

# POSTERS

Abstracts



EuroSciCon Joint Events on

## Plant Science, Tissue Engineering and Parasitology

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## CAN *SCHISTOSOMA* BE DETECTED BY ENVIRONMENTAL DNA?

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**S**chistosomiasis is a parasitic disease that causes severe pathology, mortality and economic loss worldwide. It is especially prevalent in Africa. These parasites require intermediate host (snails) in their life cycles. This pathogen continues to extend to new geographical regions despite significant advances in control. To fully understand the dynamics of schistosomiasis transmission, integration of the ecological factors that affect both humans and freshwater snails is needed. New diagnostic methods that target extra-organismal environmental DNA (eDNA) can offer better identification of human-infecting *Schistosoma* in epidemiological studies. These methods could also provide more sensitive detection in low population densities of the target organisms. Therefore, this study aimed to design and test real-time qPCR probes and primers for *Schistosoma mansoni*, *S. haematobium* and *S. japonicum* by amplifying species-specific amplification. The developed primers were tested on microcosm eDNA samples with confirmed *S. mansoni* presence in the cultures of gastropods. These developed primers and probes successfully identified the presence of *Schistosoma* within eDNA from the natural environment (Tanzania), thereby indicating that eDNA monitoring is a valid method for the detection of *Schistosoma* in freshwater bodies.

### Biography

Hind A Alzaylaee has completed her Masters' Degree from Taif University, Saudi Arabia and she is currently doing her PhD at University of Bristol. She is a Lecturer of Parasitology, Princess Nourah Bint Abdulrahman University, Saudi Arabia.

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# MAGNETIC FIELD EFFECT ON GERMINATION, SEEDLING GROWTH AND PHYSIOLOGIC CHARACTERS OF ENGLISH MARIGOLD (*CALENDULA OFFICINALIS L.*) UNDER SALINITY CONDITION

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The aim of this study was to investigate the effect of salinity levels (0, 75 and 150 mM) and the duration of treatment of seeds with magnetism (non-treatment of seeds with magnetism, treatment of seeds with magnetic intensity of 20  $\mu$ T for 5, 10, 15 min) on germination and growth of seedlings in English marigold. This study was conducted under laboratory conditions based on a completely randomized design in Depako Lab. In most traits, the interaction of salinity levels and magnetic treatment duration was significant. In the absence of magnetic treatment, salinity of 75 mM significantly reduced the seedling length (43.5%), seedling dry weight (28.3%), seedling dry weight (30.4%) and germination percentage (59.1%). In salinity of 150 mM, due to the lack of germination of seeds of English marigold, seedling growth characteristics were also zero, while the magnetic treatment improved germination and seedlings growth in marigold under salinity conditions and reduced the negative effect of salinity on these traits. However, in most of the traits, the magnetic treatment of seeds did not have a significant effect on zero salinity. It seems that the magnetic treatment of seeds with an effect on alpha-amylase activity improves the transfer of seed reservoirs and improves seedlings growth in the marigold at salinity conditions. Both salinity treatments and magnetic treatment of seeds have a significant effect on alpha amylase activity. In general, the magnetic treatment of seeds for 15 minutes was the best treatment to improve germination and seedling growth of English marigold in non-stress conditions and salinity. The results of this study showed that the use of magnetism can reduce the effect of soil salinity on the growth and yield of plants, in addition, it is necessary to investigate the potential of magnetism in reducing soil salinity in the next researches.

## Biography

Behruz Rahimzadeh has completed his primary, secondary education in Tabriz. He has completed his Bachelors and Master's degree from East Azerbaijan province universities. Since 2011, he has been working in the Green Landscaping Organization of East Azerbaijan. He is a Member of the Agricultural Engineering Academy, Member of the Green Space Organization of the Province, Member of the Association of Students of the Azad University and Member of the Committee for reviewing the Tabriz Green Environment Convention, 1397. He is currently working as a Senior Greenhouse Contractor in the field of green space. He has published two conference proceedings.

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# A SMALLER ROOT SYSTEM WITH ENHANCED BIOMASS ACCUMULATION AND TRANSPORTER EXPRESSION IN FOXTAIL MILLET [*SETARIA ITALICA* (L.) BEAUV.] UNDER LOW NITROGEN

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**F**oxtail millet (FM) [*Setaria italica* (L.) Beauv.] is an important grain and forage crop well adapted to nutrient-poor soils. Studies related to its adaption to nutrient limitation are rare. How FM adapts to low nitrogen (LN) at the morphological, physiological, and molecular levels remains worth studying and to date, little is known about that. Low nitrogen (LN) led to lower chlorophyll contents and N concentrations, and higher root/shoot and C/N ratios and N utilization efficiencies in FM variety Yugu1 under hydroponic culture. A smaller root system as indicated by significant decreases in total root length; crown root number and length; and lateral root number, length, and density; was in contrast to enhanced biomass accumulation in the root under LN. Increased average diameter of the LN root, potentially favourable for wider xylem vessels or other anatomical alterations favourable for nutrient transport facilitated enhanced carbon allocation towards root. Consistent with smaller root system IAA and CKs levels were lower whereas higher levels of GA may promote root thickening under LN. Further, up-regulation of *SiNRT1.1*, *SiNRT2.1*, and *SiNAR2.1* expression and nitrate influx in the root and that of *SiNRT1.11* and *SiNRT1.12* expression in the shoot probably favoured nitrate uptake and remobilization as a whole. Lastly, more soluble proteins accumulated in the N-deficient root likely as a result of increases of N utilization efficiencies. Such excessive protein-N was possibly available for shoot delivery. Thus, FM may preferentially transport carbon toward the root facilitating root thickening/nutrient transport and allocate N toward the shoot maximizing photosynthesis/carbon fixation as a primary adaptive strategy to N limitation.

## Biography

Faisal Nadeem has completed his Masters' degree in Agriculture-Soil Science from Pakistan and then got admitted to China Agricultural University, Beijing, China for PhD. He is in final year of his PhD and his research focuses on morphological, physiological and molecular response of foxtail millet to different nitrogen regimes. He has published one research article as first author and the other as second author during his PhD tenure so far.

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# WHOLE GENOME WIDE ASSOCIATION ANALYSIS OF MAIZE (*ZEA MAYS L.*) IN RESPONSE TO GLUTAMINE

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**M**aize (*Zea mays L.*) plays a significant role in global food security and energy consumption. Nitrogen (N), an important macronutrient, exists in soils heterogeneously both as inorganic and organic form. Amino acids, a crucial organic nitrogen source for plants, are not only the structural components of biological proteins but also have biological functions to regulate hormone synthesis, nitrogen transport, resistance to external abiotic stress, and root morphology. Glutamine is an important intermediate in plant nitrogen metabolism. The nitrogen absorbed by plants from the soil is first converted to glutamine, and then to other biomolecules vital for various biological activities of plants. In addition, glutamine as a metabolite of NO is a negative signal for plants to absorb nitrate and induce nitrogen metabolism. Therefore, understanding the molecular and physiological mechanisms of amino acid absorption and transport in maize, using emerging biotechnological and analytical methods, is of great significance. There are large differences in the genomes of maize, which provide a good opportunity to study them using genome-wide association analysis (GWAS). With the development of high-throughput sequencing technology and the application of correlation analysis of plants, genetic basis of amino acid absorption in maize can be further analysed. Use of GWAS combined with genomics and metabolomics and other big data analysis methods for the analysis of amino acid absorption and metabolism in maize becomes worth studying. This study used 510 maize inbred lines under control and glutamine treatment. We found phenotypic indicators related trait of maize varied broadly. There was 2.23-8.96 fold change and 7.76-18.48 fold change SPAD value, shoot dry weight, root dry weight, total dry weight, root length and root to shoot ratio as a whole under control and glutamine treatment, respectively. A set of 27229 high quality SNPs were used to perform GWAS on the diverse panel of 510 maize inbred lines. 6 genetic loci were identified in control whereas 5 genetic loci were identified in glutamine treatment at  $p \leq 3.35 \times 10^{-6}$  significant level.

## Biography

Huilan Luo has completed her Undergraduate degree from Shihezi University and Masters' degree from China Agriculture University. Currently, she is a PhD student in Department of Plant Nutrition, College of Resources and Environmental Sciences, China Agricultural University, China.

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# KERNEL ABORTION IS COUPLED WITH CIS-REGULATION OF ZMAAP2 AND ZMLHT1 BY ZMPHRS IN THE PHOSPHATE LIMITED MAIZE EAR

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**M**aize grain yield is depending on ear development and kernel number which is associated with the crucial silking stage. However, low P-caused consequences in developing maize ear remains elusive. Morphological alteration in the Pi deficient ear showed less kernel number but no less longitudinal growth. A cut down of soluble proteins, free amino acids and variation in hormone profiling, especially a significant drop in IAA accumulation were examined in low P feed ear as well. Transcriptional analysis uncovered 847 genes differentially expressed, with 423 genes induced and 424 genes repressed, which were characterized of genes related to hormones, transcription factors and the dominating repression of genes in transport category. To further investigate if those genes are directly bound to P deficiency, 313 out of 847 genes were found harbouring PHR1 Binding Sequence (P1BS) and featured in the pathways of N metabolism, lipid metabolism and photosynthesis indicating the wide spectrum consequences caused by Pi deficiency and the direct cross-talk between phosphorus and nitrogen signalling pathway. Furthermore, with yeast one-hybrid assay and EMSA, we detected the interaction between ZmPHR1 and P1BS at the promoter region of two amino acid transporters, ZmAAP2 and ZmLHT1, which function in transport of several key amino acids, and ZmPHR1 represses the expression of ZmAAP2 and ZmLHT1, consolidating the potential interaction between N and P. Taken together, these results suggest that the kernel abortion caused by Pi deficiency was associated with hormones variation and may intensively connected with the overall down regulation of genes in transport and especially the alteration of N metabolism.

## Biography

Ruifeng Wang is pursuing his PhD (third year) from Department of Plant Nutrition, China Agricultural University. His research works are focused on the molecular basis of phosphate deficiency to the developing maize ear and characteristics of AUX/IAA involved in the root development under nitrogen limitation. Recently, he is doing analysis of fine mapping genes related to maize root architecture traits with a RIL population. He has published three papers in reputed journals as a co-author of which one is focused on maize ear development under nitrogen deficiency and the other two on the root morphological modification of foxtail millet under N and Pi deficiency respectively. His interest is on plant genomics and molecular breeding.

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# INVESTIGATION OF SPATIOTEMPORAL DISTRIBUTION OF NITROGEN, HORMONE ACCUMULATION, AND EXPRESSION PATTERNS OF RELATED GENES IN THE MAIZE (*ZEA MAYS L.*) EAR UNDER LOW NITROGEN

**Xiaoting Liu, Yaoru Xie and Xuexian Li**

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**N**itrogen (N) is a crucial factor limiting the yield of maize, particularly by affecting the kernel number during the silking stage. Insufficient supply of nitrogen can lead to poor ear development and a decrease in the number of grains per ear. Phytohormones, as information transmission substances, play an essential regulatory role in the process of crop growth and development, especially during grain filling (i.e. the development and enrichment of endosperm). Therefore, studying the control mechanisms of maize cob and kernel development under nitrogen stress has important practical significance for improving maize yield. Field experiments were conducted using maize inbred line B73 to study the nitrogen and hormone accumulation and related gene expression characteristic to the maize cob development under low nitrogen condition. We found that low nitrogen significantly inhibited the growth of maize ears and led to baldness consistent with previous reports. At silking stage, the dry weight and nitrogen concentration of different parts of the maize ear followed the order as lower part > middle part > upper part, whereby reducing N concentration significantly in middle part. Furthermore, low nitrogen significantly reduced the accumulation of dry matter in the lower part of the corn cob; decreased auxin and abscisic acid concentrations in all parts of the corn ear without affecting cytokinins and gibberellin concentrations. Importantly, the expression patterns of hormone synthesis/signal transduction genes in the upper, middle and lower parts of the maize ear showed complex variations in rapidly developing maize ear under low nitrogen stress.

## Biography

XiaoTing Liu is a PhD scholar in The Key Laboratory of Plant-Soil Interactions, MOE, Department of Plant Nutrition, China Agricultural University, Beijing, China. During her Masters' degree, she used the promoters specifically expressed in the root surface to overexpress the mutant proteins that are free of phosphorylation to observe whether the nitrogen efficiency of transgenic rice can be improved. Her primary research work during PhD is the spatiotemporal distribution of nitrogen, hormone accumulation, and expression patterns of related genes in the maize (*Zea mays L.*) ear under low nitrogen.

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# ZMCCD8B ENCODES A CAROTENOID CLEAVAGE DIOXYGENASE REGULATING THE PLANT RESPONSE TO PHOSPHORUS LIMITATION

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In our study, overexpression of *ZmCCD8b*, a paralogous gene of *ZmCCD8a*, in *Arabidopsis* mutant *max4* failed to rescue the mutant phenotype. However, we found *ZmCCD8b* expression is significantly higher in silk, while relative higher in ear and root under low phosphorus (LP) condition reciprocal to control (sufficient nutrients) reaching the highest level on the tenth day. The expression was stronger in pericycle of the meristematic zone and the elongation zone as revealed by *In situ* hybridization. Protoplast amplification showed that this gene was localized to the plastid. We used virus induced gene silencing (VIGS) technology to silence *ZmCCD8b* and the results indicated that in contrast to GFP control, the carotenoid contents significantly reduced in leaves of *Zmccd8b* lines. In contrast to increased phosphorus concentration in root whereas, decreased phosphorus concentration in shoot, root biomass of *Zmccd8b* silenced lines non-significant. While shoot dry weight decreased significantly as compared to GFP control, contributing to an increased root to shoot ratio of *Zmccd8b* silenced lines. Furthermore, *ZmCCD8b* may affect the expression of *PHO2* and *PHO1* through PHRs. Additionally, *ZmPht1;1* and *ZmPht1;6* were down-regulated whereas, *ZmPht1;3* and *ZmPht1;13* were up-regulated in *Zmccd8b* silenced lines under LP conditions. Yeast one-hybrid and EMSA experiments verified that *ZmPHR1*s can regulate *ZmCCD8b* through P1BS element. Transcriptome analysis indicated increased expression of genes related to stress and signal transduction in *ZmCCD8b* heterologous overexpression lines.

## Biography

Zhong yanting, is pursuing his PhD from College of resources and environmental sciences, China Agricultural University, Beijing, China. His research broadly focuses on Molecular Biology of Plant Nutrition, and currently working on Phosphorus

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# PHOSPHORUS LIMITATION ENLARGES THE ROOT SYSTEM ALONG WITH CONTRASTING EXPRESSIONS OF PHOSPHATE AND NITRATE TRANSPORTERS IN FOXTAIL MILLET (*SETARIA ITALICA* L.)

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Foxtail millet is being studied as a model crop for cereals owing to its adaptability to nutrient-poor soils. We previously found a smaller root system with an enlarged root diameter in foxtail millet that facilitates nutrient transport under nitrogen limitation. How foxtail millet responds to phosphate limitation (LP) remains unaddressed. In this study, LP seedlings of the sequenced variety Yugu1 were grown under hydroponic culture. LP plants had significantly lower P concentrations and displayed higher levels of anthocyanin accumulation in leaves. A more extensive root system was developed primarily via stimulation of lateral root proliferation regarding the number, density, and length in foxtail millet under P limitations. Preferential biomass accumulation in the root under LP ensured carbon provision for root expansion and resulted in significant increases in the total and specific root length, which substantially extended the absorptive surface of P in the growth medium. Elevation of auxin and gibberellin concentrations might serve as an internal boost supporting root architectural re-patterning under LP. Not a just morphological adaptation, up-regulation of expression of *SiPHT1;1* and *SiPHT1;4* in roots and that of *SiPHT1;2* in roots and shoots preconditioned adaptive enhancement of P uptake and translocation under LP. By a more extensive root system of LP plants, internal nitrogen surpluses occurred as indicated by higher concentrations of nitrogen in roots and dramatic increases in free amino acids in shoots and roots. Such nitrogen surplus 'signals' tended to switch down expression of nitrate transporters *SiNRT2.1* and *SiNAR2.1* in the root and that of *SiNRT1.11* and *SiNRT1.12* in the shoot to reduce nitrate mobilization towards or within the shoot. Together, our work provided new insights into the adaption of a critical cereal crop to LP and its innate connections with nitrogen.

## Biography

Zeeshan Ahmad is a PhD Scholar under CSC Scholarship in The Key Laboratory of Plant-Soil Interactions, MOE; Department of Plant Nutrition, China Agricultural University, Beijing, China. His primary research work is on foxtail millet (*Setaria italica* L.). How it responds to nutrient limitations especially Nitrogen and Phosphorus. He has published two papers in *Frontiers in Plant science*. He is also researching maize (mutant lines) response to LP at vegetative stage under hydroponic conditions. He has also published one paper in *Pakistan Journal of Agricultural Sciences* while working with rice (*Oryza sativa* L. response to Potassium. Xuexian Li is his supervisor during his PhD research.

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# EVALUATION OF FIFTH FUNCTIONAL TRAITS OF NEW DUAL PURPOSE FLAX GENOTYPES IN COMPARISON TO STANDARD VARIETIES

**Katarzyna Wielgusz, Marcin Praczyk**

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**A** comparative experiment of two dual purpose flax breeding lines with selected standard varieties were conducted. The aim of the study was to assess the economic value of genotypes obtained as a result of breeding conducted at the Institute of Natural Fibres and Medicinal Plants in Poznań, Poland.

Dual purpose varieties, which combine the traits of fiber flax and linseed in a single genotype, make it possible to increase the economic viability of flax cultivation. The initial variety in these cultivars, depending on the main purpose, may be either oily forms in which the straw and fiber yield is increased, or fibrous forms in which the seed yields are to be significantly increased.

In the presented experiment, two breeding lines of dual purpose flax (R-3/59/10 and R-26/62/10), as well as linseed varieties: Bukoz, Szafir, Jantarol and Amon were evaluated in terms of plant height, total yield, straw yield, seed yield, thousand seeds weight and *fusarium* resistance. The assessment of resistance was carried out in a field where 5 species of fungi of the genus *fusarium* were introduced to the ground before sowing. These were the most common species causing plant infestation (*fusarium* wilt).

The obtained results showed a high value of the R-3/59/10 line in terms of all tested functional traits, in the context of its use as a dual purpose form. Further breeding work will therefore refer to the registration of line R-3/59/10 as a new variety of cultivated flax.

## Biography

Dr Katarzyna Wielgusz. Master of Biological Sciences, doctor of agricultural sciences, field: gardening, specialty: phytopathology. The PhD thesis on: Effect of Biopreparations and Substances on Decrease in Occurrence of Flax Fusarium wilt (*Fusarium oxysporum* Schlecht.f. sp. lini Bolley). Deputy head at the Department of Breeding and Agriculture Technology for Fibrous and Energy Plants. She leads the work on non-chemical methods of plant protection. Among others she has been involved in several projects on organic crop growing, technologies of non-food crops growing and processing for sustainable development. She has been working on EU Framework Projects: COST Action 628, 4FCrops, Crops2Industry, FIBR, Norwegian Grant SiliSol and several national projects. Author and co-author of 40 publications, 1 patent.

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## Applied Science, Biofuels & Petroleum Engineering

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# MALARIA AND HEPATITIS-B CO-INFECTION IN RELATION TO SELECTED HAEMATOLOGICAL PARAMETERS AMONG ATTENDEES OF TWO HEALTH FACILITIES IN PORT HARCOURT, NIGERIA

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**A** cross-sectional study was conducted among subjects from modern primary health centre (MPHC), Eneka and Braithwaite Memorial Specialist Hospital (BMSH) in Port Harcourt, to determine malaria and hepatitis-B co-infection in relation to selected haematological parameters. 700 subjects of different ages and both sexes were included in the study after ethical approval was obtained from Rivers State Ministry of Health, Port Harcourt. Consent forms were issued to get subjects consent before questionnaires to obtain their demographic data. The uninfected subjects were used as control. Four milliliters (4 ml) of blood was taken from each subject by vein-puncture; 2 ml each was dispensed into EDTA and plain bottles for analysis. The samples were used to assay for full blood count (FBC), hepatitis-B surface antigen (HBsAg), hepatitis-B envelop antibody (HBeAb), hepatitis-B core antibody (HBcAb) and hepatitis-B virus (HBV) markers using standard techniques whereas EDTA bottled blood was for malaria parasite identification using Giemsa staining technique. The overall prevalence rates of malaria (27.0%), HBV (5.1%) and co-infection (1.9%) in Port Harcourt were as stated. The females have higher prevalence of malaria (16.1%) than the males (10.9%) while the males have higher prevalence of HBV (3.0%) and co-infection (1.0%) than the female (2.1%) and (0.9%) respectively; although not statistically significant ( $P \geq 0.05$ ). The prevalence of malaria was statistically highest among children 47.7% (6-11) and 48.4% (0-5) years ( $P \leq 0.05$ ). HBV was highest among subjects of age brackets 8.2% (24-29 years) and 9.2% (30-35 years) accordingly. The packed cell volume was significantly affected by malaria ( $P \leq 0.05$ ) but neither hepatitis-B nor co-infection ( $P \geq 0.05$ ). The HBV markers result showed that while HBsAg occurred among all subjects that were positive for HBV, HBeAb was completely absent; this is an indication of an on-going or previous infection with hepatitis-B virus.

## Biography

Wokem G N has obtained her PhD from University of Port Harcourt, Nigeria and postdoctoral studies from Federal Medical Laboratory Science School, Nigeria. She is an Associated Professor of Parasitology and Public Health, a licensed Histopathologist with Medical Laboratory Science Council of Nigeria (MLSCN), the South Zonal Coordinator of Parasitology and Public Health of Nigeria (PPSN) and Formal Head, Department of Medical Laboratory Science, Rivers State University, Nigeria. She has presented papers in many national and international conferences with more than 50 papers published in reputed journals and have been serving as an Editorial Board Member of repute.

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# CONTAMINATION OF SOILS IN PUBLIC AREAS OF YEREVAN BY EGGS AND SPORES OF PARASITES

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The aim of our work was to study the soil cover in public places of Yerevan for the contamination of eggs and spores of parasites which are dangerous for health of animals and people and to give a veterinary and sanitary assessment. This full-scale sanitary research of the soil in Yerevan was first held in 2015-2016. The study was subjected to soil samples taken from 28 urban parks and squares belonging to 12 administrative districts. Studies of soil samples was carried out at research center for soil science, agro chemistry and melioration named after H Petrosyan, as well as in the laboratory of parasitic studies of the Department of Epizootology and Parasitology of the National Agrarian University of Armenia. The same samples were examined in a comparative manner by two methods: Romanenko and the method, used to detect helminth eggs, ticks and intestinal protozoa spores in soil. As a result of studies conducted during 2015-2016, eggs of the following helminths were found in soil samples, taken from different administrative districts of Yerevan: nematodes: *Toxocara canis*, *Toxascaris leonina*, *Trichocephalus vulpis*, *Uncinaria stenocephala*, *Ancylostoma caninum*; cestodes: *Dipylidium caninum*, *Taenia Hydatigena*, *Diphylobotrium latum*; as well as spores of parasitic protozoa *Neospora caninum*, *Cystoisospora Canis*. Eggs of nematodes of species *Ancylostoma duodenale*, *Ascaris lumbricoides*, *Trichocephalus trichiurus*, and of the simplest spores *Entamoeba histolytica* and *Balantidium coli* were found from pathogens inherent only to humans. It should be noted that the conditions necessary for the development of cestodes *Diphylibothrium latum* in our country are absent. Soil contamination of 12 administrative districts of Yerevan with eggs and spores of parasites is caused by the increase in the number of domestic animals in the city, the lack of planned preventive measures in the city and the corresponding propaganda among the urban population.

## Biography

Hovhannes Naghashyan from 2009 to present is Head of Department of Epizootology and Parasitology of National agrarian University of Armenia. In 2002 was Awarded the title of Professor of veterinary medicine. 1994 - Awarded the degree of doctor of veterinary Sciences. 1982 - Institute of pharmacology and toxicology (Ukraine) Awarded the degree of doctor of biology. 1968 – 1974 - Veterinary faculty of Yerevan Zooveterinary. 2001-2009 – Professor of Department of Epizootology and Parasitology of State agrarian University of Armenia.

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# POLYCAPROLACTONE (PCL) BASED SYNTHETIC BIOPOLYMERS FOR MODERN SCAFFOLD-BASED TISSUE ENGINEERING

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**W**ith the progress of technological advancement, biopolymers have drawn great attention in the modern regenerative therapy. Scaffold-based tissue engineering approach is truly promising in repairing and/or regenerating diseased/damaged tissues/organs. Natural biopolymers mimic the properties of natural extracellular matrices (ECM) of tissues. However, the natural biopolymers inherit a number of limitations that include large batch-to-batch variation, limited resources, poor mechanical performance etc. Synthetic biopolymers overcome these limitations offering a range of benefits such as enormous availability, high processability, controllable biomechanical properties and so on. Because of favourable viscoelastic property and low melting temperature, PCL has been one of the most contributing biopolymers to the field of tissue engineering. In this study, a set of polycaprolactone (PCL)-based biopolymers were employed to develop a scaffold family to cater for various tissue engineering applications as per necessity. The scaffolds were fabricated using a customized desktop robot based rapid prototyping (DRBRP) technique. Morphological and mechano-chemical characterizations were performed using scanning electron microscope (SEM) and in vitro degradation test, respectively. The biocompatibilities of the fabricated scaffolds were also tested via cell culture study. The results demonstrated great potential of the PCL-based synthetic biopolymers for advanced scaffold-based tissue engineering therapy.

## Biography

Md Enamul Hoque is a Professor in the Department of Biomedical Engineering at the Military Institute of Science and Technology (MIST), Dhaka, Bangladesh. Previously, he served a number of key positions in some other global universities prior to joining MIST including Head of Department, Biomedical Engineering at King Faisal University (KFU), Saudi Arabia and also Founding Head of Bioengineering Division, Department of Mechanical, Materials and Manufacturing Engineering at the University of Nottingham Malaysia Campus (UNMC). He received his PhD from the National University of Singapore (NUS), Singapore in 2007. He also obtained his PGCHE (Post Graduate Certificate in Higher Education) from the University of Nottingham, UK in 2015. He is a Chartered Engineer (C Eng) certified by the Engineering Council, UK and Fellow of Higher Education Academy (FHEA), UK. So far, he has authored three books, edited three books and co-authored 19 book chapters. He has also published around 160 technical papers in referred journals and international conference proceedings. His publications have attracted about 900 citations. His major research interests include the areas of biomaterials, bio-composites, tissue engineering, stem cells, rapid prototyping technology, nanotechnology, nanomaterials.

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Abstracts



EuroSciCon Joint Events on

## Plant Science, Tissue Engineering and Parasitology

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# THE CONSTRUCTION AND CLINICAL APPLICATION OF TISSUE ENGINEERED BONE

**Guoxian Pei**

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**T**issue engineering construct has already been used to repair some kinds of bone defect in clinical patients, but repairing massive segmental bony defect in tibia more than 10 cm still has not been reported. We describe a case of patient, who received a tissue engineered bone graft for repairing the 12 cm bone defect in his right tibia and report the findings after 36 months of follow-up. The recipient, a 35-year-old man from China, had his leg severely injured in Sep' 2014 and got massive tibia defect after one year of treatment using external fixator. Implantation of tissue engineered bone graft was done in Aug' 2015. A  $\beta$ -tricalcium phosphate ( $\beta$ -B-TCP) scaffold was custom-made according to the shape of the bone defect area. The patient got bone marrow aspiration and 15 ml bone marrow was used for isolation and proliferation to get enough autologous bone menchymal stem cells (BMSCs) with serum-free stem cell medium to avoid immune rejection.  $3.4 \times 10^6$  cells were seeded onto the  $\beta$ -B-TCP scaffold and then implanted into the bone defect area after two weeks of co-culture. Laboratory blood examination was used to observe the immune rejection or infection. The radiography and three-dimensional computed tomography (CT) were used to detect the bone repair effect. No major complications and no obvious immune rejection or infection occurred after the surgery. After 3, 6, 12, 24 and 36 months, radiography showed bone defect gradually repaired with time, and bone repair effect was satisfactory. The patient was allowed to gradually regain limb function after 12 months. After 36 months, the patient recovered a full function of the lower extremity without any support. Our promising results suggest the clinical safety and effectiveness of tissue engineered bone for repairing massive weight-bearing tibia bone defect more than 10 cm and our treatment procedure might be an option for those patients with weight-bearing massive bone defect.

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# A NOVEL NATURAL AND PATIENT-DERIVED BIOMATERIAL FABRICATED USING THE POLYPHENOL OLEUROPEIN AND THE AUTOLOGOUS BLOOD-DERIVED PLASMA FOR POTENTIAL APPLICATIONS IN BONE TISSUE ENGINEERING

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To provide a bioactive and biocompatible environment for new bone formation, several materials have been used to mimic the bone-forming components. In this context, we examined the feasibility of the natural polyphenol oleuropein as a cross-linking agent for fabricating a polymerizable biomaterial for potential applications in bone tissue engineering. Oleuropein is a natural-occurring iridoid glycoside especially found in the leaves and fruits of the olive tree (*Olea europaea*). It is well known for its antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory activities. More importantly, it has also been shown that oleuropein is able to induce bone formation and inhibits bone resorption as well as bone tissue demineralization. Interestingly, the conversion of oleuropein glucoside to its aglycone by the enzyme  $\beta$ -glucosidase exhibits interesting cross-linking properties which make it a suitable cross-linking agent for biomedical applications. Here, the autologous patients' derived-plasma and aglycone oleuropein were used to fabricate a novel biomaterial. We invented a novel formulation in which autologous patients derived plasma is used as protein substrate that is able to polymerize when is mixed with the aglycone oleuropein. By using L929 mouse fibroblasts and dental pulp stem cells (DPSCs), we demonstrated that this biopolymer is nontoxic and biocompatible. Importantly, plasma-derived biomaterial contains biochemical signals necessary for tissue repair and regeneration and can be easily generated from patient's autologous blood without the potential risk of foreign body reaction or infection. Although this biomaterial shows interesting potentiality, several investigations are required to elucidate the potential of this novel biomaterial for bone tissue engineering applications.

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# ADVANCING FRONTIERS IN 3D BONE BIOPRINTING

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**T**hree-dimensional (3D) bioprinting of cell-laden biomaterials has been used to fabricate tissue-engineered constructs to mimic the anatomical complexity and precise deposition of different types of cells. Bioinks used in bone bioprinting include water-swollen hydrogels loaded with bioactive ceramics and cell and growth factors. These materials facilitate the production of mineralized extracellular matrix (ECM). A critical summary of the recent advances made with various types of bioink used for bone bioprinting will be presented. Major challenges, such as the vascularity, clinically relevant size and mechanical properties of 3D printed structure that must be addressed to successfully use the technology in a clinical setting are discussed. Emerging approaches to address these problems are reviewed, and future strategies to design biomimetic 3D printed structures are proposed.

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# MULTIMATERIAL AND MULTISCALE BIOFABRICATION APPROACH TO REPRODUCE THE 3D COMPLEXITY OF NATURAL TISSUE

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**A** biological tissue is a composite material with bottom-up hierarchical structure that is closely related to its heterogeneous function. The extracellular matrix modulates biochemical and biophysical signalling and its rigidity is an important micro-environmental parameter that regulates the spatiotemporal dynamics of intercellular signalling. For this reason, many studies are focused on fabricating scaffolds processed at multiple scales with structural and mechanical properties that are optimal for eliciting specific response or mimic those found naturally. These scaffolds have to present large surface areas that have appropriate topology and biochemical cues (e.g. ligands) at the nanoscale for tissue adhesion, while also exhibiting integral porosity to allow for the exchange of molecules that maintain cellular function. In this talk, the use of a multiscale and multimaterial process will be presented to develop 3D *in vitro* model that can mimic the 3D complexity of natural tissue. These novel 3D *in vitro* models can be used for the study of physio-pathological condition and for the analysis of effects on cell activities of different biomolecule and/or drugs.

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# IMPLEMENTATION OF CHITOSAN BASED HYDROGEL FOR CARTILAGE REPAIR

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**C**artilage disease and injury are major health issues: osteoarthritis alone afflicts approximately 8.5 million people in UK. Regardless the magnitude of the problem, though, clinical success has been limited partly due to the fact that the mechanical conditions of the tissue *in vivo* do not provide a favourable environment for the regeneration of new cartilage in the defective site. Consequently, an integer mechanical and biochemical environment should be inserted on the diseased area to stimulate the growth of the tissue. This could be achieved via the use of a thermosensitive hydrogel that could gel by a change in temperature, thus giving the possibility of producing the hydrogel under injection, which is a minimal invasive administration. The present study concentrated on the development and dynamic characterization of a novel hydrogel composed of chitosan and  $\beta$ -glycerophosphate ( $\beta$ -GP) for the replacement and repair of diseased articular cartilage. The gelling parameters of the composition were optimized towards improving the hydrogel's viscosity, while the mechanical properties were evaluated in terms of rheology (viscosity flow curves and flow behaviour), sensitivity to temperature changes, storage and loss moduli via the use of Anton Paar Physica MCR 301 and TA Instruments DSC Q2000.

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# NOVEL ULTRASHORT SELF-ASSEMBLING PEPTIDE BIOINKS FOR 3D CULTURE OF MUSCLE MYOBLAST CELLS

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**T**he ability of skeletal muscle to self-repair after a traumatic injury, tumour ablation or muscular disease is slow; limited, and declines steeply with age. Tissue engineering of functional skeletal muscle using 3D bioprinting technology is promising for creating tissue constructs that repair and promote regeneration of damaged tissue. Hydrogel scaffolds used as biomaterials for skeletal muscle tissue engineering can provide chemical, physical and mechanical cues to the cells in three dimensions thus promoting regeneration. Herein, we have developed two synthetically designed novel tetramer peptide biomaterials. These peptides are self-assembling into a nanofibrous 3D network, entrapping 99.9% water and mimicking the native collagen of an extracellular matrix. Different biocompatibility assays including MTT, 3D cell viability assay, cytotoxicity assay and live-dead assay confirmed the biocompatibility of these peptide hydrogels for mouse myoblast cells (C2C12). Immunofluorescence analysis of cell-laden hydrogels revealed that the proliferation of C2C12 cells was well-aligned in the peptide hydrogels compared to the alginate gelatin control. These results indicate that these peptide hydrogels are suitable for skeletal muscle tissue engineering. Finally, we tested the printability of the peptide bioinks using a commercially available 3D bioprinter. The ability to print these hydrogels will enable future development of 3D bioprinted scaffolds containing skeletal muscle myoblasts for tissue engineering applications.

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# CHARACTERISATION OF *ASPERGILLUS FLAVUS* ISOLATED FROM MAIZE

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**A***sperrgillus flavus* is the main producer of carcinogenic aflatoxins in agricultural commodities such as maize. This fungus produces aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and aflatoxin B<sub>2</sub> (AFB<sub>2</sub>), being the most relevant in crops and this can result in economic losses. The aim of this study was to investigate four strains of *A. flavus* field for the production of aflatoxin B<sub>1</sub> and aflatoxin B<sub>2</sub>. The strains: 3909, 3911, 3951 and 3955 are isolated from Lydenburg in Mpumalanga were morphologically identified at ARC-Plant Protection Research Institute and were further characterised by Polymerase Chain Reaction (PCR) and Sanger sequencing of the internal transcriber subunit regions: ITS-5.8-ITS2. The strains were analysed for the presence of genes encoding AFB<sub>1</sub>, targeting both regulatory (*aflR*, *aflS*) and structural genes (*aflD*, *aflM*, *aflO*, *aflP* and *aflQ*). To determine the actual production of aflatoxin B<sub>1</sub> and B<sub>2</sub> of the four strains, a reverse high performance liquid chromatographic (HPLC) instrument was used. All the four strains amplified 600bp of ITS-5.8-ITS2 rDNA region. Similarly, all of seven genes for aflotoxin B1 were detected in four strains with expected band sizes. Aflatoxin production was present in strain 3911 and 3955 for AFB<sub>1</sub> and AFB<sub>2</sub> and in strain 3951 only AFB<sub>1</sub> while strain 3909 revealed negative aflatoxin (AFB<sub>1</sub> and AFB<sub>2</sub>) production. The results may contribute to development of reliable molecular techniques for detection of aflatoxigenicity as well as illustrating the complexity of local fungal communities associated with maize.

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# EVALUATION OF GENETIC DIVERSITY AMONG MAIZE GENOTYPES USING SSR MARKERS

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**M**aize has become a significant crop all over the world owing to its potential for fulfilling food demand of people. Present study was carried out to evaluate genetic variations among maize genotypes through SSR markers. For genetic diversity analysis 15 genotypes of maize collected from different research institutes of Punjab Pakistan. Several growth and quality parameters were also examined such as fresh root weight, fresh shoot weight, dry shoot weight, dry root weight, crude protein, crude fat, ash content, acid detergent fiber and neutral detergent fiber. On the basis of reproducibility and high PIC value, 22 SSR primers were selected for DNA finger printing of all collected genotypes. Binary method was used for band scoring. The polymorphism information content (PIC) value of primers was calculated to estimate extent of genetic differences. It was calculated that polymorphism information content ranged from 0.1 to 0.8. Only three markers umc1122, umc1568 and umc1988 provided highly polymorphic values which were 0.8, 0.5 and 0.6 respectively. Unweighted Paired Group Method using Arithmetic Averages (UPGMA) cluster analysis has grouped the genotypes into three major clusters. Cluster analysis revealed that maximum genetic distance was found 3.16 between Sgd 2002 and Golden, while minimum genetic distance was observed 1.414 between Fsd maize and Neelam. The identified clusters may find useful to plan crossing program in maize breeding. This study concluded SSR as a good source for genetic characterization. It was cleared from phylogenetic analysis that genetic diversity among above mentioned genotypes was limited since all these genotypes were collected from nearby areas in province of Punjab and there were no exotic genotype. On the other hand, data recorded for growth and quality parameters subjected to statistical analysis and results showed all maize genotypes had considerable genetic differences for all growth and quality traits and maximum genetic variability was examined for fresh shoot weight, fresh root weight, dry shoot weight, dry root weight, crude protein, crude fat, acid detergent fiber (ADF), neutral detergent fiber (NDF) and ash content in Sgd 2002 (13.5g), Agaiti 72 (18g), Fsd maize 2018 (6.8g), Agaiti 72 (8.7g), EY 1098 (5.1g), YHM (1.9g), Pearl maize (30g), Agaiti 2002 (49g) and MS 2010 (30g) respectively. These findings of genetic diversity analysis among maize genotypes with the help of SSR markers as well as on basis of growth and quality traits may be consider for further evaluation and selection as parents in breeding programmes.

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# DEVELOPMENT OF A NOVEL VEGETABLE FARMING CHAMBER UTILIZING ZERO-WATER COOLING AND NATURAL LIGHTING

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Typical greenhouses are based on collecting maximum amount of light and eventually maximum heat accumulate inside and enormous cooling power is needed to optimize the microclimate especially under middle Eastern dry hot environment. Fadel et al (2013) have concluded that the water used in greenhouse cooling equals the amount of water consumed in the irrigation of organic tomatoes under UAE local conditions. Since the climatic conditions of this part of the world comprise an excessive amount of light and heat most of the year, a novel growth room is developed and tested under local conditions utilizing ground heating/cooling effect where the GHE was laid at 2.5 m depth. To eliminate using precious water in microclimatic cooling, the concept focused on blocking both light and heat where the growth room was made of insulated 10 cm thick sandwich panel equipped with two large automatically controlled windows to allow ambient air and light in when the surrounding conditions are within the set range of both temperature and humidity levels. A fuzzy logic controller was designed to control linear actuators to open the windows and in-line cooling fans to draw air into the growth room. To minimize energy consumption, natural light is collected using a sun tracking solar collector and transmit sunlight via fibre optics cables into the room while if the light is not enough additional LED is used and controlled by the Arduino microcontroller. Results showed that the controller can maintain the greenhouse temperature on most days of the year utilizing the combination of partially closed windows and cooling fans with zero water cooling at all. However, in summer the controller was working in pre-cooling mode and further cooling unit is needed to reduce the greenhouse temperature by about 6.8 °C amplitude on average compared to 10.8 °C when the GHE is not utilized.

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# POLLINATORS IN PARADISE: CONSERVATION OF THE ENDANGERED HAWAIIAN YELLOW-FACED BEES

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In Oct' 2016, seven species of Hawaiian yellow-faced bees became federally protected under the Endangered Species Act. Endemic *Hylaeus* (yellow-faced bees) are pollinators which have coevolved with native plants and support native Hawaii ecosystems functions since before humans arrived on the islands. Plants which had coevolved with these endemic Hawaiian bees and depend on the bee for pollination are also endangered. The habitats which the bee and plants depend on are becoming unsuitable due to erosion, pollution, development, and fragmentation. *Hylaeus anthracinus* were the only native yellow-faced bee species found in significant numbers on Oahu during recent targeted surveys and during this study. Although once widespread, *H. anthracinus* are now apparently restricted to small fragments of endangered, coastal habitat on Oahu. Immature *H. anthracinus* were reared from egg to healthy adult in a controlled setting. Data gathered through nest dissections and immature rearing facilitated the design of artificial nest sites. Artificial nest sites were developed, established and monitored weekly. Artificial sites were successfully nested in by *H. anthracinus* and were tested as conservation tools. *Hylaeus anthracinus* nesting and foraging ecology observations were described as well as opportunities these tools may hold for additional pollinator conservation management. Invasions by ants were quickly identified as a primary reason for the bees decline. The nests in artificial nest sites were protected with a non-toxic, sticky barrier (Tanglefoot) that ants were unable to cross if applied correctly and reapplied often. A citizen science project is being developed to spread awareness of this endangered native pollinator and enlist the help of the Hawaiian community and visitors to Hawaii in conservation efforts.

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# ETHNOBOTANICAL USES OF FLORA OF SATHAN GALLI DISTRICT MANSEHRA, KP, PAKISTAN

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**T**his study was designed keeping in view the greatest importance of the study area in context of its plant biodiversity and location. The study area falls in Western Himalaya show rich floristic diversity. This is first ever ethnomedicinal exploration from the investigated area. Area was visited frequently during 2014 to 2015 to collect medicinal flora of the study area. Semi-structured questionnaire method was used to gather ethnobotanical information from each site. Information about the local uses of the plants such as medicinal, timber, fodder and fuel wood etc. were got through random sampling by interviewing 300 individuals including nomads, local inhabitants from different age group and gender. Separate questionnaire was completed from plant harvester, retailers, seniors, plant merchants and local therapists (Hakims). The data was gathered and analysed by using MS Excel, 2013. The study revealed that the indigenous peoples of the area exploited 86(51.19%) species as traditional medicinal plants, 136(80.95%) species for fodder, 48(28.57%) for fuel wood, 28(16.66%) for timber woods, 07(4.16%) for wild vegetable and 02(1.19%) for ethno-veterinary therapies. Similarly, 17(10.11%) species are for wild edible fruits, 2(1.19%) species for making agricultural tools, 1(0.59%) species for fencing field borders. It was observed that local peoples used plant species for curing different ailments such as toothache, backache, headache, body pain, abdominal pain, rheumatism, indigestion, wound healer, cough, expectorant and tonic. These plants species as a whole or its parts are used to make remedy for treatment of diseases at home. Community of the study area is poor and those living in the isolated partition depend on the use of therapeutic plants and prepared crude drugs in form of juice, paste, infusion, decoction, water extract and powder pills.

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# FLOWER CROPS: A POTENTIAL SOURCE OF NUTRACEUTICAL COMPOUNDS

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Flower crops are considered as rich source of various nutraceutical compounds that are being commercially exploited worldwide. These bioactive compounds play a significant role in plant as well as human health. Polyphenols, flavonols, pigments etc. are the important bioactive compounds that are found in most of the plant species. Among them, plant pigments holds the prime position as they serve many functions in plants such as they provide protection from temperature, UV radiation, low water potential etc. Moreover, these pigments also possess antioxidant properties. Nowadays, synthetic pigments dominate the market world over, however due to increase awareness among people about the ill effects of these synthetic pigments on human health and environment, focus is oriented towards maximum utilization of natural products as they are extracted from natural sources, which are pure and safe for human use and more importantly ecofriendly in nature. Plant pigments are classified into four major classes viz. anthocyanins, carotenoids, betalains and chlorophylls. Anthocyanins are flavonoid pigments which are responsible for imparting red, purple, pink and blue colouration to the plants. In flower, rose petals are rich source of anthocyanins and wide diversity of anthocyanins is found in roses. Petunia is another ornamental crop rich in anthocyanin pigments. Carotenoids are fat soluble pigments which imparts yellow, orange and red colours in plants and animals. Marigold is also one of the richest source of carotenoids especially lutein which possess antioxidant activities and moreover intake of lutein helps in curing age related macular diseases. Carotenoids also find its commercial applications in food colouring industries. Another group of pigments, betalains which is sub divided into two sub-groups, betacyanin responsible for red-violet colouration and betaxanthin imparts yellow to orange pigments. Bougainvillea is also one of rich source of betalains and floral bracts are the economic part. The Division of Floriculture and Landscaping, ICAR-IARI has initiated research work on characterization of major pigments i.e. anthocyanins in rose, petunia and chrysanthemum; carotenoids in marigold and chrysanthemum and betalains in bougainvillea. The estimation of antioxidant properties in these crops has also been done. Standardization of the drying methods for higher retention of pigments has been done in marigold and bougainvillea. Moreover, histological studies of rose genotypes have been done.

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# MORPHOLOGICAL CHARACTERIZATION AND PLANT BASED CONTROL OF LERNAEA CYPRINACEA COPEPOD FISH PARASITE

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**L**ernaea, a copepod parasite commonly known as (Anchor worm) contamination is a major sickness issue experienced in fish culture in the Indian subcontinent. Different developmental stages in parasites and numerous associated impacts of full scale and small scale, environment impact the parasitic fauna of fish. The present study reveals the external morphology and control the highly infested copepod parasite *Lernaea cyprinacea* which have contributed disease problems to the aquaculture industry in Pakistan. The external morphometric parameters i.e. full body length, length of cephalothorax, length of thorax region, length of abdomen, length of dorsal and ventral head region, length of dorsal and ventral anchors, length of dorsal anterior and dorsal posterior anchor, length of 1st and 2nd maxillae, length mandible, length of dorsal and ventral antennule were measured and statistically analysed for mean, standard deviation, standard error, coefficient of variability, confidence interval (95%) and analysis of variance. To determine anti-copepods potential of ethanol extracts of leaves of *Carica pappaya*, *Eucllyptus camaldulensis* and *Grevillea robusta*, 10 infected fish with three average copepod parasites were placed in each glass aquarium treated with different concentrations (10 ppm, 25 ppm, 50 ppm). The survival percentage of *L. cyprinacea* copepod was observed after 6 hrs, 12 hrs, 24 hrs, 36 hrs, 48 hrs, 60 hrs and 72 hrs. The results were analysed by using one way Anova and Tukey test. Among all extracts bioassays, the maximum activity was observed in extracts of *Grevillea robusta*, *Carica pappaya*, *Eucllyptus camaldulensis*. To estimate the toxicity level of each plant, extract was calculated by determining LC<sub>50</sub> and LC<sub>90</sub>. To estimate the protein (mg/g), carbohydrate (mg/g) and lipid mg/dl (cholesterol) level in effected copepods were estimated by Lowry's method, phenol sulphuric acid method by biochemistry analyser. Protein, carbohydrate and lipid contents of copepod parasite were decreased as compared to control.

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# EFFECTS OF KUDOA ANATOLICA (CNIDARIA: MYXOZOA) ON THE MEAT QUALITY OF ATHERINA HEPSETUS (ATHERINIDAE)

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Some species of the myxozoan genus *Kudoa* are of significant concern to marine aquaculture due to their negative impacts in the musculature and meat quality of fish hosts. Recent studies showed that some species also pose a threat to human health when ingested in raw fish. *Kudoa anatolica* has recently been described from several organs including muscles of Mediterranean sand smelt *Atherina hepsetus* collected from Sinop coasts of the Black Sea and this study focused on its effects on the meat quality of host fish. In this research study, fish were divided into four groups according to infection intensities of *K. anatolica* in fish muscles, uninfected (A), slightly infected (B), moderately infected (C), highly infected (D), and the crude protein, crude lipid, crude ash, water, fatty acids and amino acids contents of each group were analysed. The crude protein, crude fat, crude ash and water content of uninfected fish meat (A) were 21.9, 2.3, 2.6 and 73.1% respectively. Total crude protein and crude lipid contents of all infected groups (B, C, D) of fish were less than that of uninfected group (A). Total amino acid and essential amino acid levels were determined at their highest values in uninfected group (A) and statistically significant decreases were found in the amount of both amino acids as the infection rate increased from B to D ( $p < 0.05$ ). Of the amino acids determined in fish meat, alanine, arginine, glutamic acid, histidine, proline and serine contents of uninfected group (A) were higher than those highly infected group (D). The amount of SFA and PUFA in group A were higher than those the infected groups B, C and D. The ratio of omega-6/omega-3 of uninfected group (A) was determined as 0.45 and decreased in the infected groups (B, C, D). We can conclude that increasing *Kudoa anatolica* infection levels decreased the nutritional composition of *Atherina hepsetus* meat and this is the first investigation proving negative impacts of a *Kudoa* species on the nutritional composition of its host fish.

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# HIGH MALARIA TRANSMISSION SUSTAINED BY ANOPHELES GAMBIAE S.L. AND STATUS OF KDR RESISTANCE MUTATION OF PLASMODIUM FALCIPARUM INFECTED SPECIMENS IN THE CITY OF YAOUNDÉ CAMEROON

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Urban malaria is becoming a major public health problem in Cameroon. In the frame of a larval control study due to be implemented in the city of Yaoundé, we conducted baseline surveys to assess malaria transmission dynamic in this city. Adult mosquitoes were collected indoor and outdoor using light traps of Center of Diseases Control and Human Landing Catches from Mar' 2017 to Mar' 2018 in 30 districts of the city. Mosquitoes were sorted by genus and identify up to the species level using polymerase chain reaction (PCR). TaqMan *Plasmodium* infection and ELISA CSP analysis were used to determine mosquito infection status. The detection of Knock down resistance (kdr) mutation was carried out on infected and non-infected *An. gambiae* s.l. using DNA-based molecular technique. A total of 218,991 mosquitoes were collected. The main malaria vectors were *An. gambiae* s.l. (n=6154) and *An. funestus* s.l. (n=229). PCR results (n=1622) revealed that 92.29% *An. coluzzii* and 7.71% *An. gambiae* s.s. constituted *An. gambiae* s.l. Amongst the 186 *An. funestus* s.l. analysed by PCR, 93.51% belonged to *An. funestus* s.s. and 2.16% to *An. leesonii*. The average biting rate of *An. gambiae* s.l. was higher outdoor than indoor (P=0.013). Seasonal variation in mosquito abundance and biting rate was recorded as well as variation according to districts. Furthermore, the *P. falciparum* infection rate was 2.15% and the annual entomological infection rate was estimated at 80.49 infective bites. There was no significant difference between the resistance allele frequencies of infected and non-infected females (P=0.49). Thus, the susceptibility of *An. gambiae* s.l. to *P. falciparum* was not affect by the presence of the resistance alleles at the kdr locus. The results provide evidence of increasing outdoor malaria risk in the city of Yaoundé and call for more actions to improve control strategies in this city.

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# MESENCHYMAL STEM CELL AS A CURE FOR BRAIN DAMAGE INDUCED BY CHRONIC TOXOCARA CANIS INFECTION IN AN EXPERIMENTAL MOUSE MODEL

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**H**uman toxocariasis is an important worldwide soil-transmitted zoonotic disease. Neurotoxocariasis is a serious condition that is linked to reduced cognitive function, behavioural alterations and neurodegenerative diseases. Unfortunately, the available drugs for treatment of toxocariasis are with variable results. Mesenchymal stem cells (MSCs) have been used in animal models and clinical trials of tissue injuries and it gave promising therapeutic results. Therefore, this study was designed using 40 *T. canis*-infected albino mice (1000 eggs/mouse, orally) and an additional control group (GI) of 10 healthy mice. The infected mice were divided into four groups (n=10). GII was the infected non-treated group (infected control), GIII was treated with albendazole at a dose of 100 mg/kg/d once orally for 5 successive days, GIV was treated with bone marrow derived MSCs at a dose of 3x10<sup>6</sup> MSCs in 0.1 mL of PBS via the tail vein, and GV was treated with albendazole + MSCs. Treatment was commenced 6 weeks p.i. and the experiment was terminated four weeks after administration of the last doses of the tested drugs. The brain tissue of each mouse was subjected for histopathological, immunohistochemical studies (caspase-3, TGF- $\beta$ ), detection of *T. canis* DNA by real-time PCR and gene expression the biomarkers of brain damage (S100B, GFAP) by RT-PCR. Moreover, homing of iron oxide-labelled MSCs in brain tissues was assessed by Prussian blue stain. The brain tissues of GII showed numerous *T. canis* larvae, significant congestion, thickening of arterioles, inflammatory infiltrate and gliosis associated with marked immunohistochemical expression of TGF- $\beta$  and caspase-3 as well as marked S100B and GFAP gene expression. Significant improvements of the previous parameters and *T. canis* DNA were recorded in all the treated groups. However, the best results were obtained with combined albendazole + MSCs therapy. Thus, MSCs could be considered in the treatment of chronic neurotoxocariasis.

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# EFFECT OF DEFORESTATION ON AVIAN PARASITICCO-INFECTIONS IN RECAPTURED BIRDS OF THE TALANGAYE RAINFOREST OF CAMEROON

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Although much is known about malaria, its transmission, its genomics, and drug interactions, relatively little is known about how rapid ecological changes affect the transmission of the disease in real time, particularly in co-infection scenarios with other parasitic infections. Given the socioeconomic patterns of malaria infection and other diseases in humans, the use of birds for the study of infectious diseases is advantageous because they represent natural populations that can be studied in both human-impacted environments and in pristine unaltered forests. In order to create a link between avian blood borne parasitic co-infections and deforestation, bird sampling was conducted in intact and degraded forest in real time by mist netting. We analyzed the prevalence variation and co-infections of four avian blood-borne parasite genera: *Plasmodium* spp, *Haemoproteus* spp, *Leucocytozoon* spp and *Trypanosoma* spp and the superfamily Filarioidea in all recaptured birds following deforestation using both microscopy and PCR techniques. After two years of sampling, a total of 1954 birds were caught belonging to 26 families and 67 species, 156 of which were recaptures. The most abundant birds recaptured were *Bleda notatus* (20.51%), *Alethe castanea* (18.59%) and *Stiphrornis erythrorhax* (8.97%). Only the *Plasmodium* genus prevalence varied significantly in the intact forest and it was highest in the fire-crested alethe (*Alethe castanea*). In fire-crested alethe and yellow-whiskered greenbul (*Eurillas latirostris*) microfilariae prevalence positively correlated with *Trypanosoma* prevalence. A negative microfilariae correlation with *Haemoproteus* prevalence and no correlation with *Plasmodium* prevalence were observed. Three morpho *Trypanosoma* species were reported (*T. everetti*, *T. anguiformis*, and *T. naviformis*). *Trypanosoma everetti* predominated of all *Trypanosoma* spp. and it was present in three avian hosts; olive sunbird (*Cyanomitra olivacea*), yellow-whiskered greenbul and fire-crested alethe. The results provide insight into the impacts of deforestation on co-infection and have implications for the study of infectious diseases in rapidly changing environments.

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# PREVALENCE AND RISK FACTORS FOR TOXOPLASMA GONDII INFECTION IN PREGNANT WOMEN FROM SINNAR STATE, SUDAN

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**T**his study accomplished during Aug' 2013 to Jul' 2014 to investigate the seroprevalence of *Toxoplasma gondii* among pregnant women at Sinnar state in Sudan. Serum samples were collected from 314 pregnant women and pretested questionnaire including risk factors administered to each patient. The serum samples were screened for anti-*T. gondii* specific IgG and IgM antibodies, using commercial detection kit. Out of 314 pregnant women, 145 (46.2%) [95% CI: 40.69-51.7] and 152 (48.4%) [95% CI: 146.47-157.53] were positive for IgG and IgM respectively, and the overall seropositivity was 222 (70.7%). There was very poor agreement between anti-*T. gondii* IgG and IgM results ( $\kappa=0.531$ ). Higher prevalence was observed in 21-30 years old group than other age groups, but there were no significant difference between age groups and IgG positivity. While significant association observed among IgM positivity and age group ( $P=0.048$ ). IgM positivity was significantly associated with cat ownership ( $P\text{-value}=0.006$ ) and also with history of eating unwashed vegetables ( $P=0.021$ ). The reactivity to IgG was in putative association with history of abortion ( $p=0.04$ ). The majority of pregnant women were secondary educated 113 (35.99%), educational level was significant  $p\text{-value}=0.000$ , 0.018 with both IgM and IgG respectively. The residence, trimester of pregnancy, eating raw meat, drinking unboiled milk, working as a peasant and dog ownership, these risk factors showed no statistically meaningful results  $p\text{-value}>0.05$ .

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