradykinesia and tremor.

Traditional treatment remains on L-dopa which allows the relief of the patients and allows them to move a little more normally. By cons, this treatment causes side effects in the medium and long term. These occur after taking L Dopa and manifest as abnormal involuntary movements.

Unlike chemical drugs that can be very aggressive (like chemotherapy), herbal medicine has no side effects. The plants used in herbal medicine are scientifically tested, and the remedies are more effective but also safe, the human body is best suited to herbal treatment that essentially chemical therapy. Natural extracts of plants contain a variety of phenolic compounds which are used in the biological activities.

Our study is concerned on the antiparkinsonian and antioxidant effect of *Quercussuber* leaves. The tested extract was obtained by ethanoic extraction, it was tested on female mice received haloperidol for 7 days, the obtained results showed a beneficial effect on cognitive function which results in an improvement of locomotor activity as well as some hematologic and histologic analysis.

The ethanol extract of *Quercus suber* is endowed with very important antioxidant power that is protective and corrective of the damages caused by haloperidol and can be considered as an antioxidant mediator.

Key words: Antiparkinsonian, *Quercus suber*, haloperidol, antioxydant